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Effect of Pb-stress on growth and mineral status of two groundnut (*Arachis hypogaea* L.) cultivars

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Abstract: Heavy metal pollution of air and agricultural soils is one of the most important ecological problems on world scale. Among the heavy metals, lead (Pb) is one of the common environmental pollutants. To investigate Pb effects on nutrient uptake, two groundnut (*Arachis hypogaea* L.) cultivars (cultivar K6 and cultivar K9) were grown in pot cultures and stressed with lead nitrate ($Pb(NO_3)_2$) at four concentrations (100, 200, 400 and 800 ppm). Pb is accumulated in roots and leaf tissues in dose dependent manner in both groundnut cultivars, which resulted in reduced root and shoot growth and lower uptake of all mineral ions tested. The content of mineral ions such as Ca, Na, Mg, Co, Cu, Ni, Zn and Mn reduced in root and leaf tissues of both cultivars due to Pb stress. But the reduction in mineral ion content was less in cultivar K6 than in cultivar K9. The deficiency of mineral nutrients correlates in a strong decrease in the contents of total chlorophyll, and anthocyanin in both cultivars, but these effects are less pronounced in cultivar K6 than in cultivar K9.

Keywords: Pb Stress, Groundnut, Mineral Nutrients

1. Introduction

Heavy metal pollution of air and agricultural soils is one of the most important ecological problems on world scale. Contaminant metal can often accumulate in considerable amounts in the plant tissue and exceed the levels that are toxic to man or animal before they produce visible phytotoxic effects [1]. These pollutants persist in the environment for a longer period of time, as they are not easily degraded by soil microorganisms and therefore, can easily be absorbed by plants [2, 3]. The magnitude of Pb contamination is high relative to that of other heavy metals due to extensive processing of Pb ore, in addition to wide usage of metal. When taken up by plants, like other metal ions, Pb interfaces with the physiology and metabolism of the plants by binding to the sulfhydryl groups of various proteins, leading to structure disruption or activity inhibition, and in certain cases causes the displacement of essential elements resulting in deficiency effects [4, 5].

Responses of plants to Pb exposure include decrease in root and shoot growth, plant biomass [6], accelerated leaf senescence [7], inhibition of chlorophyll biosynthesis, inhibition of seed germination, a wide range of adverse effects on growth and metabolism of plants [8], influence the net photosynthetic rate and respiration, and alternate permeability of cell membrane [9]. Many of the observed activates of Pb appear to be indirect as a result of mineral imbalance within the tissues. Significant changes in nutrient contents as well as in internal ratios of nutrients occur in plants under Pb toxicity [7]. In most cases Pb blocks the entry of cations (K, Ca, Mg, Zn, Cu and Fe) and anions (NO_3^-) [9]. Pb can also alter the activities of the key enzymes of various metabolic pathways such as the photosynthesis, Calvin cycle, nitrogen metabolism, and sugar metabolism [10].

Legumes are reported to be tolerant to several heavy metals [11]. There has been considerable interest in finding legume species which are able to colonize in metal-enriched soils for use in land reclamation or for crop production on marginal soils [1, 12]. Despite the importance of legume crops in maintaining soil fertility and the conflicting reports on the effects of heavy metals, this study was undertaken with groundnut to evaluate the which cultivar is able to resist Pb stress relatively.

Groundnut, (*Arachis hypogaea* L.) is a drought tolerant,

economic and oil seed legume crop. In the present study, we have investigated the effect of Pb stress on mineral content and its consequences on biomass, chlorophyll and anthocyanin content in two high yielding groundnut cultivars (cultivar K6 and cultivar K9).

2. Materials and Methods

2.1. Plant Growth Conditions and Pb Stress Treatments

Groundnut (cultivar K6 and K9) seeds were procured from Regional Agricultural Research Station, Acharya NG Ranga Agricultural University (ANGRAU), Kadiri, India, was sown in earthen pots containing air dried red soil and farmyard manure in 3:1 proportion. The pots were kept under natural photoperiod (12-14 hours and temperature 28 ± 4 °C) in the botanical garden and were irrigated once a day with water. After germination, seedlings were thinned to three per pot and maintained for 14 days. 14-day-old plants were subjected to Pb stress once by adding 0 (control), 100, 200, 400 and 800 ppm of Pb solution using lead nitrate ($Pb(NO_3)_2$). Both control and treated pots were irrigated daily with tap water. Care was taken while adding water slightly less than field capacity (approximately 300 ml) to avoid leaching out of solution from treated pots. After 10 days of stress imposition, the plants were uprooted carefully; the leaves and roots were separated, flash frozen in liquid nitrogen and stored at –80 °C until further use.

2.2. Determination of Growth Parameters

The plants were carefully uprooted from pots and washed thoroughly with running tap water. Plant growth was determined by measuring the length of the root and shoot system. The dry weight (DW) was measured after the shoots and roots were dried at 80 ºC to constant weight. Leaf area was measured by using leaf area meter (Li-Cor, Li3100 USA).

2.3. Determination of Pb and Nutrient Elements

Plants were uprooted and separated into root and leaves, these were thoroughly washed with tap water followed by 0.2% detergent solution (Tween-20) to remove the waxy / greasy coating on the sample surface. Again, these samples were washed with 0.1 N HCl followed by thorough washing with plenty of water and rinsed finally with double distilled water. These samples were dried at 80 ºC in a hot air oven for 24 hours and powdered using mortar and pestle. Oven dried powder sample (0.5 g) of roots and leaves was taken in a 50 ml boiling test tube and 5 ml of concentrated nitric acid (70%) was added and incubated at room temperature overnight. The next day, 5 ml of HNO_3 and H_2O_2 mixture 10: 4 (v: v) was added to it and placed on a mantle (REMI) till all the white fumes evaporated and the thick white residue was left out in flask. It was allowed to cool and volume was made up to 25 ml using ultra pure water. These diluted samples were used for elemental analysis using ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrophotometer) (iCAP-6000 Series, Thermo Scientific, UK). Replications were maintained and their average was used for calculations. The concentrations of elements were expressed as mg or µg/g dry weight.

2.4. Determination of Total Chlorophyll Content

The chlorophyll content was estimated according to the method described by Hiscox and Israelstam [13]. 0.1 g of leaf bits of both control and stressed plants were placed in a test tube containing 7 ml DMSO (Merck) and chlorophyll was extracted at 65 ºC for 30 min. The liquid was transferred to a 15 ml graduated test tube and the volume was made to 10 ml with DMSO. The absorbance was measured at 645 nm, 663 nm in a UV-Spectrophotometer (Shimadzu UV-1800) against DMSO as blank. Total chlorophyll content was calculated following the formula used by Arnon [14].

2.5. Determination of Anthocyanin Content

Anthocyanins were extracted from 0.5 g of leaves with 10 ml of mixture of n-propanol: HCl: H_2O (18:1:81 v/v/v). The samples were heated in boiling water bath for 30 min and they were incubated for 24 h in the dark at 4 °C. Extracts were filtered and optical density was measured at A_{535} and A_{663} anthocyanin contents were calculated according to Lange *et al.* [15] and were expressed as $A_{535}\ g^{-1}$ fresh weight after correction for Chlorophyll.

2.6. Data Analysis

All data were analyzed using the SPSS (Statistical Package for the Social Sciences) version 16.0. Data presented here are mean values and standard deviation (±SD). One-way ANOVA was carried out using Post hoc multiple comparison from the Duncan's test at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Effect of Pb Stress on Plant Growth

Growth inhibition is a common response to heavy metal stress and is also one of the most important indices of heavy metal tolerance of plants. Pb is not generally considered to be an essential element for plant growth. The effect of Pb on seedling growth seems to be different with regards to plant species, cultivars organs and metabolic processes [9]. Groundnut cultivars grown in different concentrations of Pb exhibited inhibition of both root and shoot growth (table 1). Growth of cultivar K6 was less affected due to Pb treatments compared with cultivar K9. After exposure to 800 ppm Pb, the root growth was inhibited to 24% and 46% in cultivar K6 and K9 respectively. Whereas, the shoot growth of both cultivars resulted less affected than root and show a decrease about 14.6% and 38% in cultivar K6 and K9 respectively.

Morphological changes such as root and shoot growth in response to Pb treatment has been studied by several investigators. It has been reported that root and shoot growth was reduced in plants [16, 17, 18] by Pb stress. The growth of legume plants grown on Pb ore tailings was reported to be drastically affected [19]. Pb also inhibited root and shoot

growth in tobacco [20] and wheat [21]. Similarly, in the present study, the root and shoot growth of cultivar K6 and cultivar K9 were inhibited by Pb stress and the reduction was found to be concentration dependent (table 1). However, the percent decrease in root and shoot growth was less in cultivar K6 than in cultivar K9, which indicates the better adaptation of former one to Pb stress. The reduction in root length and shoot length under Pb stress may be due to the inhibition in cell elongation process [22] or due to reduced mitotic activity as observed in lupin roots system [23].

3.2. Effect of Pb on Plant Biomass

Increased Pb metal concentration significantly reduced the biomass of two groundnut cultivars (table 1). Pb induced root and shoot biomass reduction in cultivar K6 was always lower than that of cultivar K9 at all stress treatments. Two cultivars differed from each other in terms of both root and shoot dry mass. At 800 ppm Pb treatment the reduction in root dry mass was estimated 13.4% and 29.9% in cultivar K6 and K9 respectively, compared to the control. Similarly, the shoot dry mass undertake a decrease compared to the control which reached 18.7% in cultivar K6 and 47.3% in cultivar K9, compared to the control.

Generally, excess concentration of heavy metals in soil results in decreasing plant biomass production. Biomass production has been considered as an index of tolerance level of plants growing on metal enriched soils. Cox and Hutchinson [24] have reported that the dry mass production in non-tolerant plant was significantly negatively correlated with log metal concentration in soil, but the same relationship with tolerant plant was positive. Similarly, in the present study, the dry mass accumulation was much less affected in tolerant cultivar K6 than susceptible cultivar K9 with increasing stress intensity. Concomitantly, Ekmekci *et al.* [18] reported that the shoot and root ratio (dry mass) was affected by increasing Pb concentrations in maize. Inhibition of fresh and dry mass accumulation under Pb stress conditions was also reported in cotton seedlings [25] and in sunflower [26].

3.3. Effect of Pb on Leaf Area

The leaf area of cultivar K9 was reduced significantly at all concentrations of Pb as compared to the control. Whereas, cultivar K6 showed no significant decrease in leaf area due to Pb treatments (table 1). After exposure to 800 ppm Pb, the decrease in leaf area reached 8.8% and 34.6% in cultivar K6 and K9 respectively compared to their unstressed plants. An increase in the metal supply resulted in inhibition of leaf area in the cotton [25], garden cress [27], and tomato [28, 16]. In contrast, in the present study, Pb stress didn't induce a significant inhibition of leaf area in cultivar K6.

3.4. Pb Accumulation in Plant Tissues

The Pb content increased in a dose-dependent manner in leaves and roots of two groundnut cultivars (figure 1). At all Pb stress a level, the Pb content was lower in leaves and roots of K6 cultivar than that of K9 cultivar. Furthermore, the Pb content was relatively higher in the roots than in leaves of both cultivars. At 800 ppm Pb there was a 4.7-fold increase in Pb content in leaves and 30.7-fold increase in the roots of cultivar K6 whereas 18-fold increase in leaves and 55-fold increase in roots of cultivar K9 was observed when compared with their respective controls.

An increase in Pb content in both cultivars was noticed with increase in the intensity of stress. The increase in the accumulation of Pb was lesser in cultivar K6 than in cultivar K9 under stress conditions. Plants absorb Pb in its soluble form from soil through roots. Based on comparative studies of metal content in plant parts Baker and Walker [29] suggested that uptake, translocation and accumulation mechanisms differed for various heavy metals and between species and genotypes [30]. It is known that the root system partially defends the above ground parts from Pb [31], as showed in the present study. Mostly, the plants with highest tolerance take-up the smallest proportion of the total soil-metal and had the lowest shoot metal concentrations [22, 32].

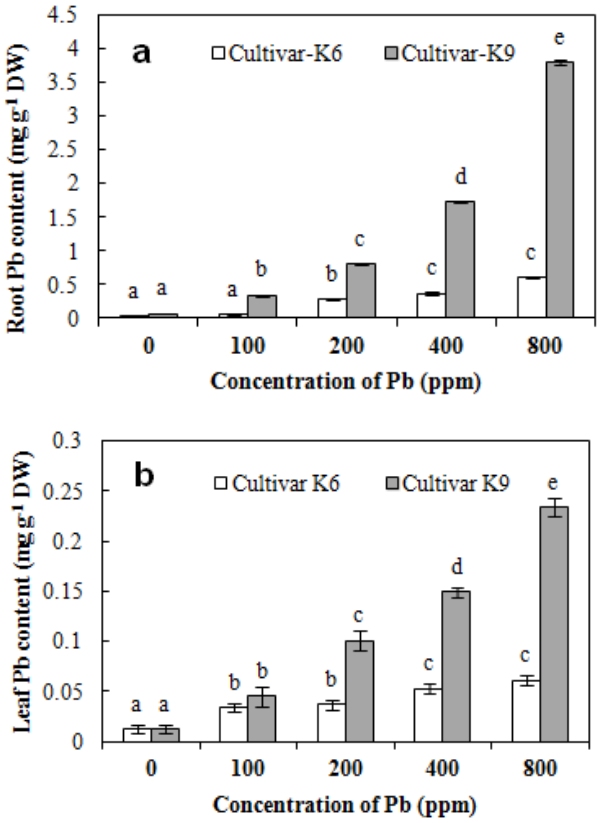

Figure 1. *Pb content in roots (a) and (b) leaves of two groundnut cultivars subjected to $Pb(NO_3)_2$ stress. The data represent the mean ±SD (n=3) of three different experiments and the same letters above the bars are not significantly different at p<0.05 (DMR test).*

In the same context, in this study although both of cultivars accumulated lower Pb levels in leaves, tolerant cultivar K6 accumulated still lower proportion of Pb by restricting the uptake and further translocation of Pb from roots to leaves. Our study also indicated that the uptake and translocation proportion of Pb metal vary greatly among these two cultivars and also indicated the retaining ability of the roots to Pb.

3.5. Effect of Pb-Stress on Chlorophyll Content

Chlorosis was associated with reduced leaf chlorophyll content. In wheat seedlings, concentrations of total chlorophyll content decreased significantly in the presence of Pb in the soil, but this response varied with the concentration of exogenous Pb [21]. After expose to 800 ppm, the reduction was about 22.7% in cultivar K6 and 38.5% in cultivar K9, compared to respective controls (table 1). The most common symptoms caused by Pb toxicity were yellowing of the leaves (chlorosis) and leaf curling was noticed at higher concentrations (400 and 800 ppm) especially in cultivar K9.

Total chlorophyll content has been reported to be one of the important indices to assess the tolerance of plants to heavy metals. A reduction in total chlorophyll content during Pb supply has been noticed in cucumber [32], radish [33] *Populus* [34] and *Vallisneria natans* [35]. In the present study, the reduction of total chlorophyll content was less in cultivar K6 suggesting the tolerance of cultivar K6 compared to cultivar K9.

Table 1. *Effect of increasing concentrations of $Pb(NO_3)_2$ on root growth, shoot growth, root dry weight (DW), shoot dry weight (DW), leaf area, total chlorophyll content (TCC) and anthocyanin content of two groundnut cultivars*

	Pb (ppm)	Root growth (cm/plant)	Shoot growth (cm/plant)	Root DW (g^{-1}/plant)	Shoot DW (g^{-1} plant)	Leaf area (cm^2)	TCC (mg/g^{-1} FW)	Anthocyanin (mg/g^{-1} FW)
Cultivar K6	0	19.18c ±1.97 (100.0)	5.74c ±0.57 (100.0)	0.082b ±0.004 (100.0)	0.325c ±0.017 (100.0)	3.60a ±0.22 (100.0)	2.34c ±0.010 (100.0)	0.225a ±0.008 (100.0)
	100	17.02b ±2.24 (88.7)	5.55bc ±0.75 (96.6)	0.085b ±0.005 (103.6)	0.327c ±0.030 (100.6)	3.62a ±0.28 (99.1)	2.26b ±0.008 (96.1)	0.236a ±0.010 (104.9)
	200	15.66ab ±1.74 (81.6)	5.29ab ±0.76 (92.1)	0.075a ±0.005 (91.4)	0.305bc ±0.018 (93.8)	3.47a ±0.35 (95)	2.21b ±0.044 (94.1)	0.305b ±0.012 (135.5)
	400	15.06a ±3.85 (78.51)	4.94a ±0.74 (86.06)	0.075a ±0.005 (87.8)	0.297b ±0.017 (91.4)	3.46a ±0.35 (94.8)	2.03b ±0.047 (86.6)	0.417c ±0.041 (185.3)
	800	14.57a ±2.62 (75.9)	4.90a ±0.73 (85.3)	0.071a ±0.006 (86.6)	0.264a ±0.036 (81.2)	3.33a ±0.47 (91.2)	1.93a ±0.041 (82.4)	0.826d ±0.025 (367.1)
Cultivar K9	0	23.37d ±3.96 (100.0)	1.07d ±0.08 (100.0)	0.077d ±0.004 (100.0)	0.338e ±0.030 (100.0)	2.66c ±0.29 (100.0)	2.44d ±0.056 (100.0)	0.600d ±0.030 (100.0)
	100	18.19c ±3.38 (77.8)	5.37d ±0.72 (82.8)	0.068c ±0.005 (88.3)	0.312d ±0.042 (92.3)	2.53c ±0.32 (95.1)	2.30d ±0.029 (94.6)	0.648e ±0.030 (108)
	200	16.62bc ±2.83 (71.1)	4.45c ±0.51 (80.03)	0.061b ±0.004 (79.2)	0.268c ±0.020 (79.2)	2.08b ±0.17 (78.2)	2.12c ±0.028 (87.2)	0.560c ±0.017 (93.3)
	400	15.89b ±3.25 (67.9)	3.94b ±0.37 (70.86)	0.057ab ±0.005 (74)	0.243b ±0.018 (71.9)	1.96b ±0.16 (73.7)	1.84b ±0.023 (75.5)	0.469b ±0.019 (78.1)
	800	12.66a ±3.06 (54.1)	3.45a ±0.64 (62.05)	0.054a ±0.004 (70)	0.178a ±0.014 (52.6)	1.74e ±0.16 (65.4)	1.55a ±0.160 (63.8)	0.317e ±0.015 (52.8)

(Percentages to control in parenthesis)
The data represent the mean ±SD (n=10) of three different experiments and the same letters after averages are not significantly different at $p<0.05$ (DMR test).

Table 2. *Effect of $Pb(NO_3)_2$ on leaf elemental profile of two groundnut cultivars.*

	Pb (ppm)	Ca (mg/g^{-1} DW)	Mg (mg/g^{-1} DW)	Na (mg/g^{-1} DW)	Cu ($\mu g/g^{-1}$ DW)	Co ($\mu g/g^{-1}$ DW)	Fe ($\mu g/g^{-1}$ DW)	Mn ($\mu g/g^{-1}$ DW)	Ni ($\mu g/g^{-1}$ DW)	Zn ($\mu g/g^{-1}$ DW)
Cultivar K6	0	25.93e ±1.35 (100.0)	10.25d ±0.06 (100.0)	0.108d ±0.015 (100.0)	18.02d ±0.18 (100.0)	4.16b ±0.09 (100.0)	0.150c ±0.013 (100.0)	53.20d ±2.35 (100.0)	8.16d ±0.08 (100.0)	0.264e ±0.011 (100.0)
	100	25.50d ±1.05 (98.3)	9.56c ±0.07 (93.2)	0.107c ±0.012 (99.0)	14.18c ±0.17 (79.7)	4.16ab ±0.03 (100.0)	0.135b ±0.012 (90.6)	50.82c ±3.72 (95.5)	8.08c ±0.08 (99.0)	0.251d ±0.017 (95.0)
	200	17.90c ±1.23 (69.0)	9.13b ±0.10 (89.0)	0.103b ±0.011 (95.4)	12.56b ±0.16 (78.7)	4.16ab ±0.03 (100.0)	0.134b ±0.009 (90.3)	50.29bc ±2.62 (94.5)	7.97bc ±0.09 (97.6)	0.248c ±0.019 (93.9)
	400	16.15b ±1.02 (62.3)	9.10b ±0.14 (88.8)	0.094a ±0.014 (87.0)	11.68a ±0.28 (64.8)	4.16ab ±0.08 (100.0)	0.133b ±0.011 (89.1)	49.22b ±4.74 (92.5)	7.86b ±0.05 (96.3)	0.244b ±0.016 (92.4)
	800	15.73a ±1.07 (60.6)	8.25a ±0.10 (80.4)	0.094a ±0.009 (87.0)	11.44a ±0.16 (63.5)	4.10a ±0.05 (98.5)	0.105a ±0.007 (70.7)	48.02a ±3.77 (90.2)	7.70a ±0.13 (94.4)	0.230a ±0.014 (87.1)
Cultivar K9	0	26.33e ±1.12 (100.0)	12.37e ±0.05 (100.0)	0.113e ±0.005 (100.0)	67.81d ±0.11 (100.0)	4.16b ±0.05 (100.0)	0.191d ±0.009 (100.0)	59.60d ±4.63 (100.0)	9.38d ±0.05 (100.0)	0.266d ±0.011 (100.0)
	100	22.07d ±1.01 (83.8)	10.17d ±0.09 (82.2)	0.104d ±0.008 (92.0)	28.61c ±0.17 (42.2)	4.13b ±0.02 (99.3)	0.151c ±0.015 (79.1)	58.13cd ±2.58 (93.0)	8.16c ±0.08 (86.9)	0.255c ±0.011 (95.8)
	200	16.14c ±1.02 (61.3)	9.06c ±0.11 (73.2)	0.092c ±0.009 (81.4)	19.04b ±0.14 (29.0)	4.10ab ±0.05 (98.5)	0.149dc ±0.002 (78.1)	57.60c ±2.61 (91.3)	7.92b ±0.08 (84.4)	0.242c ±0.011 (90.9)
	400	15.72b ±1.07 (59.3)	8.38b ±0.05 (67.7)	0.088b ±0.004 (78.0)	18.66b ±0.19 (27.5)	4.08ab ±0.02 (98.0)	0.144b ±0.006 (75.4)	53.70b ±3.82 (90.5)	7.86b ±0.05 (83.8)	0.238b ±0.011 (89.4)
	800	7.72a ±1.13 (29.7)	8.20a ±0.06 (66.3)	0.084a ±0.004 (47.1)	14.10a ±0.32 (20.7)	4.08a ±0.01 (98.0)	0.131a ±0.002 (68.4)	51.04a ±4.37 (82.2)	7.65a ±0.05 (81.5)	0.227a ±0.015 (85.3)

(Percentages to control in parenthesis)
The data represent the mean ±SD (n=3) of three different experiments and the same letters after average values are not significantly different at $p<0.05$ (DMR test).

Table 3. *Effect of $Pb(NO_3)_2$ on root elemental profile of two groundnut cultivars.*

	Pb (ppm)	Ca (mg/g^{-1} DW)	Mg (mg/g^{-1} DW)	Na (mg/g^{-1} DW)	Cu ($\mu g/g^{-1}$ DW)	Co ($\mu g/g^{-1}$ DW)	Fe ($\mu g/g^{-1}$ DW)	Mn ($\mu g/g^{-1}$ DW)	Ni ($\mu g/g^{-1}$ DW)	Zn ($\mu g/g^{-1}$ DW)
Cultivar K6	0	21.61e ±0.41 (100.0)	13.89d ±0.28 (100.0)	0.45a ±0.011 (100.0)	33.78d ±1.41 (100.0)	2.24b ±0.081 (100.0)	1.50c ±0.015 (100.0)	42.56c ±0.24 (100.0)	10.01d ±0.08 (100.0)	0.156e ±0.007 (100.0)
	100	19.70d ±0.63 (91.2)	13.26c ±0.17 (99.5)	0.44c ±0.014 (99.5)	33.78c ±1.53 (100.0)	2.16ab ±0.085 (96.4)	1.44b ±0.184 (96.0)	41.92c ±0.41 (98.5)	9.78c ±0.12 (97.8)	0.137d ±0.009 (87.8)
	200	18.66c ±0.41 (86.3)	11.67b ±0.36 (97.3)	0.43b ±0.015 (97.3)	31.06b ±1.80 (97.9)	1.81ab ±0.122 (80.8)	1.36b ±0.039 (90.2)	37.33bc ±0.69 (87.7)	9.01bc ±0.44 (90.1)	0.132c ±0.008 (84.6)
	400	17.60a ±0.80 (81.5)	11.72b ±0.11 (95.8)	0.43b ±0.013 (95.7)	30.93a ±1.36 (91.5)	1.78ab ±0.08 (79.5)	1.26b ±0.021 (83.2)	34.16b ±0.64 (80.2)	8.08c ±0.08 (80.8)	0.129b ±0.012 (82.7)
	800	17.05b ±0.45 (83.0)	11.63a ±0.08 (95.3)	0.42a ±0.015 (95.3)	29.52a ±1.26 (87.3)	1.62a ±0.046 (72.3)	1.24b ±0.003 (77.5)	33.01a ±0.44 (77.5)	7.97a ±0.09 (79.7)	0.124a ±0.008 (79.5)
Cultivar K9	0	23.46a ±0.60 (100.0)	16.52e ±0.26 (100.0)	0.51e ±0.013 (100.0)	34.66d ±2.38 (100.0)	3.49b ±0.046 (100.0)	2.38a ±0.015 (100.0)	57.12d ±0.49 (100.0)	10.67d ±0.16 (100.0)	0.192d ±0.009 (100.0)
	100	20.73c ±0.33 (88.3)	14.84d ±0.27 (89.5)	0.46d ±0.012 (89.5)	33.73c ±1.88 (97.3)	2.61ab ±0.046 (74.8)	1.97c ±0.011 (95.2)	54.40cd ±0.08 (95.2)	10.05c ±0.17 (94.1)	0.173c ±0.008 (90.1)
	200	19.45b ±0.43 (83.0)	14.51c ±0.10 (87.8)	0.36c ±0.013 (70.0)	33.20b ±1.67 (95.8)	2.58ab ±0.046 (73.9)	1.8bc ±0.044 (92.2)	52.66c ±0.76 (92.1)	8.93b ±0.20 (83.7)	0.172b ±0.006 (89.6)
	400	19.74c ±0.75 (84.2)	13.95b ±0.19 (84.5)	0.35b ±0.018 (68.3)	31.28b ±1.66 (90.2)	1.84b ±0.081 (52.7)	1.60c ±0.022 (73.1)	41.78b ±0.56 (73.1)	7.81b ±0.28 (83.7)	0.158b ±0.006 (82.3)
	800	18.01a ±0.53 (76.7)	12.11a ±0.21 (73.3)	0.32a ±0.015 (63.6)	28.26a ±1.38 (81.5)	1.36b ±0.081 (38.9)	1.48a ±0.030 (60.1)	37.45a ±1.97 (65.4)	6.66a ±0.17 (66.6)	0.101a ±0.008 (52.6)

(Figures in parenthesis are per cent values)
The data represent the mean ±SD (n=3) of three different experiments and the same letters after averages values are not significantly different at $p<0.05$ (DMR test).

3.6. Effect of Pb-Stress on Anthocyanin Content

Kumar *et al.,* [36] suggested that in plants, the synthesis of anthocyanins makes it an effective strategy against ROS generation due to Pb stress, but interestingly lower concentrations of Pb stimulated synthesis of anthocyanins more than higher concentrations of Pb. Anthocyanin concentration increase in leaves of both the cultivars exposed to increasing Pb concentrations. However, this increase was always higher in cultivar K6 than in cultivar K9. This indicates that there is a strong correlation between the level of anthocyanins and the presence of heavy metals in the growth medium (table 1).

3.7. Effect of Pb on Mineral Contents

Table 2, 3 showed that high Pb concentrations in the soil cause a reduction of most macro-elements (Ca, Mg and Na). microelements (Cu, Fe, Mn, Ni and Zn) are also reduced in both the cultivars. By contrast, Co concentrations are not significantly changed in leaves of both the cultivars. Thus, we can conclude that the uptake of all the measured nutrients is reduced by Pb treatment. In order to better visualize the effects of Pb, the data have been expressed as the percentage change relative to dry weight and for total amounts per plant. It is known that Pb physically blocks the access of many ions to their absorption sites on the roots [37], thus inhibiting their uptake. However, the very large reductions of ionic content observed in the present study can hardly result from just an inhibition of ion uptake, and they probably also result from additional ion leakage from the plants. In all cases, cultivar K6 appears less affected than cultivar K9 by Pb treatment. Ca and Cu ion concentration seems very sensitive to Pb treatments, and the lowest Pb concentration already has very large effects. This observation also applies to Ca, Mg, Na, Co, Mn, Ni and Zn in roots in the case of cultivar K9, whereas in cultivar K6 these levels are only slightly disturbed by 400 ppm Pb.

4. Conclusions

Our results demonstrated that Pb treatment even at low concentrations induces large disturbances in ion uptake by plants, which results in serious metabolic changes (e.g., in photosynthetic capacity) (unpublished data) and finally in a strong inhibition of cultivar K6 as a result of the deleterious effects of Pb on plant growth. Two groundnut cultivars exhibit different sensitiveness to Pb treatment, and cultivar K6 appears more resistant. Future experiments will be aimed at searching for the mechanisms responsible for the improved protection of cultivar K6 against the deleterious effects of Pb.

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