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Effects of Lead on Different Growth Attributes of *Petunia hybrida*L.

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Abstract:
Background*: Production of ornamental plants is a rapidlyexpandingtradeworldwide and has greateconomicpotential. Soil of the urban areas isoftenenrichedwithheavymetalscomingfromdifferentpotential sources and ornamental plants in these areas are usuallyexposed to suchmetal pollution. Amongheavymetals, lead has receivedconsiderable importance due to itsextremetoxicity and persistencebehavior. High level of lead (Pb) in environment leads to more accumulation of thistoxicmetal in plants.*
Materials and Methods*: The aim of this study was to investigate the effect of lead on ornamental plant Petunia hybrida*L. *through pot experiment. In this research three different lead concentrations i.e. 10ppm, 20ppm and 30ppm were used to check the effect of lead on selected plant. These selected lead concentrations were given to plant in the form of lead nitrate [Pb (NO_3)]. Lead effect was investigated on different growth attributes of Petunia hybrida* L. *Plant performance under lead stress was monitored through parameters such as chlorophyll and carotenoid content, ascorbic acid content, relative water content, above and below ground biomass, plant height, leaf area and number of leaves. Amount of lead taken up by above and below ground parts of the plant was also examined through atomic absorption spectrophotometer.*
Results*: Results showed that chlorophyll, carotenoids and biomass were significantly affected by all three lead treatments. However, applied lead concentrations had no effect on ascorbic acid, relative water content, plant height, leaf area and number of leaves of Petunia hybrida*L. *Plant did not exhibit any sign of visible injury due to lead stress. Lead concentration was highest in root as compared to above ground parts of the plant. Overall plant showed stable growth under all three lead treatments.*
Conclusion:*This study indicated that Petunia hybrida* L.*can tolerate lead in soil up to the level of 30ppm.*
Key Word*: Ornamental plants, lead pollution, plant growth, chlorophyll, 10ppm, 20ppm, 30ppm*

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I. Introduction

Ornamental plants are mainly grown for their aesthetic beauty. Worldwide, production of ornamental plants is a rapidly expanding trade and has great economic potential[1,2].

Due to industrialization and urbanization, soil of the urban areas is often polluted with heavy metals. In urban areas, ornamental plants are often grown in commercial buildings, private yards, institutional grounds, parks, gardens and along roadsides. These plants are usually exposed to such type of metal pollution coming from different potential sources. Among heavy metals toxic to plants, lead (Pb) is of particular concern due to its persistence and numerous potential sources[3]. High level of lead (Pb) in environment leads to more accumulation of this toxic metal in plants. Lead (Pb) accumulation in plants is responsible for broad range of changes on growth of plants[4,5,6]. However, different plants show different tolerance level towards lead pollution. Some plants show toxicity symptoms even at low concentrations, while others can tolerate even very high concentration of lead (Pb). Few studies [7,8,9] have explored the effects of lead pollution on different ornamental plants but to our knowledge none has studied the lead toxicity on *Petunia hybrida* L.

Petunias are popular ornamental plants that belong to the family solanaceae (nightshade family). Petunias show excellent growth in full sunlight but they also have the ability to grow in shade. In Pakistan, Petunias are available in variety of shapes like star, picotee etc. The most familiar hybrid series is Falcon F1; however Ultra series is also equally common. Flowering season of Petunias is from December to May. In Pakistan, Petunias are commonly grown plants along road side as well as in commercial buildings, private yards, institutional grounds, parks and gardens[10].

This study was conducted with the aim of finding out the effects of lead on different growth attributes of *Petunia hybrida* L. through pot experiment. The objectives of this study were a) To check the tolerance level of *Petunia hybrida* L. towards lead stress b) To look whether the selected plant species can accumulate lead or not c) To highlight the potential benefits of this plant towards pollution reduction and landscape improvements in our cities.

II. Material And Methods

A pot experiment was conducted to study the effects of lead (Pb) on growth of *Petunia hybrida*L. Soil was filled in twenty-four earthen pots which were about 25cm in diameter and 10cm in height. Seedlings of *Petunia hybrida*L. (similar in size, shape and at the same growth stage) were transplanted into prepared pots (three seedlings per pot). Six pots were used as control where no lead treatment was given; similarly, six pots were used for each selected lead concentration.

Before the start of the experiment, physical and chemical properties such as electrical conductivity, pH, and moisture content of this soil were analyzed. Lead treatments (10ppm, 20ppm and 30ppm) were given in the form of lead nitrate [$Pb(NO_3)$].

During the growth of the plants different parameters such as number of leaves, leaf area, plant height, ascorbic acid, chlorophyll and carotenoids content was analyzed after every two weeks. At the time of harvest, relative water content as well as above and below ground biomass was also studied.

Determination of chlorophyll and carotenoid content

Chlorophyll and carotenoid content was determined according to Arnon method[11]. 0.5 g of fresh leaf sample was grinded with 10ml of 80% acetone. This mixture was then transferred into graduated tube and centrifuged at 2500rpm for 10 minutes. A portion of this extract was taken into cuvette and absorbance was recorded at 645, 663 and 480 nm using UV spectrophotometer. Chlorophyll was estimated using the following formula:

Chlorophyll 'a' (mg/ml) = (0.0127) × (A.663) – (0.00269) × (A.645)

Chlorophyll 'b' (mg/ml) = (0.0229) × (A.645) – (0.00468) × (A.663)

Total chlorophyll (mg/ml) = (0.0202) × (A.645) +(0.00802) × (A.663)

Carotenoid content was determined using the formula of Kirk and Allen[12].

Carotenoid (mg/g) = A.480 + (0.114 × A.663 – 0.638 ×A.645)

Measurement of number of leaves and plant height

Number of leaves was measured by simply counting the leaves of each plant. Plant height of each plant was measured by using measuring tape from the base of the plant to the tip of the last leaf [13].

Measurement of leaf area

For measurement of leaf area, graphical method was used. For this purpose, leaf was carefully removed from the plant and placed on a graph. Then outline of leaf was carefully drawn on the graph paper and area of leaf was measured by counting the number of grid covered by leaf [14].

Determination of ascorbic acid

Titration method was used for estimation of ascorbic acid in leaves of Petunia hybrida L. according to the method of Reiss [15].

Determination of biomass

Fresh biomass of each plant was determined immediately on weighing balance after harvest. For determination of dry biomass, plants were dried in hot air oven at 65oC for 48 hours and after that their weight was determined on weighing balance[16].

Determination of relative water content

The relative water content (RW C) was also determined according to the formula of Chen et al.[17].

Relative water content (%) = Fresh weight-(Dry weight)/(Fresh Weight) x 100

Estimation of lead content in above and below ground biomass

After harvest, lead analysis in plants was carried out using atomic absorption spectrophotometer according to the method of Ansari et al.[18].

Statistical analysis

For all parameters means were calculated and Analysis of Variance (ANOVA) was performed to check the significant differences between treatments using IBM SPSS Statistics version. 20. All figures were made in Microsoft Excel 2007.

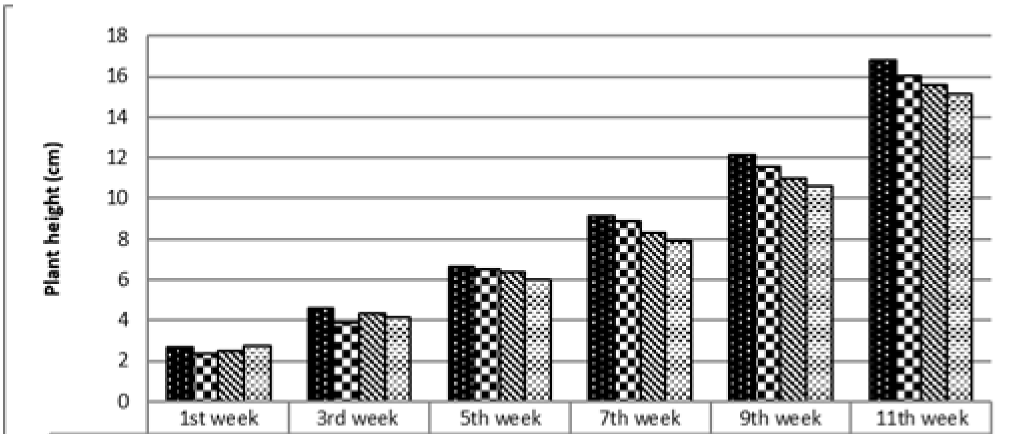
III. Result

Properties of selected soil

The electrical conductivity, moisture content and pH of the soil were 139 □ S/cm, 9.53% and 6.8 respectively.

Effects of lead on plant height, number of leaves and leaf area.

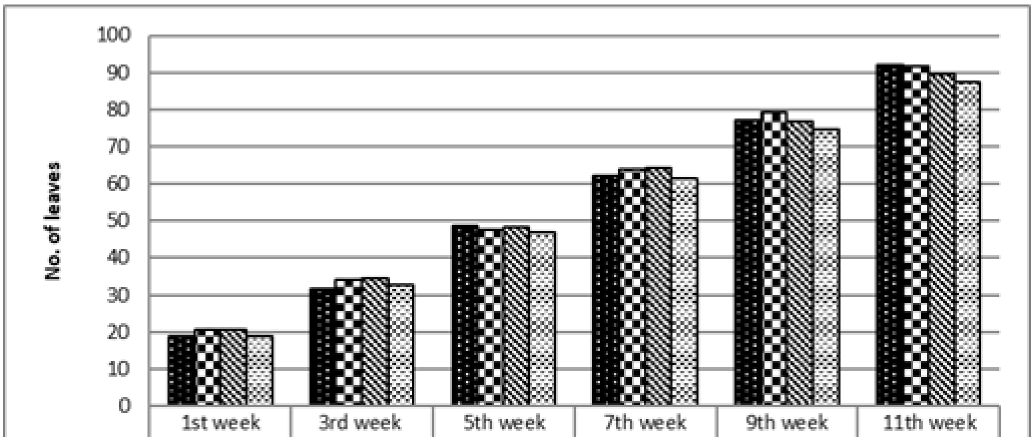
Figures 1, 2 and 3 show effects of lead on plant height, number of leaves and leaf area of *Petunia hybrida*L. respectively. All these parameters in control plants were somewhat higher than treated plants. However, this difference between treated and control plants was not statistically significant (Table no 1).



	1st week	3rd week	5th week	7th week	9th week	11th week
Control	2.694444444	4.583333333	6.666666667	9.138888889	12.11111111	16.77777778
10ppm	2.361111111	3.861111111	6.527777778	8.861111111	11.58333333	16.02777778
20ppm	2.5	4.333333333	6.388888889	8.277777778	11	15.58333333
30ppm	2.722222222	4.138888889	6.027777778	7.944444444	10.58333333	15.13888889

Figure no1: Effect of lead on plant height

Figure no 2: Effect of lead on number of leaves



	1st week	3rd week	5th week	7th week	9th week	11th week
Control	18.77777772	31.55555556	48.66666667	62.05555556	77	91.88888889
10ppm	20.77777778	34.05555556	47.44444444	63.83333333	79.38888889	91.61111111
20ppm	20.44444444	34.44444444	48.22222222	64.16666667	76.66666667	89.44444444
30ppm	18.72222222	32.83333333	47.05555556	61.44444444	74.61111111	87.33333333

Figure no 3: Effect of lead on leaf area

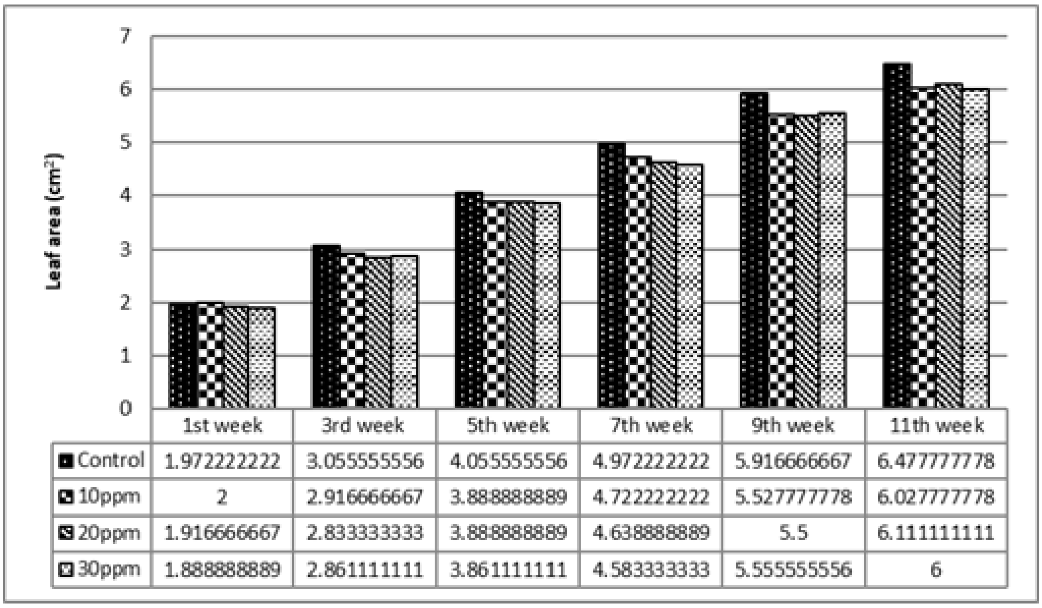


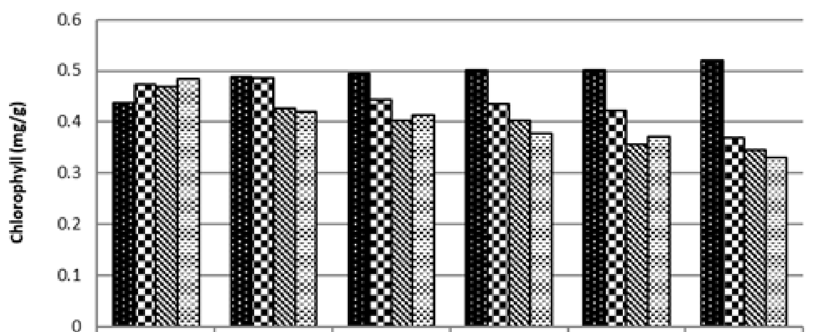
Table no 1: ANOVA results for effect of lead on plant height

Parameters	Lead in soil (ppm)	Mean (cm)	Std. Deviation	F	df	p
Plant height	Control	8.66	5.18	0.036	23	0.990
	10ppm	8.20	5.07			
	20ppm	8.01	4.75			
	30ppm	7.75	4.55			
Number of leaves	Control	54.99	27.55	0.010	23	0.999
	10ppm	56.18	27.09			
	20ppm	55.56	26.07			
	30ppm	53.66	25.81			
Leaf area	Control	4.41	1.71	0.039	23	0.989
	10ppm	4.18	1.54			
	20ppm	4.14	1.59			
	30ppm	4.12	1.57			

Effects of lead on chlorophyll and carotenoid content

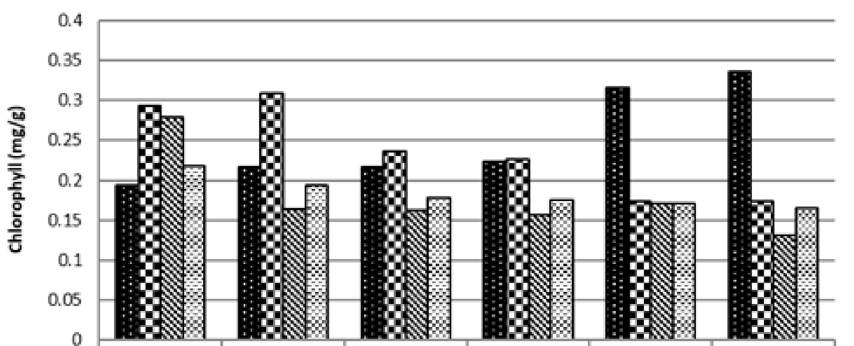
Results show that amount of chlorophyll ‘a’, chlorophyll ‘b’; and total chlorophyll decreased at all three lead concentration (Figure no 4, 5 and 6). Carotenoid content of *Petunia hybrida* L. increased with growth of plant for both control and 10ppm, but at 11th week amount of carotenoids was less in plants under 10ppm lead. While for both 20ppm and 30ppm, crotenoid content decreased with plant growth (Figure 7). ANOVA results indicate significant decrease in chlorophyll and carotenoid content (Table no 2) of *Petunia hybrida*L.

Figure no 4: Effect of lead on chlorophyll 'a'



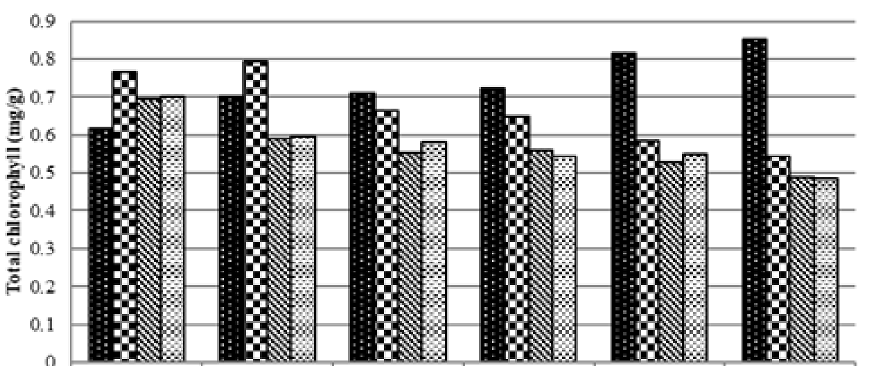
	1st week	3rd week	5th week	7th week	9th week	11th week
Control	0.4376	0.4891	0.49535	0.50195	0.501166667	0.519216667
10ppm	0.472716667	0.486966667	0.444166667	0.435416667	0.421333333	0.369333333
20ppm	0.46835	0.4275	0.403766667	0.403733333	0.356683333	0.345033333
30ppm	0.484266667	0.41945	0.413083333	0.377533333	0.370183333	0.331333333

Figure no 5: Effect of lead on chlorophyll 'b'



	1st week	3rd week	5th week	7th week	9th week	11th week
Control	0.193533333	0.217016667	0.216483333	0.2243	0.31535	0.336483333
10ppm	0.293816667	0.308616667	0.236516667	0.226383333	0.174033333	0.173633333
20ppm	0.278583333	0.164266667	0.162216667	0.15695	0.171608333	0.1312
30ppm	0.21775	0.194233333	0.177916667	0.176083333	0.170483333	0.164766667

Figure no 6: Effect of lead on total chlorophyll



	1	2	3	4	5	6
A	0.618633333	0.700066667	0.7109	0.724116667	0.815116667	0.8541
B	0.765	0.7942	0.6654	0.6499	0.584233333	0.542266667
C	0.69545	0.591016667	0.55295	0.55945	0.527016667	0.4895
D	0.7002	0.596	0.5802	0.5433	0.5485	0.4845

Figure no 7: Effect of lead on carotenoid content

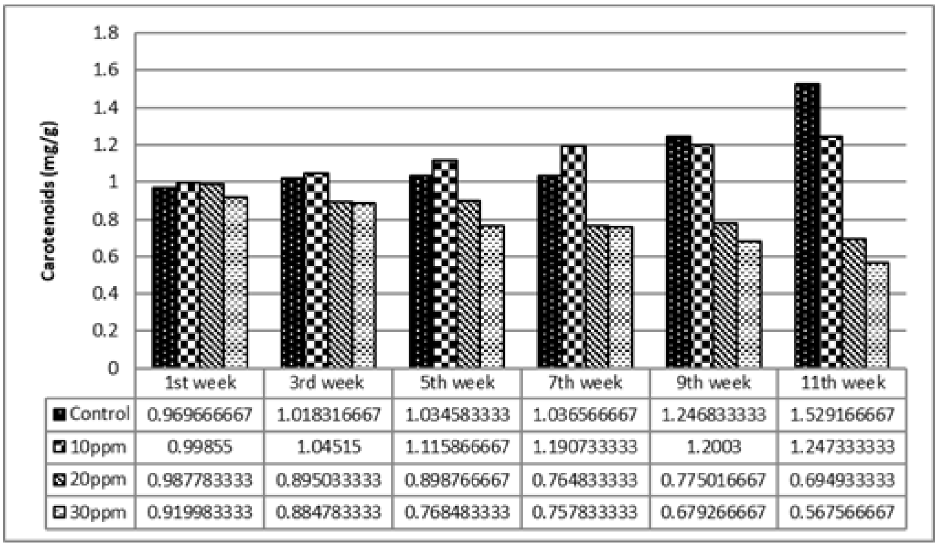


Table no 2: ANOVA results for effect of lead on chlorophyll ‘a’; chlorophyll ‘b’; total chlorophyll and carotenoid content

Parameters	Lead in soil (ppm)	Mean (mg/g)	Std. Deviation	F	df	p
Chlorophyll‘a’	Control	0.49	0.027	6.035	23	0.004
	10ppm	0.43	0.041			
	20ppm	0.40	0.045			
	30ppm	0.39	0.055			
Chlorophyll‘b’	Control	0.25	0.059	3.272	23	0.043
	10ppm	0.23	0.057			
	20ppm	0.17	0.051			
	30ppm	0.18	0.019			
Total chlorophyll	Control	0.73	0.084	3.587	23	0.032
	10ppm	0.66	0.099			
	20ppm	0.66	0.098			
	30ppm	0.56	0.070			
Carotenoid content	Control	1.13	0.213	11.060	23	0.000
	10ppm	1.13	0.096			
	20ppm	0.83	0.108			
	30ppm	0.76	0.130			

Effects of lead on ascorbic acid and relative water content

Ascorbic acid and relative water content in *Petunia hybrida*L.under lead stress has been given in Figure no 8 and 9 respectively. Lead did not cause any significant decrease in ascorbic acid and relative water content of above and below ground biomass (Table no 3).

Figure no 8: Effect of lead on ascorbic acid content of *Petunia hybrida* L.

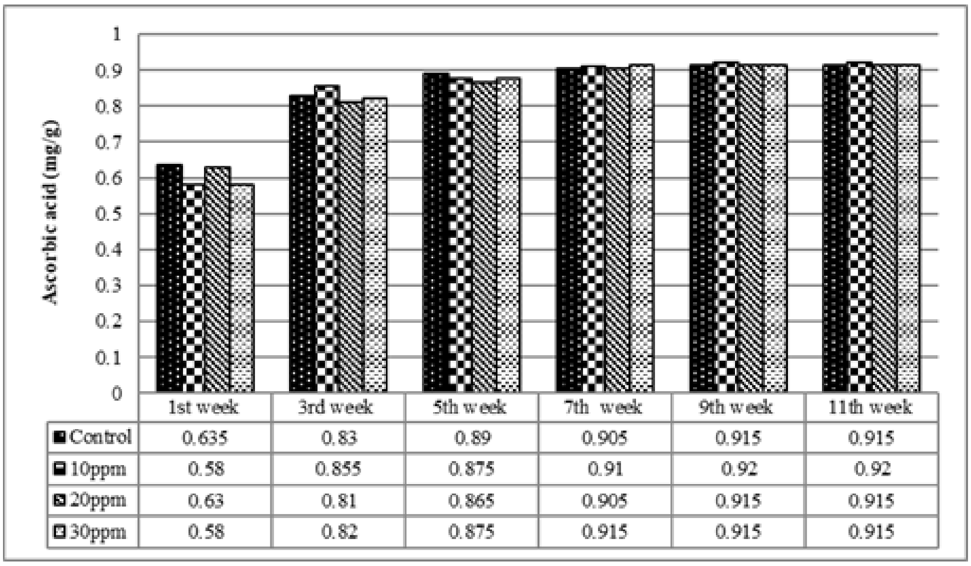


Figure no 9: Effect of lead on relative water content of *Petunia hybrida* L.

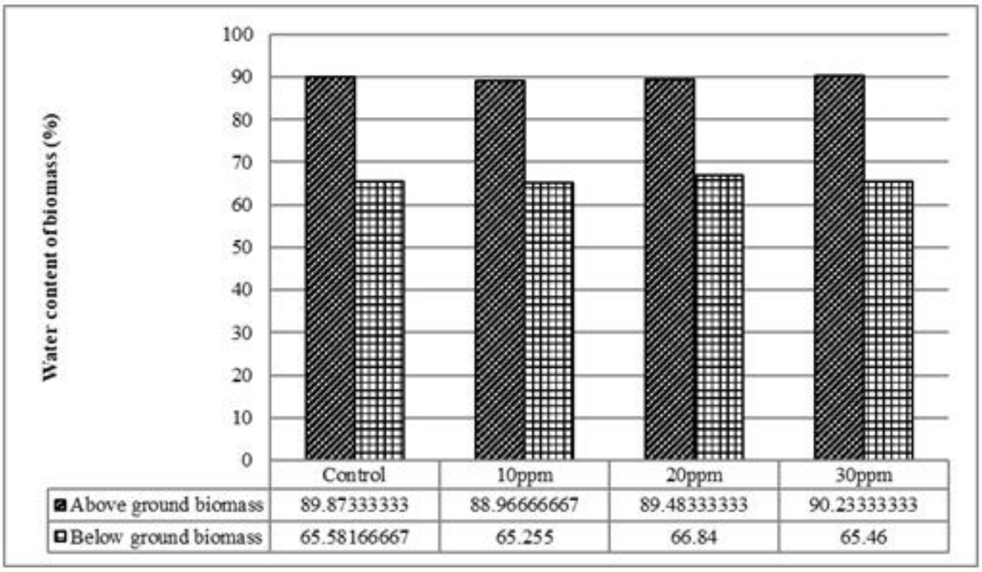


Table no 3: ANOVA results for effect of lead on ascorbic acid and relative water content

Parameters	Lead in soil (ppm)	Mean (mg/g)	Std. Deviation	F	df	P
Ascorbic acid	Control	0.84	0.099	0.010	23	0.999
	10ppm	0.84	0.120			
	20ppm	0.84	0.100			
	30ppm	0.83	0.199			
Relative water content (%) of above ground biomass	Control	88.96	1.43	1.662	23	0.207
	10ppm	89.87	0.98			
	20ppm	90.23	0.75			
	30ppm	89.48	0.81			
Relative water content (%) of below ground biomass	Control	65.25	4.14	0.243	23	0.865
	10ppm	65.58	2.84			
	20ppm	65.46	4.75			
	30ppm	66.84	1.66			

Effects of lead on above and below ground biomass

Plants growing in 10, 20 and 30ppm lead concentration showed a significant decrease in fresh weight of both above and below ground biomass (Table no4) with respect to control. Figure no 10 show percentage reduction in fresh weight of above and below ground biomass. Like fresh weight, plants treated with selected lead concentration also exhibited significant decrease in dry weight of both above and below ground biomass (Table no 4) as compared to control. Percentage reduction in dry weight of above and below ground biomass has been exhibited in Figure no 11.

Figure 10: Percentage reduction in fresh weight of above and below ground biomass with respect to control

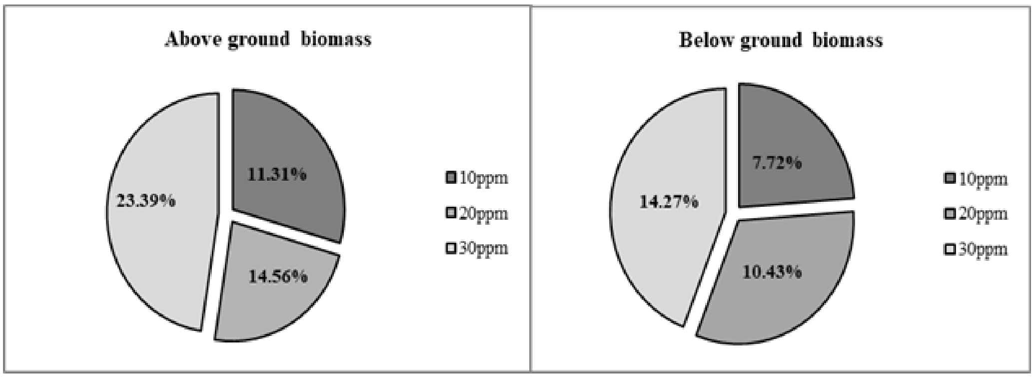


Figure 11: Percentage reduction in dry weight of above and below ground biomass with respect to control

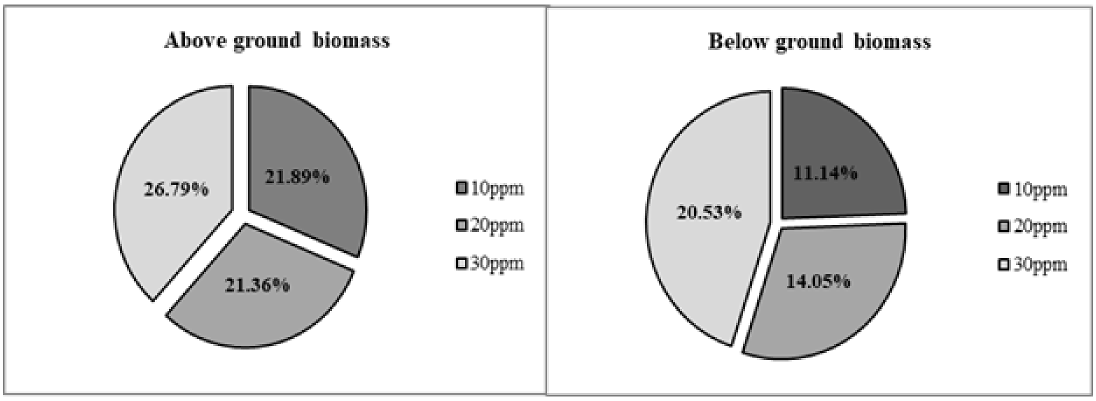


Table no 4: ANOVA results to assess the percentage reduction in fresh and dry weight of above and below ground biomass with respect to control

Parameters	Lead in soil (ppm)	Mean (g)	Std. Deviation	F	df	p
Fresh weight of above ground biomass	Control	51.90	3.17	9.607	23	0.000
	10ppm	44.34	5.41			
	20ppm	46.03	4.71			
	30ppm	39.76	1.14			
Fresh weight of below ground biomass	Control	0.43	0.017	4.636	23	0.014
	10ppm	0.39	0.016			
	20ppm	0.38	0.028			
	30ppm	0.37	0.031			
Dry weight of above ground biomass	5.71	0.682	5.71	8.813	23	0.001
	4.46	0.512	4.46			
	4.49	0.611	4.49			
	4.18	0.386	4.18			
Dry weight of below ground biomass	Control	0.15	0.013	6.603	23	0.003
	10ppm	0.14	0.014			
	20ppm	0.13	0.013			
	30ppm	0.12	0.009			

Uptake of lead by *Petunia hybrida*L.

Amount of lead accumulated by above and below ground plant parts has been presented in Table no 5. Results indicate that lead content was high in treated plants as compared to control. Furthermore, lead concentration was more in roots as compared to above ground parts of the plant.

Table no 5: Lead accumulation by *Petunia hybrida*L.

Lead in soil (ppm)	Lead taken up by above ground parts (mg/kg)	Lead taken up by root(mg/kg)
Control	0.307	0.8891
10ppm	10.81	12.44
20ppm	16.11	19.32
30ppm	17.58	22.70

IV. Discussion

Contamination of soil with lead is of particular concern because it may pose harmful effects (such as effects on biomass, photosynthesis, water content, enzymatic activity, mineral content etc.) on plants growing in it[19]. Lead enters into the soil from a variety of sources like sewage sludge application, mining and smelting, fertilizers etc.[20,21]. In this study lead effect was evaluated on *Petunia hybrida*L. up to eleven weeks after exposing it to selected concentration of lead applied as lead nitrate $[Pb(NO_3)]$. In phytoremediation studies, lead nitrate salt is commonly used due to its high solubility [22].

The electrical conductivity and moisture content of the soil was 139 µS/cm and 9.53% respectively. pH of the selected soil was found to be 6.8. pH is an important property of soil that has a great impact on mobility and bioavailability of heavy metals to plants. If pH of the soil is below 7.0 then it increases the solubility and mobility of nickel, lead and mercury in the soil and consequently these metals are readily taken up by plants from such type of soil. pH of the soil selected for this study was 6.8, which means that this soil will allow more uptake of lead by plants [7].

Chlorophyll content is quite essential for the photosynthetic process of plant. Lead at high concentration causes reduction in chlorophyll content of plants[23]. It has been suggested that this reduction in chlorophyll content occurs due to reduced uptake of Mg^{2+}, $Fe^{2+}$and Zn^{2+} by plant under lead stress [24]. Furthermore, lead also causes inhibition of ALA-dehydratase and protochlorophyllide reductase which play a key role in chlorophyll synthesis[25]. In our study, lead caused significant reduction in chlorophyll a, b and total chlorophyll content of *Petunia hybrida*L. Similar effect has been reported by Bibi and Hussain[26]. They studied the effect of 25 mg/kg and 50 mg/kg of lead on two black gram (*Vigna mungo*) cultivars through pot experiment. They observed that at both lead concentrations, amount of chlorophyll a, b and total chlorophyll decreased significantly in both cultivars of black gram (Vigna mungo). The sensitivity of chlorophyll a, b and total chlorophyll was in the order of chlorophyll a > total chlorophyll > chlorophyll b. Similarly, Hussain et al.[27] studied the effect of lead on photosynthetic pigment in two mash bean [*Vigna mungo* (L.) Hepper] cultivars i.e. Fs-1 and Mash-97 by exposing them to 20 or 40 mg/L of lead. Results of this study showed that chlorophyll a, b and total chlorophyll was reduced significantly at both these lead treatments. However, reduction was more at higher lead concentration. Reduction in chlorophyll content has also been reported for bean (*Phaseolus vulgaris* L.) seedlings due to lead toxicity [28].

Like chlorophyll, carotenoid content of treated plants was also affected by selected lead concentrations. These results were in accordance with the results obtained by Bibi and Hussain[26] on two black gram (*Vigna mungo*) cultivars. They found that both cultivars showed gradual decrease in carotenoids content under 25 mg/kg and 50 mg/kg of lead.

Results indicate that selected lead treatments did not affect plant height in our study. Data about the effect of lead on height of other plants shows that some plants have the ability to tolerate lead contamination in soil. Ghani[29] studied the effect of lead on two maize (*Zea mays* L.) varities i.e. Neelam and Desi by exposing them to 10, 20 and 30ppm of lead concentration. Results of this study showed that lead did not affect shoot growth in both varieties selected for the experiment.

Like plant height of *Petunia hybrida*L., number of leaves also remained unaffected by selected lead concentrations in soil. Sometimes lead even causes an increase in number of leaves. Such increase in number of leaves has been described by Ratushnyak et al.[30] in *Pisum sativum* L. which may be an adaptive reaction towards lead toxicity.

Results showed that leaf area was similar for control as well as for lead treated plants at 11th week of experiment which means that selected lead concentrations did not affect leaf area of the plant selected for our study. Similar results were found by Ratushnyak et al.[30]. They studied effect of lead on growth parameters of *Pisum sativum* L. They found that leaf area of *Pisum sativum* L. remained unaffected by given lead treatment.

Ascorbic acid (vitamin C) is an important chemical component of plants. It is an antioxidant that is present in almost all cell components. Ascorbic acid helps to protect the plants from oxidative injuries as well as from damaging effects of different pollutants like heavy metals, ozone and salinity[31]. During exposure of plants to pollution, ascorbic acid helps to maintain cell membranes stability and attack free radicals that can cause oxidative damage[32]. There is a direct relationship between amount of ascorbic acid present inside the plants and their vulnerability to pollution[33]. It also acts as a cofactor for many enzymes that are involved in different fundamental processes of plants. Ascorbic acid also controls flowering time and beginning of senescence in plants[31,34]. Results show that lead did not affect ascorbic acid content of *Petunia hybrida*L. during the course of our study. Similar results have been presented by Zenginand Munzuroglu[28]. They found that in bean (*Phaseolus vulgaris* L .) seedlings, applied lead treatments did not affect ascorbic acid content rather it was increased in a dose-dependent way when they were grown under 1.5, 2.0 and 2.5mM lead concentrations. Gupta et al.[35] also reported that in *Zea mays* seedlings, amount of ascorbic acid increased with increase in lead level when they were exposed to different lead concentrations (0-200 µM) under hydroponic condition.

Under heavy metals stress, plant biomass is an important indicator for characterizing the health of plant[36]. Plant biomass is considered as the tolerance index for plants that are growing in heavy metals contaminated soil[37]. Lead (Pb) pollution in soil leads to reduced biomass production by plants. This reduction in biomass occurred due to disturbance in photosynthesis and nitrogen metabolism caused by excess lead in soil[38]. In our study, fresh and dry weight of *Petunia hybrida*L. was significantly affected by lead treatments. Similar results about lead effect on plant biomass have been described by various studies. Zhao et al.[39] conducted a study to find out the effect of lead on tomato plants. They found that plant biomass decreased as lead concentration increased in soil. Similarly, McComb et al.[40] reported decreased root and shoot biomass in *Sesbaniaexaltata* (coffee weed) due to lead toxicity. Hussain et al.[23] reported decrease in fresh and dry weight of root and shoot in *Zea mays* under lead stress. Toxic effect of lead on root and shoot biomass has also been reported by Azad et al.[41] in sunflower and by Bharwana et al.[42] in cotton seedling.

Water is quite essential to perform many functions within plants. Water status of plant is considerably affected by lead stress[43]. Excess amount of lead causes disruption of transpiration intensity as well as root system of plant. This disruption leads to reduced uptake of water by affected plants. Under such situation, aboveground parts of plant are unable to receive sufficient amount of water[44].These results indicate that selected lead concentrations had no effect on water content of *Petunia hybrida*L. Similar study conducted on water hyacinths [*Eichhorniacrassipes*(Mart.)] showed that relative water content increased considerably with increasing lead concentration up to the level of 400 mg/L as compared to control[16].

Results about uptake of lead by *Petunia hybrida*L. indicate that amount of lead was highest in the root as compared to above ground parts of plant. This high concentration of lead in root was due to the fact that roots are the primary site of lead accumulation from soil. Lead translocation occurs within plant but amount of lead that moves into aerial parts of the plant is somewhat lower than the roots. Different studies show high concentration of lead in root as compared to shoot and leaves. Reddy et al.[45] reported that lead concentration was more in roots than leaves in case of Horse gram (*Macrotylomauniflorum* (Lam.) Verdc.)and Bengal gram (*Cicer arietinum* L.)grown under 0, 200, 500 and 800 ppm lead concentrations. Similarly, Israr et al.[36] also found out that lead accumulation was more in root as compared to shoot in seedlings of *Sesbaniadrummondii*. Cenkci et al.[46] reported that in seedlings of fodder turnip (*Brassica rapa* L.), amount of lead in shoot and root increased gradually with increase in concentration of lead in liquid medium.

Lead pollution is a common problem due to different anthropogenic activities. Lead is quite injurious for plants. So, any illegal discharge of this toxic pollutant into the environment must be controlled through regulatory measures. Moreover, lead pollution problem in urban environment can be lowered through plantation of lead tolerant plants as they can sequester significant amount of lead in their tissues. Since Petunia hybrida L. can accumulate considerable amount of lead in its tissue so it can be helpful in cities to reduce the problem of lead pollution.

V. Conclusion

Overall under lead stress, plant did not show any visible symptoms of injury. Lead treatments did not affect number of leaves, plant height, leaf area, ascorbic acid and relative water content of selected plant. However, above and below ground biomass, chlorophyll, and carotenoids content was significantly affected by all three selected lead concentration. Despite having such disturbances, overall selected plant showed healthy growth. These results about *Petunia hybrida*L. suggest that this plant has the ability to tolerant lead in soil up to the level of 30ppm.

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