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Lead toxicity and Spermine as affecting the Chemical Composition and Growth of *Solidago canadensis* L. cv. 'Tara' plant

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Abstract

Solidago canadensis L. cv. 'Tara' as important flower production and traditional medicine. Heavy metals such as lead (Pb) have harmful effects on ornamental plants. So, this study aimed to reduce the toxic effect of Pb on *Solidago canadensis* plant through spermine(SPM) application. Plants were grown in treated soils with different Pb levels (0, 400, and 800 ppm) and sprayed with spermine at 0, 1 and 2 mM. Plant height, leaves number, total leaf area, stem diameter, number of inflorescences, length of inflorescences stem, fresh & dry weights of shoot, root & inflorescences and flowering period were recorded as growth parameters. Photosynthetic pigments, antioxidant enzymes, total sugars, total phenols, free amino acids, proline, minerals (N, P, K, Pb) and protein as chemical contents. Results showed that Pb at 800 ppm resulted in the lowest values for most tested parameters, but increased Pb level in shoots and roots. Spermine treatments especially 2mM were highly significant in most parameters under Pb conditions, except Pb concentration reduction as compared to the control. Results provided evidence that spermine minimized the adverse effects of lead (Pb) stress and could play an essential role in providing stress tolerance .

***Key words*:** *Solidago canadensis*; lead; Spermine; growth parameters, chemical contents.

1. Introduction

Recently, floriculture has become a lucrative agribusiness, so should be optimized the production conditions of these plants. *Solidago canadensis* L. cv. 'Tara' (*S. canadensis*) which belongs to the Asteraceae family is originally from North America and Mexico. It is a wild weed, but also it used in landscaping, as perfect cut flowers with high longevity harvest and as dried flowers [1]. *S. canadensis* is rapidly colonized of many due to the high reproductive ability of its underground parts, rapid spreading and seed germination [2] and used an antibacterial [3].

Soil is exposed to a wide range of contaminats such as heavy metals, pesticides, petroleum products, or excessive fertilizer use. Lead (Pb) is one of the most critical toxic heavy metals in the environment. It has important physical and chemical properties. Previous studies illustrated that Pb contamination caused damage to plant root system and decreased transpiration strength. Also, Pb resulted in disorder in the composition of both protein fraction and lipid membrane. [4]. In previous studies, there are numerous lands polluted with heavy metals such as lead [5,6].

Polyamines (PAs) are aliphatic nitrogenous bases of low molecular weight including two or more amino groups, and have strong biological activity [7, 8]. PAs are essential for normal cell development and differentiation which present in all living organisms and represent many physiological processes of critical importance. Furthermore, the main polyamines in plants are putrescine (Put), spermidine (SPD) and spermine (SPM) that involved in many physiological and biochemical processes, including plant development and growth, cell differentiation, cell division, flowering, immunity to senescence, embryogenesis rooting, and DNA replication and synthesis of nucleic acid [9, 10, 11] . PAs have been widely studied for exploring their role in conferring tolerance to abiotic stresses (drought, salt, and metal toxicity) in the last decades. Additionally, final

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product of the biosynthetic pathway of polyamine is SPM and ubiquitously present in most organisms [12].

Therefor the aim of this work, exploit the soil contaminated with different levels of lead in the production of plant high economic important such as *Solidago canadensis* plants as cut flowers by supporting with foliar with spermine through evaluating plant growth, flowering parameters. In addition chemical consentient such as some antioxidant enzymes activities and proline.

2. Materials and Methods

Experimental: experiment was carried out at the National Research Centre (NRC) greenhouse, Giza, Egypt, during two successful seasons began in January 2018 and ended in July of the same year and replicated during the same period 2019. *S. canadensis* rooted cuttings at 5 cm length with eight to nine leaves per cutting obtained from "Floramax" Company, Mansoria, Giza. Were grown in plastic pots (10cm) and after 21 days transplanted into clay pots in the first week of January in both seasons. Filled pots with 30 cm in diameter with 10 kg of clay and sand at the ratio of (1:1, v/v). NPK fertilization with krystalon (18:18:18) was applied three times to the soil every 21 days after transplanting at the rate of 0.5 g/pot. During both seasons, *S. Canadensis* plants were grown in treated soils with different Pb levels (0, 400, and 800 ppm Pb) prepared from lead nitrate [Pb $(NO_3)_2$] and sprayed with SPM at 0, 1 and 2 mM. SPM was sprayed three times, started at day 21 from transplanting, and repeated every 15 days.

Plants of *S. canadensis* were grown from 9 pm to 3 am under normal temperatures (22–23°C) (at the rate of 15 watts m^{-2}) with cyclic lighting of 15 min on and 15 mint off under natural temperature and controlled day length (16 to 18 lighting hour/day) using Tungsten lamps for prolonged day length. Placed the lamps were at 2 m from the soil surface, *Solidago canadensis* plants still rosette , when short day length and temperature are less than 12 h and 15º C, the effect of day length of flower formation is greater than the impact of cold temperatures. Thus, a year-round production program of *Solidago canadensis* with the application of lighting and heating is feasible [13]. Express Plugs, also who mentioned that the solidago keeps producing flower stalks by using of lighting and heating (temperature over 10°C and light 16 h) while the stems reach to 35-45 cm or your target stalks length, stop lighting and then *S. canadensis* grows generative and forms its flowers.

2.1. Soil properties

Physical and chemical analysis of the experimental soil in Table (1) which taken from Research and Production Station, National Research Centre, Nobarya Site, Beheara Governorate , Delta Egypt determined according to [14].

Table 1 Physical and chemical analysis of the experimental Soil

Physical properties		Clay	Silt	Sand		Texture			
		2.8	14.6	82.6		Sandy loam			
Chemical analysis	pH	EC ds\m	Soluble Cations Meq/L					Soluble Anions(Meq/L)		
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Co_3	HCO_3	Cl^{-}	SO_4^{--}
	7.85	0.39	0.8	0.5	2.5	0.1	-	-	2.50	1.4

Electrical conductivity (EC); deciSiemens per meter (dS/m); Milliequivalents per liter (Meq/L).

2.2. Vegetative parameters:

Vegetative parameters recorded (plant height (cm), leaves number /plant, stem diameter (cm), Total leaf area/plant (cm^2), fresh and dry weights of shoot and root/plant (g) for each treatment.

2.3. Flowering parameters:

Number of inflorescences/plant, Inflorescences length stem (cm), fresh and dry weight of inflorescences /plant (g) and flowering period (day)) were recorded.

2.4. Determination of photosynthetic pigments:

Chlorophyll a & b (Chl a & b) and total carotenoids were estimated in fresh leaf samples according to Saric *et al*. [15] .

2.5. Extraction and determination of antioxidant enzymes activities:

Enzyme extraction was done in fresh leaves as described by Mukherjee and Choudhuri [16]. The activity of both Catalase (CAT) EC 1.11.1.6 and peroxidase (POX) EC 1.11.1.7 were determined according to the method of Kar and Mishra [17] .Superoxide dismutase activity (SOD) EC 1.15.1.1 was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a method was determined by Marklund and Marklund [18].

2.6. Determination of total sugars, free amino acid and total Phenols:

Ethanol extract was prepared by cutting 2.5 g of fresh shoot into small pieces, then it was crushed in porcelain mortar using about 25 ml of 80% ethanol and then was boiled for 10 minutes after filtrated through a sintered glass silica-filter. Finally, residue was adjusted to 50 ml using ethanol 70%.

Total sugars were estimated using ethanol extract, phenol, sulphuric acid reagent method described by Dubois *et al*. [20]. Analysis of free amino acids was measured by spectrophotometer (JWNWAY 6315) at 570 nm, using glycine as the standard described by Moore and Stein [21]. Total phenols were measured by colorimetric method, using a folin-Ciocalteau reagent, calculated by spectrophotometer at 650 nm and using Gallic acid as the standard was described by Swain and Hillis [22].

2.7. Determination of Proline:

Proline content was measured in fresh leaves using the method was determined by Bates *et al*. [19].

2.8. Determination of minerals and protein:

P (%) was assessed using the ammonium molybdate method according to Snell and Snell [23]. K (%) was determined in the digested solution described by Chanpman and Pratt [24] and the results were represented as g/100g D.W. of the plant. Using atomic absorption spectroscopy (PerkinElmer 100 B, US), Pb (ppm) was estimated by Cottenie *et al*. [25]. Nitrogen contents were described by the modified Kjeldahl method as determined by Cottenie *et al.* [25]. Protein content was calculated as multiply N% by 6.25 Mariotti *et al*. [26].

2.9. Statistical analysis:

In this experiment, 2 factors were considered, Pb at 0.0, 400, 800 ppm and SPM at 0.0, 1.0 and 2.0mM. For each treatment there were five replicates, each of which had ten pots; in each pot three individual plants were planted. The experimental design followed a split split design. According to Snedecor and Cochran [27], the averages of data were statistically analyzed by using 2 ways analysis of variance (ANOVA). Significant values determined according to p-values ($p<0.05$ = significant, $p<0.01$ = moderate significant and $p<0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program.

3. Results:

3.1. Vegetative parameters:

Data presented in Tables (2&3) showed that the harmful effect of Pb on vegetative parameters[plant height (cm), leaves number/plant, total leaf area/plant (cm^2), stem diameter (cm), fresh and dry weights of shoot and root (g)] increased as increasing its concentration. Whereas, the lowest values [45.0, 38.2(cm); 37.0, 31.3/plant; 175.6, 128.0/plant (cm^2); 0.3, 0.3 (cm); 8.2, 5.7; 3.5, 2.5; 3.6, 1.8 and 1.7, 0.9 (g)] during first and second seasons, respectively were obtained from plants exposed to Pb (800 ppm), except fresh and dry weights of root in second season had non-significant. On the other hand, the greatest vegetative parameters were obtained from plants treated with 2 mM SPM without Pb with values of [88.0, 81.3(cm); 65.0, 60.0/plant; 837.2, 708.6/plant (cm^2); 0.7, 0.6 (cm); 18.7, 14.6; 7.7, 6.5; 8.8, 5.2; 4.1, 2.7(g)] during the first and second seasons, respectively. Plants treated with SPM x Pb resulted in higher values of different vegetative parameters than those treated with Pb alone. The changes were highly significant with SPM or Pb treatments except changes in stem diameter in second season and fresh weight of shoot & root in the first season were significant for Pb treatment. The interactions between SPM and Pb resulted in moderate significant variations of both fresh and dry weights of the shoot in first and second seasons. Variations in fresh weight of shoot in first season, fresh and dry weights of root in both seasons were non- significant for SPM x Pb treatments.

3.2. Flowering parameters:

Data in Table (4) indicated that the addition of lead (Pb) in the soil caused a significant reduction in all of the tested flowering parameters (number of inflorescences/plant, length of inflorescences stem (cm), fresh and dry weights of inflorescences (g) /plant and flowering period (day)). Plants added with a higher dose of Pb (800 ppm), recorded the minimum values for all the tested flowering parameters (13.0, 14.9; 80.3, 62.7 cm; 6.4, 5.2 g; 1.6, 1.3 g and 48.8, 49.7days) during first and second seasons respectively. These results clearly showed a negative impact on flower development, with soil adding lead to *Solidago canadensis* plants.

Furthermore, foliar spray of SPM at 2mM resulted in the greatest values (22.3, 25.1; 106.5, 90.7 cm; 11.8, 10.4 g; 3.4, 3.1 g and 62.2, 61.8 days in first and second season respectively). The interaction between Pb

doses and SPM applications, revealed that the greatest values were calculated from plants treated with SPM at 2mM alone without Pb or with Pb, followed by plants sprayed a low concentration of SPM at 1mM comber with control plants or plants treated with Pb alone.

3.3. Photosynthetic pigments:

Data presented in Table (5) indicated that plants treated with Pb treatments produced highly reduction of photosynthetic pigments (chl a, chl b, and total carotenoids mg\g F.W.). However, plants treated with Pb at (800ppm) gave the lowest values (0.6; 0.3 and 0.5 mg/g F.W.) compared with control. The highest values (1.3; 0.7 and 0.8 mg/g F.W.) resulted from plants sprayed with SPM at 2 mM . The changes in chl a, and chl b were highly significant for SPM or Pb treatments, while SPM x Pb was moderately significant in chl aand non- significant in chl b. Variations in total carotenoids were significant with SPM or Pb and non- significant for their interactions.

Table 2 Impact of Pb, SPM and their interactions on plant height, leaves number, total leaf area and stem diameter.

Treatments		Plant height (cm)		leaves number /plant		Total leaf area /plant (cm^2)		Stem diameter (cm)	
		Seasons							
SPM mM	Pb ppm	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.0	0.0	48.7±0.6	44.0±1.0	50.6±0.6	45.9±0.2	339.8±4.1	246.5±0.5	0.5±0.1	0.4±0.1
	400	46.5±0.5	39.5±0.5	38.0±1.0	32.0±1.0	190.9±5.4	131.4±5.3	0.4±0.0	0.3±0.0
	800	45.0±0.0	38.2±0.4	37.0±1.0	31.3±0.4	175.6±5.0	128.0±4.3	0.3±0.1	0.3±0.0
Overall 0.0		46.7±1.6	40.6±2.7	41.9±6.6	36.4±7.1	235.4±78.6	168.6±58.5	0.4±0.1	0.3±0.0
1.0	0.0	67.5±0.5	61.5±0.5	62.5±0.5	56.5±0.5	505.6±4.4	397.2±5.1	0.6±0.1	0.6±0.0
	400	52.3±0.7	46.5±1.0	42.0±1.0	41.7±0.8	289.4±6.0	265.3±2.4	0.5±0.0	0.5±0.0
	800	49.6±0.5	44.4±1.0	40.7±0.8	32.5±0.5	264.9±6.9	208.3±3.2	0.6±0.0	0.5±0.0
Overall 1.0		56.5±8.4	50.8±8.1	48.4±10.6	43.6±10.5	353.3±114.8	290.3±84.0	0.6±0.0	0.5±0.0
2.0	0.0	88.0±0.5	81.3±0.3	65.0±1.0	60.0±1.0	837.2±13.5	708.6±10.0	0.7±0.1	0.6±0.1
	400	78.3±0.8	71.0±1.0	59.0±1.0	55.0±1.0	435.0±6.5	417.6±18.4	0.6±0.0	0.6±0.0
	800	76.5±0.5	64.5±0.5	52.5±0.0	47.0±1.0	371.5±5.0	325.5±3.5	0.6±0.0	0.5±0.1
Overall 2.0		80.9±5.4	72.3±7.4	58.8±5.5	54.0±5.7	547.8±218.2	483.9±173.5	0.6±0.0	0.6±0.0
Overall Pb	0.0	68.1±17.1	62.3±16.2	59.4±6.7	54.1±6.4	560.9±219.5	450.7±204.2	0.6±0.1	0.5±0.1
	400	59.1±14.7	52.3±14.3	46.0±10.0	42.8±10.0	305.3±106.4	270.3±125.8	0.5±0.1	0.5±0.1
	800	57.0±14.8	49.0±11.9	43.7±6.7	36.9±7.6	270.7±85.1	221.7±84.7	0.5±0.1	0.5±0.1
F-ratio									
SPM		9753.0***	4175.0***	965.3***	1209.9***	4755.8***	3791.8***	126.2***	177.1***
Pb		1083.6***	759.4***	944.5***	1177.3***	4799.4***	2183.8***	12.7***	10.7*
SPM *Pb		163.8***	68.1***	70.3***	73.6***	450.5***	256.0***	2.9*	1.0 N.S

Spermine (SPM), Lead (Pb), SPM*Pb= interaction, 1st= First season(2018), 2nd= Second season (2019), NS Non-significant,*Significant, **Moderate significant, ***Highly significant, values are given as Means ±SD

Table 3 Impact of Pb ,SPM and their interactions on fresh and dry weights of shoot and root.

Treatments		Fresh weight of shoot (g)		Dry weight of shoot (g)		Fresh weight of root (g)		Dry weight of root (g)	
		Seasons							
SPM mM	Pb ppm	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.0	0.0	11.7±0.3	7.3±0.4	4.9±0.5	3.2±0.3	5.1±0.6	2.5±0.6	2.5±0.3	1.3±0.5
	400	8.7± 1.3	5.9±0.5	3.7±0.1	2.6±0.0	4.2±0.2	2.3±0.9	1.9±0.1	1.2±0.1
	800	8.2± 1.1	5.7±0.4	3.5±0.0	2.5±0.1	3.6±0.3	1.8±0.1	1.7±0.1	0.9±0.0
Overall 0.0		9.5± 1.8	6.3±0.8	4.0±0.7	2.8±0.4	4.3±0.8	2.2±0.6	2.0±0.4	1.1±0.3
1.0	0.0	14.3± 3.1	9.3±0.9	6.0±0.2	4.1±0.0	6.1± 1.6	3.6± 1.3	2.8±0.0	1.9±0.1
	400	13.4± 2.1	8.7± 1.8	5.6±0.1	3.9±0.1	6.0±0.1	3.6±0.5	2.8±0.3	1.8±0.2
	800	13.3±0.3	7.5±0.7	5.6±0.3	3.4±0.1	5.9±0.6	3.3±0.0	2.8±1.0	1.7±0.0
Overall 1.0		13.7± 2.0	8.5± 1.3	5.8±0.3	3.8±0.3	6.0±0.8	3.5±0.7	2.8±0.5	1.8±0.1
2.0	0.0	18.7±0.8	14.6±0.2	7.7±0.4	6.5±0.2	8.8± 1.8	5.2±0.9	4.1±0.0	2.7± 1.0
	400	17.4±0.9	13.0±0.5	7.4±0.1	6.4±0.2	7.1±0.1	4.0±0.5	3.3±0.4	2.0±0.1
	800	15.8± 2.5	9.7±0.3	6.6±0.2	4.3±0.0	6.3±0.9	3.9±0.6	2.9±0.3	2.0±0.1
Overall 2.0		17.3± 1.9	12.5± 2.2	7.2±0.5	5.7± 1.1	7.4±1.5	4.4±0.9	3.4±0.6	2.2±0.6
Overall Pb	0.0	14.9±3.4	10.4±3.3	6.2± 1.2	4.6± 1.5	6.6±2.1	3.8±1.5	3.1±0.7	2.0±0.8
	400	13.2±4.0	9.2±3.2	5.5± 1.6	4.3±1.7	5.8±1.3	3.3±0.9	2.7±0.6	1.7±0.4
	800	12.4±3.6	7.6±1.8	5.2±1.4	3.4±0.8	5.3±1.4	3.0±1.0	2.4±0.8	1.5±0.5
F-ratio									
SPM		49.4***	145.7***	392.1***	1133.7***	26.1***	20.9***	26.8***	19.1***
Pb		5.2*	28.9***	39.4***	195.3***	5.2*	2.6 N.S	7.4**	2.7 N.S

SPM *Pb	0.7 N.S	5.0**	6.1**	52.1***	1.3 N.S	0.7 N.S	1.6 N.S	0.6 N.S	

Spermine (SPM), Lead (Pb), SPM*Pb= interaction, 1st= First season(2018), 2nd= Second season (2019), NS= Non-significant,*Significant, **Moderate significant, ***Highly significant , values are given as Means ±SD

Table 4 Impact of Pb , SPM and their interactions on flowering parameters.

Treatments		No. of inflorescences/plant		Inflorescence stem length (cm)		F. W. of inflorescences (g)		D.W. of inflorescences (g)		Flowering period(day)	
		Seasons									
SPM mM	Pb ppm	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0.0	0.0	20.2± 1.0	22.7±1.1	73.0±1.0	65.8±0.3	8.5±0.2	6.3±0.0	2.0±0.1	1.5±0.1	50.2±0.8	55.0±0.9
	400	12.8±0.3	16.0± 1.0	69. 7±0.6	58.8± 1.9	7.2±0.2	4.1±0.1	1.5±0.1	0.9±0.1	43.5±0.5	50.3±0.6
	800	11.4±0.2	12.2±0.8	64.5± 0.9	51.2±0.3	4.6±0.2	3.0±0.1	0.9±0.1	0.6±0.0	41.3±0.6	45.0±1.1
Overall 0.0		14.8± 4.1	17.0± 4.7	69.1± 3.8	58.6± 6.4	6.8± 1.7	4.5± 1.5	1.5±0.4	1.0±0.4	45.0± 4.0	50.1± 4.4
1.0	0.0	25.0± 1.0	28.3±0.6	96.0± 1.0	82.4±0.2	13.3±0.2	12.2±0.1	3.5±0.1	3.2±0.1	63.3±0.6	65.7±0.6
	400	17.5±0.5	18.5±0.5	87.5±0.5	70.5±0.5	9.6±0.1	7.5±0.1	2.5±0.1	2.0±0.0	55.3±0.6	61.0± 1.0
	800	12.7±0.6	15.0± 1.0	79.8± 1.0	61.4±0.5	6.3±0.1	5.5±0.2	1.5±0.1	1.4±0.0	50.2±0.8	50.0± 1.2
Overall 1.0		18.4±5.4	20.6±6.0	87.8±7.0	71.4±9.1	9.7±3.1	8.4±3.0	2.5±0.9	2.2±0.8	56.3±5.8	58.9±7.0
2.0	0.0	31.1±0.1	35.2±0.8	113.5± 0.9	103.2± 0.7	15.7±0.2	14.5±0.2	4.8±0.1	4.7±0.3	70.2± 1.8	67.5± 1.5
	400	20.7± 1.5	22.7±0.6	109.5±0.5	93.3±0.3	11.5±0.2	9.5±0.2	3.2±0.1	2.6±0.1	61.5± 0.9	63.8± 1.0
	800	15.0±0.1	17.5±0.5	96.5±0.5	75.5±0.5	8.3±0.1	7.1±0.1	2.3 ±0.1	2.0±0.0	55.0± 0.9	54.0± 1.0
Overall 2.0		22.3±7.1	25.1±7.9	106.5±7.7	90.7 ±12.2	11.8±3.2	10.4±3.3	3.4±1.1	3.1±1.3	62.2±6.7	61.8±6.1
Overall Pb	0.0	25.4±4.8	28.7±5.5	94.2±17.6	83.8±18.2	12.5±3.2	11.0±3.6	3.4±1.2	3.1±1.4	61.2± 8.8	62.7±5.9
	400	17.0±3.5	19.1±3.0	88.9±17.3	74.2±15.2	9.4± 1.9	7.0±2.4	2.4±0.7	1.8±0.8	53.4±7.9	58.4±6.2
	800	13.0± 1.6	14.9±2.4	80.3±13.9	62.7±10.6	6.4± 1.6	5.2± 1.8	1.6±0.6	1.3±0.6	48.8±6.1	49.7± 4.0
F-ratio											
SPM		219.2***	241.3***	5010.4***	4086.4***	2094.0***	5008.5***	921.8***	707.6***	869.9***	320.4***
Pb		629.8***	729.8***	702.6***	1756.8***	2991.4***	4919.9***	862.5***	562.5***	444.3***	383.3***
SPM *Pb		18.6***	17.8***	36.4***	68.4***	107.2***	229.8***	52.5***	52.6***	10.3***	8.4***

Spermine (SPM), Lead (Pb), SPM*Pb= interaction, 1st= First season(2018), 2nd= Second season (2019), NS= Non-significant,*Significant, **Moderate significant, ***Highly significant , values are given as Means ±SD

Table 5 Impact of Pb, SPM and their interactions on phtosynthetic pigments and antioxidant enzymes

Treatments		Chl a (mg/g F.W.)	Chl b (mg/g F.W.)	Total carotenoids (mg/g F.W.)	Antioxidant enzymes (unit/ g F.W. min)		
SPM mM	Pb ppm				POX	CAT	SOD
0.0	0.0	0.7±0.1	0.4±0.0	0.6±0.1	2.3 ±0.0	15.9 ±0.1	6.0 ±0.1
	400	0.6±0.1	0.3±0.1	0.5±0.2	2.5 ±0.0	20.0 ±0.1	6.5 ±0.0
	800	0.6±0.0	0.3±0.1	0.5±0.1	2.6 ±0.0	17.5 ±0.1	7.1 ±0.1
Overall 0.0		0.7±0.1	0.3±0.1	0.5±0.1	2.5 ±0.2	17.8 ±0.8	6.5±0.4
1.0	0.0	1.0±0.1	0.6±0.1	0.7±0.0	2.4 ±0.0	20.5 ±0.1	7.1 ±0.1
	400	0.8±0.0	0.5±0.0	0.6±0.3	2.5 ±0.1	23.9 ±0.1	7.6 ±0.1
	800	0.8±0.0	0.3±0.0	0.6±0.1	3.0±0.0	18.6 ±0.4	7.7 ±0.1
Overall 1.0		0.9±0.1	0.5±0.1	0.6±0.2	2.6 ±0.3	21.0 ±0.1	7.5 ±0.3
2.0	0.0	1.3±0.0	0.7±0.0	0.8±0.0	2.8 ±0.0	22.7 ±0.1	7.5 ±0.1
	400	0.9±0.1	0.5±0.0	0.6±0.0	3.1 ±0.0	25.6 ±0.2	7.7 ±0.1
	800	0.9±0.0	0.4±0.0	0.6±0.1	3.2 ±0.0	19.5 ±0.2	11.9 ±0.1
Overall 2.0		1.0±0.2	0.6±0.1	0.7±0.1	3.1 ±0.2	22.6 ±0.3	9.1 ±0.3
Overall Pb	0.0	1.0±0.3	0.6±0.1	0.7±0.1	2.5 ±0.2	19.7 ±0.6	6.9 ±0.5
	400	0.8±0.1	0.4±0.1	0.6±0.2	2.7 ±0.3	23.2 ±0.9	7.3 ±0.6
	800	0.7±0.1	0.3±0.1	0.5±0.1	2.9 ±0.2	18.5 ±0.9	8.9 ±0.8
F-ratio							
SPM		85.1***	70.8***	4.2*	921.2***	1900.8***	1594.8***
Pb		45.1***	70.2***	4.3*	541.2***	1892.7***	1020.5***
SPM *Pb		5.1**	3.2*	0.2 N.S	67.9***	184.6***	514.3***

Spermine (SPM), Lead (Pb), SPM*Pb= interaction, 1st= First season(2018), 2nd= Second season (2019), NS= Non-significant,*Significant, **Moderate significant, ***Highly significant, values are given as Means ±SD

3.4. Antioxidant enzymes activities:

Activities of antioxidant enzymes (POX, SOD and CAT) were enhanced due to the application with Pb and /or SPM (Table 5). The treatment of SPM at 2 mM x Pb at 800 ppm resulted in the greatest production of POX (3.2 unit/ g F.W. min) and SOD (11.9 unit/ g F.W. min) compared with other treatments. Furthermore, the highest rate (25.6 unit/ g F.W. min) of CAT enzyme produced from SPM at 2 mM x lead at 400 ppm treatment. The increases in antioxidant enzymes rates were highly significant for SPM, Pb and their interactions.

3.5. Total sugars:

SPM and Pb treatments were increased total sugars synthesis in the shoot of plants (Table 6). The highest synthesis of total sugars (16.7 mg/g F.W.) was resulted from the treatment of (2mM) SPM without Pb compared with other treatments. Variations in total sugars values were highly significant with Pb or SPM but non- significant in the interaction between Pb and SPM treatments.

3.6. Free amino acids:

Data in Table (6) revealed that Pb treatments led to a lack of free amino acid formation but, the adding of SPM with /without Pb led to increase free amino acids content in the shoot. Plants treated with SPM at 1mM x Pb at 0.0 ppm gave the highest value (7.2 mg/g F.W.) of free amino acids. Changes in free amino acid were highly significant for Pb and the interaction between SPM x Pb and more significant for SPM alone.

3.7. Total phenols:

The treatment SPM at 1 mM without Pb produced the minimum amount of phenols which was 0.9 mg/g F.W. (Table 6) in the shoot**.** The maximum amount of phenols with the value of 1.9 mg/g F.W. in response to (2mM) SPM x (800 ppm) Pb. Variations in phenols were highly significant with SPM, Pb and their interactions. Phenolics increased by increasing Pb levels.

3.8. Proline concentration:

Data in Table (6) demonstrated that treatment of Pb at 800 ppm without SPM gave the highest accumulation (23.8 µ mol /g) of proline compared with others. While the lowest accumulation (4.7 µ mol/g) of proline with control. Proline accumulation was highly significant for Pb, SPM and SPM x Pb treatments.

3.9. Mineral contents and protein:

Accumulation of N, P, K, protein (%) and Pb (ppm) in shoot and root were illustrated in Table (7). The highest rates of N and protein percentages in shoot have resulted from SPM at 2mM x 0.0 ppm Pb with values 2.3% and 14.1%, as well as the highest rate of N % in root was 0.8 % resulted from SPM at 2mM x0.0 ppm Pb and control. In shoot, the highest percentage of P (0.3%) was recorded from the treatments of SPM (1 &2mM) without Pb and SPM at 2mM with lead at 800 ppm, whereas treatments of SPM (1 &2mM) without Pb, SPM at 2mM with Pb at 400 & 800 ppm gave the highest value 0.2% in root. Changes in mineral contents and protein were highly significant for SPM or Pb treatments. Variations of Pb in shoot, P and K in root were highly significant but N, protein, P in shoot and Pb in root were moderate significant, while K in shoot and N in root were non-significant for the interactions of SPM and Pb. Application of SPM increased N, P, K in shoot and root and Protein in shoot, but decreased Pb concentration in both shoot and root.

4. Discussion:

Applying with Pb reduced different growth parameters because it effects on stomata or mesophyll cells, regulation of the water status, miner nutrition, respiration and photosynthesis which reflected on photochemical and biochemical activities of the plant [28, 29].Other studies reported that using of Pb decreased plant growth parameters, chlorophyll accumulations, lamellar organization in the chloroplast, cell division, root growth and root morphological disorders [30]. After exposure to Pb, the inhibition of root growth may be due to a reduction in Ca in tips of roots and reduction cell elongation [31].In *Allium sativum* root plant, exposure to Pb at 48 to 72 h showed swelling of mitochondrial, loss of cristae, vacuolization of endoplasmic reticulum, dictyosomes and, and injured plasma membrane and deep colored nuclei [32]. In this trial, external adding of SPM affects positively different vegetative parameters, might be attributed to polyamines are involved in many physiological and biochemical processes, including plant development and growth, cell differentiation, cell division, flowering, immunity to senescence, embryogenesis rooting, and DNA replication and synthesis of nucleic acid. [9], as well as stress management and plant biostimulant newly invented [33]. Also, Kubiś [34] suggested a positive correlation between amine groups in reducing reactive oxygen species. Hence, SPM application on *solidago canadensis* plants

under Pb treatment can be a form of avoiding the effect of Pb stress by reducing the oxidative stress effect.

Table 6 Impact of Pb, SPM and their interactions on total sugar, free amino, total phenols and proline.

Treatments		Total sugar (mg/g F.W.)	Free amino acids (mg/g F.W.)	Total phenols (mg/g F.W.)	Proline (μ mol / g)
SPM mM	Pb ppm				
0.0	0.0	13.1±0.5	6.4±0.5	1.2±0.0	4.7±0.1
	400	10.7±0.0	5.3±0.1	1.3±0.1	19.9±0.2
	800	9.0±0.5	4.7±0.5	1.3±0.0	23.8±0.2
Overall 0.0		10.9±1.8	5.4±0.8	1.3±0.1	16.1±0.8
1.0	0.0	14.1±0.1	7.2±0.0	0.9±0.1	5.7 ±0.1
	400	11.6±0.0	6.2±0.0	1.4±0.0	9.5 ±0.1
	800	9.8±0.0	5.0±0.5	1.7±0.1	13.6 ±0.3
Overall 1.0		11.8±1.9	6.1±1.0	1.3±0.4	9.6 ±0.4
2.0	0.0	16.7±0.2	4.7±0.5	1.2±0.0	6.4 ±0.2
	400	13.4±0.4	6.4±0.1	1.6±0.0	12.0 ±0.2
	800	11.9±0.1	5.6±0.0	1.9±0.1	14.3 ±0.2
Overall 2.0		14.0±2.1	5.5±0.8	1.6±0.3	10.9 ±0.4
Overall Pb	0.0	14.6±1.6	6.1±1.2	1.1±0.2	5.6 ±0.8
	400	11.9±1.2	5.9±0.5	1.4±0.1	13.8 ±1.4
	800	10.2±1.3	5.1±0.5	1.6±0.3	17.2 ±1.6
F-ratio					
SPM		305.6***	11.5**	87.1***	3394.0***
Pb		598.3***	26.9***	273.3***	10140.6***
Spm *pb		2.4NS	27.0***	43.2***	1279.4***

Spermine (SPM), Lead (Pb), SPM*Pb= interaction, 1st= First season(2018), 2nd= Second season (2019), NS= Non-significant,*Significant, **Moderate significant, ***Highly significant, values are given as Means ±SD

Table 7 Impact of Pb, SPM and their interactions on N, P, K, Pb and protein.

Treatments		Shoot				Root				Protein% in shoot
SPM mM	Pb ppm	N%	P%	K%	Pb ppm	N%	P%	K%	Pb ppm	
0.0	0.0	1.7±0.1	0.2±0.0	1.2±0.0	90.9±7.9	0.8±0.0	0.1±0.0	0.7±0.0	105.0±5.0	10.3±0.3
	400	1.2±0.0	0.2±0.0	0.9±0.1	144.3±0.7	0.6±0.0	0.1±0.0	0.6±0.0	166.0±3.0	7.5±0.0
	800	1.1±0.1	0.2±0.0	0.6±0.1	176.6±3.6	0.5±0.0	0.1±0.0	0.5±0.0	217.0±3.0	6.9±0.6
Overall 0.0		1.3±0.3	0.2±0.0	0.9±0.3	137.3± 37.7	0.6±0.1	0.1±0.0	0.6±0.1	162.7±48.7	8.2±1.6
1.0	0.0	2.0±0.1	0.3±0.0	1.5±0.2	53.2 ± 0.0	0.8±0.1	0.2±0.0	0.9±0.0	65.0±5.0	12.2±0.3
	400	1.3±0.0	0.2±0.0	1.2±0.0	131.5±3.5	0.7±0.0	0.1±0.0	0.8±0.0	137.5±2.5	8.2±0.1
	800	1.2±0.1	0.2±0.0	0.9±0.0	156.5±3.5	0.6±0.0	0.1±0.0	0.7±0.0	177.5±7.5	7.6±0.5
Overall 1.0		1.5±0.3	0.2±0.0	1.2±0.3	113.7± 46.7	0.7±0.1	0.1±0.0	0.8±0.1	126.7±49.6	9.3±2.2
2.0	0.0	2.3±0.0	0.3±0.0	1.8±0.1	46.0± 3.0	0.8±0.0	0.2±0.0	1.1±0.0	53.5±1.5	14.1±0.1
	400	1.6±0.0	0.2±0.0	1.5±0.1	120.0± 2.0	0.7±0.0	0.2±0.0	0.9±0.0	129.5±0.5	10.0±0.1
	800	1.5±0.0	0.3±0.0	1.0±0.1	146.0± 3.0	0.6±0.0	0.2±0.0	0.8±0.1	162.5±0.5	9.3±0.3
Overall 2.0		1.8±0.4	0.3±0.0	1.5±0.4	104.0± 45.0	0.7±0.1	0.2±0.0	0.9±0.2	115.2±48.2	11.1±2.2
Overall Lead	0.0	2.0±0.3	0.3±0.0	1.5±0.3	63.4± 21.3	0.8±0.0	0.2±0.0	0.9±0.2	74.5±23.7	12.2±1.6
	400	1.4±0.2	0.2±0.0	1.2±0.3	131.9± 10.7	0.7±0.0	0.1±0.0	0.7±0.1	144.3±16.7	8.6±1.1
	800	1.3±0.2	0.2±0.0	0.9±0.2	159.7 ±13.8	0.6±0.0	0.1±0.0	0.7±0.1	185.7± 24.7	7.9±1.2
					F-ratio					
SPM		187.7***	65.2***	54.4***	193.7***	24.4***	355.5***	320.1***	373.3***	187.7***
Pb		462.7***	29.4***	83.0***	1627.6***	207.7***	139.5***	166.1***	1919.2***	462.7***
SPM *Pb		4.9**	5.4**	0.7NS	10.3***	1.8NS	56.3***	12.4***	5.0**	4.9**

Spermine (SPM), Lead (Pb), SPM*Pb= interaction, 1st= First season (2018), 2nd= Second season (2019), NS= Non-significant,*Significant, **Moderate significant, ***Highly significant, values are given as Means ±SD

Influence of Pb treatments on flower parameters was mentioned by Rajeev and Neena [35] on sunflower when used Pb at nitrate at 0.25 mM flowering delayed and size of head decreased. Effect of lead may be due to its effect on glutathione content that was important in flowering timing in *Arabidopsis thaliana* and it is regulated by photosynthetic synthesized ATP, therefore directly correlated with photosynthetic efficiency of plants [36]. Hadi and Aziz [37] reported that plants produce fewer flowers at high concentrations of Pb and the same results were recorded by Badawy *et al* .[38] on *Zinnia elegans* plants . Also, Tatte *et al*. [39] showed that the exogenous SPM, SPD and Put applications can improve polyamines content, enhance the quality of flowers and,

delay their senescence, increase quality and prolonged shelf life by three days in cut rose flowers. In addition, higher concentrations of Put and SPM caused an increase in flower & buds numbers and increase the average of floral diameter of *Dendrobium nobile* and chrysanthemum plants [40, 41]. These results harmony with amaranth plant when applied with SPD showed alleviated the negative effects of Hg^{2+} and Cr^{6+} heavy metal stress treatment [42]. When treated wheat plants with SPM (at 1mM) decreased the effects of Cd^{2+} and Cu^{2+} on lipid peroxidation and restored SOD activity [43]. Although *solidago canadensis* plants with SPM treated produced high quality inflorescences under Pb stress condition, this is consistent with our experiment.

Photosynthesis is adversely influenced by Pb toxicity, which consequences from deformed chloroplast ultrastructure, restrained synthesis of chlorophyll, plastoquinone and carotenoids, obstructed electron transport, prevented activates of Calvin cycle enzymes, as well as deficiency of CO_2 as a result of stomata closure of *Zinnia elegans* and *Helianthus annus* [44, 45]. Qufei and Fashui [46] noted that Pb accumulated in PS II damaged its secondary structure inhibiting energy transfer among amino acids within PS II protein- pigment complex, and reduced energy transport from tyrosine residue to chlorophyll a. These results are in contract with Djukic *et al*. [47] on *Ailanthus altissima* and Dey and Mondal [48].In addition, Polyamines can organize the structure and action of membranes implicated in photosynthetic processes. Applications of polyamines preserve the stability of chloroplast membranes and prevent the degradation of chlorophyll. Also, they reduce the activity of protease enzyme and prevent chlorophyll degradation, polyamines, especially spermidine, prevent the production of enzymes involved in ethylene production and prevent the production of free radicals that degrade chlorophyll [49]. Photosynthesis is one of the primary processes most affected by a biotic stresses. Additionally, Photosynthic pigments are known to serve a variety of purposes, and are thus critical to function and health of the plant, through relative concentration of these pigments can vary significantly depending not only on species but also on surrounding environmental factors [50]. Thus, enhanced effect of SPM on ultrastructural features of chloroplast may be another reason for pigment retention in plants under stresses [51].These results are harmony with many reports that have indicated that plants tolerate of stress by the capability to enhance the synthesis of polyamines upon encountering the stress [52], or exogenous applied such as *Helianthus annuus* plants [53].

Antioxidant enzymes of higher plants increased the ability to scavenge the toxic active oxygen which accumulates under stress conditions. Diwan *et al*. [54] suggested that increasing SOD, POX and CAT may be protecting the plant from damage induced by Pb toxicity. CAT and POX are key enzymes in the detoxification of H_2O_2 so enzymes protective can prevent the accumulation of O_2 and H_2O_2 effectively and limit membrane lipid peroxidation caused by free radicals in *Brassica juncea* plants. Li *et al*. [55] found significantly increased activities of SOD and POX in *Zoysia japonica* plants when applied SPD.

The increase of proline level in the presence of Pb is confirmed by Posmyk *et al*. [56] who reported that a high level of proline under heavy metals stress may be the response to leaf damage or symptom of stress. Yang *et al*. [57] suggested that the sensitive plants when exposed to high lead concentration are associated with a higher level of proline accumulation, [58] on *Anethum graveolens*. Tajti *et al*. [43] reported that polyamines pretreatment could accelerate metal chelation, preserve hormonal balance and promote an antioxidant defense. Antioxidative mechanisms in plants under metal stress are regulated by exogenously applied of polyamines to mitigate the over reactive oxygen species (ROS) production [59]. Under mercury(Hg) toxicity, the exogenous application of PAs induced stress tolerance and Proline accumulation in water hyacinth leaves (*Eichhornia crassipes*) [60].PAs and other biomolecules an interaction role to retrieve of metal stress, including interaction with osmolyte (Proline, glycine betaine(GB), compatible solute , macromolecules (DNA and RNA) which contribute in reducing ROS generation, scavenging and signaling, enhancing antioxidant metabolisms, signaling role with other signaling molecules and regulating ion channels [61]. These results agree with many reports by Ahanger *et al*. [62] on *Vigna angularis*.

Decrease of sugars content in plants exposed to stress conditions probably corresponded with the photosynthetic inhibition or stimulation of respiration rate [63]. Mohamed and El-Tanany [64]

suggested that stressful plant is unable to provide the energy needed to convert sugars to carbohydrates. On the other hand, some studies reported that soluble sugars concentration increased markedly upon raising a biotic stress that plant exposed to it, the mechanism of plant to relieve the negative effects of stress, also sugars accumulation helps to maintain the stability of the membrane through preventing and protect membrane fusion [65]. The interaction between Pb levels x spermine treatments affected total sugars of *S. canadensis* plants. These results in agreement with Wang *et al*. [66] on *Celosia argentea* plants under copper stress.

Amino acids have many roles as an osmolyte, regulation of ion transport, modulating stomatal opening, and detoxification of heavy metals, synthesis and activity of some enzymes, gene expression, and redox-homeostasis [67]. Pb stress reduced the accumulation of free amino acids and the results are harmony with the findings of several researchers who reported that lead stress decreased the concentration of free amino acids such as *Albizia proceera* [68], tea plants [69] and *Zinnia elegans*[38]. Polyamines play an essential role in the chelation of various metals like Zn, Cd, and Pb, thus enhanced metal tolerance [70]. Foliar spray of SPM had a considerable effect on the concentration of free amino acids in fresh shoot of *Solidago canadensis* plant. They connect to negatively charged proteins, nucleic acids and phospholipids by ionic and hydrogen bonds through their amino and imino groups and participate in zygote polarity establishment, apical axis formation, cell layer differentiation and establishment of the meristem [71, 72].

One of the metal detoxification mechanisms is the synthesis /or exudation of metal chelating agents. Chelate with metal excluding it from the root apex or the ligand- metal complex detoxify metals internally, organic acids and phenolics are included among these ligands [73]. On *Ficus nitida* plant, Ahmed *et al*. [74] indicated that phenols are generally thought to prevent oxidative damage by scavenging active oxygen species and breaking the radical chain reaction during lipid peroxidation. The amount of phenols increases in plants sprayed with spermine at 2mM without lead compared to plants treated with lead alone. These results are in harmony with Roghieh and Nashmin [75] on tobacco plants they reported that phenolics metabolism was greatly influenced by exogenous polyamines (Put and SPD at 0.5 mM). Orabi *et al*. [76] reported that phenolic compounds are a key component in antioxidant response elements, which showed greater production under use of spermine. The interaction between Pb and SPM treatments, recorded the highest amount of phenols compared to other treatments. These results harmony with Choudhary *et al.* [77] on *Raphanus sativus* plants under Cr stress, they found applied 1 mM SPM enhanced accumulation of osmolyte (Protein, GB, and Phenol). Phenolics recently have received considerable attention as bioactive components in plants, especially their role as antioxidant agents. There is sufficient evidence of induction of phenolic metabolism in plants as a response to multiple stresses [78]. Phenolic, especially phenylopropanoids and flavonoids are oxidized by peroxidase (POX) and act directly in scavenging molecular species of active oxygen, such as H_2O_2 which if in excess cause oxidative cellular damage [79].

Decrease of N under Pb stress may be due to inhibition of uptake and transport of NO^{3-} and the reduction of NR activity [80]. Also, same results agree with Ashraf *et al*. [81] on canola showed decrease uptake of N and P with Pb applied. Lamhamdi *et al*. [82] reported that decrease of minerals content with applied Pb probably result from additional ion leakage from plants. Chatterjee *et al*. [83] recorded that decrease of K with increased Pb concentration may be these two ions compete to get in plant by the same K channels. Sharma and Dubey [84] reported that the evasion of K^+ from roots by the effect of lead on K^+- ATP ase and –SH groups of cell membrane proteins. These results are in accordance with Badawy *et al*. [38] on *Zinnia elegans.* Decrease of protein % under increased Pb concentration harmony with Palma *et al*. [85] who suggested in *L. polyrrhiza* that decreased content of protein probably by promoting protein degradation process as a result of increased protease activity. This results harmony with Shu *et al.* [86] on *Jatropha curca* L. plants.

Effect of SPM on elements indicated with many reports found that polyamines interpose for the root process, and the exogenous ameliorate root structure by increasing the percentage of narrow and hairy roots and reducing thick roots. These changes progress the assimilation of elements and increase their concentrations within plants. On the other hand, polyamines can edict as a further source of nitrogen for the plants and improve their growth [87, 88]. These results are in harmony with Yousefi *et al* [89] on *Rosa hybrida* that polyamine

application has a significant effect on leaf NPK at 1Mm SPM and with increased SPM concentration had no significant.

In many states, nitrogen increases the amount of plant dry matter and that way it rebate the concentration of elements in plants [90]. These results agree with many searches such as Rady and Hemida [91], they reported that applied spermidine or SPM at 2 mM of seed treatment until 6 h of *T. aestivum* plant under Cd stress, increased protein and, starch content. But, it showed decreased Pb concentration in shoot and root of plants with applied SPM treatment. These results agree with Groppa *et al*. [92] on wheat plants under cadmium and copper stress suggested that SPM treatment may be a certain antioxidant action by protecting the tissues from metals-prompt oxidative damage.

It can be concluded that Pb concentration in soil especially at high levels (800 ppm) had hazardous effects on growth parameters and chemical compositions of *S. Canadensis* plants. On the other hand, *S. Canadensis* plants can tolerance this harmful effect response to applied SPM as a foliar spray on plant especially concentration 2mM spermine. Therefore, soil contaminated with Pb can be used to grow *S. Canadensis* plants, which are of economic importance cut flower by using SPM and taking into considering account the concentration of Pb in soil as well as the concentration of SPM.

5. Conflicts of interest

There are no conflicts to declare.

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