

Response of *Leuceana leucocephala* Seedlings Grown under Lead Pollution to Phosphorin Application in Sandy Soil

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Abstract: Effect of lead pollution and phosphorin on growth and chemical constituents of *Leuceana leucocephala* seedlings in sandy soil were studied in 2004 and 2005 seasons in National Research Centre, Dokki, Cairo. Seeds were inoculated with “Phosphorin” using 0 and 10 g of carrier agent (peat moss). Pb at 0, 25, 50 and 100 ppm were used for irrigation water. All vegetative growth characters decreased as the lead concentrations increased in both seasons. Increasing the concentration of Pb increased Chlorophyll (a), (b) and carotenoids content as well as Pb ppm and K percentages. Soluble sugars, non-soluble sugars, total sugars, N and P percentage, Zn and Mn ppm decreased by increasing Pb concentrations at each season. On the other hand, phosphorin application gave the highest growth parameters, Chlorophyll (a), (b) and carotenoids content. Also, soluble sugars, non-soluble sugars, total sugar, N, P and K percentages as well as Zn ppm in both seasons. But, Pb and Mn ppm decreased by inoculation compared with the control. Generally, phosphorin overcome the harmful effect of pollution up to 100 ppm Pb. The results suggest that *Leuceana leucocephala* seedlings benefited from inoculation with phosphorin especially under Pb pollution in sandy soil.

Key words: *Leuceana leucocephala* • phosphorin • lead pollution • growth

INTRODUCTION

Leuceana leucocephala is native of central America, occurs as a many branches shrub and tall single trunked tree. It is also as protein forage plant. This plant has good potentials to be planted in the poor soils.

Heavy metals are generally found naturally only at very low concentrations. Elevated concentrations are commonly associated with pollution from human activities. Many plants are sensitive to heavy metals, those which are tolerant are generally tolerant of most heavy metals. Several researchers have investigated the effect of heavy metals on the growth of different plants. Ragab [1] on *Cucumis melo* L., Liu *et al.* [2] on *Brassica juncea* and Azza [3] on *Dalbergia sissoo* found that the great positive effect on plants fresh weight especially on roots than shoots. Chen *et al.* [4] on *Vigna radiata*, *Medicago sativa*, *Pinus elliotii* and *Populus* spp, Abbaas [5] on *Casuarina glauca* L., *Taxodium disticum* L. and *Populus nigra* L. and Azza [3] on *Dalbergia sissoo* reported that all growth characters was decreased progressively with increasing rate of Pb in solutions. Azza [3] on *Dalbergia sissoo* showed that, Chlorophyll (a), (b) and carotenoids content was

increased in response to heavy metals pollution. Sayed [6] on safflower, found that, Pb pollution due to a reduction in sucrose and glucose contents. Liu *et al.* [2] on *Brassica juncea* and Azza [3] on *Dalbergia sissoo* noticed that, the uptake of Pb increased with increasing soil Pb. Abbaas [5] on *Casuarina glauca* L., *Taxodium disticum* L. and *Populus nigra* L. and Azza [3] on *Dalbergia sissoo* mentioned that N and P percentages decreased with increasing Pb. But, increasing the concentration led to increasing K percentage.

Considering the effect of *Bacillus megatherium* inoculation, previous studies have indicated that phosphorus deficiency is one of the most important factors limiting plant growth. Luheurte and Barthelin [7] found that plant development as not stimulated by inoculation with *Bacillus megatherium* in a soluble P deficient medium and root growth significantly increased by inoculation of maize plant with *Bacillus megatherium* for 5 ppm of soluble P. The effect of bacterial inoculation on growth of plants depend on the amounts of available P present in soil. On the other hand, increasing microbial activities in the rhizosphere raised available nutrients content in soil and played special role by decomposing organic substances or transforming inorganic substances

to available nutrients for the plant growth and PH of all soils. The role of microorganisms in the production of forms of phosphorus (P) which are available to plants, have been examined and Oberson *et al.* [8] reported that, a high positive correlation between phosphatase activity and residual P. Shalan *et al.* [9] on roselle, Azza [10] on *Parkinsonia aculata*, Rashed [11] on *Anethum graveolens*, *Coriandrum sativum* and *Petroselinum sativum* and Turkey *et al.* [12] on *Foeniculum vulgare* found a significant increase in plant height, number of leaves, fresh and dry weight of vegetative growth as a result of using biofertilizers.

Chemical constituents may be induced by biofertilizer treatments, this was indicated by Gomaa and Abou-Aly [13] on *Pimpinella anisum*, Gad [14] on *Foeniculum vulgare* and *Anethum graveolens*, Shalan *et al.* [9] on roselle, Rashed [11] on some aromatic plants and Turkey *et al.* [12] on *Foeniculum vulgare*.

Thus, the aim of the present investigation was to study the response of *Leuceana leucocephala* to phosphorin under Pb in the irrigation water in sandy soil.

MATERIALS AND METHODS

This investigation was carried out during 2004 and 2005 seasons. The plants were experimentally treated in the National Research Centre, Dokki, Cairo, Egypt, to study the response of *Leuceana leucocephala* seedlings grow under lead pollution to phosphorin treatment in a sandy soil.

The physical and chemical characteristics of the used soil are determined according to pipette method of Piper [15] and presented in Table 1.

Plant materials and procedures: *Leuceana leucocephala* Fam. Memoaceae, the seeds were sown on 25th March of the 2004 and 2005 seasons. Five seeds were sown in pots (30 cm diameter). The plants were thinned, after 30 days from sowing, each pot contained one plant only. Half of the pots were biofertilized with phosphorin (*Bacillus megatherium* phosphorus dissolving bacteria produced as biofertilizer by Ministry of Agriculture, Egypt "Biofertilizer Lab") at 0 and 10 g peatmoss/pot. The irrigation regime began after 30 days from thinned. The average height of seedlings was 20-25 cm.

Basic dressing and treatments: The experiments were sit in factorial experiment in completely randomized design with 6 replications. The treatments used as follows: four Lead levels (0, 25, 50 and 100 ppm) and two inoculation treatments (Uninoculation and inoculation with phosphorin).

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 g per pot in four doses. The plants were fertilized after 4, 8, 16 and 20 weeks from sowing.

Leads as lead acetate ($\text{CH}_3\text{COO})_2\text{Pb}_3\text{H}_2\text{O}$ was used after dissolving in tap water and added as surface irrigation. Irrigation regime repeated twice every week till the end of the season (25th November).

Data recorded: Stem length (cm), stem diameter (mm.), leaves number/plant, root length (cm), fresh and dry weight of plant organs in g (leaves, stem and root) were recorded at the end of season. These data are statistically analyzed according to Snedecor and Cochran [16] using the least significant different (LSD) at 5% level. Chlorophyll (a), (b) and carotenoids content was determined in fresh leaves according to Saric *et al.* [17]. The following data were determined. Soluble, non-soluble and total sugar percentage was determine in the dry leaves according to Dubios *et al.* [18]. Nitrogen percentage was determined by the modified microkiel Dahl method and described by Pregl [19]. Phosphorus percentage as estimated according to King [20]. Potassium percentage was determined by Flame photometer Model Carl-Zeiss according to the method of Richard [21]. The elements (Pb, Zn and Mn) were determined by Atomic Absorption described by Chapman and Pratt [22].

RESULTS AND DISCUSSION

Growth characters: Tables 2 and 3 mentioned that stem length, stem diameter, root length and leaves number/plant were greatly significant reduced by the application of the tested concentrations of Pb compared with the control in the two seasons. The reduction was associated positively with the increase in the concentration. The same result was obtained by Azza [3]

Table 1: Physical and chemical analysis

Coars sand %	Fine sand %	Silt %	Clay %	pH	CaCO ₃ %	Organic matter (%)	EC dSm ⁻¹
57.7	29.3	9.1	3.9	8.1	7.3	0.26	3.1
Cations meq/L				Anion meq/L			
Ca ⁺	Mg ⁺	Na ⁺	K ⁺	CO ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
3.5	10.12	1.35	0.61	—	1.74	0.35	15.11

Table 2: Stem length (cm) and Stem diameter (mm) of *Leuceana leucocephala* as affected by phosphorin and Pb in irrigation water in the 2004 and 2005 seasons

Characters	Stem length (cm.)						Stem diameter (mm)					
	Phosphorin (g) First season			Phosphorin (g) Second season			Phosphorin (g) First season			Phosphorin (g) Second season		
	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
Lead (ppm)												
0	96.31	111.75	104.03	113.31	126.11	119.71	12.3	15.80	14.05	13.1	15.45	14.28
25	91.73	105.45	98.59	106.73	117.31	112.02	10.7	13.95	12.33	11.2	14.20	12.70
50	83.74	92.11	87.93	87.11	98.31	92.71	7.4	9.60	8.50	7.1	10.10	8.60
100	58.31	71.77	65.04	56.67	75.31	65.99	3.3	5.70	4.50	3.6	5.30	4.45
Mean	82.52	95.27		90.96	104.26		8.43	11.26		8.75	11.26	
LSD 0.05												
Pb (A)		9.41			9.85			1.53			1.54	
Phosphorin (B)		6.66			6.95			1.08			1.09	
(AxB)		13.31			13.93			2.16			2.18	

Table 3: Leaves number/plant and root length (cm) of *Leuceana leucocephala* as affected by phosphorin and Pb in irrigation water in the 2004 and 2005 seasons.

Characters	Leaves number/plant						Root length (cm)					
	Phosphorin (g) First season			Phosphorin (g) Second season			Phosphorin (g) First season			Phosphorin (g) Second season		
	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
Lead (ppm)												
0	35.73	47.91	41.82	39.31	51.91	45.61	23.71	28.31	26.01	24.74	30.11	27.43
25	33.11	45.31	39.12	38.74	48.31	43.53	23.11	26.51	24.81	22.67	26.86	24.77
50	21.71	30.24	25.98	26.37	43.36	34.87	15.31	19.23	17.27	16.73	21.19	18.96
100	11.53	19.69	15.61	13.13	21.19	17.25	9.61	11.36	10.49	8.05	13.67	10.86
Mean	25.52	35.74		29.43	41.19		17.94	21.35		18.05	22.96	
LSD 0.05												
Pb (A)		5.87			5.82			2.53			2.68	
Phosphorin (B)		3.56			2.15			1.79			1.9	
(AxB)		7.12			6.62			3.58			3.8	

Table 4: Fresh weight of leaves, stem and root/plant (g) of *Leuceana leucocephala* as affected by phosphorin and Pb in irrigation water in the 2004 and 2005 seasons

Characters	Leaves						Stem						Root					
	Phosphorin (g)			Phosphorin (g)			Phosphorin (g)			Phosphorin (g)			Phosphorin (g)			Phosphorin (g)		
	First season			Second season			First season			Second season			First season			Second season		
Lead (ppm)	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
0	34.31	46.37	40.34	38.83	43.61	44.22	27.13	35.18	31.16	25.31	36.67	30.99	15.31	18.83	17.07	16.75	19.99	18.37
25	30.17	44.51	37.34	36.71	42.53	39.62	24.51	31.11	27.81	25.05	33.36	29.21	13.67	17.61	15.64	15.34	19.51	17.43
50	22.18	30.02	26.10	20.74	31.75	26.25	13.31	19.37	16.34	14.45	22.13	18.21	8.71	10.31	9.51	8.93	11.73	10.33
100	14.36	17.11	15.74	13.61	17.75	15.68	7.75	12.26	10.01	9.11	13.35	11.23	5.31	8.56	6.94	5.96	8.87	7.42
Mean	25.26	34.50		27.47	33.91		18.18	24.48		18.48	26.38		10.75	13.83		11.75	15.03	
LSD 0.05																		
Pb (A)		2.53			3.38			2.68			2.39			0.48			0.89	
Phosphorin (B)		1.66			2.1			1.9			1.69			0.6			0.63	
(AxB)		3.32			4.2			3.8			3.38			1.19			1.25	

Table 5: Dry weight of leaves, stem and root/plant (g) of *Leuceana leucocephala* as affected by phosphorin and Pb in irrigation water in the 2004 and 2005 seasons

Characters	Leaves						Stem						Root					
	Phosphorin (g)			Phosphorin (g)			Phosphorin (g)			Phosphorin (g)			Phosphorin (g)			Phosphorin (g)		
	First season			Second season			First season			Second season			First season			Second season		
Lead (ppm)	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
0	17.75	23.56	20.66	19.23	25.17	22.20	13.53	21.31	17.42	13.91	20.34	17.17	10.11	13.37	11.74	10.35	14.45	12.40
25	14.79	23.51	19.14	16.15	22.54	19.35	11.06	18.75	14.91	10.26	18.55	14.41	10.01	13.15	11.58	9.13	12.17	10.65
50	10.51	13.67	12.09	11.15	14.49	12.82	6.12	11.73	8.93	7.17	12.51	9.84	5.17	7.71	6.44	4.37	6.95	5.66
100	6.76	7.96	7.36	6.66	9.35	8.01	3.51	6.71	5.11	3.95	6.97	7.44	2.19	4.91	3.55	2.43	3.89	3.16
Mean	12.45	17.18		13.30	17.89		8.56	14.63		8.82	14.59		6.87	9.79		6.57	9.37	
LSD 0.05																		
Pb (A)		1.16			1.52			1.06			1.41			1.75			0.87	
Phosphorin (B)		0.82			1.07			0.75			0.91			1.11			0.62	
(AxB)		1.64			2.15			1.51			2.81			2.22			1.23	

Table 6: Chlorophyll (a), (b) and carotenoids (mg/g F.W.) of *Leuceana leucocephala* as affected by phosphorin and Pb in irrigation water in the 2004 and 2005 seasons

Characters	Chlorophyll (a)						Chlorophyll (b)						Carotenoids					
	Phosphorin (g)						Phosphorin (g)						Phosphorin (g)					
	First season			Second season			First season			Second season			First season			Second season		
	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
Lead (ppm)																		
0	1.169	1.316	1.243	1.187	1.263	1.225	0.071	0.111	0.091	0.035	0.123	0.097	0.212	0.375	0.294	0.237	0.362	0.300
25	1.256	1.451	1.354	1.373	1.431	1.402	0.091	0.156	0.124	0.101	0.156	0.129	0.271	0.481	0.376	0.312	0.453	0.383
50	1.371	1.563	1.467	1.451	1.611	1.531	0.115	0.187	0.151	0.121	0.191	0.156	0.323	0.522	0.423	0.356	0.553	0.459
100	1.731	1.811	1.771	1.634	1.761	1.698	0.15	0.216	0.366	0.155	0.221	0.188	0.441	0.565	0.503	0.482	0.612	0.547
Mean	1.382	1.535		1.411	1.517		0.107	0.168		0.103	0.173		0.312	0.486		0.347	0.495	

and Abbaas [5]. The previous characters increased significantly by inoculation with *Bacillus megatherium*. This may be due to the increase in nitrogen content in the soil as a result of N fixation and phosphorus from phosphate dissolving bacteria as well as growth promoting substances such as indole acetic acid and gibberellins produced by organisms used. These results are in line with those of Gad [14]. The interaction between inoculation and Pb concentrations indicated that the stem length, stem diameter, root length and leaves number/plant were more than that obtained from the plant irrigated with the different levels of Pb alone in both seasons.

Data in Tables 4 and 5 showed that the fresh and dry weight of all plant organs were significant decreased as received the used Pb in the irrigation water. The weight of plant organs gradually decreased with increasing Pb concentrations comparing with the control in both seasons. This conclusion is confirmed by Pendias and Pendias [23], who reported that inhibitory effect of Pb may be due to this interaction with other elements and environmental factors. Lead had a toxic effects on several processes such as photosynthesis, mitosis and water absorption. Also subcellular effects of Pb on plant tissues are related to the inhibition of respiration through disturbance of electron transfer. Lead is strongly bound to cell walls, resulting in an increase in tissue wall turgidity. Also, the Zinc-lead antagonism adversely affects the translocation of each element from roots to top, this lead to height shortening. Bersin [24] mentioned that, the effect of Pb on some enzymes involved in the metabolism of N in plant, the marked decrease of N concentration may be one of the reasons that cause reduction of the weight. These results agreed with Azza [3] and Abbaas [5]. The inoculation with *Bacillus megatherium* significant increased the weight significantly in all plant organs compared with the control in the two seasons. These increases in root fresh weight may be attributed to the activity of the free-living bacteria, *Bacillus megatherium* found in the

rhizosphere of roots as phosphate dissolving bacteria, which save the available phosphate. These bacteria proved to be able to produce auxins and other plant growth substances in the plant rhizosphere [25]. The increase in root length, fresh and dry weight of roots may be cause increase of root size leading to increase absorption of nitrogen, phosphorus and potassium as well as trace elements, which reflexes on plant growth. Generally, the increase of fresh and dry weight may be due to the production of bacterial growth hormones and dissolved phosphate by *Bacillus megatherium*. This result is in agreement with those obtained by Turkey *et al.* [12]. Inoculation with phosphorin increased the weight even under lead stress compared with the lead treatments without inoculation.

Chemical constituents: Table 6 revealed that Chlorophyll (a), (b) and carotenoids content increased by Pb applications compared to the control in both seasons. This increment occurred coinciding with the increasing Pb concentration. This result was confirmed by Azza [3]. The values of Chlorophyll (a), (b) and carotenoids were increased in the two seasons with biofertilizer treatments. Similar trend was observed by Turkey *et al.* [12]. Dealing with Pb concentrations and inoculation interactions, the data indicated that the combination of both factors on Chlorophyll (a), (b) and carotenoids was more effective than the effect of each factor tested alone.

Data in Table 7 indicate that, increasing Pb concentration gradually decreased soluble, non-soluble and total sugar percentage as compared with the control in the two seasons. This result agreement with Azza [3]. Inoculation with phosphorin increased soluble, non-soluble and total sugars in both seasons compared with uninoculation plants. This result may be indicate indirect effect of biofertilizer on sugar, it increase the plant growth, consequently sugars metabolism increased. This result was confirmed by Azza [3]. Regarding the interaction between inoculation and Pb treatments, the data indicated that soluble, non-soluble and total sugars

Table 7: Soluble, non-soluble and total sugar percentage of *Leuceana leucocephala* as affected by phosphorin and Pb in irrigation water in the 2004 and 2005 seasons

Characters	Soluble sugar %						Non-soluble sugar %						Total sugar %					
	Phosphorin (g)						Phosphorin (g)						Phosphorin (g)					
	First season			Second season			First season			Second season			First season			Second season		
	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
Lead (ppm)	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
0	5.62	8.31	6.97	4.83	7.67	6.25	8.08	11.81	9.95	9.93	14.04	11.99	13.7	20.12	16.91	14.76	21.71	18.24
25	5.13	7.77	6.45	4.36	7.45	5.91	7.08	9.09	8.09	8.35	11.18	9.77	12.21	17.67	14.94	12.71	18.63	15.67
50	3.67	5.34	4.51	3.71	6.56	5.14	6.07	8.79	7.43	6.48	9.55	8.02	9.74	14.13	11.94	10.19	16.11	13.15
100	3.01	4.83	3.92	2.78	4.11	3.45	4.55	6.98	5.77	4.59	7.89	6.24	7.56	11.81	9.69	7.37	12.00	9.69
Mean	4.36	6.56		3.92	6.45		6.45	9.17		7.34	10.67		10.80	15.93		11.26	17.11	

Table 8: Nitrogen, phosphorus and potassium percentage of *Leuceana leucocephala* as affected by phosphorin and Pb in irrigation water in the 2004 and 2005 seasons

Characters	N%						P%						K%					
	Phosphorin (g)						Phosphorin (g)						Phosphorin (g)					
	First season			Second season			First season			Second season			First season			Second season		
	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
Lead (ppm)	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
0	3.31	4.21	3.67	3.67	4.79	4.73	0.241	0.369	0.305	0.296	0.391	0.343	0.48	0.86	0.67	0.41	0.85	0.63
25	3.01	3.85	3.43	3.13	4.27	3.70	0.221	0.315	0.268	0.271	0.352	0.312	0.67	1.02	0.85	0.6	1.11	0.86
50	2.52	3.07	2.80	2.69	3.21	2.95	0.199	0.237	0.218	0.198	0.293	0.246	0.81	1.23	1.02	0.77	1.26	1.02
100	2.13	2.69	2.41	2.37	2.86	2.62	0.151	0.229	0.190	0.11	0.251	0.181	0.99	1.35	1.67	0.93	1.47	1.20
Mean	2.74	3.46		2.97	3.78		0.203	0.288		0.219	0.322		0.74	1.12		0.68	1.17	

Table 9: Lead, zinc and manganese ppm of *Leuceana leucocephala* as affected by phosphorin and Pb in irrigation water in the 2004 and 2005 seasons

Characters	Pb ppm						Zn ppm						Mn ppm					
	Phosphorin (g)						Phosphorin (g)						Phosphorin (g)					
	First season			Second season			First season			Second season			First season			Second season		
	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
Lead (ppm)	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean	0	10	Mean
0	0.081	0.022	0.052	0.06	0.029	0.045	0.316	0.473	0.395	0.281	0.414	0.348	0.461	0.161	0.311	0.373	0.193	0.283
25	1.213	0.660	0.937	1.161	0.783	0.972	0.283	0.371	0.327	0.253	0.366	0.310	0.333	0.143	0.238	0.325	0.175	0.25
50	1.791	0.922	1.357	1.831	0.961	1.369	0.251	0.325	0.289	0.231	0.317	0.274	0.272	0.121	0.197	0.291	0.151	0.221
100	2.173	1.261	1.717	2.081	1.34	1.711	0.187	0.282	0.235	0.182	0.265	0.224	0.246	0.11	0.178	0.235	0.125	0.18
Mean	1.315	0.716		1.283	0.778		0.259	0.363		0.237	0.341		0.328	0.134		0.306	0.161	

percentage were more than that obtained from the plant irrigated with the different levels of Pb in both seasons. This means that inoculation had an antagonistic effect to the Pb in irrigation treatments.

Tables 8 and 9 showed that, increasing Pb concentrations decreased N percentage in leaves. Bersin [24] mentioned that, the decrease in N percentage in leaf may be due to the effect of Pb on some enzymes involved in the metabolism of N in plant. This result agreed with Azza [3] and Abbaas [5]. Phosphorus percentage gradually decreased with increasing Pb concentration as compared with the control. The reduction of P percentage is possibly due to the precipitation of Pb as $Pb_3(PO_4)_2$ in soil. In this respect, data revealed that, increasing Pb has decreased Zn and Mn contents in both seasons compared with the control. On the other hand, biofertilization with *Bacillus*

megatherium increases N, P% and Zn content in leaves compared with nonbiofertilized ones. These results may be attributed to the synergistic effect of inoculation with biofertilization on N, P and Zn in leaves. But, inoculation application has decreased Mn content in both seasons. Moreover, the interaction between inoculation and Pb leads to a positive effect on N, P and Zn content in leaves, the increase of Zn content concentrations may be rendered to the slight decrease of soil pH stimulated by *Bacillus megatherium* additions as well as to the antagonistic relationship between Mn and Zn since biofertilizer addition decreased Mn and this may stimulate the increase of plant Zn.

Data showed that, K percentage and Pb content increased with increasing Pb concentration in leaves in both seasons. This result may be indicate that there is a relationship between Pb and K. In this respect,

inoculation increased K% and but decreased Pb content in the two seasons compared with the control. Interaction between inoculation application and Pb in all concentrations increased K percentage and decreased Pb content compared with Pb treatments alone in both seasons.

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