Recent Advancements in Bioremediation of Metal Contaminants

Satarupa Dey
Shyampur Siddheswari Mahavidyalaya, India

Biswaranjan Acharya
School of Computer Engineering, KIIT University (Deemed), India

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List of Contributors

1.	**Riya Dutta**	Microbiology and Microbial Biotechnology laboratory Department of Botany and Forestry Vidyasagar University Midnapore, lndia.	Biofertilizers and Sustainable Development
	Hiran Kanti Santra	Microbiology and Microbial Biotechnology laboratory Department of Botany and Forestry Vidyasagar University Midnapore, lndia	
	Debdulal Banerjee	Microbiology and Microbial Biotechnology laboratory Department of Botany and Forestry Vidyasagar University Midnapore, lndia	
2.	**Arpita De**	Department of Botany, Government Science College, Autonomous, Bangalore, Karnataka, India.	An Overview of Multifarious Potential of Biofertilizer
3.	**Suchhanda Ghosh**	Department of Botany, Shri Shikshayatan College, 11, Lord Sinha Road, Kolkata, India.	Heavy Metal Bioremediation of Agricultural Soils for Sustainability and Food Safety
	Satarupa Dey	Department of Botany,Shyampur Siddheswari Mahavidyalaya, Ajodhya, Howrah, West Bengal, India.	
4.	**Suchismita Chatterji**	Nabadwip Vidyasagar College, Nabadwip, West Bengal, India.	Prospects of Agriculture in Near Future
5.	**Priya Mondal**	Centre for Interdisciplinary Studies,186A, Kalikapur Canal Road, Ekatre 2nd Floor, Kolkata, lndia.	Agronomic and Nutraceutical Properties of Indigenous Rice Varieties
	Souvik Datta	Centre for Interdisciplinary Studies,186A, Kalikapur Canal Road, Ekatre 2nd Floor, Kolkata, lndia.	
	Debal Deb	Centre for Interdisciplinary Studies,186A, Kalikapur Canal Road, Ekatre 2nd Floor, Kolkata, lndia.	

6.	**Preeti Agarwal**	Department of Botany, Gargi College, Sirifort Road, New Delhi, lndia.	Plant Nutraceuticals: An Emerging Approach for Better Health Management
	Reema Mishra	Department of Botany, Gargi College, Sirifort Road, New Delhi, lndia.	
	Samira Chugh	Department of Botany, Gargi College, Sirifort Road, New Delhi, lndia.	
	Geeta	Department of Botany, Gargi College, Sirifort Road, New Delhi, lndia.	
7.	**Chandana Paul**	Department of Microbiology, St. Xavier's College, Park Street, Kolkata, lndia.	An Overview of Endophytic Bacteria in the Production of Bioactive Compounds of Nutraceutical Importance
	Madhumita Maitra	Department of Microbiology, St. Xavier's College, Park Street, Kolkata, lndia.	
	Nirmalendu Das	Department of Botany, Barasat Govt. College, Barasat, 24 Parganas (N), India.	
8.	**Dipan Adhikari**	Department of Botany (UG and PG), Hooghly Mohsin College, Chinsurah, Hooghly, West Bengal, lndia.	A Curtain Raiser on Natural Supplements Being Targeted Choice of Surveillance As Potential Anti-Viral Drugs : Neutraceutical Supplementations Vis-à-Vis Immune Boosters
9.	**Anubhuti Kawatra**	Department of Microbiology, Maharshi Dayanand University, Rohtak, Haryana, India.	Plants As Antiviral Agents
	Reema Mishra	Department of Botany, Gargi College, Sirifort Road, New Delhi, lndia.	
	Aparajita Mohanty	Department of Botany, Gargi College, Sirifort Road, New Delhi, lndia.	
	Pooja Gulati	Department of Microbiology, Maharshi Dayanand University, Rohtak, Haryana, India.	

10.	**Susmita Nad**	Department of Microbiology, Bankura Sammilani College, Bankura, West Bengal lndia.	Herbal Drug: A Natural Bioactive Formulation & its Scope Against Viral Diseases
	Asish Mandal	P.G. Department of Botany, Ramananda College, Bishnupur, Bankura, West Bengal, lndia.	
	Pradeep Kumar Das Mohapatra	Department of Microbiology, Raiganj University, Raiganj, Uttar Dinajpur, West Bengal, lndia.	
	Arindam Ganguly	Department of Microbiology, Bankura Sammilani College, Bankura, West Bengal lndia.	
11.	**Smita Ray**	PG Department of Botany, Bethune College, Kolkata, West Bengal, lndia.	Medicinal Plants : A Boom Towards Antiviral Drug Development
	Sritama Mukherjee	PG Department of Botany, Bethune College, Kolkata, West Bengal, lndia.	
	Seemanti Ghosh	PG Department of Botany, Bethune College, Kolkata, West Bengal, lndia.	
12.	**Samira Chugh**	Department of Botany, Gargi College, Sirifort Road, New Delhi, lndia.	Common Indian Medicinal Plants with Antidiabetic Potential
	Geeta	Department of Botany, Gargi College, Sirifort Road, New Delhi, lndia.	
	Preeti Agarwal	Department of Botany, Gargi College, Sirifort Road, New Delhi, lndia.	
	Reema Mishra	Department of Botany, Gargi College, Sirifort Road, New Delhi, lndia.	
13.	**Arnab Kumar Ghosh**	Department of Biochemistry, School of Life Science mand Biotechnology Adamas University, Kolkata, India.	Anti-Inflammatory Activities of the Eudicots in Triphala
14.	**Himani Yadav**	Department of Botany, University of Delhi, Delhi, India.	Potential of Gymnosperms As Source of Medicinal Products
	P. L. Uniyal	Department of Botany, University of Delhi, Delhi, India.	
15.	**Anjana Singh**	Department of Botany, Deshbandhu College, Kalkaji, University of Delhi, New Delhi, lndia.	Ethnobotanical and Medicinal Importance of Bryophytes
	Shelly Sinha	Department of Botany, Rabindra Mahavidyalaya, Champadanga, Hooghly, West Bengal, India.	

16.	**Priyanka De**	Postgraduate, Department of Biotechnology, St. Xavier's College(Autonomous), 30 Park Street (30 Mother Teresa Sarani), Kolkata, India.	Enigma of Indian Tradition of Healing: A Phytomedicinal Perspective
17.	**Ashim Chakravorty**	Biotechnology: Plant Tissue Culture Unit Department of Botany, Sripat Singh College, Jiaganj, Murshidabad, West Bengal, lndia.	Pteridophytes A Treasure Trove of Medicine for Human Health and Their in Vitro Conservation
18.	**Chandreyee Dey**	PG Department of Botany, Bethune College, Kolkata, West Bengal, lndia.	An Overview of Multifarious Importance of Pteridophytes
	Sritama Mukherjee	PG Department of Botany, Bethune College, Kolkata, West Bengal, lndia.	

Chapter 8

Ricinus communis: A Potent Lead (Pb) Accumulator

Raikamal Pal
Shri Shikshayatan College, India

ABSTRACT

Contamination of soil and ground water with heavy metals is a great threat to human health, vegetation, and wildlife. Pb is the second most hazardous substance according to ATSDR. The main sources of Pb entering an ecosystem are atmospheric Pb (mainly from automobile emission), paint chips, fertilizers, and pesticides and Pb acid batteries or other industrial Pb products. Phytoremediation could provide sustainable techniques for metal remediation. Roots of Ricinus communis were found to accumulate maximum amount of Pb (275.12mg/kg dry wt,). Depending on soil Pb content, the concentration of Pb in shoots of Ricinus communis *also varied. In most cases only a small part of Pb was translocated in the aerial parts. In 95% of the plant samples collected, the root Pb concentration are much greater than those of the shoot lead content, indicating low mobility of Pb from roots to the shoots. Their ability to accumulate higher amounts of Pb in their roots and considering their rapid growth rate and biomass, this plant has the potential for removal of Pb from contaminated soil.*

INTRODUCTION

Indiscriminate use of different heavy metals has been increased due to rapid urbanization.Heavy metals cannot be destroyed or degraded as they occur as natural constituent of earths crust. These heavy metals enter the body system through food, air, and water and bio-accumulate over a period of time. (UNEP/GPA,2004).

In todays industrialized society heavy metals are ubiquitous environmental contaminants. Heavy metal pollution in soil differ from air or water pollution as heavy metals retain much longer than any other component of the biosphere. (Lasat., 2002)

Heavy metal contaminants in soils emitted through metalliferous mining and smelting, metallurgical industries, sewage sludge treatment, warfare and military training, waste disposal sites, agricultural fertilizers and electronic industries (Alloway1995). For example, mine tailings rich in sulphide minerals

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may form Acid Mine Drainage (AMD) through reaction with atmospheric oxygen and water, and AMD contains elevated levels of metals that could be harmful.

Wild life and human exposed to high level of these heavy metals has adverse effects on both.Natural and anthropogenic activities both are responsible for the heavy metal emission into the environment. Mining operations are the main anthropogenic sources which causes heavy metal emission.(Battarbee et al.,1988; Nriagu, 1989). Even long after mining activities have ceased, the emitted metals continue to persist in the environment. Heavy metals are emitted both in elemental and compound (organic and inorganic) forms in the environment. Various former and present mining sites,foundries and smelters, combustion by products are the anthropogenic sources of emission. These metals dissolve with rain water leached out in sloppy areas, and are carried by acid water downstream or run-off to the species in water or as an integral part of suspended sediments (dissolved species in water have the greatest potential of causing the most deleterious effects). These heavy metal rich sediments may then be accumulated in river bed sediments or seep into the underground water and thus contaminate water from underground sources, particularly wells; and the extent of contamination will depend on the nearness of the well to the mining site. Wells which are located near mining sites have been reported to contain heavy metals at levels that exceed drinking water criteria (Garbarino et al., 1995; Peplow, 1999).

Table 1. United State Environmental Protection Agency (USEPA) maximum contamination levels for heavy metal concentration in air, soil and water

Heavy Metal	Max.Conc.in air (mg/m^3)	Max.Conc. in Sludge (Soil)(mg/kg or ppm)	Max.Conc in Drinking water (mg/L)	Max Conc, in water supporting aquatic life(mg/L or ppm)
Cd	0.1-0.2	85	0.005	0.008$^{\delta}$
Pb	--	420	0.01$^{\pi}$(0.0)	0.0058$^{\delta}$
Zn^2	1.5*	7500	5.00	0.0766 $^{\delta}$
Hg	--	<1	0.002	0.05
Ca	5	Tolerable	50	Tolerable > 50
Ag	0.01	--	0.0	0.1
As	--	--	0.01	--

(Value in bracket is the desirable limit; WHO ; 1adapted from U.S. – OSHA; 2 EPA, July 1992; _USEPA, 1987; Georgia Code,1993; Florida Code, 1993; Washington Code, 1992; Texas Code, 1991; North Carolina, 1991; *1 for chloride fume, 5 for oxide fume; - - no guideline available).

In the 3rd world countries importance has been given mainly for the establishment of the industries but the issues of protection of environment remain neglected. Thus a number of factories were developed in a unplanned manner, it increases generation affluents from the factories. These affluents are often mixed with heavy metals.The problems of urbanization, population explosion and the increased use of automobiles have become very common. It is well known that environmental pollution is a product of urbanization and technology, and other associated factors of population density.

Depending on the type of industries in the vicinity different metals such as As, Pb, Cd, Cu, Cr, Ni etc are deposited in the soil. Among these some metals are needed for biological function such as Cu, Zn whereas Pb, Cr, As, Hg have no known biological role. All these metals, when present in very low concentration have no or little effect on living organism, but when the level crosses the threshold value these metals affect the biological system. Among these heavy metals Pb, Cd, Cr are more harmful to both plants and animals and these heavy metals are mostly widespread. According to ATSDR (2005) Pb, Cd, Cr stands 2nd, 7th and 77th in position respectively.

BACKGROUND

Lead (Pb)

Pb imposes serious threat to the health of children and wildlife as it is an extremely toxic heavy metals (EPA 2005). The main sources of Pb poisoning include lead paint and old gasoline spills (PbBrCl, 2Pb-BrCl.NH4Cl) resulting in dust and soil contamination of food and water (Xintaras 1992)

Physical Properties

Lead is a main-group element with the symbol **Pb** (from Latin: *Plumbum*) and atomic number 82. Lead has the highest atomic number of all the stable elements, 82 and its atomic weight is 207.2.

Pb is dense, ductile, very soft, highly malleable, bluish white metal with poor electrical conductivity. Metallic lead has a bluish white to silvery shine after been freshly cut, but it soon turns to a dull greyish colour when exposed to air . Liquid lead has a shiny silvery lustre.

Metallic lead is attacked (oxidized) only superficially by air, forming a thin layer of lead oxide that protects it from further oxidation. The metal is not attacked by sulfuric or hydrochloric acids. It dissolves in nitric acid with the evolution of nitric oxide gas to form dissolved Pb(NO3)2.

Exposure Routes

Lead is a common environmental pollutant. Household dust, soil, water, and commercial products, lead in air are the routes of exposure of lead. Environmental contamination includes industrial use of lead, such as is found in facilities that process lead-acid batteries, lead wire or pipes, and metal recycling and foundries. Battery recycling workers are at high risk for lead exposure. Occupational exposure is the main cause of lead poisoning. Facilities that produce a variety of lead-containing products; these include radiation shields, ammunition, certain surgical equipment’s, plumbing, circuit boards, jet engines, and ceramic glazes are the causes of lead exposure for the people working in those industries. Lead miners and smelters, plumbers and fitters, auto mechanics, glass manufacturers, construction workers, battery manufacturers and recyclers, firing range instructors, and plastic manufacturers are at great risk for lead exposure in addition. Other occupations that present lead exposure risks include welding,

Lead paint is a major route of lead exposure in children as some lead compounds are colourful and are widely used in paint. Deteriorating lead paint can produce dangerous lead levels in household dust and soil. Deteriorating lead paint and lead-containing household dust are the main causes of chronic lead poisoning. Colourful toys used by children are extremely harmful for them as deteriorating lead can be easily ingested. However, removing lead paint from dwellings, e.g. by sanding or torching, can create lead-containing dust and fumes. Special precautions must be taken when removing lead paint.

Contaminated food, water or alcohol are also the source of lead exposure. Even ingestion of certain home remedy medicines may also expose people to lead or lead compounds. Fruits and vegetables grown in lead contaminated sometimes may be the source of lead ingestion in human. Soil is contaminated through particulate accumulation from lead in pipes, lead paint and residual emissions from leaded gasoline that was used before the Environment Protection Agency issue the regulation around 1980.

Inhalation is the second major pathway of exposure, especially for workers in lead-related occupations. Almost all inhaled lead is absorbed into the body, the rate is 20–70% for ingested lead; children absorb more than adults.

Effects of Lead Toxicity on Plants

Among the heavy metals, lead is a potential pollutant that readily accumulates in soils and sediments. Lead gets easily absorbed and accumulated in different plant parts although it is not an essential element for plants. Depending on different parameters such as pH, particle size and cation exchange capacity of the soils as well as by root exudation and other physico-chemical parameters determines the uptake of lead by plants. A number of toxicity symptoms in plants e.g. stunted growth, chlorosis and blackening of root system is caused by excess Pb accumulation Pb inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability. Mechanisms of Pb-detoxification include sequestration of Pb in the vacuole, phytochelatin synthesis and binding to glutathione and amino acids etc. Pb tolerance is associated with the capacity of plants to restrict Pb to the cell walls, synthesis of osmolytes and activation of antioxidant defence system. Remediation of soils contaminated with Pb using phytoremediation and rhizofiltration technologies appear to have great potential for cleaning of Pb contaminated soils.

Effects of Lead Toxicity on Animals

Lead can also be found listed as a criteria pollutant in the United States Clean Air Act section 108. Lead that is emitted into the atmosphere can be inhaled, or it can be ingested after it settles out of the air. It is rapidly absorbed into the bloodstream and is believed to have adverse effects on the central nervous system, the cardiovascular system, kidneys, and the immune system.

Lead is a poisonous metal that can damage nervous connections (especially in young children) and cause blood and brain disorders. Long-term exposure to lead or its salts (especially soluble salts or the strong oxidant PbO2) can cause nephropathy, and colic-like abdominal pains. The effects of lead are the same whether it enters the body through breathing or swallowing. Lead can affect almost every organ and system in the body. The main target for lead toxicity is the nervous system, both in adults and children.

The Importance and Hazard of Lead as a Rhizospheric Contaminant

Lead imposes a serious threat to the health of children and wildlife as it is an extremely toxic heavy metal. (EPA 2005). The main sources of Pb poisoning include lead paint and old gasoline spills (PbBrCl, 2PbBrCl.NH4Cl) resulting in dust and soil contamination of food and water (Xintaras 1992).

Elemental Pb is insoluble and the most water soluble forms of Pb compounds are lead acetate (2 mg/ml), lead chloride (0.009 mg/m1), and lead nitrate (5 mg/ml). Atmospheric Pb mostly exists as PbSO4 and PbCO3. Many plants have a strategy of Pb exclusion such as *Thlaspi praecox,* which hyperaccumulates Cd and Zn but exclude Pb (Vogel-Mikus et al. 2005).

Pb isa hyperaccumulator as it is cited in different references.. It has been reported that *Sesbania drummondii,* a leguminous shrub, and several *Brassica* species can accumulate significant amounts of Pb in their roots (Blaylock et al. 1997; Sahi et al. 2003; Wang et al. 2001), and *Piptathertan miliacetall,* a grass, accumulates Pb directly correlating to soil concentrations without symptoms of toxicity for 3 weeks (Garcia et al. 1998). Sahi et al. (2002) have noted that S. *drummondii* can tolerate Pb levels up to 1500mg/L and accumulate 40g/kg shoot dry weight. *Brassica juncea* showed reduced growth at 645 mg/gm Pb in the soil substrate, but can accumulate 34.5 gm/kg shoot dry weight, although significant shoot accumulation is not observed until Pb reaches saturation levels in the roots. Most of the shoot accumulation was found in stems and not leaves suggesting that Pb is relatively insoluble (Kumar et al. 1995).

Metal-contaminated soil can be remediated by chemical, physical or biological techniques (McEldowney et al. 1993). Chemical and physical treatments irreversibly affect soil properties, destroy biodiversity and may render the soil useless as a medium for plant growth. These remediation processes can be costly. Phytoextraction is one of the lowest cost techniques for contaminated soil remediation among the listed remediation techniques. There is a need to develop suitable cost-effective biological soil remediation techniques to remove contaminants without affecting soil fertility.

For metal remediation phytoremediation could provide a sustainable technique. The idea that plants can be used for environmental remediation is very old and cannot be traced to any particular source.

OBJECTIVE OF THE STUDY

Phytoremedial potential of wild plants growing in Pb contaminated sites were studied in this chapter. Pb is chosen as the metal of interest because it is listed as the 2nd most hazardous substance in the list of CERCLA(ATSDR2007). The aim of the study was to find out a plant which is tolerant and can accumulate considerable amount of Pb in its parts, so that it can assist in remediation of contaminated soil. For this the desirable qualities of the candidate plant should have the following properties:

1. The plant should have high biomass.
2. Plant should have rapid growth rate.
3. Can tolerate high amount of Pb in soil.
4. Can accumulate significant amount of Pb in its parts.

MATERIAL AND METHOD

Field Work

Eleven different contaminated sites in and around Kolkata were surveyed which were variously contaminated either by some industrial pollutants or by some anthropogenic activities. This eleven sites are spread in three districts Kolkata, Howrah and North 24-Pargans, These three districts have various large and small scale industries and population pressure is maximum in these areas. Sites were selected by taking into account the probable presence of Pb contamination in these areas. Pb was selected to study accumulation pattern in plant and soil from contaminated sites because Pb stands second most hazardous substance according to ATSDR (2007) as well as it was not possible to study all the heavy metals present in those contaminated site due to time constrains. So the study was focused on Pb accumulation pattern and tolerance mechanism of the plants grown luxuriantly in these six contaminated sites. So, the abundance of different plant growing in those areas were studied.

Determination of Relative Abundance

Quadrate Study : All the species of higher plants of any community can be classified in one or other life form. The ratio of life forms of different species in term of no. of percentages in any floristic community is called biological spectrum or phytoclimatic spectrum.

Method: A given plot of area in eleven locations was chosen and was studied for biological spectrum. The area was about 4mt X 4mt. (Raunkiar, 1934)

Table 2. Location of Study Sites

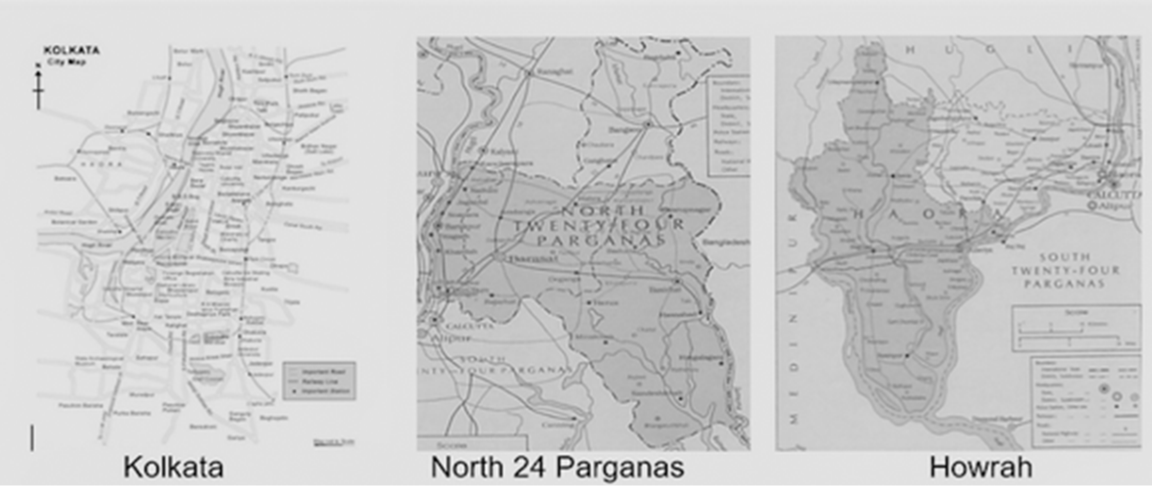
Location No.	Area of Collection	Sources of contaminants
I	Paint Industries, Howrah	Effluents and chemical waste from industries.
II	Habra I, North 24 Parganas	All kind of Anthropogenic and environmental waste.
III	Habra II, North 24 Parganas	-Do-
IV	Habra III, North 24 Parganas	-Do-
V	Habra IV, North 24 Parganas	-Do-
VI	Habra V, North 24 Parganas	-Do-
VII	Battery production plant, Shyamnagar	Mainly effluents from a battery manufacturing unit.
VIII	Tannery Industries, East Kolkata	Chemical waste from leather tanning unit.
IX	Keshtopur Canal,Kolkata	All possible human and animal waste, along with the vehicular discharge.(Municipal sewage)
X	Bantola Leather Industries	Chemical used in leather industries.
XI	EM Bypass, Kolkata	Road side eateries, automobile discharges and waste from few human settlings.

Kolkata North 24 Parganas Howrah

Biomass Measurement

Biomass of most abundant species was measured by weighing method. It has been shown that the biomass of certain plants such as *Ricinus communis* is much greater than those of other species which were seem to be most abundant by quadrate method. As higher biomass is needed for more accumulation of toxic metal i.e. lead and thus it helps in phytoremediation process, therefore in most cases *Ricinus communis* has been selected for metal assay.

Figure 1. **Area map showing study sites of three different districts**



Kolkata North 24 Parganas Howrah

Collection of Sample

Samples were collected in zip lock packets to eliminate possibilities of incorrect sampling. Plants were collected in the shrub stage . Leaves of the shrub stage were collected as follows: Old /senescent leaves, young leaves and emergent leaves .Root were also collected 20cm below ground level.

Digestion of Soil and Plant Sample

Protocol for digestion of soil and plant sample for heavy metals

- In 0.5gm of oven dried soil & tissue, 12ml concentrated HNO3 were added and allowed to stand overnight.
- Heated in hot plate until production of red nitrogen dioxide (NO2) fumes stopped and a watery mass is left.
- 4ml of perchloric acid was added after the beaker was cooled in room temperature.
- Heated again at 70ºC to 80ºC and evaporated until a white mass is left.
- It was filtered through Whatman 42 ash less filter paper and the volume was made up to 50ml with double distilled water / deionized water.

Lead was estimated in the samples at the Department of Geology, Jadavpur University by AAS analysis following digestion using the method described in the Perkin Elmer handbook for Atomic Absorption Spectroscopy.

The results of metal assay have been given in table no.14. From the result *bioaccumulation factor* and *translocation factor* was determined. A plants ability to accumulate metals from soils can be estimated using the *bioaccumulation factor*, which is defined as the ratio of metal concentration in the shoots to that in soil.

A plants ability to translocate metals from the roots to the shoot is measured using the translocation factors, which is defined as the ratio of metal concentration in the shoots to the roots.

Soil Analysis: Soil parameters such as organic carbon, cation exchange capacity, electric conductivity and pH were measured.

Organic Carbon Content Measurement:

1gm of soil was taken in 500ml conical flask. To it 10 ml 1N Potassium dichromate was added. Then 20 ml concentrated H2SO4 was added in each flask.

After that, the flask was incubated for at least 1 hour. After 1hour 200ml distilled water and 5ml phosphoric acid and 1ml DPA were added. Then it was titrated against Mohr salt.

Measurement of CEC

25ml of 0.05 (N) HCl was added to 1gm of soil sample. After vigorous shaking with glass rod it was left for 20 minutes. Then it was centrifuged for 10minutes at 3000-4000 rpm. The supernatant was discarded. Then distilled water was added to the tube, and filtered with Whatman No 1 filter paper. Now few drops of Ag NO3 was added to it to check the Cl removal. If it becomes turbid (Cl present) the filtrate was discarded, boiling water was added to the filter paper and the test was repeated again. After complete removal of Cl the filter paper was taken, to it 25 ml Barium acetate was added and the filter paper was washed into the beaker so that the entire filtrate comes into the beaker. Then it was incubated for 1 hour, to it 10 drops of Thymol Blue was added, and titrated against 0.05 (N) NaOH.

Measurement of pH

1gm of soil was dissolved in 30 ml distilled water. Then it was kept for 15 minutes. After that, pH of that solution was measured.

RESULT AND DISCUSSION

Study of Relative Abundance

Observation: The following tables and graphical representation shows the frequency of different groups of plants.

From quadrate study it was found that the castor oil plant, *Ricinus communis*, is a plant species of the family Euphorbiaceae, the sole member of the genus *Ricinus*, castor oil plant can reach a height of 2-3 meter in a year. Castor establishes itself easily as apparently native plant and can often be found on waste land. It is a fast growing perennial shrub. This fast growing plant has been found to grow luxuriantly in severely heavy metal contaminated soils of Kolkata and suburbs.

Table 3. Showing frequency and % of biomass of plants from Location 1 (Shalimar)

Plant Species	Family	No. of Plants	Weight of plants	Frequency of Plants(A)	Percentage of Biomass(B)
Tilanthera Sp	Amaranthaceae	45	0.34	71.42	1.6
Ricinus communis	Euphorbiaceae	5	15.5	7.9	72.85
Ipomoea sp	Convovulaceae	7	0.74	11.1	3.5
Solanum sp	Solanaceae	6	4.64	9.5	21.85

A=Frequency of plants =x/åX where, x =no. of plants for a particular sp.
åX=total no of plants in a(4mt x 4mt) plot
B= % of biomass =w/åWx100 where, w=cumulative weight of the particular sp.
åW=total weight of plants

Table 4. Showing frequency and % of biomass of plants from Location 2 (Habra I)

Plant Species	Family	No. of Plants	Wt of plants	Frequency of Plants	% of Biomass
Solanum sp	Solanaceae	10	9.5	41.66	43.7
Eupatorium sp	Asteraceae	5	0.157	35.71	0.72
Ricinus communis	Euphorbiaceae	3	12	04.16	54.64
Sida sp	Malvaceae	6	0.09	25.00	0.43

Table 5. Showing frequency and % of biomass of plants from Location 3 (Habra II)

Plant Species	Family	No. of Plants	Wt. of plants	Frequency of Plants	% of Biomass
Oxalis sp	Oxalidaceae	40	0.143	66.66	0.5
Abutilon indica	Malvaceae	6	10.9	10.00	38.07
Parthenium hysterophorus	Asteraceae	10	0.719	16.66	2.5
Ricinus communis	Euphorbiaceae	4	16.8	6.66	58.37

Table 6. Showing frequency and % of biomass of plants from Location 4 (Habra III)

Plant Species	Family	No. of Plants	Wt. of plants	Frequency of Plants	% of Biomass
Tilanthera sp	Amaranthaceae	25	0.31	60.97	1.7
Croton sp	Euphorbiaceae	8	0.76	19.51	4.1
Solanum nigrum	Solanaceae	4	5.2	9.7	28.0
Cephalandra sp	Cucurbitaceae	2	0.186	4.87	1.0
Ricinus communis	Euphorbiaceae	2	12	4.87	64.5

Table 7. Showing frequency and % of biomass of plants from Location 5(Habra IV)

Plant Species	Family	No. of Plants	Wt. of plants	Frequency of Plants	% of Biomass
Ricinus communis	*Euphorbiaceae*	3	12.3	5.17	91.7
Tilanthera sp	*Amaranthaceae*	40	0.34	68.96	2.54
Croton sp	*Euphorbiaceae*	10	0.62	17.24	4.7
Sida sp	*Malvaceae*	5	0.12	8.62	0.9

Table 8. Showing frequency and % of biomass of plants from Location 6 (Habra V)

Plant Species	Family	No. of Plants	Wt of plants	Frequency of Plants	% of Biomass
Oxalis sp	*Oxalidaceae*	25	0.102	47.16	0.4
Abutilon indicum	*Malvaceae*	5	8.44	9.43	33
Ricinus communis	*Euphorbiaceae*	3	15.6	5.66	60.79
Cyperus sp	*Cyperaceae*	20	0.205	37.73	0.8

Table 9. Showing frequency and % of biomass of plants from Location 7 (Exide Shyamnagar)

Plant Species	Family	No . of Plants	Wt of plants	Frequency of Plants	% of Biomass
Sida sp	*Malvaceae*	7	0.067	15.21	1.68
Abutilon indica	*Malvaceae*	5	1.428	10.86	35.7
Tilanthera sp	*Amaranthaceae*	30	0.12	65.21	3.0
Solanum nigrum	*Solancaeae*	4	2.4	8.69	59.52

Table 10. Showing frequency and % of biomass of plants from Location 8 (Tannery)

Plant Species	Family	No. of Plants	Wt of plants	Frequency of Plants	% of Biomass
Abutilon sp	Malvaceae	6	5.66	35.29	27.27
Ricinus communis	Euphorbiaceae	3	12.6	17.64	60.60
Sida sp	Malvaceae	8	2.50	47.05	12.12

Table 11. Showing frequency and % of biomass of plants from Location 9 (Kestopur)

Plant Species	Family	No. of Plants	Wt. of plants	Frequency of Plants	% of Biomass
Clerodendron inerme	Verbenaceae	5	9.07	14.28	40.89
Tilanthera sp	Amaranthaceae	25	0.226	71.42	1.02
Solanum nigrum	Solanaceae	2	1.45	5.71	6.54
Ricinus communis	Euphorbiaceae	3	11.4	8.57	51.53

Table 12. Showing frequency and % of biomass of plants from Location 10(Bantola)

Plant Species	Family	No. of Plants	Wt.of plants	Frequency of Plants	% of Biomass
Cephalandra indica	Cucurbitaceae	2	0.54	22.22	1.50
Sida sp	Malvaceae	4	2.16	44.44	6.01
Ricinus communis	Euphorbiaceae	3	12	33.33	92.48

Table 13. Showing frequency and % of biomass of plants from Location 11 (Science City)

Plant Species	Family	No. of Plants	Wt of plants	Frequency of Plants	% of Biomass
Tilanthera sesillis	Amaranthaceae	45	0.46	46.39	4.10
Tridax procumbens	Asteraceae	10	2.5	10.3	22.83
Ricinus communis	Euphorbiaceae	2	8.2	2.06	73.05

DETERMINATION OF BIOMASS

The biomasses of different plants are given below.

Next, relative biomass of the plants were measured (Table 14) and a comparative account of percentage of biomass from different contaminated sites (Fig 2) indicates that *R.communis* has the highest amount of biomass among all these plants species. (51.53% in Lx1 to 92.48% in Lx2).L

Figure 2. Comparative account of % of biomass from different contaminated locations

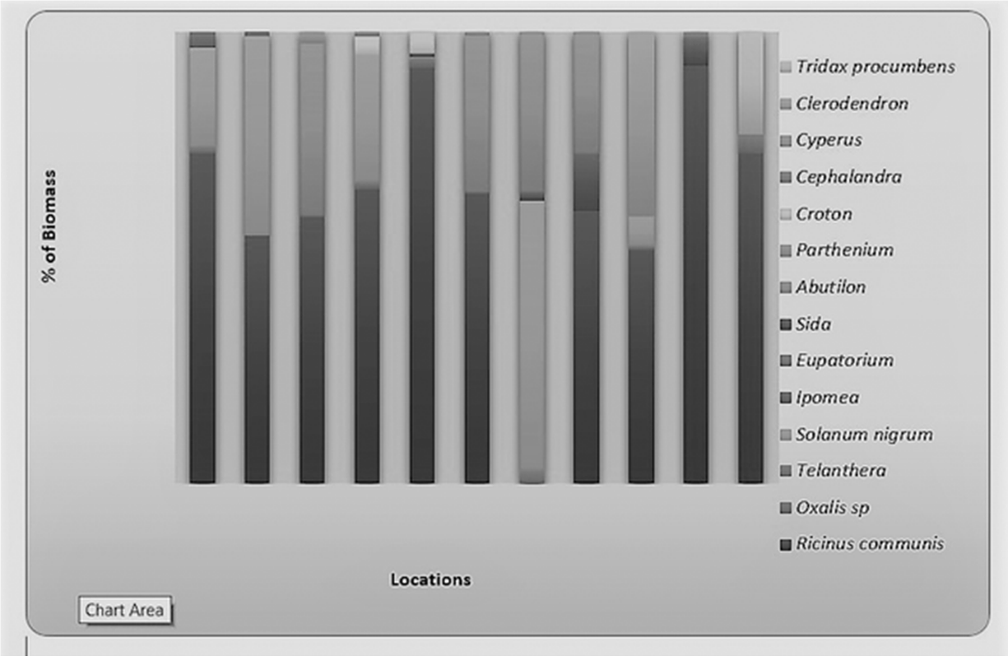


Table 14. Relative height and biomass of plants collected from contaminated sites

Location	Name of the Plant *Sp*ecies	Plant Height (inches)	Biomass (Kg)
I **(Paint Industries, Howrah)**	*Ricinus communis*	47	3.16
	Solanum nigrum	12	0.6
	Tilanthera sp	6	0.02
	Ipomoea sp	16	2.0
II **(Habra I, North 24 Parganas)**	*Ricinus communis*	59	3.0
	Eupatorium sp	18.3	1.3
	Solanum sp	16	0.8
	Sida sp	11	0.5
III **(Habra II, North 24 Parganas)**	*Ricinus communis*	63	4.2
	Oxalis sp	3	0.2
	Abutilon indica	30	0.7
	Parthenium hysterophorus	35	0.6
IV **(Habra III, North 24 Parganas)**	*Ricinus communis*	44	3.3
	Tilanthera sp	8	0.04
	Croton sp	41	2.0
	Solanum nigrum	21	0.8
V **(Habra I V, North 24 Parganas)**	*Ricinus communis*	61	4.2
	Tilanthera sp	11	0.2
	Croton sp	34	0.6
	Sida sp	12	0.3
VI **(Habra V, North 24 Parganas)**	*Ricinus communis*	67	5.1
	Cyperus sp	17	0.3
	Oxalis sp	5	0.2
	Abutilon indicum	25	0.8
VII **(Battery production plant, Shyamnagar)**	*Solanum nigrum*	11	0.5
	Tilanthera sp	6	0.02
	Sida sp	24	0.5
	Abutilon indica	31	1.2
VIII **(Tannery Industries, East Kolkata)**	*Abutilon sp*	34	1.0
	Sida sp	20	0.5
	Ricinus communis	60	4.3
IX **(Keshtopur Canal, Kolkata)**	*Ricinus communis*	56	3.7
	Clerodendron inerme	41	2.7
	Tilanthera sp	6	0.2
	Solanum nigrum	34	0.8
X **Bantola Leather Industries**	*Ricinus communis*	58	5.0
	Cephalandra indica	41	0.7
	Sida sp	23	0.86
XI **(EM Bypass, Kolkata)**	*Ricinus communis*	49	4.2
	Tilanthera sesillis	9	0.3
	Tridax procumbens	20±1,b	0.2±0.1,a

Table 15. Lead concentration (mg /Kg) in soil, root and shoot samples from different lead contaminated sites

Location	Name of the Plants species	Amount of Lead (Pb) content (mg/Kg)			Translocation Factor (TF) Shoot/root	Bio Accumulation Factor (BF) Shoot/Soil
		Soil	**Root**	**Shoot**		
I	*Ricinus communis*	2484*	275.12**	71.87**	0.26	0.028
	Solanum nigrum	Do	Nil	8.73	Nil	0.003
	Clerodendron inerme	Do	5.714	7.97	1.39	0.003
II	*Ricinus communis*	120.42*	48.94**	6.7	0.13	0.055
III	*Ricinus communis*	69.88	16.46**	14.77	0.89	0.211
IV	*Ricinus communis*	46.9	31.18**	5.16	0.16	0.110
V	*Ricinus communis*	55.52	4.62	4.2	0.90	0.075
VI	*Ricinus communis*	39.23	Nil	2.2	Nil	0.056
VII	*Solanum nigrum*	1400*	6.43	7.5	1.16	0.005
	Tilanthera sp	Do	0.122	5.10	41.83	0.003
VIII	*Tilanthera sp*	128*	334.16**	4.3	0.012	0.033
	Ricinus communis	Do	53**	2.4	0.04	0.0003
IX	*Ricinus communis*	14.5	2.69	7.22	2.77	0.497
X	*Ricinus communis*	3.67	Nil	8.5	Nil	2.31
XI	*Ricinus communis*	13.78	11.46**	2.31	0.20	0.167

** Lead concentration is than more than normal in these samples
[Normal concentration of lead is 10mg/kg] (Kabata,Pendias,2001)

Focus of the study was to find out a suitable plant which can be utilized for remediation of Pb contaminated soil. For a potential candidate to be a phytoremediating *sp*ecies the plant s should have a) high biomass, b) rapid growth, c) abundance of growth in metal contaminated sites, d) tolerance and accumulation capacity of metals.

CONCENTRATION OF LEAD IN SOIL

Concentration of Pb in soil varied from location to location (Table -5) depending on the nature of the surrounding environment. Maximum Pb concentration in soil was identified from location I (2484mg/Kg soil), located in the vicinity of paint factory . The site was used as a dumping ground for the factory waste, as Pb is main component of paint, waste from this factory released huge load of Pb in soil no other sites studied recorded such a high amount of Pb in soil. The next highest concentration in soil was recorded from location VII (1400mg/kg soil),near a car-battery manufacturing unit. Lowest concentration of soil Pb was recorded from location X (3.67 mg/kg soil) housing a leather processing unit .Similar leather located near East Kolkata (location VIII) recoded much higher amount of Pb concentration in soil (128 mg/kg soil) .This clearly showed that effluents treatment can reduce the pollution level much significantly. According to Kabata-Pendias (2001) the allowable limit of Pb in uncontaminated soil is 10mg/kg. Taking this value as reference, all the locations (except LX) can be termed as contaminated. From the study it is observed that LI and LVII are highly contaminated, LVIII and II are moderately contaminated and LIII,V, IV, VI, IX and XI are poorly contaminated by Pb. The results clearly indicate that Pb is mainly added to soil by industrial activities.

CONCENTRATION OF Pb IN PLANTS:

Plants (most frequent with significant biomass) were collected from all the eleven experimental sites and assayed for the presence of Pb in them.In most of the sites *Ricinus communis* plants showed highest biomasss and found to be the most significant species of that quadrat (except LX). Therefore Pb accumulation potential of *R. communis* and the next dominant members *Tilanthera philoxiroides* and *Solanum nigrum* were measured. It is observed from the study that Pb accumulation in plants is not dependant on soil Pb content linearly, rather it depends on some other edaphic factors (such as soil pH, organic carbon content etc.) regulating the bio-availability of Pb to the plants. Roots of *Ricinus* was found to accumulate more Pb in comparison with the shoot. Roots of *Ricinus* were found to accumulate maximum amount of Pb (275.12mg/kg dry weight) (Table 14). In LI no detectable amount of Pb was recorded in roots of *Ricinus.* Depending on soil Pb content the concentration of Pb in shoots also varied. In most cases only a small part was translocated in the aerial parts . In 95% of plant samples, the root Pb concentration are much greater than those of the shoot Pb contents, indicating low mobility of Pb from the roots to the shoots. Maximum shoot Pb accumulation was also observed (71.87mg/kg dry weight) in plants collected from LI . According to Baker this plant cannot be considered as a hyper accumulator, as these plants cannot accumulate more than 1000mg/kg dry weight of Pb in their aerial parts, b)do not have a TF value more than 1, c) the concentration of Pb in shoots is not 50- 100 times more than that in plants from non-polluted areas (5mg/kg).

Other than *Ricinus, Solanum nigrum* and *Tilanthera* sp., were also collected from LI, LVII and LVIII, but it was observed that, Pb accumulation in this two plants were not very significant. In LI where soil Pb content were very high, *Solanum* roots showed no accumulated Pb and *Tilanthera* showed very low amount of Pb accumulation (5.7mg/kg dry weight) which are significantly less than the *Ricinus* roots. Reevis and Brooks (1983) reported only four hyperaccumulator species of Pb. Among them *Thalspi rotundifolium* spp. *Cepaeifolium* were found to accumulate 8200mg/kg Pb in shoots. Bary and Clark (1978) found Pb concentration up to 20,000 mg/kg in shoots of *Minuarita verna* grown on a metal mining complex in Yorkshire . But Sieghardt (1987) reported that only 814mg/kg Pb was found to accumulate in the shoots of same sp. While Pb concentration in roots were more, This findings clearly shows that Pb accumulation by the plants is an innate quality of the plants and different population of the same sp may show variable accumulation potential.

SOIL PROPERTIES

As metal bioavailabilty dependant on other edaphic factors, soil pH, organic carbon (OC) content, EC and cation exchange capacities (CEC) of all the experimental sites as well as soil used in pots for growing *Ricinus* plants in Departmental garden were determined. The different parameters clearly indicate how these factors regulate the Pb bio availability to the plants.

In the experimental sites soil pH varies from slightly acidic (5.4in L VI) to neutral (7.9 in LI). Salinity of the soil is reflected by the EC value and value greater than 4 is considered as saline. Here lowest EC value (0.26)was recorded from L V and highest EC value (3.16) was recorded from L III . Organic carbon content values varies from a lowest 0.60 (LI) to a highest 6 (L VI & LIV) (Table-6) . CEC values also varied from sites to sites (6m.e/100gm -49.8m.e/100gm).The combination of elevated soil pH and high organic content may have played a role in limiting the metal availability, resulting in low uptake of Pb by the plants (Jung and Thornton, 1996, Rosselli et.al 2003). From the data it is observed that soil pH values are positively correlated for translocation of Pb in both in root (r=+ 0.42) and in shoot (r=+0.38) .Whereas CEC values are also positively correlated for translocation of Pb in both in root (r=+ 0.21) and in shoot (+0.31) but OC value are negatively correlated for translocation in root (r= – 0.36) and in shoot r= - 0.30 .Soil used in pots to grow experimental plants showed near neutral pH (7.3), organic carbon content was less (0.25%) and CEC value was 6. Low pH and high organic content increase bioavailability of Pb to the plant.

Table 16. Showing soil properties of contaminated sites

Location	pH	EC(ms)	Organic Carbon (%)	CEC (m.e./100gm)
Control	7.4±0.1,cd	1.20±0.1,d	0.42±0.28,a	7±1,a
I	7.7±0.2,cd	2.16±0.1,e	0.7±0.1,b	37.54±0.02,f
II	6.74±0.1,bc	0.423±0.001,b	0.834±0.001,a	16±1,c
III	5.86±0.01,ab	3.16±0.1,g	5±1,b	28.85±0.035,d
IV	5.63±0.15,a	0.54±0.01,c	6±1,b	9.7±0.2,b
V	8.00±1,d	0.26±0.1,a	0.845±0.001,a	49.6±0.15,g
VI	5.4±0.01,a	3.03±0.01,f	6±1,b	35.5±0.173,e

Phytoextraction Efficiency of *Ricinus communis*

Assuming that Pb phytoextraction follows a linear pattern, the quantity of Pb extracted per hectare per year (QPb: kg Pbha^{-1}y^{-1}) can be expressed as

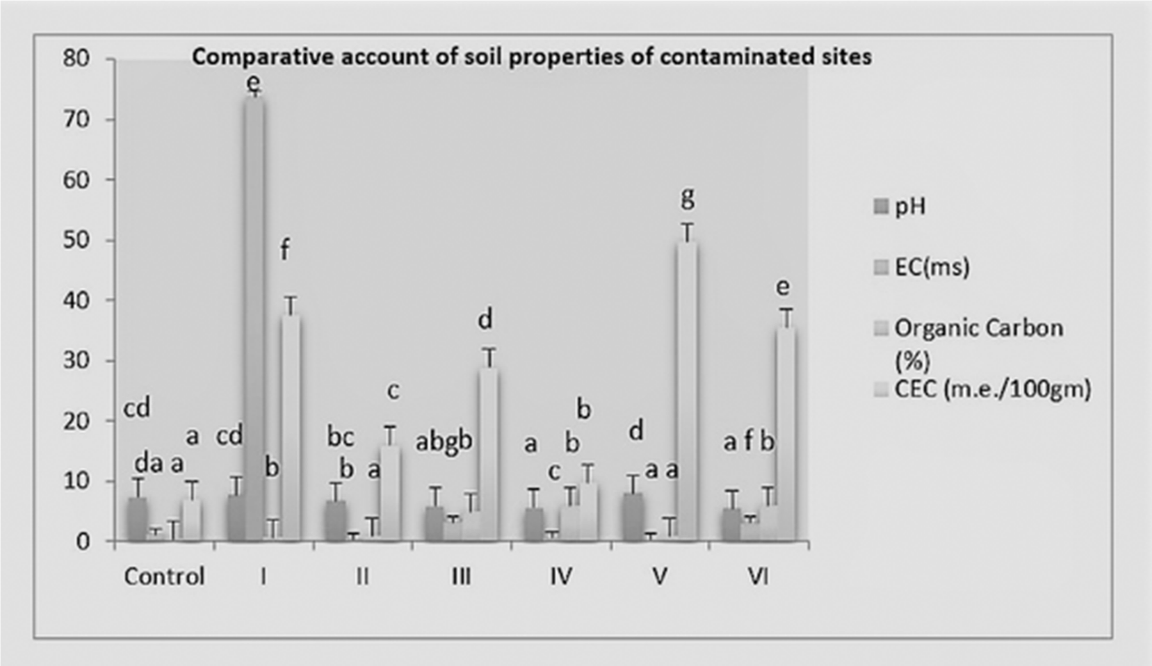
$QPb = (10^{-3} X bDW X D) X (10^{-6} X [PB]DW) X C$

[bDw – Dry weight of plant biomass per plant (g plant^{-1}DW) ; D – Density of plant per hectare ;

[Pb]DW – Total Pb concentration measured in shoots (mg Pb kg^{-1} DW) ; C – Number of plantsper year](Arshad et al,2008)

By applying this equation, from the present study it was calculated that the quantity of Pb extracted per hectare per year (QPb: kg Pbha^{-1}y^{-1}) is **3.426625 kg Pbha^{-1}y^{-1}** considering the parameters from Location I because it has highest Pb concentration in soil (2483 mg/kg); (bDw = 3.16 kg/plant, D = 3125 plants/hectare, [Pb]DW= 347 mg Pb kg^{-1} DW, C = 1plant /year).Using an average soil density of 1.2x10^{3}/m^{3}, the weight corresponding the top soil sheet of 1 m^{2} surface would be 120kg, therefore the quantity of Pb that correspond to 1 hectare of the contaminated top soil termed S (total Pb quantity in

Figure 3. comparative account of soil properties of different Pb contaminated sites
**The data represents mean plus minus sign SD of three independent replicas. Values carrying different letters are significantly different at p<0.05. Means sharing letters within a column are not significantly different by Tukey multiple range test(P<0.05).*



the top soil) is 2970Kg Pb, therefore the no. of years necessary for the *Ricinius* plants to perform total Pb extraction was calculated by the following formula t= S x10^3/QPb. Therefore the time estimated for total soil remediation is **865.89 years** for *R.communis* grow in L I.

CONCLUSION

In this study accumulation, tolerance and phytoremedial potential of wild plants growing in Pb contaminated sites were studied. Pb is chosen as the metal of interest because it is listed as the 2nd most hazardous substance in the list of ATSDR 2007. For this reason eleven Pb contaminated sites were screened and after quadrat study it was found that *Ricinus communis* is the most commonly growing plant in all these sites, it also has rapid growth rate and have high biomass. That is why *R. communis* was selected as the most suitable plant for phytoremediation. It was found from the study that the plant *R. communis* can tolerate very high amount of Pb in soil (2484 mg/kg). As the plants do not accumulate more than 100mg/kg dry weight Pb in their shoots they cannot be considered as hyperaccumulator but their ability to accumulate high amount of Pb in their roots and considering their rapid growth rate and biomass these plants have the potential for removal of Pb from Pb contaminated soil.

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