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Influence of lead applications and biofertilizer (phosphorine) on vegetative growth and chemical composition of *Sterculia acerifolia* L. seedlings

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Abstract : The experimental trials were carried out during two successive seasons, 2014 and 2015 at green house of the National Research centre , Cairo, Egypt. The purpose of this study is to investigate the influence of lead concentrations (0, 30, 60 and 20ppm) and Phosphorine Biofertilizer (0.10 gm/pot) on vegetative growth and chemical composition of *Sterculia acerifolia* L. Lead at low concentration (30ppm) increased all growth parameters and all chemical constituents values. But lead at high concentrations (60 and 90 ppm) decreased all growth characters (plant height,number of leaves, length of roots, stem diameter, fresh and dry weight of leaves, stems and roots. On the contrary, all the previous characters as well as phosphorus %, uptake of phosphorus and potassium and carbohydrates % increased by inoculating plants by phosphorine compared with the control and other treatments lead at 60 ppm gave the highest value of pigments content (chlorophyll (a), (b) and carotenoids. Additionally, all interactions treatments increased growth characters, nitrogen %, phosphorus %, potassium %, lead ppm and uptake of N, P, K compared with control and high concentrations of lead (60 and 20 ppm).

It could be recommended to use lead concentration up to 30 ppm to induce the growth characters and chemical constituents and to inoculate plants grown in regions polluted with lead with phosphorine to overcome the hazardous and destructive effects of lead high concentrations.

Introduction

The *sterculia acerifolia*, A. Cunn is from the family Sterculaceae, it is deciduous medium-sized tree that reaches up to 30m in height, it does not have thorns. It is based in Australia. It has bright red flowers shaped like a bell that usually cover the whole tree when it has not any leaves. The tree is planted all over the world for its beauty. The tree can reach up to 40 meters in height only in its original warm environment, otherwise it normally grows to 20 meters. It is deciduous and sheds the leaves in dry seasons. The tree flowers in the end of spring and its flowers are scarlet red with 5 petals that are fused and produce fruits shaped like pods of dark brown color. The tree is planted for their ornamental value, for their timber and also used in Charcoal production and fuel [1].

There are heavy metals in the soil usually at low levels, but because of the output of industry, agriculture and other waste, such as cities' waste, the pollution it is best to measure the plants' content of these toxic and hazardous elements (Pb, Cd, Cu, Zn, Hg), due to the accumulation and toxicity, which leads to the serious effect of not only the plant but also on human health.

And plants contain these elements because of their existence in the soil, water, air, and some of these plants do not bear the high levels of these elements. The problem of heavy metals comes from their tendency to accumulate in human organs over time and the existence of these elements lead to the occurrence of metabolic disorders.

These main elements (Fe, Zn, Cu) lead to of undesirable effects[2] while toxic elements (Cd, Pb, Ni, Cr) result in significant problems as cadmium leads to Pyelonephristis, Oesteomolica and lead also causes cancerous and renal tumors[3] Iron, copper and zinc are considered less toxic than these two elements but become dangerous when they increase to an impermissible limits. Many studies have shown that lead, cadmium, chromium, nickel, zinc and copper reach toxic levels of concentrations in pollution -prone lands.

Lead is a very harmful element to humans and plants. It comes basically from lead mines, motor vehicle emissions, and the use of sludge and compost. The highest allowed concentration of lead in nutrients is 1mlgm/kg and the long exposure to lead may result in an increase in lead levels in the body and many dangerous symptoms like anemia, abdominal pain, nausea, vomiting and paralysis. [4] the constant exposure may also lead to kidney failure, infertility and increases the chances of miscarriage and birth defects.

In the last two decades, Advanced Science and Major countries have become more interested in organic and bio-agriculture (organic-farming) as the two terms cover a big variety of techniques that have been developed, tried and applied in many research centers. This is because the country aims at increasing the agricultural areas and the productivity of each unit area to fill the food gap. The used studies have achieved the highest productivity through using organic farming that limits the use of agricultural chemicals that are used as compost or insecticides in new cultivated lands to avoid pollution, reduce cost and save irrigation water. Compost and organic fertilizers are considered cheap nutrient sources that replaces mineral compost that causes environmental pollution for the soil and water alike when used excessively[5].

Phosphorus is essential for plant growth essential elements, less crops is often due to phosphorus deficiency more of any other element deficiency. Therefore, farmers are used to adding the annual phosphoric fertilizers, but those are quickly turned into dissolved phosphorous in Egyptian lands because the soil fixes it before the plant is able to use it making the Egyptian lands a phosphorous bank[6].

Microbiology scientists produced vaccines from the organisms that have a significant role in making the phosphorous available it he soil giving the plant its required phosphorous, one of those organisms is phosphorine vaccine that contains bacteria specialized in dissolving undissolved phosphorous in the soil.

Phosphorine is a bio-fertilizer that can be used for all crops. The inability to use the phosphorous fertilizers resulting from the alkaline nature of the Egyptian soil, phosphorine is used instead. Reasons for using the bio-phosphorine fertilizer are because of the active bacteria it contains that turn Tricalcium phosphate, present in Egyptian soils in high concentrations as a result of the use of phosphate fertilizers, into monocalcium phosphate. In the right conditions, the bacteria usually multiply and spread on the rhizosphere and provides it with phosphorous necessary for different stages of plant growth.

Benefits of using phosphorine include reducing the use of Phosphate fertilizers and cost, benefiting from the phosphate fixed in the soil, improving the characteristics of the soil and restoring the microbial balance in the soil, increasing the rhizosphere and its ability for uptake and thus it becomes a reason for production increase, reducing environmental pollution, resisting some plant diseases in the soil through hormones and stimulants that it produces[7].

Materials and Methods

This experiment was carried out during 2014 and 2015 seasons. The plants were experimentally treated in National Research centre, Dokki, Cairo, Egypt, to study the response of *Starculia acerifolia* seedlings grow under lead pollution to phosphor in treatment in sandy soil. The physical and chemical properties of the tested soil were determined according to the methods for[8].

Table(1): Some physical and chemical properties of used soil:

Field capacity (%)		**Particle size distribution**					
		Soil texture	**Clay (%)**	**Silt (%)**		**Sand (%)**	
20.1		**sandy loam**	**3.6**	**25.6**		**70.8**	
Chemical properties							
Soluble cobalt (ppm)		O.M (%)	CaCO3 (%)		pH(1:25)	Ecdsm-1	
0.49		0.23	3.57		7.9	1.2	
Soluble Anions (meqL-1)				Soluble cations (meqL-1)			
SO_4^-	Cl^-	HCO_3^-	CO_3^-	Na^+	K^+	Mg^{++}	Ca^{++}
4.28	0.65	1.5	-	1.87	0.162	2	2.4
Available micronutrients (ppm)				available (mg/100g)		Total N (mg/100g)	
Cu	Zn	Mn	Fe	K	P		
4	1.44	2.61	4.47	21	13	15.1	

Plant materials and procedures: The seedlings of *sterculia acerifolia* fam. Sterculaceae (one-year-old, 15-20 cm height and 5-7 compound leaf/plant in average) were used. The plants were obtained from the Forestry Department, Horticulture Research Institute, ARC. The experiment was open field conditions through the two seasons. The seedlings were transplanted on March 15th 2014 and 2015 for the first and second seasons respectively. Seedlings were transplanted in 30 cm pottery pots (one seedling/pot) filled with 12 kgm sandy soil. Half of the pots were biofertilized with phosphorine (*Bacillus megaterium* var Phosphaticum*)* phosphorus dissolving bacteria produced as biofertilizer by Ministry of Agriculture, Egypt "Biofertilizer Lab") at 0 and 10 gm peatmoss/pot. Basic dressing and treatments: The experiments were sit in completely randomized design with 5 replications. The treatments used as follows: four lead levels (0,30,60 and 90 ppm) and two inoculation treatments (uninoculation and inoculation with phosphorine). The available commercially fertilizer used through this experimental work was kristalon (N: P:K, 19: 19: 19) produced phayzon Company, Holand. The used rate of fertilizer was 5.0 gm per pot in four doses. The plants were fertilized after 4,8, 16, 30 weeks for transplanting. Leads as lead carbonate $PbCO_3$ was ued after dissolving in top water and added as surface irrigation. Irrigation regime repeated twice every week till the end of the season (15th November).

Data recorded: plant height (cm), number of leaves/plant, stem diameter(cm), root length (cm) fresh and dry weight leaves, stems and roots (g) were recorded at the end of season. These data are statistically analyzed according to[9] using the least significant different (L.S.D.) at 5% level. The chemical analysis: chlorophylls a, b and carotenoids were extracted from fresh leaves (mg/g according to the producer achieved by[10]. Carbohydrate were extracted promdring (powdered) according to[11] and estimated the total carbohydrate clorometrically by the phenol sulphoric acid method[12]. Nitrogen percentage was determined by the modified microkieldahl method and described by [13]. Phosphorus percentage as estimated according to[14]. Potassium percentage was determined by Flame photometer Model Car-Zeiss according to the method of[15]. Lead were determined by Atomic Absorption described by[8].

Results and Discussion

Vegetative growth:

Data in Table (2) mentioned that plant height, leaves number/plant root length and stem diameter increased with Pb application at 30 ppm. The increments were (5.29%) plant height, (8.24%) number of leaves,(1.67%) root length and (8.33%) stem diameter compared with the untreated plants. The same parameters were decreased with Pb treatments at 60 and 90 ppm compared with the other treatment. The decrease were (4.77 and 15.48%) plant height, (11.76 and 32.94%) leaves number/plant, (11.82 and 34.62%)

root length and (23.61 and 9.72%) stem diameter comp. with the untreated plants at 60 and 90 ppm respectively. The same results was obtained by [16] on same timber trees,[17] on *Dalbergia* sisso and [18] on *Leuceana leucocphala*. On the contrary, the previous characters increased significantly by inoculation with *Bacillus megatherium*. The increment with phosphorine treatment were (27.15%) plant height, (47.76%) leaves number/plant, (80.77%) and (31.94%) stem diameter compared with the control. The above can be attributed to the nitrogen content and phosphorus increment in the soil which is a result of nitrogen fixation and the phosphorus produced by phosphate dissolving bacteria as well as the substances that promote growth such as indole acetic acid and gibberellins produced by the used organisms[19]. Regarding the effect of interaction between adding different concentrations of Pb (30, 60 and 90 ppm) plus adding phosphorine. The data indicated that, all interaction treatments significantly increased plant height, leaves number/plant, root length and stem diameter compared with Pb treatments individual and the control. In addition, data reported that the highest plant and the greatest leaves number/plant were obtained with Pb at 30 ppm + phosphorine. The highest root length and the thickest stem were obtained when plants treated with 60 ppm pb+phosphorine. The les increase of the previous characters were obtained by 90 ppm Pb+phosphorine.

Table (2): Influence of lead applications and bio-fertilizer (phosphorine) on vegetative growth and chemical composition of *Sterculia acerifolia* L. seedlings

Characters / Treatments	Plant height(cm)	No. of leaves	Length of root(cm)	Stem diameter(cm)
Control	77.5	42.5	39.0	0.72
Phosphorine 10gm	98.5	75.0	70.5	0.95
Pb 30gm	81.6	46.0	45.5	0.78
Pb 60gm	73.8	37.5	34.6	0.55
Pb 90gm	65.5	28.5	25.5	0.65
Pb 30gm+ Phosphorine 10gm	93.0	66.5	55.4	0.85
Pb 60gm+ Phosphorine 10gm	91.0	57.5	67.7	0.90
Pb 90gm+ Phosphorine 10gm	88.4	52.0	47.0	0.83
L.S.D at 5%	7.08	4.65	4.33	0.07

Table(3): Influence of lead applications and bio-fertilizer (phosphorine) on fresh and dry weight of leaves stems and roots of *Sterculia acerifolia* L. seedlings

Characters / Treatments	F.W of leaves	F.W of stems	F.W of roots	D.W of leaves	D.W of stems	D.W of roots
Control	34.73	32.48	156.86	9.72	10.43	54.74
Phosphorine 10gm	58.43	60.10	268.34	17.53	20.44	99.28
Pb 30gm	41.72	45.60	174.55	11.80	14.87	61.44
Pb 60gm	31.53	25.55	144.81	8.54	8.07	50.25
Pb 90gm	30.74	29.58	135.27	8.51	9.47	46.13
Pb 30gm+ Phosphorine 10gm	49.05	53.94	201.30	14.52	17.96	72.87
Pb 60gm+ Phosphorine 10gm	51.08	55.60	244.32	14.92	18.74	89.42
Pb 90gm+ Phosphorine 10gm	45.94	47.70	196.82	13.23	15.74	70.46
L.S.D at 5%	4.25	3.8	16.89	1.16	1.28	5.79

The results obtained in Table (3) showed that Pb at 30 ppm increased fresh and dry weight of all plant organ compared with other treatments. But Pb at 60 and 90 ppm decreased stem, leaves and roots fresh and dry weight compared to the control.

The fresh weight decrease by Pb at 60 and 90 ppm were (9.21 and 11.49%) stem, (21.34 and 8.93%) leaves and (7.68 and 15.97%) roots respectively compared to the control. The reduction of dry weight were (12.14 and 12.45%) stem, (22.63 and 9.20%) leaves and (8.20 and 15.73%) roots, respectively compared with the untreated plant. [2]has shown that the inhibiting effect of lead may be attributed to the interaction between those elements and environmental factors and that lead has a toxic effect on the many processes such as photosynthesis, mitosis and water uptake. Also the subcellular affects of lead on plant tissue is due to respiration inhibition through electron transport disturbance. Lead binds strongly to cell walls leading to the turgidity of tissue walls. In addition, the antagonism of lead-zinc affects the translocation of each element and its transport from roots to top adversely causing height reduction. [21]said that the significant nitrogen concentration reduction after nitrogen metabolism the plant might be one of reasons of weight reduction. Those results agree with those of [16,17].

On the other hand, phosphorine treatment increased all fresh and dry weight of stem leaves and roots as compared to the other treatments.

The fresh weight increment were (68.24%) stem, (85.04%) leaves and (71.07%) roots as compared to the control. The increment of dry weight were (80.35%) stem, (95.97%) leaves and (81.37%) roots compared with the untreated plants.

The activity of the free *Bacillus megatherium* bacteria might be behind that increase. The bacteria is present in the area around the roots (rhizosphere) like phosphate dissolving bacteria that saves available phosphate. These bacteria were proved to be capable of producing auxins and other growth substances in the rhizosphere in *Pennisetum amercanum* L.[22].

Concerning the effect of interactions treatments, Data in Table (3) demonstrated early that Pb at 30, 60 and 90 ppm + phosphorine gave the highest fresh and dry weight of all plant organs (stem, leaves and roots) compared to the other treatments. It is obvious from the data that fresh and dry weight plant organs were markedly decreased when plants were treated with the following forms, 60 ppm Pb + phosphorine giving (47.08 and 53.50%) leaves, (71.18 and 79.67%) stem and (55.76 and 63.35%) roots respectively and 30 ppm Pb + phosphorine giving (41.23 and 49.38%) leaves, (66.07 and 72.20%) stem and (28.33 and 33.12%) roots respectively compared with the untreated plants. This is in comparable with results of[17] on *Leuceana leucocephala* seedlings.

Chemical composition:

1-Carbohydrates content (% D.W.):

The results in Table (4) cleared that, plants inoculated with phosphorine had leaves, stem and roots containing more carbohydrates (35.60, 32.75 and 30.50%) respectively followed by plants treated with phosphorine + Pb 30 ppm giving (34.20, 30.88 and 29.66%), respectively, compared with the control and other treatments. On the other hand, the lowest values of carbohydrates content were recorded by control plants in leaves, stem and roots.

2- Pigment content:

Data in Table (4) revealed that, lead at 60 ppm showed the highest content of chlorophyll-a, b and carotenoids (3.687, 1.934 and 4.334 mg/g F.W.) followed by lead at 90 ppm (3.679, 1.815 and 4.290 mg/g F.W.) then lead at 30 ppm (3.632, 1.727 and 4..248 mg/g F.W.) I the leaves respectively. While, the lowest content of chlorophyll-a (3.243 mg/g F.W.) were determined in the leaves of the plants treated with phosphorine + Pb 90 ppm. But the lowest content of chlorophyll-b and carotenoids were recorded, when plants were treated by phosphorine + Pb 30 ppm.

Table (4): Influence of Lead applications and biofertilizer(phosohorine) on Carbohydrates% and Pigments content mg/gm f.W of *Sterculia acerifolia* L. seedlings (Average of two seasons 2014 and 2015).

Characters / Treatments	Carbohydrates%			Pigments content mg/gm f.W		
	leaves	Stems	Roots	Chlorophyll (a)	Chlorophyll (b)	Carotenoids
Control	26.30	25.20	4.80	3.569	1.399	4.206
Phosphorine	35.60	32.75	30.50	3.411	1.535	4.186
Pb 30 ppm	30.65	29.10	27.95	3.632	1.727	4.248
Pb 60 ppm	29.20	28.35	26.80	3.687	1.934	4.334
Pb 90 ppm	27.40	27.22	26.12	3.679	1.815	4.290
Phos + Pb 30 ppm	34.20	30.88	29.66	3.254	1.060	4.077
Phos + Pb 60 ppm	30.50	29.30	28.15	3.538	1.372	4.221
Phos +Pb 90 ppm	8.68	27.88	27.42	3.243	1.205	4.176

Phos= Phosphorine

Table (5) : Influence of Lead applications and bio-fertilizer(phosohorine) on Nitrogen, Phosphorus and Potassium % and Lead ppm of *Sterculia acerifolia* L. seedlings (Average of two seasons 2014 and 2015).

Characters / Treatments	Nitrogen%			Phosphorous %			Potassium %			Lead ppm		
	Leaves	Stem	Roots	leaves	Stem	Roots	Leaves	Stem	Roots	leaves	Stem	Roots
Control	0.48	0.35	0.28	0.35	0.31	0.21	0.45	0.40	0.35	0.08	0.18	1.00
Phosphorine	1.30	0.65	0.55	0.71	0.65	0.48	1.1	0.85	0.55	0.16	0.44	1.60
Pb 30 ppm	0.75	0.62	0.43	0.56	0.52	0.40	0.85	0.80	0.58	0.30	1.80	3.73
Pb 60 ppm	0,65	0.53	0,40	0.46	0.35	0.31	0.65	0.59	0.47	0.43	2.10	5.59
Pb 90 ppm	0.55	0.43	0.36	0.38	0.28	0.22	0.59	0.52	0.40	0.58	3.20	7.40
Phos + Pb 30 ppm	0.95	0.75	0.51	0.68	0.60	0.53	0.95	0.88	0.63	0.95	4.7	10.20
Phos + Pb 60 ppm	1.10	0.83	0.58	0.60	0.56	0.45	0.86	0.78	0.58	1.40	5.5	18.70
Phos +Pb 90 ppm	0.80	0.64	0.46	0.53	0.40	0.35	0.73	0.65	0.55	1.90	7.9	27.40

Phos= Phosphorine

3-Nutrient content:

The data presented in Table (5) showed that the nitrogen percentage in leaves increased in plants inoculated by phosphorine (1.3%) followed by plants treated by phosphorine + Pb 60 ppm with (1.10%). The lowest value of N% (0.48%) was recorded with the untreated plants. Results in stem, reveal that the average of N% ranged from 0.35% to 0.83%. The plants inoculated with phosphorine gave the highest of N% (0.83%) followed by those treated by phosphorine + Pb 30 ppm with (0.75%). The lowest value of N% (0.35%) was recorded with control plants.

The roots nitrogen percentage reached to the highest value when plants were treated with phosphorine + Pb 60 ppm giving (0.58%). Whereas, the lowest value of nitrogen content were recorded with untreated plants.

Data presented in Table (5) showed that, phosphorine only or with lead at 30 and 60 ppm gave the highest value of P% (0.71, 0.68 and 0.60%) in leaves respectively and (0.65, 0.60 and 0.56%) in stem, respectively. But the highest value of P% in roots were recorded when plants treated with phosphorine + Pb 30 ppm followed by phosphorine giving (0.53 and 0.48%) respectively. The lowest values of P% in leaves, stems and roots were recorded with untreated plants giving (0.35, 0.31 and 0.21%) respectively.

The results in Table (5) indicated that the highest content of K percentage in leaves (1.1% D.W.) was recorded on plants inoculated with phosphorine. But in stems and roots the highest values were recorded with plant treated with phosphorine + Pb 30 ppm giving (0.88 and 0.63% D.W.) respectively. Compared with the other treatments. Whereas, the lowest values of potassium content was observed in control plants giving (0.45, 0.40 and 0.35% D.W.) in leaves, stems and roots respectively.

The results in Table (5) reported that, the average content of lead ranged from (0.08 to 1.90 ppm) in leaves, (0.18 to 7.9 ppm) in stems and (1.00 to 27.40 ppm) in roots. The plants treated with phosphorine with phoshorine + Pb 90 ppm gave the highest lead content in leaves, stems and roots. Whereas, the lowest lead content in every plant organs (leaves, stem and roots) was obtained in control plants.

Results in Table (6) revealed that, the highest N uptake in leaves was obtained by plants inoculated with phosphorine (227.90 mg) followed with phosphorine + 60 ppm Pb (164.12mg) compared with other treatments and the control.

Table (6): : Influence of Lead applications and bio-fertilizer(phosohorine) on Nitrogen, Phosphorus and Potassium uptake of *Sterculia acerifolia* L. seedlings (Average of two seasons 2014 and 2015).

Characters / Treatments	Nitrogen (mg)			Phosphorus (mg)			Potassium (mg)		
	Leaves	Stem	Roots	Leaves	Stem	Roots	Leaves	Stem	Roots
Control	46.60	36.5	153.27	34.o2	32.3	114.9	43.7	41.7	191.6
Phosphorine	227.90	132.8	546.00	124.20	132.8	476.5	192.2	173.7	546.0
Pb 30 ppm	88.50	92.1	264.2	66.00	77.3	245.8	100.3	118.9	356,4
Pb 60 ppm	55.51	42.8	201.00	39.30	28.2	155.8	55.5	47.6	236.2
Pb 90 ppm	46.80	40.7	166.40	32.30	26.5	101.7	50.3	49.2	185.0
Phos + Pb 30 ppm	137.94	134.7	371.60	98.70	107.8	386.2	137.9	158.0	459.0
Phos + Pb 60 ppm	164.12	155.5	418.64	89.50	104.9	402.39	128.1	146.2	518.6
Phos +Pb 90 ppm	105.84	100.7	324.1	70.10	62.9	246.6	96.6	10.2	387.5

Phos= Phosphorine

The same result was obtained with N uptake in roots. The highest value of N uptake was recorded by plants inoculated with phosphorine (546.00 mg) followed by phosphorine + 60 ppm Pb (418.64) compared with control and other treatments. Whereas, the hgiehst value of nitrogen uptake in stem was resulted by plants treated with phosphorine + 60 ppm Pb followed by phosphorine + 30 ppm Pb compared with the control and the other treatments. Generally, the lowest value of nitrogen uptake was recorded with untreated plants.

Data also reported that, the highest values of phosphorus uptake in all plant organs (leaves, stem and roots) was observed in plants inoculated with phosphorine (124.20, 132.8 and 476.5 mg) respectively, as compared to the control. On the other hand, the lowest values of phosphorus uptake resulted in plants treated with 90 ppm Pb in leaves, stems and roots (32.30, 26.5 and 101.7 mg) respectively, compared with other treatments and the control.

Similar trend was noticed in potassium uptake, the greatest values of potassium uptake was obtained by plants inoculated with phosphorine in all plant organs (192.2 mg) leaves, (173.7 mg) stems and (546.0mg) roots compared with the control and other treatment. However, the smallest values of potassium uptake were recorded in untreated plants. These results were in the same line of the findings of [18] on *Leuceana leucocephala*

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