Acceleration of Lead Phytostabilization by Maize (*Zea mays*) in Association with *Gliricidiasepium* Biomass

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***Abstract*—** *Soil where shooting practices are conducted is extremely contaminated with heavy metals, exclusively by Pb, due to the bullets and bullet fragments. These extreme concentrations of Pb, generate an unfavourable surroundings for agriculture and human health through phytoaccumulation. This study was conducted to assess the possibility of particular bio-amendment, phytostabilization on the reduction of bioavailable Pb in such contaminated soil. Biomass (BM) produced from Gliricidiasepium was used to see its ability to be used in soil remediation together with Maize (Zea mays) as phytostabilizer. A pot experiment was conducted with Maizeby adding BM at three different percentages, 1, 2.5 and 5% (w/w). Soil without amendments served as the control and arranged in a complete randomized design. By maize, translocation rate of heavy metals into crop were determined. After sixth week, grown maize were harvested and analyzed followed by digestion with con.*HNO_3*. The most significant immobilization (*$p<0.05$*) was indicated by treatment 5% BM for Pb than control. Metal translocation towards plant decreased with increasing application rate of soil amendment,Gliricidia woody BM. Determination of Plant factor (PF) and Translocation factor (TF) indicates that PF>TF in maize. For the treatment 5% BM, PF and TF for Pb are 1.22 and 0.15 respectively. Thereby maize can be considered as a potential phytostabilizer. At the same time efficiency of phytostabilizing nature of maizecan increase together with the application of soil amendment – BM.*

***Keywords*— *Amendment, Biomass, Immobilization, Phytostabilizer, Translocation factor*.**

I. INTRODUCTION

Soils polluted with heavy metals have become common across the world due to increase in geologic and anthropogenic activities [1]. Some of these happenings include mining and smelting of metals, burning of fossil fuels, use of fertilizer and pesticide, exhaust of auto mobiles and effluent from battery industries [2, 3]. Some heavy metals such as Co, Cu, Fe, Mn and Zn are required in minute quantities by organism; however, excessive amount of these elements can become harmful to organism. Other heavy metals such as Pb, Cd, Hg and As don’t have any beneficial effect on organism and are thus regarded as the keypressures since they are very detrimental to both plant and animal [3, 4].

Lead is a non-essential water soluble heavy metals that contaminates food chain by phytoaccumulation in plant parts as it is easily mobile in soil and bioavailable eventually transisting and accumulating in top consumers of food chain i.e. human. Pb backlog in environment causes harmful effects to both plants and animals. Plants growing on these soils show a reduction in growth, performance and yield [1]. This has been recorded by Oancea[5]. So the problems which are raised by the contamination of heavy metal in the eco system can be partially solved by the approaches of bioremediation technologies. It is an economically feasible method when associate with other treatment systems[6]. In recent times, phytoremediation act as an emerging technology, which involves rising of plants in metal polluted soil [7].

This phytoremediation mechanism includes phytoextraction, phytostabilization and phytovolatilization[1]. Plants which act as phytostabilizers accumulate metals in roots than plant shoots [6]. The efficiency of phytostabilization depends on the plants used together with soil amendments [1]. Biomass (BM) of Glirecidiasepium as soil amendment, which is easy to handling, inexpensive, and most importantly aren’t toxic to plants and eco system.

Thereby, the objective of the study was to explore the potentiality of Maize (Zea mays) as phytostabilizer together with soil amendment - *Gliricidiasepium*BM to clean the agricultural sites which are affected by Pb contamination.

II. MATERIALS AND METHODS

2.1 Soil sample collection

The pre-characterized bulk surface sample of contaminated soil was collected from Diyadhalawa, Sri Lanka for the experiments. These soil samples are contaminated with heavy metals such as Pb and Cu due to shooting practices by forces. The collected soil samples were thoroughly mixed and standardized into one sample, air dried and sieved mechanically to particle size of < 2 mm of prior to experiments [8].

2.2 Biomass production

BM was produced by using dried *Gliricidiasepium* chips which obtained from a Dendro power industry at Thiruppane, Anuradhapura district, Sri Lanka which was ground in a blender and sieved to < 1 mm of particle size prior carrying out tests.

2.3 pH and Electrical Conductivity (EC) of BM

pH and EC of BM was measured in 1:20 suspensions BM-to-water using a digital pH meter (702SM Titrino, Metrohm, Swiss) and EC meter (Orion 5 star meter, Thermo Scientific), respectively after shaking in a horizontal shaker at 100 rpm constant shaking rate for 4 h.

2.4 Experimental set up

The air dried and 2 mm sieved contaminated soil samples were used in pot experiment to assess the potentiality of BM as soil amendment to immobilize the bio available toxic Pb in contaminated site. Untreated soil was used as control. Pot mixture was prepared by mixing 250 g of soil with BM separately according to the mass fraction of 1.0, 2.5 and 5.0 % (w/w). After filling of potting mixture, these filled pots were placed in a controlled dark room and the mixtures were allowed to achieve equilibrium during two weeks of time. After the equilibration period the pots were transferred into a poly tunnel and they were kept for six weeks.

2.5 Soil analysis

2.5.1 pH and EC

The pH and EC of the soils were measured using a digital pH meter (702SM Titrino, Metrohm, Swiss) and EC meter (Orion 5 star meter, Thermo Scientific), respectively after shaking in a horizontal shaker at 100 rpm constant shaking rate for 4 h.

2.6 Plant analysis

All maize plants were harvested at the end of the sixth week from the date of planting. Harvested plants were rinsed with tap water and followed with deionized water. Roots and shoots were separated. Both shoot and root lengths were measured after blotting with filter paper. Then plant tissues were dried in an oven at the temperature of 60^0C for 48 hrs for the further analysis including determination of dry matter content. The total contents of Pb in root and shoot of the maize were analyzed separately by AAS after digestion with 10 ml of concentrated HNO_3 acid in a closed poly phenyl vessel temperature controlled microwave digester system (Milestone ETHOS PLUS labstation with HRP-1000/10S high pressure segmented rotor).

2.6.1 Calculation of translocation and plant factor

The following equations were used to calculate the both plant factor or bioaccumulation factor (BAF) and translocation factor

$$\text{Plant factor (PF)} : \frac{\text{Concentration of metal in root}}{\text{Concentration of metal in soil}}$$

$$\text{Translocation factor (TF)} : \frac{\text{Concentration of metal in shoot}}{\text{Concentration of metal in root}}$$

2.7 Statistical Analysis:

The experimental design was set up in a Complete Randomized Design (CRD) with three treatments and each was triplicated. Difference among treatments was tested with analysis of variance (ANOVA) and mean separation were conducted using DNMRT procedure using SAS 9.1 software.

III. RESULTS AND DISCUSSION

3.1 pH and EC of BM

BM showed a weakly acidic pH of 6.8 (in 1:20 ratio of BM to water). Furthermore, increase in pH values has the capability to immobilize heavy metals and mobilization of oxy-anions in heavy metals contaminated soils [9, 10]. Harter stated that soil pH is the major factor affecting metal availability in soil [11]. BM has the net surface charge of negative, The expansive peak at 3359 cm^{-1} in the spectrum of feedstock (BM) specifies the presence of –OH stretching due to strong hydrogen bonding [12]. This will help to bind up the metal cations like Pb^{2+} from the contaminated soil together with its surface [13, 14]. In case of EC, that has the value of 99. 0µS/cm.

TABLE 1
pH and EC of BM

Treatments	EC (µS/cm)	pH (water)
BM	99. 0	6.8

3.2 Soil analysis

3.2.1 pH and EC of soil

The pH values of all the treatments were laid between 5.62 - 6.71 (Table 2) and did not show any significant difference at 5% probability level. But results have shown a slight increase in pH with the applied concentration of BM. In the treatment levels 5% BM had high pH and showed statistically significant when associate with control.

EC values of the all treatments fall between 28.52 - 78.60 (Table 2). It showed little increasing pattern as pH and if we compare the treatments the maximum was achieved by the treatment level of 5% BM and this is highly statistically significant among the other applications.

TABLE 2
Effect of BM application on soil pH and EC

Treatments	pH	EC (µS/cm)
S (Control)	5.62 ^{d}	28.52 ^{d}
1% BM	6.36 ^{c}	34.30 ^{c}
2.5% BM	6.53 ^{b}	45.83 ^{b}
5% BM	6.71^{a}	78.60 ^{a}

Means with the same letter are not significantly different

3.3 Plant analysis

3.3.1 The consequence of BM on the growth of maize plants

Maize plants which were grown in control soil exhibited low biomass production when compared to the plants that had received 1.0, 2.5 and 5.0% BM applications due to common symptoms of heavy metal phytotoxicity. Two weeks after seed germination, signs of metal toxicity in the aerial parts of maize plants were noticeable in the control soil.

Maize plants in 1% BM amended soil became collapsed by third weeks onwards after germination and 2.5% BM by fourth week. However, plants in 5% BM amended soils showed a great resistivity against heavy metals stress. The plant biomass production with increasing application rate of Gliricidiawoody BM showed a significant increase *($p<0.05$)* compared to the control.

Maize grown in control soil showed the least dry weight compared to the other treatments. That may be due to the phytotoxicity of heavy metals which inhibiting the growth of plants. Plants grown in 5% BM amended soils exhibited a 3.8-fold raise in biomass (Root+Shoot) compared to the control plants. This may be due to the higher release of nutrients by 5% than the 1% BM into soil by the BM degradation (Figure1). Therefore, biomass of plants indicated a significant effect of BM with its application on the growth of plants. These findings agreed with other recent studies[15] andthe higher plant productivity is encountered when BM is applied and attributed this enhancement to the immobilization of heavy metals in soil.

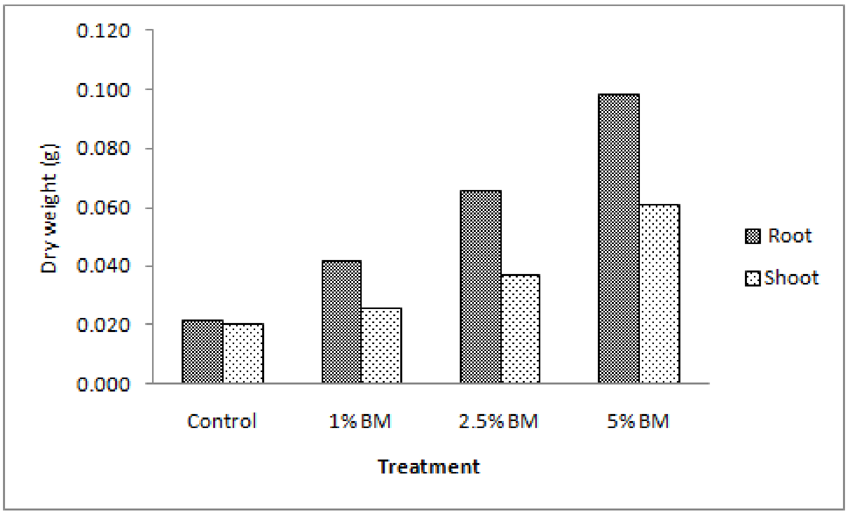

FIGURE 1: EFFECT OF BM ON THE DRY WEIGHT OF ROOTS AND SHOOTS OF PLANTS IN CONTAMINATED SOIL

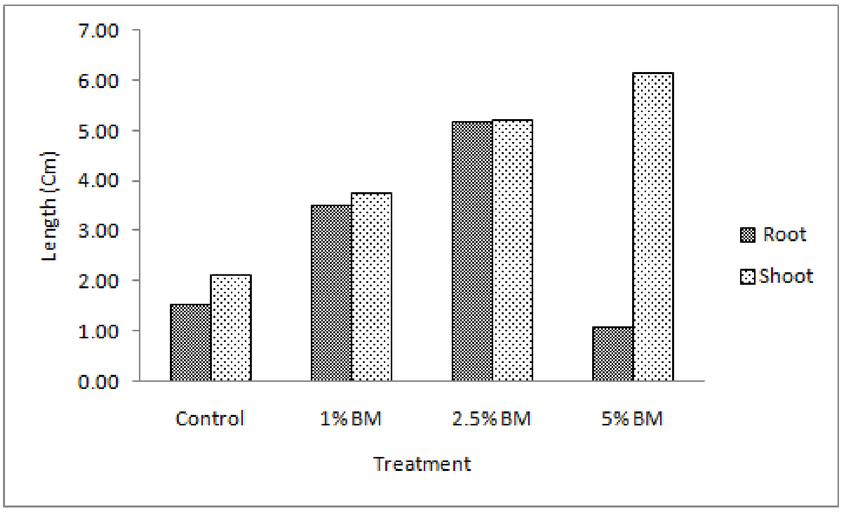

FIGURE 2: EFFECT OF BM ON THE LENGTH OF ROOTS AND SHOOTS OF PLANTS IN CONTAMINATED SOIL.

3.3.2 Accumulation and translocation of Pb in plants

Generally, two tactics have been suggested in using plants to extract heavy metals from contaminated soils. First approach is the use of plants with extraordinary ability to accumulate the pollutant known as “hyperaccumulators” and second is the tolerant plants [7]. A plant's ability to accumulate metals from soils can be estimated using the PF or bioaccumulation factor (BAF), which is defined as the ratio of metal concentration in the roots to that in soil and the plant's ability to translocate the metals from the roots to the shoots is measured using the TF, which is defined by way of the ratio of metal concentration in the shoots to the roots [12].

Enrichment occurs when a contaminant is accumulated by a plant is not degraded rapidly, resulting in an accumulation in the plant [16, 17,18, 19]. By comparing PF and TF, we can compare the ability of maize with different treatments in taking up metals from soils and translocating them to the shoots. If it is tolerant plant, it may tend to restrict soil–root and root–shoot transfers, and therefore have much less accumulation in its biomass.

All the plants treated with different treatments showed the ability to accumulating heavy metals in the roots, which means plants have higher PF than TF. But some of them had low TF and PF values, which means limited ability of heavy metal accumulation and translocation by the plants (Table 3).Among these, control plant had the highest TF. Normaland phytotoxic concentrations of Pb, were reported by Levy which were 0.5–10 and 30–300mg k -1 [20]. Almost all plants in different treatments showed heavy metal concentration higher than the normal or phytotoxic levels. These results may indicate that maize growing on the contaminated soil with heavy metals were tolerant for such metals. Restriction of upward movement from roots to shoots can be reflected as one of the tolerance mechanism [21].

Phytostabilization can be used to minimize migration of contaminants in soils [22]. This process uses the ability of plant roots to modify the environmental conditions via root exudates. Plants can immobilize heavy metals through absorption and accumulation which are occurred through roots, adsorption onto roots, or precipitation within rhizosphere. This process reduces metal mobility and leaching into ground water, and also reduces metal bioavailability for entry into the food chain. In addition to these, application of BM as bioamendmnets also induce the immobilization of heavy metals especially by 5% BM. Metals accumulated in the roots are considered relatively stable as far as release to environment is concerned. So maize has the ability to be used as phytostabilizer.

TABLE 3
PLANT AND TRANSLOCATION FACTORS OF MAIZE FOR PB AND CU IN CONTAMINATED SOIL

Treatments	Plant factor	Translocation factor
Control	5.81	0.04
1% BM	1.69	0.12
2.5% BM	3.60	0.05
5% BM	1.22	0.15

3.3.3 Effect of BM on uptake of Pb by maize

Bioaccumulation of Pb in maize as phytostabilizer grown in BM amended and unamended soils are exhibited in Figure 3. Soil amendments used in phytostabilization should have the ability to inactivate heavy metals and thus reduce metal uptake [23]. BM applications significantly reduced the uptake of Pband its bioaccumulation in maize than the control together with its application rate. The highest heavy metal concentrations were found in shoots of maize grown in control soil. Minimum accumulated concentrations of Pb were exhibited in 5% BM treatment. With increasing BM application (i.e. 1, 2.5 and 5%), the bioavailable concentrations of Pb had been reduced compared to the control (Figure 3).

The decline nature of accumulated concentrations in plants that had achieved was due to greater immobilization of Pb in heavy metal contaminated soil via sorption and adsorption mechanisms. The retention potential of these metals in mesopores of the BM could be increased with BM application rates and due to negative surface charge of BM [11]. Due to increase in soil pH also reduce the concentration of Pb by precipitation [24] after the application of BM. Hence the reduction of toxic levels of these heavy metals in the existence of BM could be endorsed to improve the patterns of essential nutrient uptake by maize which resulting high biomass production.

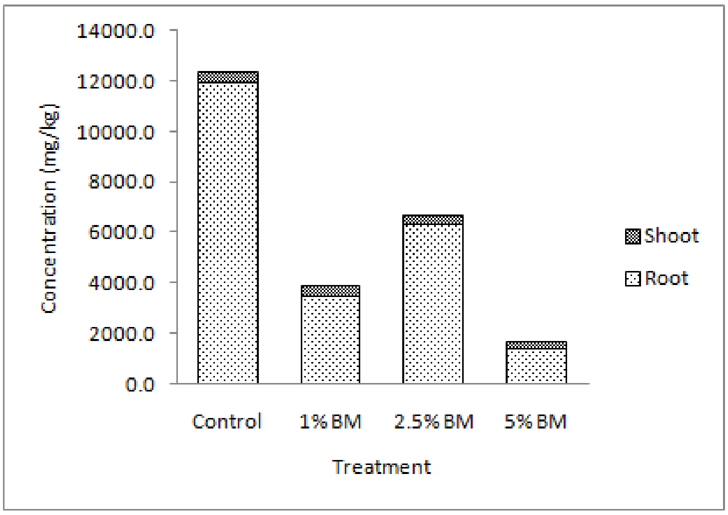

FIGURE 3: ACCUMULATED CONCENTRATIONS OF PB IN ROOTS AND SHOOTS OF MAIZE GROWN ON BM AMENDED AND UNAMENDED SOIL.

IV. CONCLUSION

Maize has been renowned as a best phytostabilizer to enhance the quality of soil, which is severely affected by accumulated Pb.Results of the presence study suggest that maize has the potential to remove the metal Pb from contaminated site significantlytogether with the application of BM as soil amendment. BM affects the behavior of Pbin contaminated soil by altering their solubility and availability. BM, which was applied at the rate of 5% was the most effective amendment when compare to other application rate of BM – 1% and 2.5%. Maize which was grown together with 5%BM had higher PF than TF. These resultsindicate that maize + 5% BMare the best treatment of phytostabilization to eradicate the metal concentration from contaminated site. These types of treatments can be carried out in field level to enrich the quality of affected soils in Sri Lanka. Furthermore, additional studies should be designed to get maximum positive result by increasing the application rate of BM above 5% together with Maize.

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