

Possible Phytoremediation of Chlor-alkali Waste by Using *Sesbania Aculeata*. Pers

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Abstract An attempt was made to decrease the toxic effect of waste soil from a Chlor-alkali factory by *Sesbania aculeata* pers. *Sesbania* is cultured in varying waste soil combinations accumulated an appreciable amount of mercury. The accumulation depends both on soil concentration and time. Alkaline pH of the waste soil combinations also decreased with time. After 35 days culture of *Sesbania*, a significant increase in the growth of rice *Oryza sativa* var IR 36 was reported over that of un-inoculated control set. It indicates that possible phytoremediation of chlor-alkali waste can be achieved with this plant.

Keywords Detoxification, Phytoremediation, Transplantation

1. Introduction

There is no gainsaying the fact that Industrialization is the key to economic growth and helps in raising the standard of living of the people. It is also hard to disagree with the fact that industries provide the society with services necessary to conduct daily life and to engage in productive activities. But the wastes of Industries are of the major non-utilized substance in many countries and these wastes contaminate the whole Biosphere. These wastes could not fully exploited due to non availability of viable technology as the viable technology must be cost effective, eco friendly and socially acceptable. The conventional processes used for waste treatment are precipitation, hydroxides/sulphides, oxidation/reduction, and ion exchange which are expansive and not eco friendly[1,2]. That is why a biological mean of pollution control is most favored today and Phytoremediation can be suggested as an effective method[3, 7] as the plant based technology is considered to be useful for cleaning up contaminated soils and waters because biological materials are cost effective, abundantly available, non toxic and biodegradable[5]. The heavy metal along with other pollutants can be accumulated biologically[6, 7] and subsequently transferred with biomagnifications in different tropic levels in food chain[8, 9].

Ganjam, situated at the bank of the river Rushikulya, about four kilometers from the point where it meets the Bay of Bengal perceived the threat of mercury pollution by the establishment of the M/S Jayashree Chemicals – a chlor-alkali factory, which releases effluent into the river, exhaust

air into the atmosphere through ventilators and the waste soil is dumped by the side of the effluent channel. Because the factory is in the coastal region, the dump waste is subjected to aerial dispersal during the summer. In monsoon, the dissolved salts, along with mercury, enter the nearby fields with runoff water and contaminate them. Some research has been conducted on mercury concentration in aquatic and terrestrial biota around chlor-alkali factories[10-12]. However reports pertaining to the accumulation and effects of mercury in crop plants are very few[13, 14] and Dash et al.[4] reported that the changes in the morphology of crop plants are induced by saturated solid factory extracts. Therefore, an attempt has been made in the present investigation to determine the possible phytoremediation by *Sesbania aculeata* pers. *Sesbania aculeata* pers is an erect, semi woody plant having 4-5ft height with nodulated root for fixation of nitrogen[15] and is known as a biofertiliser in some of the regions of Orissa state of India.

2. Materials and Methods

2.1. Analysis of the Waste Soil

The waste soil of the chlor-alkali factory is periodically removed and dumped as heaps by the side of the effluent channel without any chemical processing for removal of its pollutants along with heavy metal before it is released. The waste soils were collected in gunny bags and kept under air in field condition for 48 hours to reduce its moisture content. The dried soil was then powdered manually and stored for use in the experiment. The powdered soil was subject to analysis for various important constituents.

The grey colored soil was alkaline in nature with a pH of 9.20 ± 0.05 . It contained 0.95g of mercury per kg of waste

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soil as analysed by cold atomic absorption technique using a Mercury Analyzer. Model No. MA 5800A, Sodium content of the waste soil analyzed by Flame Photometer, It was 6 g per kg of the waste soil and chloride content was 18g per Kg. Potassium and phosphate contents of the waste soil were almost equivalent to that of a nutrient solution being 55 and 60 mg per kg of the waste soil respectively.

2.2. Preparation of Culture Pots

The dried and powdered waste soil was passed through a fine mesh and was used for the preparation of culture pots. Varying concentrations of the waste soil was prepared with normal garden soil (pH 6.44 ± 0.02) ranging from 10 per cent to 60 per cent at an interval of 10 per cent. Final weight of the soil combinations prepared was 4Kg. They were kept in earthen pots of equal size in triplicate to use as the medium to culture *Sesbania* seedlings. The pots were watered equally. The waste soil combinations and water were mixed thoroughly and allowed to settle. Seeds of *Sesbania* were sown in the pots containing varying contaminated waste soil combinations.

2.3. Analysis for Mercury

After 7, 14, 21, 28, 35 days of transplantation the *Sesbania* plants were removed, washed thoroughly, blotted and analyzed for mercury accumulation. The values obtained were subjected to statistical analysis [16] and the levels of significance were determined.

2.4. Analysis for Possible Detoxification

To assess the degree of detoxification seedlings of *Oryza sativa* var. IR 36 were transplanted in the waste soil combinations after the growth and decomposition of *Sesbania* (35 days). Two control sets containing varying waste soil combinations (freshly prepared) were also run for comparison along with the set containing varying waste soil combinations without *Sesbania* growth to study possible microbial activity due to native and contaminated microbes. Height and tiller number of the seedlings were studied at the time of maximum tillering stage.

3. Results

3.1. Mercury Accumulation by Root

Mercury accumulation by root of *Sesbania* seedlings was found to be dependent on both waste soil percentage and time (Table 1). Mercury accumulation was found to increase with increase in waste soil from 10 per cent to 60 per cent and also time period after transplantation from 7 days to 35 days. In the control set with only garden soil mercury accumulation was not reported. After 7 days transplantation of the *Sesbania* seedlings mercury uptake increased from $4.86 \pm 0.06 \mu\text{g g}^{-1}$ fresh weight in 10 percent to $13.85 \pm 0.05 \mu\text{g g}^{-1}$ fresh weights in 60 per cent waste soil combination. A highly significant correlation was obtained between waste

soil and mercury uptake by root ($r = 0.992$, $p < 0.01$). 14 days after transplantation of *Sesbania* seedlings, mercury accumulation increased from $7.11 \pm 0.11 \mu\text{g g}^{-1}$ in 10 percent to $18.27 \pm 0.07 \mu\text{g g}^{-1}$ fresh weight in 60 per cent waste soil combination. The correlation ($r = 0.991$) between waste soil combinations and mercury uptake by root was highly significant ($P < 0.01$) (Table 2). After 21 days transplantation of the *Sesbania* seedlings mercury uptake increased from $9.16 \pm 0.06 \mu\text{g g}^{-1}$ fresh weight in 10 percent to $21.34 \pm 0.04 \mu\text{g g}^{-1}$ fresh weights in 60 per cent waste soil combination. A highly significant correlation was obtained between waste soil and mercury uptake by root ($r = 0.990$, $p < 0.01$). 28 days after transplantation of *Sesbania* seedlings, mercury accumulation increased from $11.00 \pm 0.05 \mu\text{g g}^{-1}$ in 10 percent to $24.00 \pm 0.05 \mu\text{g g}^{-1}$ fresh weight in 60 per cent waste soil combination. The correlation ($r = 0.982$) between waste soil combinations and mercury uptake by root was highly significant ($P < 0.01$) (Table 2). After 35 days transplantation of the *Sesbania* seedlings mercury uptake increased from $12.85 \pm 0.05 \mu\text{g g}^{-1}$ fresh weights in 10 percent to $25.61 \pm 0.06 \mu\text{g g}^{-1}$ fresh weights in 60 per cent waste soil combination. A highly significant correlation was obtained between waste soil and mercury uptake by root ($r = 0.971$, $p < 0.01$).

Table 1. Mercury accumulation by root ($\mu\text{g} / \text{gm} / \text{Fw}$) of *Sesbania*. Values are mean of 3 samples \pm standard deviation

Days of Culture	Percent waste soil combination					
	10	20	30	40	50	60
7	4.86 ± 0.06	6.22 ± 0.02	9.10 ± 0.10	10.68 ± 0.08	12.43 ± 0.03	13.85 ± 0.05
	7.11 ± 0.11	10.65 ± 0.05	12.42 ± 0.10	14.18 ± 0.06	17.00 ± 0.05	18.27 ± 0.07
14	9.16 ± 0.06	13.00 ± 0.50	14.45 ± 0.95	16.25 ± 0.05	19.27 ± 0.07	21.34 ± 0.04
	11.00 ± 0.05	13.35 ± 0.05	18.10 ± 0.10	19.65 ± 0.05	22.22 ± 0.02	24.00 ± 0.05
21	12.85 ± 0.05	17.05 ± 0.05	21.00 ± 0.85	22.44 ± 0.04	24.16 ± 0.06	25.61 ± 0.06

Table 2. Bivariate correlation coefficient values for Mercury uptake by root and shoot with Percentage of waste soil of *Sesbania*

Days	Variable	Correlation values	Significance level ($p <$)	Degrees of freedoms
7	Root	0.994	0.01	5
	Shoot	0.996	0.01	5
14	Root	0.991	0.01	5
	Shoot	0.986	0.01	5
21	Root	0.990	0.01	5
	Shoot	0.996	0.01	5
28	Root	0.982	0.01	5
	Shoot	0.818	0.01	5
35	Root	0.971	0.01	5
	Shoot	0.984	0.01	5

3.2. Mercury Accumulation in Shoot

Table 3 depicts accumulation of mercury in shoot of *Sesbania* seedlings. The accumulation of mercury in shoot was dependent on amount of waste soil and time period. It increases with increased waste soil and time period. It increases with increasing waste soil from 10 per cent to 60 per cent and from 7 days after transplantation to 35 days after transplantation. Highly significant correlations were observed between mercury uptake by shoot and varying waste soil combinations.

After 7 days transplantation of the *Sesbania* seedlings mercury accumulation in shoot increased from $2.46 \pm 0.06 \mu\text{g g}^{-1}$ fresh weight in 10 per cent to $7.65 \pm 0.15 \mu\text{g g}^{-1}$ fresh weight in 60 per cent waste soil combination. The correlation between mercury uptake in shoot and waste soil combinations was highly significant ($r = 0.996$, $P < 0.01$) (Table 2). 14 days after transplantation, mercury accumulation increased from $3.88 \pm 0.07 \mu\text{g}$ in 10 per cent to $8.22 \pm 0.20 \mu\text{g}$ in 60 per cent waste soil combination per g fresh weight of shoot. A highly significant ($P < 0.01$) correlation ($r = 0.986$) was obtained between mercury accumulation and waste soil combination. After 21 days transplantation of the *Sesbania* seedlings mercury accumulation in shoot increased from $5.10 \pm 0.05 \mu\text{g g}^{-1}$ fresh weight in 10 per cent to $9.90 \pm 0.10 \mu\text{g g}^{-1}$ fresh weight in 60 per cent waste soil combination. The correlation between mercury uptake in shoot and percent waste soil combinations was highly significant ($r = 0.996$, $P < 0.01$) (Table 2). 28 days after transplantation, mercury accumulation increased from $6.20 \pm 0.10 \mu\text{g}$ in 10 per cent to $10.45 \pm 0.15 \mu\text{g}$ in 60 per cent waste soil combination per g fresh weight of shoot. A highly significant ($P < 0.01$) correlation ($r = 0.818$) was obtained between mercury accumulation and waste soil combination. 35 days after transplantation, mercury accumulation increased from $7.00 \pm 0.09 \mu\text{g}$ in 10 per cent to $11.15 \pm 0.05 \mu\text{g}$ in 60 per cent waste soil combination per g fresh weight of shoot. A highly

significant ($P < 0.01$) correlation ($r = 0.984$) was obtained between mercury accumulation and waste soil combination.

Table 3. Mercury accumulation by shoot ($\mu\text{g} / \text{gm} / \text{Fw}$) of *Sesbania*

Days of Culture	Percent waste soil combination					
	10	20	30	40	50	60
7	2.46 ± 0.06	3.11 ± 0.02	4.43 ± 0.13	5.65 ± 0.15	6.50 ± 0.10	7.65 ± 0.15
14	3.88 ± 0.07	4.34 ± 0.09	5.59 ± 0.09	6.90 ± 0.10	7.85 ± 0.04	8.22 ± 0.20
21	5.10 ± 0.05	5.88 ± 0.09	7.10 ± 0.05	8.00 ± 0.15	9.25 ± 0.12	9.90 ± 0.10
28	6.20 ± 0.10	7.18 ± 0.05	8.25 ± 0.05	9.10 ± 0.03	10.00 ± 0.11	10.45 ± 0.15
35	7.00 ± 0.09	8.29 ± 0.09	9.11 ± 0.06	10.10 ± 0.10	10.85 ± 0.13	11.15 ± 0.05

3.3. Height and Tiller Number of Rice after *Sesbania* Growth

In the control set (35 days), at maximum tillering stage height of the seedlings decreased from 48.00 ± 1.67 cm in garden soil to 20.67 ± 1.53 cm in 30% waste soil combinations (Table 4). In 40%, 50%, 60% waste soils rice seedlings failed to survive. Following growth and decomposition of *Sesbania* after 35 days rice seedlings survived in 40%, 50%, and 60% waste soil but also there was growth of the seedlings. In the treated set however reduction in survivability of rice seedlings started from 40% waste soil combinations. In 40% waste soil combination 7 out of 12 transplanted survived, in 50% waste soil 5 survived and in 60% only one survived. However heights of the rice plants decreased from the garden soil. Similarly at maximum tillering stage tiller number of the seedlings decreased from in garden soil to 30% waste soil combinations (Table 5). In 40%, 50%, 60% waste soils rice seedlings failed to survive. Following growth and decomposition of *Sesbania* after 35 days rice seedlings survived in 40%, 50%, and 60% waste soil but also the tiller numbers increased.

Table 4. Analysis of variance (f test) for mercury uptake by root and shoot.

Variable	Source of variances	ss	Mean Squares	DF	Calculated 'f value	Significance
Mercury uptake by root	1. Between % solid waste	1387.487	277.497	5	6506.216	0.01
	2. Between days	1323.043	330.761	4	7755.030	0.01
Mercury uptake by Shoot	1. Between % solid waste	243.228	48.646	5	174.272	0.01
	2. Between days	220.445	55.111	4	197.435	0.01

Table 5. Height & Tiller number of rice before after 35 days of growth and decomposition of *Sesbania*.

Percentage of solid waste without growth and decomposition of <i>Sesbania</i> .							
	0	10	20	30	40	50	60
Height	48.00 ± 1.67	33.35 ± 1.94	23.44 ± 2.78	20.67 ± 1.53	--	--	--
Tiller number	10.84 ± 1.01	3.29 ± 0.67	1.38 ± 0.25	1.07 ± 0.58	--	--	--
Percentage of solid waste with growth and decomposition of <i>Sesbania</i> . After 35 days							
	0	10	20	30	40	50	60
Height	50.48 ± 2.11 (5.17)	47.09 ± 3.01 (41.20)	34.92 ± 2.11 (48.98)	28.39 ± 1.62 (37.35)	27.88 ± 2.80	21.33 ± 4.00	15.00
Tiller number	15.08 ± 1.10 (43.89)	11.83 ± 1.79 (259.57)	7.50 ± 1.69 (418.12)	6.08 ± 1.75 (469.22)	4.40 ± 1.88	2.00 ± 1.00	1.00

Figures in the parentheses indicate percent increase over respective control.

4. Discussion

According to Chaney[17], the accumulation of metal depends on several factors like plant variety, plant parts, plant age, amount of metal in soil, soil pH, organic matter content, characteristic of metal, presence and absence of competing ions, cations exchange capacity of soil and phosphate content of the soil. According to Nasu et al[18] the absorption of metal ions by plants is also influenced by the kind of co existing ions (metals and base cations) and it also differs from one metal to another. It was also found that the higher atomic weight ions like Hg were concentrated more effectively by plants than lower atomic weight ions like Cd and Ni[19].

The accumulation and biological conversion (Methylation) of mercury by producer organisms in aquatic and terrestrial ecosystems[20] is a matter of great concern. Roger,[21] reported methylation of divalent mercury in agricultural soil, degree of methylation directly proportional to the concentration of mercury in the soil and the exposure time. The experiment discussed above shows that the accumulation of mercury by *Sesbania* is dependant on both on time and concentration of waste soil.

Thakur,[22] opined that plants can be used to remediation environmental media *in situ*. These findings led to the conclusion that the heavy metal pollution can be minimized through cost effective approaches of environmental management through plants. Phytoremediation involves two aspects to clean up the environment like use of metal accumulating plants to remove toxic metals and use of plant roots to eliminate the bioavailability of toxic metals & *Sesbania* for these purposes fits best. It can also be used as a bio-monitoring agent for Hg pollution as well. This is probably due to the production of some phytosiderophores[23] or some biosurfactants[24] produced by the microorganisms present in the root nodules and rhizosphere of *Sesbania*. The rice plants (IR 36) grown in waste soil of M/S Jayashree chemicals show decrease in growth and also decrease in biochemical variables like Protein, DNA and RNA. After the growth and decomposition of *Sesbania* suitable for the growth of rice plants (IR 36) indicates that possible phytoremediation of chlor-alkali waste can be achieved with this plant.

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