***Original Research Article***

# Effect of Lead Contamination on morphological attributes and biomass allocation of*Bambusabalcooa*(Roxb.)

## Abstract

Pb contamination in the agricultural soil by anthropogenic activities, industrialization and modernization poses a great concern to human health. A pot experiment was carried out to evaluate the effect of Pb concentrations (0, 150, 500, 1000, 1700 mg $kg^{-1}$) of lead nitrate on *Bambusabalcooa* plantlets grown in earthen pots placed in a fenced enclosure. For each concentration, three replicates were taken and to reduce the heterogeneity in the results, pots were placed in randomized manner. Thereafter, plantlets were transplanted in the pots and grown in the natural condition, only rainfall was controlled by covering the fenced enclosure with polythene sheet.The result showed that a higher concentration of Pb caused a minimum increase the number of culm (0%),nodes (25%), leaves (58.53%), length of internode (19.04%), plant height (32.89%) and leaf area (62.91%). Biomass of the plant ranged from 5.33± 0.57 to 37.33±6.42g and moisture content was found maximum in Pb 500 mg $kg^{-1}$ (38.78%) throughout the experiment. Findings of the study indicates that *B. balcooa* can tolerate the elevated level of Pb toxicity and can be used for remediation purpose.

**Keywords:***Bambusabalcooa*, Phytoremediation, Lead, Soil, Environment

## 1 Introduction

Lead (Pb) is a persistent, highly noxious and non-disintegrative heavy metal after arsenic which comprises 0.002% of Earth's crust (Zulfiqar et al., 2019). In the environment, Pb have various origin as it can arise from natural processes such as rock weathering, volcanic eruptions, forest fires, and soil-forming or can be originated from anthropogenic processes such as industrial waste, fertilizer applications, smelting, and sewage disposal. Excessive Pb concentration causes deleterious effects in plants such as decline in photosynthetic rate, chlorophyll synthesis, disturbed Calvin cycle, closed stomata due to deficiency of $CO_2$, growth inhibition which is inseparably connected with cell division, limits the mineral nutrition and water balance and enzyme activities. It also brings the alteration in lipid composition and chlorophyll b content (Collin et al., 2022). At higher concentration

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sunflower plants showed reduction in height, number of leaves and dry matter of the plant (Hung et al., 2014). Collin et al. (2022) recorded 4.5 folds higher Pb concentration in Blackberry plant’s leaves than the fruits.And blackberry contain 71% more Pb content that was exceeding 29 times more of the WHO threshold. The consumers are at high risks who take 100g fresh blackberries which consist 8.51 mg Pb. Opeolu et al. (2010) reported lesser number of flowers in sunflower under high concentration of Pb and in soybean, histological changes in leaves such as thin blade leaf, minified xylem and phloem in vascular bundle, reduction in diameter of xylem vessels were observed under Pb contamination (Hadi and Aziz, 2015). The increasing amount into the environment causes harmful effects on climate and human health and also known as protoplasmic poison (Hadi and Aziz, 2015; Lathwal et al., 2023a). Maximum acceptable intake of Pb in human food is about 25 $\mu g\, kg^{-1}$ of human body weight per week. In soil, Pb content may occur up to 10ppm (Agrawal, 2009) but for human maximum acceptable permissible range is 0.003mg/l (WHO, 2017) and 0.005mg/l by Rani et al. (2023). To overcome these issues, remediation of the soil is needed and many approaches are available to reduce the contaminants from the soil but phytoremediation is one of the best remediation strategies.

Phytoremediation is the process of remediation in which plants are used to restore the degraded land either by heavy metal (such as Pb) or by soil erosion and other cause. Grasses are more suitable for the remediation of the heavy metal contaminated soil due to their large biomass, easy to multiply and wide spread in short time (Malik and Biswas, 2012). Among the grasses, bamboo is a widely distributed, fast growing, renewable, and environment enhancing resource which has great potential toenvironmental conservation (control soil erosion, rehabilitation, carbon sequestration, water conservation and biodiversity conservation)and improve poverty alleviation. Extensive rhizome-root system and accumulation of bamboo leaf mulch serves as an efficient agent in preventing soil erosion, runoff reduction, facilitates infiltration, moisture conservation and accumulation of contaminants in the plant parts. These special characteristics make bamboo a good candidate for the phytoremediation of heavy metal contaminated soil (Lathwal et al., 2023b).The objective of the current study is to evaluate the efficiency of *Bambusabalcooa* in the remediation of Pb contaminated soil and their effects on the growth parameter of the plants.

## 2 Material and Methods

### 2.1 Test plant

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*B.balcooa*, a perennial, hardy,tropical clumping and multipurpose bamboo was chosen as the test plant for this study. It is indigenous to the North East India, Bangladesh, Laos, Myanmar, Nepal, and Vietnam (Rajput et al., 2020). It can be found up to an altitude of 600 m in various climate conditions and found throughout India. It can grow up to 25 cm height and a diameter of 1-15cm. The leaves are dull and greyish-green. The average estimated carbon storage in the above ground biomass is 65.3 tons/ha (Nath et al., 2018). It has ability to grow on any soil type but prefer low pH, heavy texture and good drainage soil. It can be cultivated by the farmersand generates 104.7 Mg ha$^{-1}$ of above-ground biomass (Devi and Singh, 2021). In Bangladesh, it hasreported that *B. balcooa* producing 120-1700 culms per hectare per year (Mulatu et al., 2016).

## 2.2 Experimental design and soil characteristics

The experiment was conducted in the Department of Botany, Panjab University, Chandigarh (India) in a well-protected dome covered with a transparent polythene sheet.

### 2.2.1 Soil collection and analysis

The soil was taken from P.N. Mehra Botanical Garden of Panjab University, Chandigarh. The collected soil was analysed for their initial physicochemical properties such as soil type, pH, cation exchange capacity, electrical conductivity, exchangeable sodium percentage, sodium absorption ratio, nitrogen, phosphorus and potassium. Initial content of the lead (Pb) was also measured through Inductively coupled plasma atomic absorption spectrometry (ICP-OES).

### 2.2.2 Plantlet collection and transplantation

Five months old*B. balcooa* plantlets that were gathered from the Forest department, Rupnagar, Punjab. For the experiment, plantlets were transplanted into earthen pots filled with with7 kg air-dried soil. Five different concentration of lead nitrate (0, 150, 500, 1000, and 1700 mg kg$^{-1}$ soil) were taken for the study. Three replicates of each concentration were used and doses were applied in aqueous form. To reduce the heterogeneity in the findings, the experiment was carried out from January to December 2021 (a period of 365 days).

## 2.3Vegetative characters

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After the application of different doses, morphological characteristics such as the number of culms, nodes, leaves, the length of the internode, the height of the plant, and the area of the leaves were counted in every 30 days.

a) Number of culms, number of leaves and nodes were counted manually.
b) The length of internodes was measured by measuring scale. Three internodes length were taken from the 2nd internode from the base of the plant and expressed in the cm.
c) Height of the plant was measured by measuring scale from the bottom of the pot to the tip of the plants and expressed in the centimetre.
d) Leaf area was measured by the scale by measuring maximum width and length of the three leaves of each replicate and measured by the following formula:

   Leaf area ($cm^2$) = (length×breadth) ×0.7 (Shi et al., 2021)
e) Biomass was calculated after uprooting the plants. The plant parts were separated as stem, root and leaves and their fresh weight (f.w.) was taken. The plant parts were oven dried at 50-60°C for 72 hours and their dry weight (d.w.) was measured in grams (g).
f) Moisture Content: Moisture content was measured with the help of fresh and dry weight and expressed in percentage (%)

   Moisture (%)=$\frac{Fresh\ weight - Dry\ weight}{Fresh\ weight} \times 100$

## Statistical Analysis

Data was statistically analysed through IBM 26 SPSS software. All the estimated values are the average of the three independent variables.Multivariate analysis of variance (three-way ANOVA) is used to assess the data at significant $p<0.05$ probability levels to examine the effects of treatment doses, days after treatment (DAT), and their interactions.

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## 3 Results and discussion

### 3.1 Physicochemical properties of the soil

Initial physicochemical characteristics of the soil indicated that the soil is sandy loam (sand (60%, silt 20%, and clay 20%), neutral pH (7.1), EC (0.33), CEC (7.0 meq/100 g), ESP (8.14meq/100g), SAR (0.048), OC (1.00%), WHC (4.11 g $g^{-1}$)Ca+Mg (1.8), N (246 kg/ha), P (12.7 kg/ha)and K (107.44 kg/ha). Initial Pb content in the soil was 9.3 mg/kg (Table

1).These characteristics showed that the soil is fertile and suitable for the growth of the selected plant (*B. balcooa)* for the experiment.

**Table 1 Physicochemical properties of the soil**

| **Sr. No.** | **Parameter** | **Value** | **Methods used for analysis** |
|---|---|---|---|
| 1. | Texture | Sandy loam soil | International Pipette Method |
| 2 | CEC (meq/100 g) | 7.0 | Centrifuge method (Ross and Kettering, 1995) |
| 3. | ESP (meq/100g) | 8.14 | Centrifuge method (So et al., 2006) |
| 4 | SAR (meq $L^{-1}$) | 0.048 | $SAR = \frac{Na}{\sqrt{\frac{(Ca+Mg)}{2}}}$ |
| 5. | EC $_{1:2}$ (dS $m^{-1}$) | 0.33 | Digital EC meter |
| 6. | pH $_{1:2}$ | 7.1 | Digital pH meter |
| 7. | OC (%) | 1.0 | Wet digestion Method (Sangmanee et al., 2016) |
| 8. | Water holding capacity (g $g^{-1}$) | 4.11 | Cylinder method (Wilke, 2005) |
| 9. | Ca+Mg (meq $L^{-1}$) | 1.8 | EDTA method (Bascomb, 1964) |
| 10. | Available N (kg $ha^{-1}$) | 246 | Kjeldahl- Distillation method (Ravi et al., 2023) |
| 11. | Available P (kg $ha^{-1}$) | 12.7 | Olsen, 1965 method (Correia et al., 2023) |
| 12. | Available K (kg $ha^{-1}$) | 107.44 | Ammonium acetate method (Kobayashi et al., 2023) |

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| | | | |
|---|---|---|---|
| 13. | Pb (mg $kg^{-1}$) | 9.3 | ICP-OES |

## 3.2 Effect of Pb on morphological traits

The following morphological characteristics, including the number of culms, leaves, nodes, length of internodes, plant height, and leaf area, were noted in order to assess the impact of lead concentration.

### 3.2.1 Number of culms

The heavy metal content had a significant impact on the number of culms ($F_{4,15}$ =3.496, $p<0.01$) as well as the days after treatment ($F_{12,15}$ =3.251, $p<0.00$) according to an ANOVA analysis. As a result, between treatments and DAT, the number of culms ranged from 2 to 4 (Fig 1).

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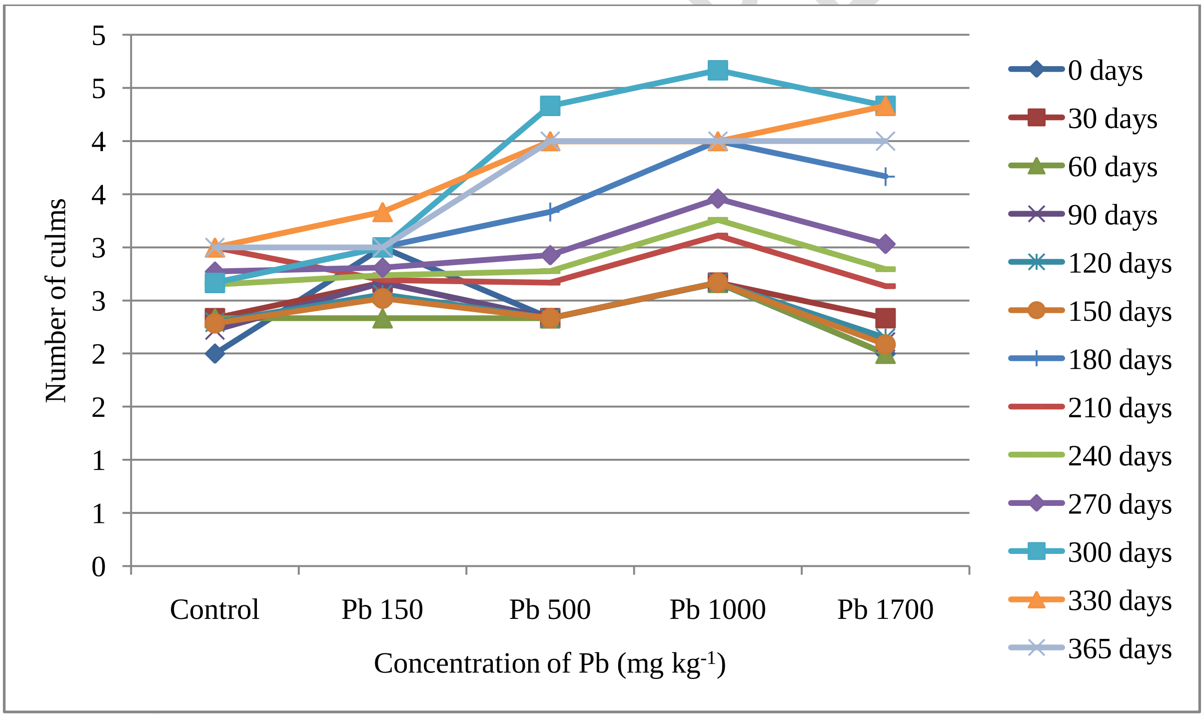

**Fig 1. Effect of Pb on number of culms**

### 3.2.2 Number of leaves

heavy metal concentrations and DAT had a significant impact on the number of leaves ($F_{4,15}$ =4.097, $p<0.004$ and $F_{12,15}$ =10.249, $p<0.000$) respectively. From initial to 210 DAT, there was an increase in the number of leaves, which thereafter dropped by 27.16%, 46.4%, 70.9%, and 66.06% at 150, 500, 1000, and 1700 mg/kg, respectively (Fig 2). The reduction in number of leaves may be due to limitation in the intake and transport of numerous minerals (Ca, Mg, p, and K) and reduction in photosynthetic activity (Chen et al. 2022).

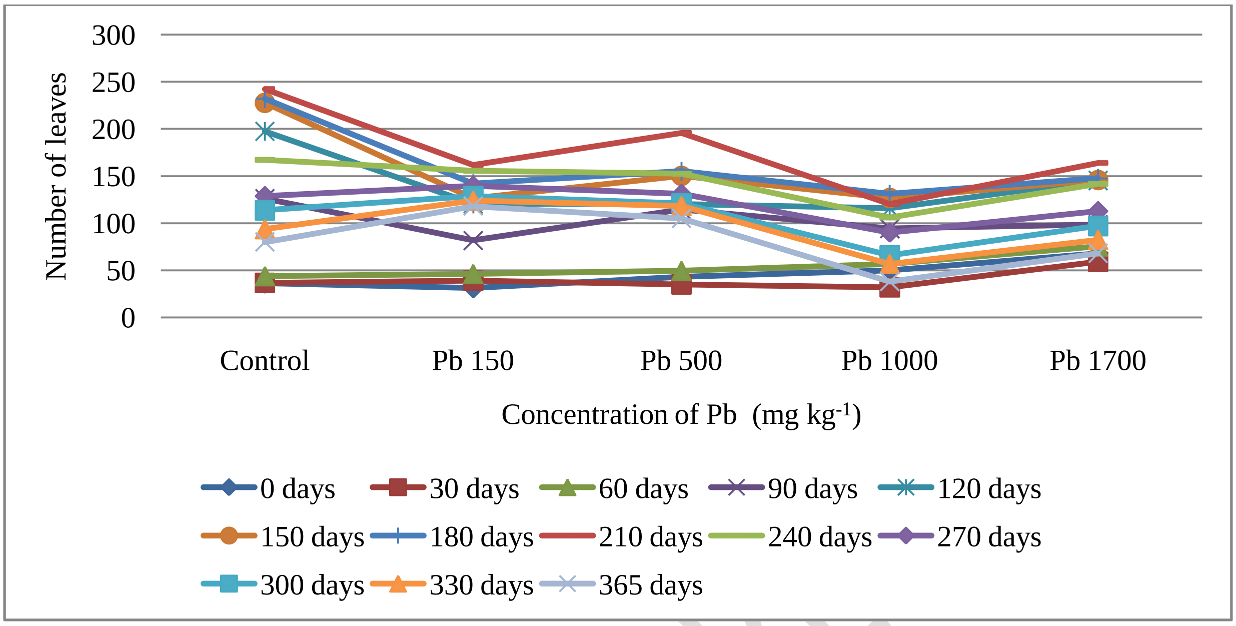

**Fig 2 Effect of different Pb concentration on number of leaves**

### 3.2.3 Number of Nodes

Pb-treated plants showed a substantial drop in the number of nodes with the increase in heavy metal content when compared to the control, which varied from 4 to 10 (Fig 3). The decrease in number of nodes may be caused by oxidative stress induced by Pb stress that leads to decrease in mitotic activity in the cell (Shehzad et al., 2023).

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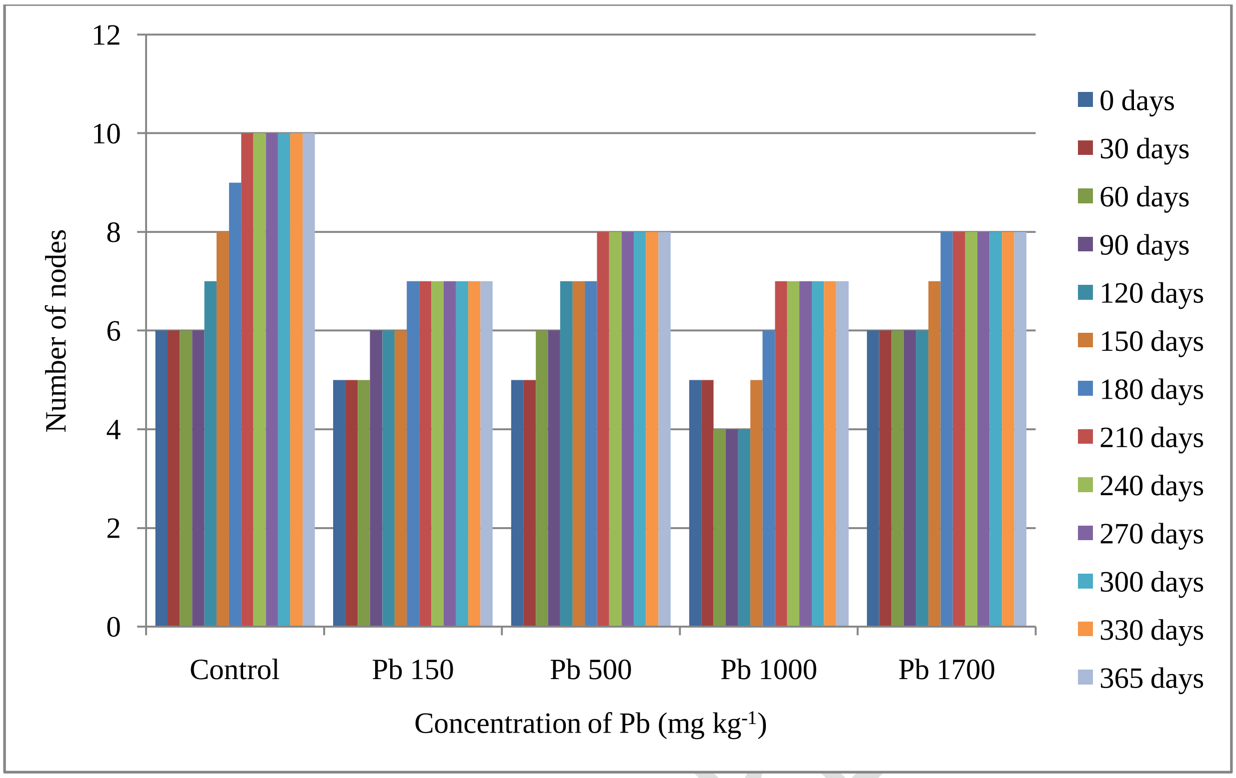

**Fig 3 Effect of various concentration on number of nodes**

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### 3.2.4 Leaf Area

Maximum and minimum increases in leaf area were recorded at 150 and 1700 mg kg$^{-1}$ (65.32±12.71 cm$^2$ and 43.01±28.80 cm$^2$respectively) (Fig 4). Statistical analysis indicated that Pb concentration had a significant impact on leaf area ($F_{4,15} = 3.214$, $p<0.01$) and DAT had a significant impact ($F_{12,15} = 16.242$, $p<0.000$). The decrease in leaf area may be caused by a reduction in gaseous exchange in the leaves, the closing of stomata, and water stress, which led to a decline in photosynthetic rate (Huihui et al., 2020). Shu et al. (2012) also reported the decrease in leaf area, root length and protein content under Pb stress.

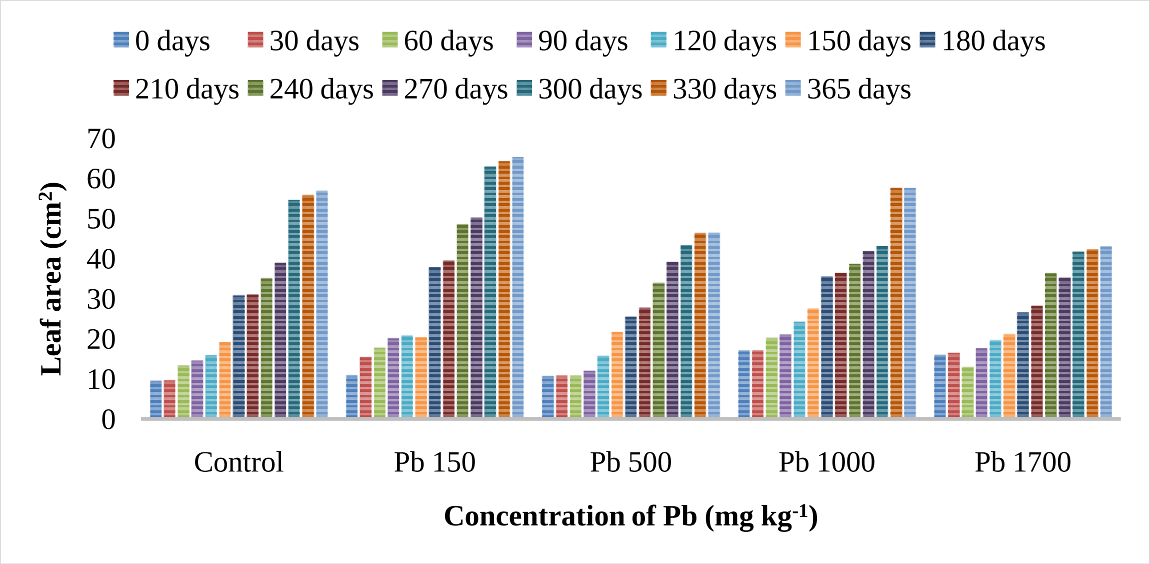

**Fig 4 Effect of different doses of Pb on leaf area**

### 3.2.5. Plant height

There was a significant change in the plant height with varying concentrations of Pb ($F_{4,15}$ =2.384, $p<0.05$) and DAT ($F_{12,15}$ = 8.739, $p<0.000$). As a result, this growth parameter values ranged from 44.36 cm to 135.66 cm in all the concentrations. the maximum and minimum increase in plant height was recorded in control and Pb 1700 mg kg$^{-1}$ (64.69% and 32.89%) respectivelywhen compared to other treatments (Fig 5). Insufficient water and nutrient availability can limit plant growth including the elongation of stems and overall plant height. Similar results were reported by Ghani et al. (2021) in *Daucus carota* where the plant height decreased with the elevated level of Pb.

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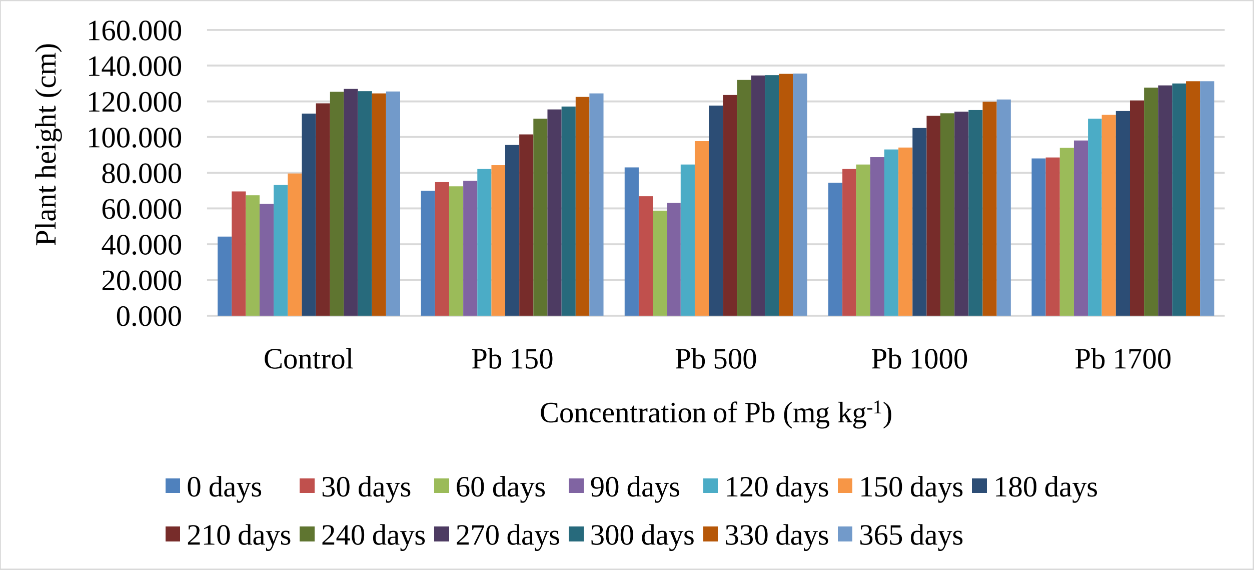

Fig 5 Effect of Pb on plant height of the plant

### 3.2.6 Internode Length

Internode length decreased with a high Pb concentration, and treatments and DAT both significantly affected internode length ($F_{4,15}$ =10.486, p<0.00) and DAT ($F_{12,15}$ = 4.784, p<0.00) (Fig 6). The most significant drop for this vegetative property was in 1700 mg $kg^{-1}$, ranging from 8.10 to 16.12 cm. Water stress due to Pb stress can lead to a reduction in cell expansion, which ultimately caused decrease in internode length (Asati et al., 2016).

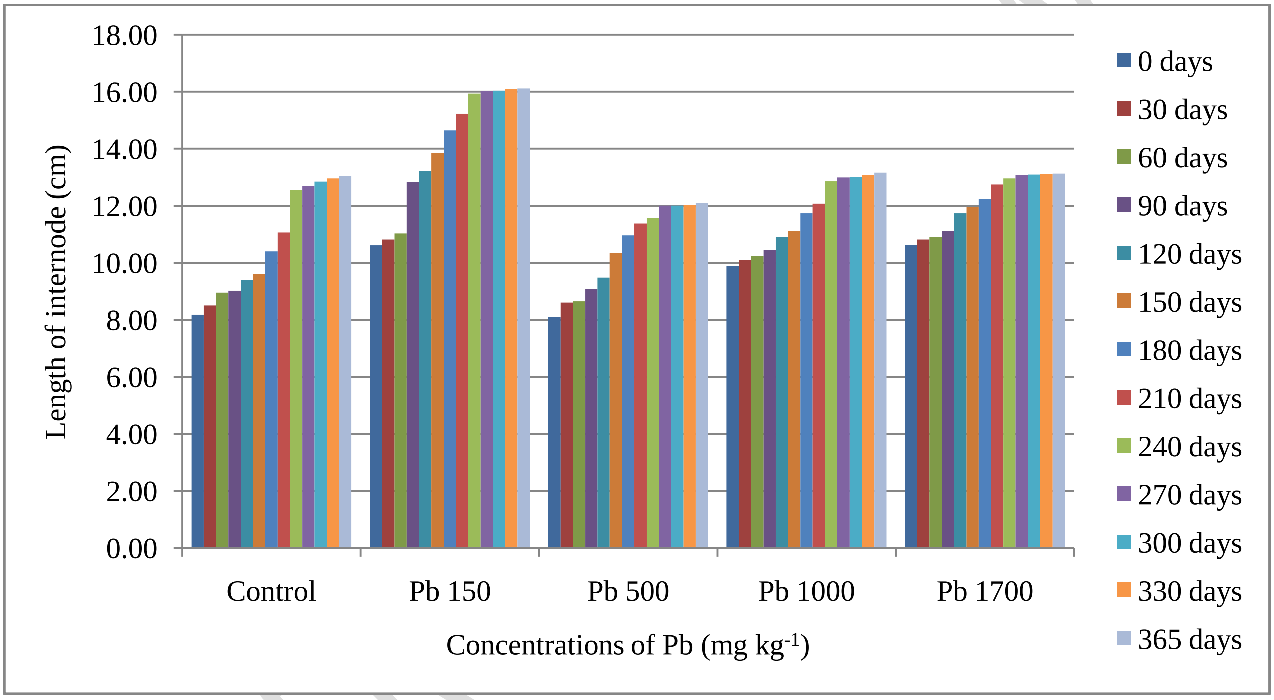

Fig 6 Effect of different Pb concentration on length of the Internode of the plant

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### 3.2.7. Biomass

Biomass was significantly influenced by the various concentration of Pb. At lower concentration biomass was positively stimulated but at higher concentration it declined. The fresh and dry root, stem and leaf biomass was ranges from 20.00-33.67g, 21.33-37.33g and 6.67-22.67g; 13.33-24.67g, 17.33-27.33g and 5.33-18.67g respectively (Table 2). At higher concentration plant may be unable to tolerate the oxidative stress caused by Pb toxicity and inhibits the cell division, cell expansion and reduction in photosynthetic activity by destroying the chloroplast structure (Kaur et al., 2014; Silva et al., 2017). In *Brassica* *napus*, Pb stress significantly reduced the plant biomass, leaf chlorophyll contents, nutrients uptake in the leaves and roots (Ali et al., 2014).

**Table 2. Data represents mean± S.D. of three replicates (n=3). Different letters superscript in column indicates the significant difference ($p<0.05$) in the biomass content under different concentration of Pb.**

| **Doses of Pb (mg/kg soil)** | **Root biomass (g)** | | **Stem biomass (g)** | | **Leaf biomass (g)** | |
|---|---|---|---|---|---|---|
| | **f.w.** | **d.w.** | **f.w.** | **d.w.** | **f.w.** | **d.w.** |
| Control | 22.33±10.5$^{ab}$ | 18.67±9.01$^{ab}$ | 21.33±5.68$^{a}$ | 17.33±3.05$^{a}$ | 10.0±2.00$^{a}$ | 8.67±1.52$^{a}$ |
| Pb 150 | 33.67±5.13$^{b}$ | 24.67±3.51$^{b}$ | 37.33±6.42$^{b}$ | 27.33±7.02$^{b}$ | 22.67±5.13$^{b}$ | 18.67±5.50$^{b}$ |
| Pb 500 | 32.67±11.71$^{ab}$ | 20.00±5.00$^{b}$ | 27.33±9.01$^{ab}$ | 22.67±6.80$^{ab}$ | 10.67±1.52$^{a}$ | 8.67±0.57$^{a}$ |
| Pb 1000 | 20.00±6.92$^{a}$ | 13.33±4.16$^{a}$ | 23.33±7.37$^{a}$ | 18.67±5.85$^{a}$ | 7.33±1.15$^{a}$ | 5.33±0.57$^{a}$ |
| Pb 1700 | 20.67±1.154$^{a}$ | 13.33±1.15$^{a}$ | 23.33±4.61$^{a}$ | 20.00±4.35$^{ab}$ | 6.67±2.51$^{a}$ | 5.33±2.08$^{a}$ |

### 3.2.8. Moisture content

Moisture content measures the water content in the plants. Under Pb stress, roots had the highest moisture content at Pb 500 mg kg$^{-1}$ (38.78%). By increasing the moisture content, plant tries to mitigate the toxic effects of the Pb stress but at higher concentration moisture content decreased (Malar et al., 2016).

**Table 3 Effect of Pb concentration on moisture content in plant parts.**

| **Concentration of Pb in soil (mg/kg)** | **Moisture content (%)** | | |
|---|---|---|---|
| | **Root** | **Stem** | **Leaf** |
| Control | 16.39 | 18.75 | 13.30 |
| Pb 150 | 26.73 | 26.79 | 17.64 |

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|  |  |  |  |
|---|---|---|---|
| Pb 500 | 38.78 | 17.05 | 18.74 |
| Pb 1000 | 33.35 | 19.97 | 27.29 |
| Pb 1700 | 35.51 | 14.27 | 20.09 |

## Conclusion

Current study highlights the toxic effect of Pb on different growth parameters of the *B. balcooa*. Pb poses the significant effect on number of culm, nodes, leaves, plant height, internode length and leaf area. The impact of Pb on these growth parameters are correlated with the biomass of the plant. Results indicated that *B. balcooa*can tolerate the higher concentration of Pb (1700mg/kg) as there is 100 % survival efficiency was recorded. Therefore, better utilization of *B. balcooa* can be implemented to remediate the Pb contaminated soil that in turn will provide a better environment.

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