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EFFECT OF EGGSHELL AND LIME ON GROWTH AND MINERAL NUTRITION OF KALMI (*Ipomoea aquatica*: CONVOLVULACEAE) GROWN ON LEAD CONTAMINATED SOIL

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Abstract

A pot experiment was conducted to evaluate the effect of Eggshell and Lime in ameliorating lead uptake to alleviate toxicity of lead in Kalmi (*Ipomoea aquatica*).The lengths, fresh and dry weights of shoot was decreased significantly in 200 mg Pb kg^{-1}soil as compared to the control. The similar significant decreasing trend in macro nutrient concentration in shoot and root was also observed. The percentage of N, P, K, S, Mg and Ca concentrations in edible parts (shoots) decreased by 7.73, 54.55, 12.50, 35.29, 17.65 and 19.61, respectively in 200 mg Pb kg^{-1} treated pots compared to the control. On the other hand, Pb concentration in shoot and root was the highest at 200 mg Pb kg^{-1} treated pots (36.14 and 111.14 mg Pb kg^{-1}) and the lowest was in the control treatment (3.48 and 14.48 mg Pb kg^{-1} pot^{-1}). Treatments of lime and eggshell had positive effects on the productivity of Kalmi and remediating the effect of lead uptake by the plants. Between the two amendments eggshell showed better efficiency in ameliorating the toxic effects of lead and increasing the macro nutrients uptake by plants. Lime and eggshell ameliorated Pb uptake of kalmi in shoot (edible parts) by 32.26 and 61.79% compared with the control and reduced root to shoot transfer (TrF) of Pb by15.15 and 30.30% in shoots and roots of kalmi compared to the untreated pots.

Key words*:* Lead; Amelioration; Egg shell; Lime; Kalmi; Contamination.

INTRODUCTION

The presence of heavy metals in soil and the surrounding environment raises serious concern about the hazardous environmental impact as a result of excessive waste and effluent discharged to agricultural lands. Bangladesh has, at present, about 30,000 large and small industrial units. They are discharging their wastes and effluents into the natural ecosystems in most cases without any treatment, thus causing environmental pollution especially with heavy metals and organic toxins. Agricultural areas are contaminated thereby, and food quality is impaired (Chamon *et al.* 2005).

Whenever toxic heavy metals are exposed to the natural ecosystem, accumulation of metal ions in human body will occur through either direct intake or food chains (Yoo *et al*. 2002). Zeng *et al*. (2007) stated that excess of Pb reduced dry weight of rice pronouncedly at harvest when the grain yield also decreased Pb toxicity causes oxidative stress and enzyme inactivation in rice plants.

The main purpose of heavy metal amelioration is to minimize the risk of these toxic compounds to human and ecological health.Organic matter amendment is preferred because of its effectiveness, inexpensive availability, and additional benefits for plant growth and soil structure (Brown *et al.* 1999).
Kalmi (*Ipomoea aquatica*) is a popular vegetable grown in Bangladesh; it is considered to be an excellent source of essential nutrients, vitamins, minerals that are essential for human health. Kalmi was used for phytoremediation that have high and fast biomass production and ability of translocation of contaminants like Pb in our research into the plant shoots. However, little work has been done to assess the nutritional benefits of eggshell. Eggshell is a solid waste, with production of several tons per day. Eggs represent a major ingredient in a large variety of products, such as cakes, salad dressings and fast foods, whose production results in several daily tons of eggshell waste and incur considerable disposal costs in the world. About 250,000 tons of eggshell waste are produced annually worldwide (Verma *et al.* 2012). Eggshell waste is widely produced from house, restaurant and bakery. Eggshell has as an important constituent pure $CaCO_3$ and a little developed porosity. Its composition has been reported

chemically (by weight) as follows: calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%) and organic matter (4%) (Stadelman 2000). Occasionally, parts of this waste are used as fertilizer, due to its high content of calcium.Many researchers studied the removal capacity of toxic heavy metals by the reused eggshell (Park *et al.* 2007 and Stadelman 2000). Considering this fact, a research work was performed to reduce lead (Pb) uptake into Kalmi and thus to minimize their entry into the food chain and to test the effectiveness of organic amendment (Eggshell).

MATERIAL AND METHODS

Soil samples (0 to 15 cm depth) were collected from Dhamrai and processed for pot experiment. The characteristics of Dhamrai soil were (according to Jackson 1962 and Black 1965): Texture-silty loam, pH- 5.5, %Organic Carbon-0.95, %Organic matter-1.64, %Moisture content-27.88, total nutrient concentrations were: %N- 0.14, %P-0.05, %K-0.80, %S-.03, %Ca-0.90, %Mg-1.45, CEC- 6.29 meq/100g, Pb–26.41mg kg^{-1}(critical level of Pb concentration for soil i.e. 100 mg kg^{-1} according to Kloke 1980).

Plastic pots were filled with 4 kg soil. Basal dose of fertilizer was added at medium rate for kalmi (4 plants/pot) (BARC 1997) and 200 mg Pb kg^{-1} was added having three replications. Eggshell (40 g pot^{-1}) (added 30 days before sowing) and lime (CaO) (17.83 g pot^{-1}) (10 ton/ha) (added just before sowing) were added as remedial measures. The treatment combinations are given in Table 1.

The pots were arranged in a completely randomized design. The plants were irrigated with distilled water whenever required. The crops were harvested at maturity.

Table 1.Treatment combinations.

Denotations	Treatment
T0	Control
T1	200 mg kg^{-1} Pb
T2	200 mg kg^{-1} Pb+ CaO
T3	200 mg kg^{-1} Pb+ Eggshell

Soil samples were digested with HCl: HNO_3 (3:1), and plant samples with a HNO_3:$HClO_4$ (5:1) mixture in closed systems (Blum *et al.* 1996). All elements were measured in the extracts by Atomic Absorption Spectroscopy (AAS).

Translocation Factor (TrF)

The Translocation Factor (TrF) of Pb in Kalmi was calculated. The study served its importance due to the dietary importance of the vegetables to humans. The root to the edible part translocation factor and its remediation by applying different amendments were calculated by the following formula (Rangnekar *et al.* 2013):

Translocation Factor (TrF) = Pb conc. in shoot / Pb conc. in root

The results of the experiment were statistically evaluated by using ANOVA (Analysis of Variance) and Duncan's Multiple Range Test in IBM SPSS statistics version 20 as outlined by Gomez and Gomez (1984). The letter was used for testing the significance of differences between mean values. The 0.05 level of probability was chosen for the statistical judgment.

RESULTS AND DISCUSSION

Growth and yield parameters

Organic amendment (eggshell) and inorganic amendment (Lime CaO) ameliorated Kalmi growth and yield parameters significantly compared to 200 mg Pb kg^{-1} treated soil. (Table 2).The length, fresh and dry weights of shoot decreased by 6.55, 1.79 and 24.05%, respectively, in 200 mg kg^{-1} Pb treated pots (T_1) compared to the control (T_0).A similar significant decreasing trend was also observed in case of root which were 20.56, 24.03 and 14.50%, respectively, for root length, fresh and dry weights, in Pb treated pots (T_1) compared to the control (T_0) (Table 2). Batti *et al.* (2013) reported that Pb reduced the morphological parameters, such as shoot/root length, shoot fresh/dry weights, number of tillers. Pb stress also decreases the photosynthetic pigments such as chlorophyll a, chlorophyll b but carotene contents were increased. Na^+, K^+ ion contents were also decreased by Pb.

Hussain *et al.* (2013) reported that seed germination, early growth seedling, root-shoot length, root-shoot fresh and dry weights, total protein content of maize were reduced by the increased lead concentrations. Such growth retardation was due to metals toxicity that resulted in damages to various physiological and biochemical processes.

Table 2. Influence of CaO and Eggshell application on the growth and yield parameters of Kalmi.

Treatment	Shoot			Root		
	Length (cm/plant)	FW (g/pot)	DW (g/pot)	Length (cm/plant)	FW (g/pot)	DW(g/pot)
T_0 (Control)	25.35 a	41.25a	6.82 b	33.90 c	5.16ab	3.38 a
T_1(200mgkg^{-1}Pb)	23.69 a	40.51a	5.18 a	26.93 a	3.92 a	2.89 a
T_2(200mgkg^{-1}Pb+CaO)	24.84 a	42.02b	9.27 c	31.50 b	5.35 b	3.80 a
T_3(200mgkg^{-1}Pb+Eggshell)	27.41 a	62.08b	9.76 c	33.06 c	5.88 b	4.19 a

Means followed by the same letter (s) in a column do not differ significantly from each other at 5% level by DMRT.

The ameliorative effect of eggshell and lime (CaO) was clearly observed in the shoot samples of kalmi. Application of lime (CaO) and eggshell increased the shoot length, fresh weight of shoot and shoot dry weight 4.85 and 15.70%, 3.73 and 53.25%, 78.96 and 88.42%, respectively, compared to 200 mg Pb kg^{-1} treated pots (T_1) (Table 2). Root length, root fresh and root dry weights in CaO and Eggshell treated pots were increased by 16.97 and 22.76%, 36.48 and 50%, 31.49 and 44.98% respectively, compared to 200 mg Pb kg^{-1} treated pots (T_1) (Table 2).The results obtained from the Table-2 indicated that, shoot and root biomass was decreased by the application of Pb, but increased by the addition of the amendments. Chamon *et al*. (2005) stated that fresh and dry weight of shoot was enhanced by 24 and 38% in lime treated pots compared with the unlimed pots. Also shoot length of wheat was significantly higher by 39% in limed pots than the unlimed pots with Tejgaon soil. Between the two amendments Eggshell ameliorated more than CaO. The positive influence of organic substances on plant growth is a well-known phenomenon, which is due to indirect effects of humic substances acting as suppliers and regulators of plant nutrients and due to direct effects of humic substances, e.g. as respiratory catalysts. The dry matter production of onion, potatoes, cabbage and lettuce was inhibited severely by Pb toxicity (Vaughan and Malccolm, 1985).

Macro nutrients and heavy metals

Due to lead application macro nutrients concentrations in kalmi shoots decreased as compared to the control (Table 4). The percentage of N, P, K, S, Mg and Ca concentrations in the shoots of kalmi decreased by 7.73, 54.55,12.50, 35.29, 17.65 and 19.61% in 200 mg kg^{-1} lead treated pots compared to the control. From the results, it was evident that the application of 200 mg kg^{-1} Pb lowers the concentration of macro nutrients in root and shoot of the plant. Many authors reported an antagonistic effect on macro nutrient uptake by rice plants due to application of Pb (Parvin, 2016; Chamon *et al*. 2005). In most cases, Pb blocks the entry of nutrients in the root system.

Heavy metal uptake was ameliorated in kalmi shoot samples by using lime and eggshell. Lime (CaO) increased the percentage of N, P, K, S, Mg and Ca in kalmi shoot significantly by 3.94, 506.67, 44.90, 72.73, 7.14, 35.37%, respectively and eggshell increased kalmi shoot by 10.34, 540, 59.18, 90.91, 21.43 and 54.88%, respectively (Table 3).

The percentage of N, P, K, S. Mg and Ca concentrations decreased by 8.94, 53.33, 10.31, 13.43, 6.25, and 17.92 respectively, in roots of Pb treated pots compared with the untreated pots. The decrease in macro nutrients concentrations might be due to the interference of Pb with the physiological function of the plant and in most cases, Pb blocks the entry of macro nutrient in the root system (Chamon *et al.* 2005). Elahi *et al.* (2010) reported that due to adverse or antagonistic effect of Pb, the uptake of macronutrients by rice and other plants decreased significantly.

The ameliorative effects of lime and eggshell were also clearly observed in the root of kalmi. Lime (CaO) increased the % N, P, K, S, Mg and Ca in the root of kalmi by 1.23, 435.71, 12.64, 75, 13.33 and 35.63%, respectively. On the other hand, egg shell increased the percentage of N, P, K, S, Mg and Ca in the root of kalmi 6.75, 457.14, 19.54, 116.67, 40 and 50.57%, respectively (Table 3).

The results obtained from Table-3 showed that both amendments increased macro nutrients concentration in kalmi. Between the two amendments, eggshell had the greater ability to increase the nutrient concentrations. Arunlertaree *et al*. (2007) reported that the chemical contents of eggshell is mainly composed $CaCO_3$ and a few other elements, i.e. S, Mg, P, Al, K and Sr. So after the application of eggshell in the polluted pots (T_2) and (T_3) the concentration of the macro nutrients increased significantly.

Table 3. Influence of lime and eggshell on macro-nutrient concentrations in Kalmi.

Treatment	Shoot (%)						Root (%)					
	N	P	K	S	Mg	Ca	N	P	K	S	Mg	Ca
T0 (Control)	2.20 a	0.33 b	0.56 b	0.17 b	0.17 a	1.02 a	1.79 a	0.21 a	0.97 b	0.20 b	0.16 ab	1.06 b
T1 (200 mg kg^{-1}Pb)	2.03 a	0.15 a	0.49 a	0.11 a	0.14 a	0.82 b	1.63 a	0.14 b	0.87 a	0.12 a	0.15 a	0.87 a
T2 (200 mg kg^{-1}Pb+ CaO)	2.11 a	0.91 c	0.71 c	0.19 bc	0.15 a	1.11 c	1.65 a	0.75 c	0.98 b	0.21 b	0.17 b	1.18 b
T3(200 mgkg^{-1}Pb+ Egg shell)	2.24 a	0.96 d	0.78 d	0.21 c	0.17 a	1.27 d	1.74 a	0.78 c	1.04 b	0.26 c	0.21 c	1.31 d

Means followed by the same letter (s) in a column do not differ significantly from each other at 5% level by DMRT.

Lead (Pb) concentration

The concentration of lead in shoot was the highest at 200 mg Pb kg^{-1} treated pot (36.14 mg kg^{-1} pot^{-1}) and the lowest was in the control treatment (3.48 mg kg^{-1} pot^{-1}). In comparison to lead treated pots and the control pots the average percentage of lead concentration was increased by 938.51% in shoots due to Pb pollution. In case of root, the concentration of lead in root was the highest at 200 mg Pb kg^{-1} treated pot which was 111.14 mg kg^{-1} pot^{-1} and the lowest was in the control treatment 14.48 mg kg^{-1} pot^{-1}. In comparison to lead treated pots and the control pots the average percentage of lead concentration was increased by 667.54% in roots due to Pb pollution.

Accumulation and distribution of Pb metals in the plant depend on plant species, the element species and bioavailability, pH, cation exchange capacity, climatic condition, vegetation period and multiple other factors (Chamon *et al.* 2005). This continuous uptake and translocation can increase the concentrations of Pb in plant tissues instead of the soil that has low metal concentrations. Such results might be attributed due to root activity, which seems to act as a barrier for translocation of metals. The green vegetables particularly the leafy vegetables uptake high amounts of heavy metals from the soil ecosystem. Normal concentrations of Pb in plants are 0.1-10 mg/kg according to Kabata-Pendias and

Pendias (1992), while toxic concentrations of Pb were found as 14.48-111.14 and 3.48-36.14 mg kg^{-1} in roots and shoots of Kalmi, respectively, in this study.

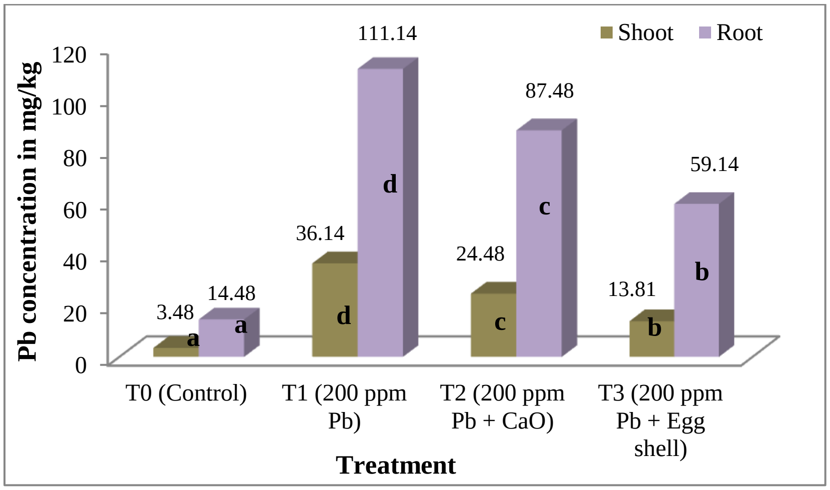

Fig. 1. Lead concentration in Kalmi as influenced by application of Lime and Eggshell

Lime decreased the concentration of lead by 32.26% in shoot and 21.29% in the root of Kalmi respectively, compared with 200 mg Pb kg^{-1} treated pots. On the contrary with Eggshell the amelioration of the lead concentration in Kalmi shoot and root was 61.79 and 46.79% respectively, compared with 200 mg Pb kg^{-1} treated pots (Fig. 1).

Lead concentration in roots and shoots ranged between 14.48-111.14 and 3.48-25.48 mg kg^{-1} pot^{-1}which was above the maximum permissible levels (10-20 mg kg^{-1}) (Lake *et al.* 1984). The highest value reduced in Pb concentration was by egg shell followed by lime in shoot and root of kalmi. This might be due to complexes or chelated by higher organic matter content of egg shell. Lee (1994) suggested that eggshell has potential possibilities to behave as adsorbents. Eggshell absorbs the heavy metals (Pb) and ameliorates the heavy metal toxicity.

Pb concentrations in all treatment differed significantly from each other in case of root and shoot. From the results it may be concluded that application of lead significantly increased the concentration of Pb in shoot and root of Kalmi (T_1), compared with the control (T_0). Saini and Gupta (2001) also reported that grain, straw and root Pb concentration increased significantly from 0.19 to 6.23, 2.11 to 11.17 and 4.96 to 21.76 µg g^{-1}, respectively when Pb levels were raised from 0 to 80 mg kg^{-1} soil, irrespective of the soil texture.

Translocation Factor (TrF)

The translocation factor of Pb is an indicator that helps to understand the mobility of Pb in plants. Translocation factor closer to 0, exhibits higher concentration of the metal retained in the roots instead of being translocated to shoots of the plant. The results showed that, at the un-amended 200 mg kg^{-1} Pb contaminated pots, the highest root to shoot Pb translocation was observed in kalmi (0.33). The translocation factors (TrF) were reduced by 15.15% and 30.30% respectively, due tothe application of lime and egg shell along with 200 mg/kg Pb treatment in Kalmi (Table 4).

Different plant parts contain different Pb metals quantities; the highest quantities were in roots and leaves, and the lowest were in fruits and seeds reported by Natasa *et al.* (2015). Metals concentration in roots indicated the degree of heavy metals accumulation in the polluted soil and shoots concentration suggested the atmospheric pollution degree.

Table 4.Root to shoot Translocation Factor (TrF) for kalmiof lead (Pb).

Treatment	Pb Conc. in mg/kg		
	Shoot	**Root**	**TrF**
To (Control)	3.48	14.48	0.24
T1 (200 mg/kg Pb)	36.14	111.14	0.33
T2 (200 mg/kg Pb+CaO)	24.48	87.48	0.28
T3 (200 mg/kg Pb+ Egg shell)	13.81	59.14	0.23

Plant roots work towards selective absorption of materials from soil and function as an obstruction for translocation of metals to above soil parts. This may be due to the reason for root to shoot movement blockage of Pb. The obtained results also indicated that all translocation factor values were less than 1 which exhibits that higher concentration of the metal retained in the roots instead of being translocated to shoots of the plant. Lead concentration in shoots of control pots were well within permissible limits (10-20 mg kg^{-1}) (Lake *et al.* 1984). The highest accumulation of Pb was found in the shoots of kalmi (edible parts), concentrations frequently exceeded the phytotoxic levels in 200 mg Pb kg^{-1} treated pots (T_1) (10-20 mg kg^{-1}) (Lake *et al.* 1984). Some plant species were found to have lower tolerance to toxic metals uptake in polluted soil as they were found to accumulate high concentrations of Ni, Cu, Pb and Zn. Similarly, different plant species grown in the same soil may contain different levels of the same elements (Ibrahim *et al*. 2014).

Lead concentration above toxic limit in plants affects the growth and productivity of many crops. Its remediation should be undertaken for protecting our environment as well as human life. So it is important to initiate appropriate research program for remediation of Pb toxicity by using different amendments which may provide a basis for better management of Pb influenced polluted soils for crop production as well as to create public awareness.

From this study it can be concluded that the two amendmends taken are suitable to decrease metal concentrations (Pb) in the harvest. Lead concentration in the shoot and root of kalmi was ameliorated significantly by applying lime (CaO) and egg shell which are easily available, affordable and have positive influence in improving soil physical and chemical characteristics. The capability of different amendments to ameliorate the toxic effects of heavy metals (Pb) depends on the site (soil) and element. At elevated levels of metal concentration in soils, the plant metal concentration may increase beyond the limiting value. Thus it should be taken care that the inflow of metals in agricultural fields needs to avoid, otherwise it will certainly contribute to accumulate metals in human tissues through food chain transfer. Through the creation of awareness and suggestion for solution of the Pb problem in Bangladesh, expense in the health sector would be saved particularly lives of children would be protected from ingestion of Pb and diseases associated with Pb toxicity. Thus, it can be concluded that chicken eggshell cannot be just considered as a waste and can be effectively used for remediation of polluted soils.

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