Salicylic Acid Enhanced Phytoremediation of Lead by Maize (Zea mays) Plant

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Abstract— *The aim of this work was to evaluate the ability of maize seeds (Zea mays) to grow and remove the lead from contaminated soil as well as to study the effect of salicylic acid on lead uptake efficiency. Seeds of maize were grown in soil spiked with different levels of lead (0, 1000, 2000, 4000, 8000 mg Pb /kg soil). After two weeks of sowing two levels of salicylic acid (0.0 and 1000 ppm) were applied to the seedling. The growth parameters were measured three times at 30 45 and 60 days after sowing, while the fresh and dry weights, of shoot and root and lead accumulation in shoot and root were done after 60 days of sowing. The results showed that, the increase in lead concentration caused a reduction in the most growth parameters. On the other hand the lead accumulation in shoot and roots was increased by increasing lead concentrations. Also the result indicated that the addition of salicylic acid has appositive influence on the all growth parameter as well as lead uptake by plant.*

Keywords—* *Salicylic acid, maize, lead, phytoremediation.

I. INTRODUCTION

Heavy meals are the major serious pollutants in our natural environment due to their toxicity, persistence and bioaccumulation problems. Trace metals in natural waters and their corresponding sediments have become a significant topic of concern for scientists and engineers in various fields associated with water quality, as well as a concern of the general public. Direct toxicity to man and aquatic life and indirect toxicity through accumulations of metals in the aquatic food chain are the focus of this concern. (Tam and Wong, 2000). An excess level of heavy metals are exposed into environment, by industrial waste and fertilizers causes serious concern in nature as they are non- biodegradable and accumulate at high levels. Heavy metal pollution is a global problem, although severity and levels of pollution differ from place to another. At least twenty metals are consider as toxic with half of them enter into the environment that poses great risks to human health (Akpor and Muchie, 2010). The common heavy metals like Cd, Pb, Co, Zn and Cr etc. are phytotoxic at both low concentration as well as very high concentration are detected in waste water. If these metals are presented in sediments then these reach the food chain through plants and aquatic animals. The most highly developed remediation methods for metal-contaminated soils are physical or chemical, such as soil washing, excavation and reburial. *Phytoremediation*, which uses plants to take up metals, is a cheap alternative technology, which is solar-driven and performed directly *in situ* (Salt *et al.*, 1998). Removing heavy metals through harvestable biomass is an efficient technique for inorganic pollutants. Plants used for this purpose should ideally combine high metal accumulation in shoots and high biomass production. Starting from the discovery of hyperaccumulator plants, which are able to concentrate high levels of specific metals in the above-ground biomass, there is now great interest in crop species which may solve the problem of the small biomass of hyperaccumulators.

Salicylic acid and other salicylates are known to affect various physiological and biochemical activities of plants and may play a key role in regulating their growth and productivity (Arberg, 1981). SA and its close analogues enhanced the leaf area and dry mass production in corn and soybean (Khan et al., 2003). Enhanced germination and seedling growth were recorded in wheat, when the grains were subjected to pre-sowing seed-soaking treatment in salicylic acid (Shakirova, 2007). Fariduddin *et al.* (2003) reported that the dry matter accumulation was significantly enhanced in Brassica juncea, when lower concentrations of salicylic acid were sprayed. However, higher concentrations of SA had an inhibitory effect. Salicylic acid causes resistance to water deficit and ameliorates the damaging effects of heavy metals, like lead and mercury (Berukova *et al.*, 2001). Therefore, the general objectives of this work is to study whether salicylic acid could be a protecting to ameliorate the influence of lead stress in maize and thereby increasing its lead tolerance, while the specific objectives are : To evaluate the effect of lead (Pb) and salicylic acid on growth of *Zea mays* plant as well as study the effect of salicylic acid on lead uptake and accumulation by *Zea mays* plant.

II. MATERIAL AND METHODS

2.1 Experiments:

The pots experiment was conduct in nursery of the Environment, natural Resources and desertification Research Institute to study the effect of lead and salicylic acid on growth and lead accumulation in maize. Seven kg of air dried soil, which consisted of a mixture of clay and sand in 2:1 ratio, The soils were spiked with five levels of lead concentrations (0, 1000, 2000, 4000 and 8000 mg kg^{-1} soil). Six maize seeds were placed in each plastic pot and then the plant were thinned to four plants per pot and were irrigated daily when needed with tap water. After 15 days of growth, the seedlings were treated with salicylic acid at two concentrations (0.0 and 1200mg/kg soil), for growth period of 60 days.

2.2 Growth parameters measurement:

Plant height (cm), number of leaves and leaf area (cm^2) were measured three time at (30, 45 and 60 days) of growth, 60 days after growth the plants were removed from the each pot and roots were washed with distilled water and separated from aerial organ. Then fresh and dry weight of roots and shoots were measured with a digital scale in grams (g),

2.3 Lead determination in plant tissues:

The fresh shoot and root of sorghum plant firstly was oven dried at 60° C for 48 hours and then was ground using electric blender. Approximately 2 g of each sample was weighed and placed into high temperature resistant crucibles. The samples were ashed in a muffle furnace at 550^{o} C. Following ashing, each sample was acid digested using 10 ml of 5N hydrochloric acid. Acid digestion occurred over 20 minute on a hot plate at 80 $^{\circ}$C. Samples were filtered and transferred to 50 mL plastic container; the containers were filled to a volume of 50 ml using distilled water and used for Atomic Absorption Spectrometry (AAS) analysis according to method described by Pearson (1981) to measure lead content in shoot and root.

2.4 Statistical analysis:

All data obtained from the effects of different treatments in the present study were analyzed by one-way analysis of variance (ANOVA) to compare the means of different treatments. Where significant F values were obtained, differences between individual means were tested using the Duncan multiples range test (DMRT) at the p = 0.05 significance level. (Gomez and Gomez, 1984)

III. RESULTS

3.1 Plant height (cm):

TABLE 1
THE EFFECT OF LEAD AND SALICYLIC ACID ON THE PLANT HEIGHT OF MAIZE SEEDLINGS AFTER 30, 45 AND 60 DAYS OF PLANTING.

	30 day	45 day	60 day
(Lead)0+(SAA) 0 ppm	62.58^{ab}	81.26^{a}	94.90^{a}
(Lead)0+ (SAA)1 ppm	64.88^{a}	89.08^{a}	93.51^{a}
(Lead)1000 + (SAA) 0 ppm	59.41^{abc}	66.48^{b}	68.06^{bc}
(Lead)1000 + (SAA)1 ppm	58.08^{abc}	70.83^{b}	74.77^{b}
(Lead)2000 +(SAA)0 ppm	53.40^{cd}	57.27^{cd}	57.83^{def}
(Lead)2000 +(SAA)1 ppm	55.87^{bcd}	68.26^{b}	69.86^{bc}
(Lead)4000 +(SAA)0 ppm	51.09^{de}	54.62^{cd}	55.71^{ef}
(Lead)4000 +(SAA)1ppm	51.32^{de}	61.71^{bc}	63.72^{cde}
(Lead)8000+(SAA) 0 ppm	45.87^{ef}	51.07^{de}	49.81^{f}
(Lead)8000+(SAA) 1 ppm	42.77^{f}	45.22^{e}	48.72^{f}

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

The results obtains in table 1 illustrate the effect of lead and salicylic acid (SAA) on plant height of maize, at four, six and eight weeks respectively. The analysis of variance showed that there were significant differences in plant height amongst the lead treatments. So, salicylic acid application had no significant effect on the plant height of maize. In accordance with control, the plant height significantly decreased with increasing in lead concentration. Generally with the exception of high lead treatment (8000ppm), the addition of salicylic acid with lead induces increasing in plant height, as compare to that without salicylic acid. The lower value of plant height (42.77 cm, 45.22 cm and 48.72) which were registered at the high lead concentration (8000 ppm) with SAA, at the all stage of plant growth, while the highest value (64.88 cm, 89.08 cm and 94.90 cm) were registered at the control with SAA.

3.2 Number of leaves/ plant

Table 2 summarizes the effect of lead and salicylic acid salicylic acid on number of leaves of maize, at four, six and eight weeks. The analysis of variance, showed that there were no significant differences in number of leaves amongst the treatments after four week, but there were highly significant differences after six and eight weeks. As comparison with control, the number of leaves per plant decreased with increasing in lead concentration. The addition of salicylic acid with lead induces increasing in number of leaves, as compare to the same lead treatment without salicylic acid. The lower value of number leaves (3.13, 4.13 and 7.73) which were registered at 8000 ppm with salicylic acid, 8000 ppm without salicylic acid and 8000 ppm with salicylic acid, after four, six and eight weeks respectively. While the highest value of number of leaves per plant (6.13, 6.47 and 7.00) were registered at 8000 ppm without salicylic acid, control with salicylic acid and control without salicylic acid after four, six and eight respectively.

TABLE 2
THE EFFECT OF LEAD AND SALICYLIC ACID ON THE NUMBER OF LEAVES OF MAIZE PLANTS AFTER 30, 45 AND 60 DAYS OF PLANTING.

	30 day	45 day	60 day
(Lead)0+(SAA) 0 ppm	4.13[a]	6.20[a]	7.00[a]
(Lead)0+(SAA)1 ppm	5.40[a]	6.47[a]	6.73[a]
(Lead)1000+(SAA) 0 ppm	4.00[a]	4.60[bc]	5.13[c]
(Lead)1000+(SAA)1 ppm	4.47[a]	5.07[b]	6.20[ab]
(Lead)2000+(SAA)0 ppm	3.80[a]	4.60[bc]	5.60[bc]
(Lead)2000+(SAA)1 ppm	4.53[a]	5.07[b]	5.33[bc]
(Lead)4000+(SAA)0 ppm	3.67[a]	4.93[b]	5.07[c]
(Lead)4000+(SAA)1ppm	4.13[a]	5.00[b]	5.47[bc]
(Lead)8000+(SAA) 0 ppm	6.13[a]	4.13[c]	5.00[c]
(Lead)8000+(SAA) 1 ppm	3.13[a]	4.67[bc]	4.73[c]

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

3.3 Leaf Area (cm^2)

The result presented in table 3 indicated the effect of lead and salicylic acid salicylic acid on leaf area of maize, at four, six and eight weeks. The analysis of variance showed that there were highly significant differences among the lead treatments after four, six and eight weeks. As comparison with control, the leaf area was decreased with increasing in lead (Pb) concentration the addition of salicylic acid with (Pb) induces increasing in leaf area, as compare to the same lead treatment without salicylic acid. The lower value of the leaf area (29.44 cm^2, 39.98 cm^2 and 37.81 cm^2) which were registered at 8000 ppm without salicylic acid, 8000 ppm with salicylic acid and 8000 ppm with salicylic acid, after four, six and eight weeks respectively. On the other hand the highest values of leaf area (69.13 cm^2, 145.41 cm^2 and 186.63 cm^2) were registered at control with SAA after four, six and eight weeks respectively.

3.4 Shoot fresh weight (g):

Table 4 illustrates the effect of lead and salicylic acid on shoot fresh weight of maize**.** Increasing concentrations of lead significantly decrease shoot fresh weight of maize as compare with control. On the other hand, application of salicylic acid cause slightly increased in shoot fresh weight of maize as compared to lead treatments without salicylic acid. The lower value of shoot fresh weight (16.43 g) registered at the lead treatment 8000 mg without salicylic acid and the higher value (92.73 g) which was observed at control with salicylic.

TABLE 3
EFFECT OF LEAD AND SALICYLIC ACID (SAA) ON LEAF AREA OF MAIZE PLANT AFTER 30, 45 AND 60 DAYS OF PLANTING.

	30 day	45 day	60 day
(Lead)0+(SAA) 0 ppm	46.48^{bc}	112.53^{b}	135.70^{b}
(Lead)0+(SAA)1 ppm	69.13^{a}	145.41^{a}	186.63^{a}
(Lead)1000+(SAA) 0 ppm	47.67^{bc}	82.68^{bc}	75.50^{cde}
(Lead)1000+(SAA)1 ppm	55.03^{ab}	89.82^{bc}	94.45^{c}
(Lead)2000+(SAA)0 ppm	54.62^{ab}	52.97^{e}	61.66^{ef}
(Lead)2000+(SAA)1 ppm	47.84^{bc}	81.36^{cd}	84.46^{cd}
(Lead)4000+(SAA)0 ppm	34.83^{c}	48.94^{e}	53.88^{fg}
(Lead)4000+(SAA)1ppm	35.74^{bc}	63.79^{de}	63.24^{ef}
(Lead)8000+(SAA) 0 ppm	29.44^{c}	49.67^{e}	43.29^{fg}
(Lead)8000+(SAA) 1 ppm	32.06^{c}	39.98^{e}	37.81^{g}

Means followed by the same letters are not significantly different at 0.05 levels of probability according to DMRT

TABLE 4
THE EFFECT OF LEAD AND SALICYLIC ACID ON FRESH AND DRY WEIGHT (G) OF SHOOT AND ROOT OF MAIZE PLANT AFTER 60 DAYS OF PLANTING.

Lead and salicylic acid	Shoot fresh	Shoot dry	Root fresh	Root dry weight
(Lead)0+(SAA) 0 ppm	58.63^{b}	11.73^{b}	15.17^{b}	6.43^{a}
(Lead)0+(SAA)1 ppm	92.73^{a}	18.55^{a}	20.13^{a}	7.23^{a}
(Lead)1000+(SAA) 0 ppm	27.73^{cde}	5.55^{cde}	13.57^{bc}	4.37^{ab}
(Lead)1000+(SAA)1 ppm	35.40^{c}	7.08^{c}	15.97^{b}	6.57^{a}
(Lead)2000+(SAA)0 ppm	25.40^{cde}	5.08^{cde}	11.10^{cde}	2.47^{b}
(Lead)2000+(SAA)1 ppm	33.47^{cd}	6.69^{cd}	14.90^{bc}	2.93^{b}
(Lead)4000+(SAA)0 ppm	21.87^{de}	4.37^{de}	9.13^{de}	2.03^{b}
(Lead)4000+(SAA)1ppm	28.00^{cde}	5.60^{cde}	11.47^{cd}	3.30^{b}
(Lead)8000+(SAA) 0 ppm	16.43^{e}	3.29^{e}	8.47^{de}	1.47^{b}
(Lead)8000+(SAA) 1 ppm	16.73^{e}	3.35^{e}	7.77^{e}	1.70^{b}

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT.

3.5 Shoot dry weight (g):

Table 4 shows the effect of lead and salicylic acid on shoot dry weight of maize**.** The analysis of variance showed that there were highly significant differences among lead treatments in shoot dry weight of maize. However, Addition of salicylic acid also increased shoot dry weight as, when compared with the lead treatments without addition of salicylic acid. The lower value of shoot dry weight (3.29 g) obtained at the lead treatment 8000 mg without salicylic acid and the higher value (18.25 g) which was registered at control with salicylic acid.

3.6 Root fresh weight (g):

The data presented in table 4 shows the effect of lead and salicylic acid on root fresh weight, the analysis of variance indicated that lead poisoning caused significant reduction in root fresh weight, So that when lead concentration increased, the amount of root fresh weight was significantly declined . Under lead and salicylic acid treatments, root fresh weight was increased when compare with lead treatment without salicylic acid treatment The lower value of root fresh weight (7.77 g) registered at the lead treatment 8000 mg with salicylic acid and the higher value (20.13 g) which was observed at control with salicylic acid.

3.7 Root dry weight (g):

The result shown in table 4 indicated the effect of lead and salicylic acid on root dry weight of maize. The analysis of variance showed that there were highly significant differences among the lead treatments in comparison with control. The root dry weight was decreased with increasing in lead concentration. The addition of salicylic acid with lead induces increasing in root dry weight, as compare to the same lead treatment without salicylic acid. The lower value of shoot fresh weight (1.47 g) registered at the lead treatment 8000 mg without salicylic acid and the higher value (7.23 g) which was observed at control with salicylic acid.

3.8 Lead (Pb) accumulation in plant tissues:

Table 5 obtains the results of the effect of lead and salicylic acid on lead accumulation in shoot and root of maize plant respectively. The analysis of variance revealed that there were significant difference among the lead treatment in lead accumulation in shoot and root of maize. Hence, the addition of salicylic acid to lead elevate the level of lead accumulation in plant when compare to the same lead treatment without salicylic acid. Plants accumulated higher lead concentrations in roots (22.55-5900 mg kg^{-1}) than shoots (3.59 – 90.62 mg kg-1). Lead concentrations in the same part (shoot or roots) of plants under different treatments were generally in the descending order of (8000, 4000, 2000 1000 and 0 mg kg^{-1}) treatment.

TABLE 5
THE EFFECT OF LEAD AND SALICYLIC ACID ON LEAD ACCUMULATION IN SHOOT AND ROOT DRY WEIGHT (MG / KG DRY WEIGHT) OF MAIZE PLANT AFTER 60 DAYS OF PLANTING.

Lead and salicylic acid	Lead in shoot	Lead in roots
(Lead)0+(SAA) 0 ppm	3.59^{f}	29.17^{c}
(Lead)0+(SAA)1 ppm	5.23^{f}	22.55^{c}
(Lead)1000+(SAA) 0 ppm	6.04^{f}	209.80^{b}
(Lead)1000+(SAA)1 ppm	16.88^{e}	265.05^{b}
(Lead)2000+(SAA)0 ppm	20.95^{cd}	373.18^{b}
(Lead)2000+(SAA)1 ppm	23.54^{cd}	332.05^{b}
(Lead)4000+(SAA)0 ppm	19.15^{cde}	972.50^{a}
(Lead)4000+(SAA)1ppm	37.03^{b}	940.83^{a}
(Lead)8000+(SAA) 0 ppm	23.08^{c}	995.83^{a}
(Lead)8000+(SAA) 1 ppm	90.62^{a}	1005.83^{a}

Means followed by the same letters are not significantly different at 0.05 level of probability according to DMRT

IV. DISCUSSION

significant difference among the lead treatment were detected in plant height, number of leaves, leaf area, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight. Similar findings were reported by (Verma and Dubey, 2003) who reported that, high concentration of lead reduces growth of rice seedling by more than 13 to 45%. (Mishra and Coundhari, 1999) who mention that Pb was decreased dry mass of root and shoot of plant. Plant biomass can also be restricted by high doses of lead exposure (Gopal and Rizvi 2008; Gichner *et al*. 2008; Islam *et al*. 2008; Piotrowska *et al*. 2009; Sing *et al*.

2010). Pb toxicity reduced the absorption of CO_2 due to reduced leaf area (Azmat *et al*., 2009). The excess lead in the nutrient medium reduces water uptake and transport (Azmat *et al*., 2006), which may affect the photosynthesis system of plant (Haider *et al*.,2006). Salicylic acid enhance the all growth parameter viz, plant height, number of leaves, leaf area, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight. This may be attributed to more Lead mobilization to the plants leading to increase lead toxicity to plants. Similar findings were obtained by (Vazirimehr and Rigi, 2014) who reported that, the application of salicylic acid to leaves of corn and soybean accelerated their leaf area and dry mass production. A role of salicylic acid in alleviating the heavy metal toxicity in plants has been reported by many workers. Mishra and Choudhuri (1999) observed that SA pre-treatment alleviated the lead and mercury induced membrane disruptions in rice. Further, exogenous salicylic acid was found to alleviate the toxic effects generated by Cd in barley (Metwally *et al.*, 2003) and in maize plants (Pal *et al.*, 2002). Significant differences were detected among the lead treatment in lead (Pb) accumulation in shoot and root. Lead accumulation in shoot and root increase with increasing Pb concentration. Accumulation of lead in shoot and root increases with addition of salicylic acid**.** Accumulation of Pb in root is higher than in shoot. This is similar with findings of Parsadoost *et al*. (2008). Once lead has penetrated into the root system, it may accumulate there or may be translocated to aerial plant parts. For most plant species, the majority of absorbed lead (approximately 95% or more) is accumulated in the roots, and only a small fraction is translocated to aerial plant parts, as has been reported in *Vicia faba, Pisum sativum,* and *Phaseolus vulgaris* (Małecka *et al.,* 2008; Shahid *et al.,* 2011),

V. CONCLUSION

Results showed that *Zea mays* have the potential to be a suitable phytoextraction species. and the greatest metal uptake was observed in the roots than in the shoot. Lead inhibited growth parameters viz. plant height, number of leaves and leaf area, and these can be used as indicator in screening for lead tolerance and hyperaccumulator genotypes. Zea mays have ability to growth and remove lead from contaminated soil up to 8000 mg Pb kg^{-1} soil. Also the results in our study indicated that SAA play a major role in modulating the plants' response to lead stress.

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