I.J.A.B.R., VOL. 2(1) 2012: 147-152 ISSN 2250 - 3579

INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

HEAVY METALS PHYTOREMEDIATION BY *PONGAMIA PINNATA* (L) GROWING IN CONTAMINATED SOIL FROM MUNICIPAL SOLID WASTE LANDFILLS AND COMPOST SUKALI DEPOT, AMRAVATI (M.S.)

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ABSTRACT

The aim of present study is to evaluate the potential of *Pongamia pinnata* (Leguminosae- Papillionoideae) for the Phytoremediation of Chromium (Cr), Copper (Cu) and Nickel (Ni). *Pongamia* can tolerate a wide range of soil types including saline, alkaline, sandy, heavy clay and rocky soils and waterlogged soils. During 2 month experiment the plants of *Pongamia pinnata* were grown in control and waste soil. Quality of waste soil have been investigated with respect to important physicochemical parameters pH, soil colour, texture, temperature, conductivity, chlorides, Na, K, etc. Vigorous growth of *Pongamia pinnata* plants has been recorded in waste soil with higher total chlorophyll content (1.99 mg chl. /g) than control (1.02mg chl./g) after 30 days. Anatomical study of T.S. of stem showed accumulation of certain pollutants from waste soil in the form of blackish deposits in cortical and vascular tissue. Secondly increase in size of xylem vessels and shrinkage of cortical cells. All recorded differences in morphological, anatomical and chlorophyll content in *Pongamia pinnata* were caused by differences of cumulative environmental conditions with dominant effects of the contamination in soil and microclimate. Heavy metal analysis of initial waste soil contains Cu (8.83 mg/0.1g), Zn (14.490 mg/0.1g), Cr (4.97 mg/0.1g), Ni (1.12 mg/0.1g), Fe (21.07 mg/0.1g), Mn (14.464 mg/0.1g), and Co (-0.120 mg/0.1g). The present study revealed that all these metal concentrations were lower down Cu (6.06 mg/0.1g), Zn (5.372mg/0.1g), Cr (0.564mg/0.1g), Ni (0.251mg/0.1g), Fe (22.175 mg/0.1g), Mn (5.372mg/0.1g), Co (-0.143mg/0.1g) except Fe after two month experiment.

KEY WORDS: *Pongamia pinnata*, phytoremediation, Sukali compost and landfill depot, Toxicity, Soil contamination, Heavy metals.

INTRODUCTION

The soil is primary recipient of solid waste (Nyles and Ray, 1999). Millions of tons of these waste from a variety of sources: industrial, domestic and agricultural, find their way into the soil. These waste ends up interacting with the soil system there by changing the physical and chemical properties(Piccolo and Mbagwu, 1997).However excessive waste in soil may increase heavy metal concentration in the soil and underground water. Heavy metals may have harmful effects on soil, crop and human health (Nyles and Ray, 1999; Smith *et al*., 1996).

Pongamia (*Millettia pinnata*), formerly known as *Pongamia pinnata*, is a tree/shrub with a broad distribution from India, through central and south-eastern Asia, Indonesia and into northern Australia. Itcan tolerate a wide range of soil types including saline, alkaline, sandy, heavy clay and rocky soils (including oolitic limestone) and waterlogged soils. In India, *Pongamia* is used in land reclamation and as a soil stabilizer. It is being trialed for 'phytocapping' landfill waste in Rockhampton (Venkatraman & Ashwath, 2009).Research has demonstrated that some plants are effective in cleaning up contaminated soil (Wenzel *et al*., 1999). Phytoremediation is a general term for using plants to remove, degrade soil pollutants such as heavy metals, pesticides, solvents, crude oil, polyaromatic hydrocarbons, and landfill leacheates for example, prairie grasses can stimulate breakdown of petroleum products. This emerging technology may offer a cost effective, nonintrusive and safe alternative to conventional soil clean up technique by using the ability of certain tree, shrub, and herb and grass sp. to remove, degrade or immobilize harmful chemicals from the soil (Chaney *et al*., 1997). Therefore, the present study was undertaken to find out the problems and prospects of Municipal solid waste and to study various physicochemical parameters and heavy metals of soil which is collect from Sukali compost depot, Amravati.

Sukali Municipal depot consists of different combination of household and commercial refuse from different zones of Amravati. The total area of Sukali depot occupied about 9.5 hectors, todayit totally polluted due to different solid waste and other contaminants. The present study investigates the potential of *Pongamia pinnata* plant to reduce the concentration of heavy metals viz. Cu, Zn, Cr, Ni and Mn and change in physiochemical parameters pH, soil colour, organic carbon, texture, temperature, conductivity, chlorides, Na. K and Ca. and chlorophyll content.

MATERIALS AND METHODS

Soil samples were collected, in June-2009from the Sukali compost and landfill depot Amravati., selected three pits was dugout and soil samples were collected from different zones with respective sampling area (fig.1).

Site A Site B Site C

FIGURE 1. Compost and landfill depot Sukali

Seeds of *Pongamia pinnata* were collected from Government Vidarbha Institute of Science and Humanities, campus Amravati in Year 2009. Selected seed were germinated in control soil (garden soil) and waste soil collect in Departmental garden in the month of June 2010 in a poly bags each containing 5kg of soil samples. Germination percentage was calculated in terms of percentage. Five plants were kept in each poly bags for experimental analysis. Experiments were carried out in triplicates.

Growth *P. pinnata* after 10 days Growth *P. Pinnata* after 1 month Growth *P. Pinnata* after 2 month

PLATE-1.*Pongamia pinnata plants grow* in control soil(right side)and waste soil (left side).

Plants were harvested individually after the completion of two month. After harvesting plants were cleaned under running tap water to remove adhering soil particles from the roots. The plant parts were sun and further Oven dried at 60^0C. The completely dried plant material is then pulverized to fine powder using a grinder. Plant ash was obtained at 450^0C for experimental analysis. For digestion 0.1g sample was dissolved in 5 ml 2N HCl and the solution was made up to volume in 50 ml volumetric flask for detection of Na, K and Ca by Flame Photometer. Analysis of heavy metals from plant material was carried out by dry ash method. This process is simple, non-hazardous and less expensive (Chapman and Pratt, 1961). The soil samples were air dried, crushed and passed through a 2mm sieve. One gram of soil sample was digested in a HNO_3 –$HCLO_4$ di- acid mixture (Jackson,1958)heating the mixture on hot plate at 150^0C. Analysis of the digest for the detection of metal ions such as Cu, Zn, Cr, Ni, Fe, Mn and Co was carried out using the atomic absorption spectrophotometer.

Moisture content in soil was calculated on the basis of oven dried sample weight(Dhyan *et al*.,1999).Soil texture was indicated by the proportion of different sized particles **(**Arora and Pathak,1989). pH of the soil was measured potentiometrically in a 1:2 or 1:5 soil- water suspension or in saturates soil paste. Electrical Conductivity measured in 1:2 or 1:5 soil-water suspensions with the help of conductivity meter. Temperature was recorded using mercury thermometer. Colour notations indicated by using Munsell`s soil colour chart. Na, K and Ca ions were determined by flame photometer (Hanway and Heidel, 1952). The organic carbon in the sample was oxidized with potassium dichromate and sulphuric acid (Walkely and Black, 1934).Calcium carbonate by titrimetric method **(**Piper, 1966**).** The chloride content of the soil was directly measured by titrimetric method (Santra *et al*., 2006).

Anatomical study, T.S. of stem and root sections were stained with saffranin and light green. These sections were examined under the compound microscope for detail study of cell structure. Staining sections were photographed with camera .Chlorophyll from leaves was extracted in 80% acetone and absorbance at 663nm and 645nm were read in a UV-Visible spectrophotometer following the procedure of Whatley and Arnon (1963).

RESULTS AND DISCUSSION

Seed Germination: Seeds of *Pongamia pinnata* were germinated in pots in week. Maximum 40 % seed germination was recorded in control soil. Whereas, in waste soil 30% seed germination under natural conditions.

Biomass Production: The plants of each pots were harvested after 30 - 60 days the biomass (fresh weight of roots and shoots) maximum production was observed in waste soil 14.58-31.9 gm. than control 13.27-19.52 gm. This may be due to various nutrients present in waste soil, it show stimulating effect on the growth and biomass production**.** The waste soil collected had a sandy texture together with the following parameters pH-8.57, moisture content- 4.32 %, Organic carbon- 43.33 %, Chlorides – 42.6mg/kg, Na- 20.5 mg/kg, K- 90 mg/kg, $CaCO_3$-75.24 %.

TABLE 1. Growth performance of *Pongamia pinnata* in field trial

Parameters	After 30 Days		After 60 Days	
	CS	WS	CS	WS
Biomass (gram)	13.27	14.58	19.52	31.9
Chlorophyll - a (mg chl./gm tissue)*	0.61	1.37	0.85	1.31
Chlorophyll - b (mg chl./gm tissue)*	0.40	0.62	0.16	0.28
Total Chlorophyll (mg chl./gm tissue)*	1.02	1.99	1.02	1.59
Na (mg/kg)**	2	4	5	6
K (mg/kg)**	330	360	310	550
Ca (mg/kg)**	12	3	25	14

***leaves, ** Plant sample**

Leaf Pigments: The gradual and significant increase in the total chlorophyll content in the plant grown in waste soil than control. However, relation between chlorophyll a and chlorophyll b content after 30-60 days in waste soil samples Chl. a = 1.37-1.31; Chl. b = 0.62-0.28 and control Chl. a= 0.61-0.85; Chl. b =0.40-0.16. Chlorophyll a and b significantly reduced after 2 month. This was due to heavy metal saturation increases in plant, after 2 month. Relation in total chlorophyll amount after 30 - 60 days the maximum in waste soil 1.99-1.59 than control 1.02-1.02 (Table 1). Della *et al*., (1998) suggested that the variation in chlorophyll content has been used in many studies in order to investigate the effect of pollutants on plants.At low concentration of metals Cu and Cd a mild increase in chlorophyll content reported by Wu *et al*.,(2003).However, as the concentration of metal increases in plants due to which chlorophyll content in plant reduced. Same type of results found in present study in one month period chlorophyll content of plant increases first and later on as the metal absorption increased because root system of plants deeply grown, showed decrease in chlorophyll content.

Anatomy: T.S. of stem showed accumulation of certain pollutants from waste soil in the form of blackish deposits in cortical and vascular tissues. Stem consist of upper layer cortical cells which is ruptured because of more secondary tissues added after maturation. 4-5 layered cortex in control soil and in waste soil found a 5-8 layered cortical tissues and vascular system contain secondary xylem and phloem tissues. Secondary xylem bears xylem vessels and medullary rays which were greatly extended in plants grown waste soil. Larger xylem cavities found in waste soil growing plant as compared to control soil growing plant. Secondary phloem 4-5 layered. T.S. of root showed accumulation of certain nutrients and metals which are beneficial for plant growth. Epidermal tissue breaks slightly due to pollutant in waste soil grown plant. Secondly increase in size of xylem vessels and shrinkage of cortical cells. Pith well developed in control but in waste soil grown plants pith region is much reduced. Kovacic and Nikolic 2005; Pandey *et al*. 2006 reported that the specific morpho-anatomical and physiological-biochemical characteristics are the result of plants adaption on environmental conditions. A high heavy metal concentration in the soil exerts negative effects on the plants (Gambus and Gorlach, 1992; Obata and Umebayashi, 1997). Plants usually adapt to high pollutant concentrations and unfavourable environmental conditions(Davis and Beckett, 1978; Wyszkowski and Wyszkowska,2003), which is likely to result in different morphology and anatomy.

FIGURE 2. T.S. Stem*P. pinnata*(Control soil)

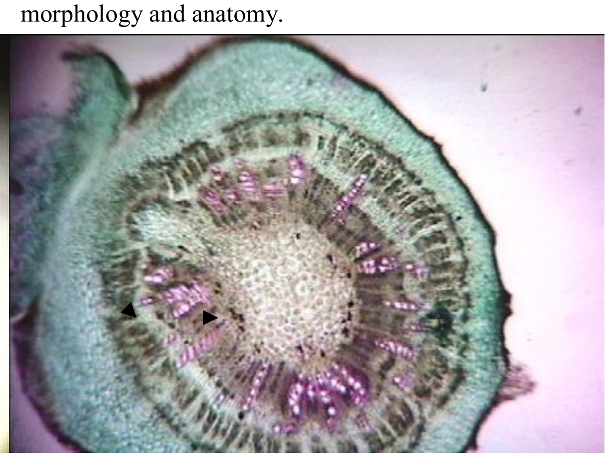


FIGURE 3. T.S. Stem *P. pinnata* (Waste soil)

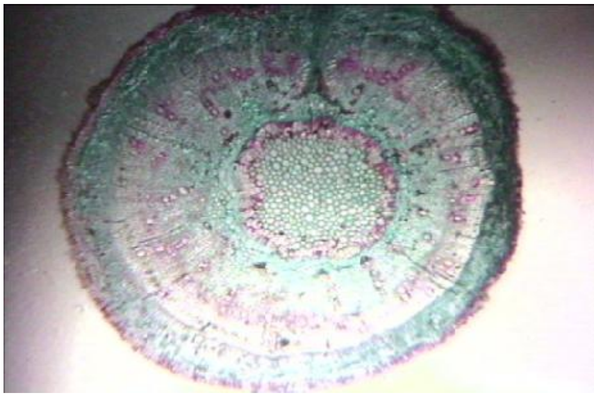


FIGURE 4. T.S. root *P. pinnata* (Control soil)

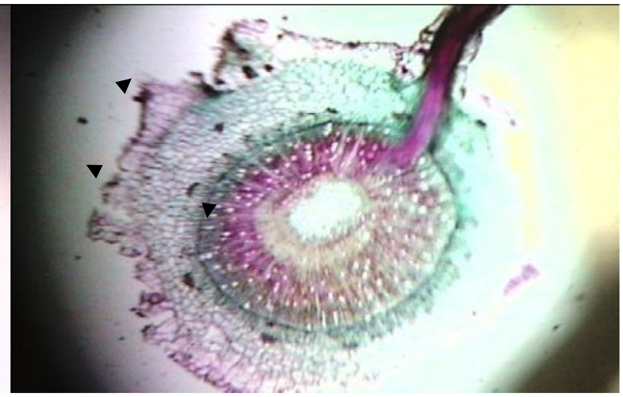


FIGURE 5. T.S. root *P. pinnata* (Waste soil)

Physicochemical analysis: Soil temperature plays an important role in many processes, which take place in the soil such as chemical reactions and biological interactions. The change of temperature will have an impact on the growth of biomass and the activity of the micro-organisms (Naranjo *et al*., 2004). The equilibrium between metal speciation, solubility, adsorption and exchange on solid phase sites is intimately connected to solution pH (Olomu *et al*., 1973; Sauve *et al*., 1997). Hence, numerous studies have found soil pH to have a large effect on metal bioavailability (Turner, 1994; McBride *et al*., 1997). Both Mn and Zn bioavailability are strongly affected by soil pH (Fergus, 1954; Turner, 1994). pH of initial waste soil (8.57) at temperature 29 ^{0} C mostly towards the alkaline side and deviated from neutral (7) to alkaline side and it decreases slightly after the *Pongamia* grown in this soil (7.38) at 31.8^{0}C. Colour of soil is useful indicator of some of the general properties of soil, such as the amount and state of organic matter and soil aeration, the colour of control soil was dark reddish brown and waste soil grayish brown, waste soil had very poor drainage or suffer from waterlogged. Moisture content in waste soil was found to be very low (4.32 %) as compared to control soil (10.13 %), wetness depends largely on the porosity of a soil, and for that reason clayey soil, which have a high porosity generally have larger water content than do sandy soils. The organic carbon content in initial waste soil (43.33 %) it is 20-30 % higher than in control soil (37.67%) after *Pongamia* grown in waste soil it totally absorbed by plant after two months, the organic carbon lowered down. The chlorides content in initial waste soil (42.6 mg/ kg) it was 20-30 % higher than control soil (28.4mg/ kg). The conductivity of initial waste soil was much higher (0.192X10^6) than control (0.128 X10^6), it become regulate after the *Pongamia* grown in waste soil (0.576X10^6).With regards to Na, K, Ca and $CaCO_3$ content, the initial waste soil contains more amount of the micronutrients than control except Ca which is higher in control soil it indicating the high productivity in the waste soil.

TABLE 2. Physicochemical analysis of *Pongamia pinnata* plants grown in Garden and waste soil after two months.

Parameters	Initial soil analysis		After two months soil analysis	
	Control soil	Waste soil	Control soil	Waste soil
Temperature (^{o}C)	25	29	29.9	31.8
pH	8.47	8.57	8.33	7.38
Colour	Dark reddish brown	Grayish brown	Dark reddish brown	Grayish brown
Moisture content (%)	10.13	4.32	11.12	7.94
Moisture correction factor (mcf)	1.10	0.11	1.11	1.07
Soil texture	Sandy loam	Sandy	Sandy loam	Sandy
Organic carbon (%)	37.67	43.33	40.79	Nil
Chlorides (mg/kg)	28.4	42.6	42.6	56.8
Conductivity µmoho/m	0.128 X10^6	0.192X10^6	0.128X10^6	0.576X10^6
Na(mg/kg)	7	20.5	5.5	10.5
K(mg/kg)	10	90	8	74
Ca(mg/kg)	700	280	550	260
$CaCO_3$(%)	22	75.24	17.76	107

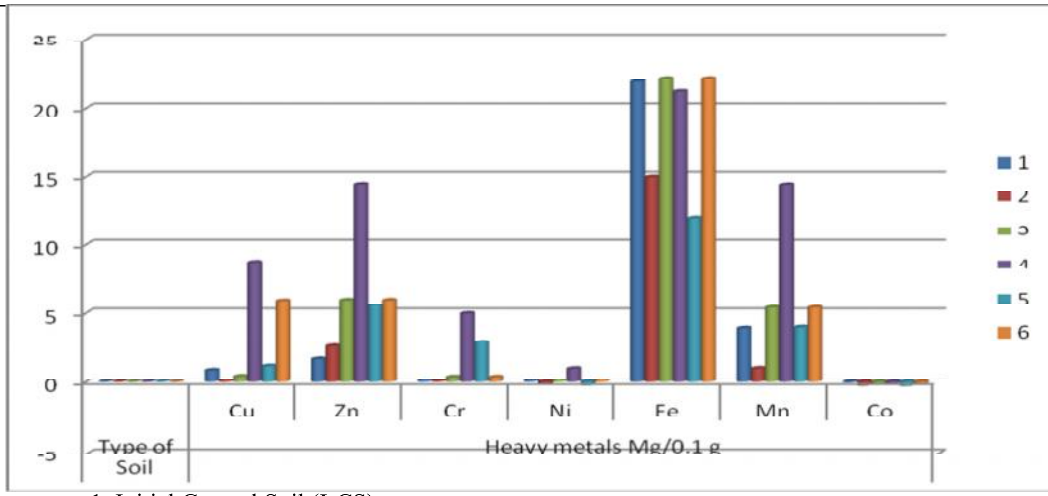
Metal Accumulation

The heavy metal content in plant and soil is presented in table 3.*Pongamia* grown in waste soil resulted to corresponding decrease in the concentration of certain heavy metal which are presented initially in Waste soil collect from landfill site. The soil concentration of all metals except Fe at harvest after (2 month), however, significantly decreased in comparison to the pre planting concentration. The *Pongamia pinnata* plant accumulates more metals from dump soil as well as control. The result showed that, highest concentration of heavy metals was recorded Cu, Zn, Cr and Mn in initial waste soil it significantly reduced due to *Pongamia pinnata* grown during the two month experiment. This shows the potential of *Pongamia* plant for remediation. With increasing metal load in dump soil, where these plants are grown, there is a greater tendency for their bio-accumulation.

It is well known that elements such as Cu, Mo, Ni, Cr, and Zn, among others, are essential for plant growth in low concentrations (Taiz and Zeiger, 1998). Nevertheless, beyond certain threshold concentrations, these same elements become toxic for most plant species (Blaylock and Huang, 2000). Studies by Peralta *et al*. (2001) on alfalfa plants found that they were able to accumulate 8 500 mg.kg-1 of Ni and 12 000 mg.kg-1 of Cu.Soil pH play a major role in the release of heavy metals as it controls the solubility and hydrolysis of metal hydroxides, carbonates and phosphates, ion-pair formation, solubility of organic matter, as well as surface charge of Fe, Mn and Al-oxides, organic matter and clay (Takalioglu *et al*., 2006).

TABLE 3. Comparison of metal phytoremediation in control and waste soil through *Pongamia pinnata*.

Type of Soil	Metals ions mg/0.1g						
	Cu^{2+}	Zn^{2+}	Cr^{3+}	Ni^{2+}	Fe^{3+}	Mn^{3+}	Co^{3+}
Amount of metals present in Garden soil (control)	1.006	1.744	0.182	0.118	21.72	4.019	-0.053
Amount of metals absorbs by Plants in Garden soil (60 days)	0.231	2.585	0.079	-0.224	14.97	1.133	-0.274
Total Metals Remains in Control soil	0.606	6.106	0.564	0.251	22.175	5.372	-0.143
Amount of metals present in Municipal Waste Soil	8.83	14.490	4.97	1.12	21.07	14.464	-0.120
Amount of metals absorbs by Plants in Municipal Waste Soil (60 days)	1.288	5.564	3.072	-0.254	11.99	4.092	-0.294
Total Metals Remains in Waste soil	6.06	6.106	0.564	0.251	22.175	5.372	-0.143



1. Initial Control Soil (I-CS)
2. Plant absorbs Metals in Control Soil (60 days) (PAM (60 days) – CS)
3. Total Metals Remains in Control soil(TMR-CS)
4. Initial Waste Soil (I-WS)
5. Plant absorbs Metals in Waste Soil (60 days) (PAM (60 days) – WS)
6. Total Metals Remains in Waste soil(TMR-WS)

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