

GROWTH, BIOCHEMICAL CONSTITUENTS AND METAL ACCUMULATION IN MUSTARD (*BRASSICA JUNCEA* L.) TREATED WITH DIFFERENT HEAVY METALS CONCENTRATIONS

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ABSTRACT

The present study evaluates biomass yield, pigment concentration, biochemical constituents and heavy metal accumulation in *Brassica juncea* L. grown under different concentrations (100, 200, 400, 600 and 800 mgkg⁻¹ of soil) of Zinc (Zn), Lead (Pb) and Cadmium (Cd). Plant growth, pigment concentrations and biochemical parameters were evaluated at 15 days interval while plant biomass yield and metal accumulation were determined at maturity. The results showed low Zn concentrations (100-400 mgkg⁻¹) increased biomass yield, chlorophyll production and biochemical contents. Higher concentrations considerably decreased growth, pigment production and biochemical parameters. Though both Pb and Cd caused decline in growth, biomass yield, carbohydrate, protein and proline contents, Cd effect was most detrimental at high concentrations. Proline content increased at lower metal concentrations but decreased with higher concentrations. Cadmium was most accumulated in plant, followed by Pb and least in Zn and the root system accumulated more metals than the shoot system. The study observed that higher concentrations of heavy metal negatively affected biomass, biochemical content with higher accumulation predominantly in the root. *B. juncea* may, therefore, be used as a model plant in studying remediation of heavy metal in contaminated soil.

Keywords: biochemical constituents, biomass yield, *Brassica juncea*, heavy metals, metal accumulation

INTRODUCTION

Brassica juncea (L.) Czern. is a member of the family Brassicaceae commonly called the mustard family which consists of 350 genera and about 3500 species. *B. juncea* (mustard) leaves are pale green, slightly hairy with leaf blades that terminate well up the petiole. The lower leaves are deeply lobed, while the upper ones are narrow. The non-clasping upper leaves distinguish it from *B. napus* and *B. rapa*; its close relatives. The mature plant is about two meters in height (Nishi *et al.*, 1980). *B. juncea* inflorescence is an elongated raceme with pale-yellow flowers.

B. juncea (mustard) is grown for spice in North America, but mainly as a source of cooking oil in Asia. As the third most important source of edible oil after soy and palm, Brassicaceae accounts for 9% of the worlds edible vegetable oil and the residuals from oil extraction could be used as livestock feed (Usfoelda *et al.*, 2010). Utilization of *B. juncea* for bio-fuel and bio-pesticides production has been reported (Kimber and McGregor, 1995; Newkirk, 2009). In comparison with its relative species (*B. napus* and *B. rapa*), which are also cultivated, mustard is more tolerant to abiotic stress and could withstand environmental stress.

Environmental pollution is a global challenge that demands serious attention and mitigation as it adversely affects the growth, quality and the sustenance of all organisms. Heavy metals in water and soil may finally reach the food chain through contaminated water and edible agricultural products. In an agricultural Determination of total chlorophyll in each treated and control plants were carried out as described by John *et*

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land contaminated with heavy metal such as Cadmium (Cd), Lead (Pb), Chromium (Cr), Nickel (Ni), which are highly toxic metals, the contaminant may accumulate in the crop tissues with a significant potential to impair animal and human health (Di Toppi and Gabrielli, 1999). Presence of heavy metals in the soil was noted to affect soil microorganisms, soil biodiversity, soil fertility and physiological indices, which resulted in reduced yield (Majer *et al.*, 2000). The application of sewage sludge, city waste and Cd-containing fertilizers could also increase heavy metal content of the soils (William and David, 1973).

Lead pollution in soils would not only affect soil microbial community and fertility, but also the physiological indices which would consequently decline the yield. Soil microbial activity is a sensitive indicator of stress in soil and has been employed in national and international monitoring programmes (Yao *et al.*, 2000). Remediation of heavy metal contaminated soil usually involves high cost and is sometimes technically difficult. New strategies that are efficient, economical, low-impact and simple in remediating elemental contaminated soil should be explored. The understanding of heavy metal absorption in plants and resistance mechanisms developed by such plants would provide information for using plants as heavy metal remediating agent in polluted soils (Zhang and Huang, 2000). The role of plants in mitigation of pollutants from the soil by accumulation of toxic elements in the harvestable parts of the plants was reported (Liu *et al.*, 2003), also, effect of Arsenic (As) on growth parameters of mustard has been studied (Gupta *et al.*, 2009). However, study of elemental accumulation using plants as bio-monitor would require plants with short life cycle. The present study, therefore, investigated the changes in chlorophyll content, metabolites production, growth and heavy metal accumulation in tissues of mustard seed (*B. juncea*) grown under heavy metal contaminations with the view of understanding *B. juncea* adaptation to environmental pollution. The results may lead to the utilization of mustard for phytoremediation and soil reclamation.

MATERIALS AND METHOD

Materials

Brassica juncea (mustard) seeds used for the study were provided by the Department of Botany, Navsari Agricultural University, Navsari, Gujarat, India. Chemicals and reagents used for this study were of high analytical grades purchased from Sigma Aldrich, USA, SD Fine Chem Ltd, India and HiMedia, India.

Methods

1. Pot experiment

The pot experiment was conducted in the net house facility of C. G. Bhakta Institute of Biotechnology (CGBIBT), Uka Tarsadia University, Bardoli, Surat District of Gujarat, India. Planting bags filled with mixture of garden soil and farm yard manure (4:1) were thoroughly watered in preparation for sowing. One week after germination, seedlings were treated with 50, 100, 150, 200 and 250 mg kg⁻¹ soil of Zinc sulphate [ZnSO₄·7H₂O], Lead nitrate [Pb(NO₃)₂] and Cadmium chloride [CdCl₂]. Five replicates were provided for each of the treatment concentrations and control in a Randomized Complete Block Design (RCBD). Moderate watering was done twice in a week. Metal solutions of selected concentrations were repeatedly applied to the soil every 21 days. Plants were harvested at 15 days interval (from day of sowing) and evaluated for shoot length, root length, leaf area, total pigment content and biochemical metabolites. Fresh and dry weight of plants were determined at maturity. The dry weight was determined by drying the plants at 70°C for 72 hours in oven and the weighing was measured using weighing balance.

2. Estimation of chlorophyll content

Determination of total chlorophyll in each treated and control plants were carried out as described by John *et al.* (2009). 100 mg of fresh leaf was kept in tubes containing dimethyl sulfoxide (DMSO) as extracting reagent.

The tubes were incubated at 65°C for 40 min. Two (2) ml DMSO was mixed with 1 ml aliquot and vortexed. Absorbance was obtained photometrically at 480, 510, 645 and 663 nm (Beckman D, USA) with DMSO as a blank.

3. Proline content estimation

Free proline content in the leaves was determined by acid ninhydrin (Bates *et al.*, 1973). Using 300 mg of fresh leaf, the amount of proline in the plant tissues was checked on absorbance at 520 nm (Cary Varian Spectrophotometer). Proline content was calculated by comparing the absorbance value against a standard curve derived from known concentrations of L-proline and expressed as $\mu\text{g}/\text{mg}$ of fresh weight. $[\mu\text{moles}/\text{g tissue} = (\mu\text{g proline}/\text{ml} \times \text{ml Toluene})/115.5]$

4. Carbohydrate content estimation

The total carbohydrate in the plant samples was determined based on a standard method (Irigoyen *et al.*, 1992). The resultant mixture was checked on absorbance at 620 nm on Epoch Micro-Volume Spectrophotometer (Biotech Instruments Inc. USA). A calibration curve was constructed with glucose in the range 20–400 $\mu\text{g}/\text{ml}$ (Sigma Aldrich, USA).

5. Protein content

Proteins were estimated (Bradford, 1976). 0.5 g of fresh leaf was homogenized in 1 ml phosphate buffer (pH 7.0), centrifuged at 5000xg for 10 min. Half ml ($\frac{1}{2}$ ml) of freshly prepared trichloroacetic acid (TCA) was added and centrifuged at 8000xg for 15 min. The debris was dissolved in 1 ml of 0.1 N NaOH and 5 ml Bradford reagent added. Absorbance was recorded photometrically at 595 nm (Beckman 640 D, USA) and bovine serum albumin was used for standard curve.

6. Estimation of Zinc (Zn), Lead (Pb) and Cadmium (Cd) Accumulation

Heavy metal concentration in the digested root and shoot of *B. juncea* was determined in hot concentration HNO_3 . Zn, Pb and Cd content were estimated using Perkin-Elmer Atomic Absorption Spectrophotometer (Analyst Model 300) equipped with an air-acetylene burner. The heavy metal content was expressed as mg/g of the dry weight of the sample.

The collected data were processed and the mean values and standard errors evaluated for Analysis of variance (ANOVA) using SPSS Ver. 16.

RESULTS AND DISCUSSION

1. Growth and biomass yield

The effect of different concentrations of Zn, Pb and Cd metals on shoot and root is presented in Tables 1-3. The response of the root and shoot system to metal concentrations of 100–400 mg kg^{-1} of soil showed enhanced growth among Zn treated plants from juvenile through fruiting stages (15–60 days after sowing; DAS) as shown in Table 1. Generally, plant required Zn in traces for essential metabolic process, but excessive heavy metals could cause decrease in growth, development and oxidative damage in plant tissues as pointed out by Prasad and Hagmeyer, (1999). The increase in growth and biomass yield of plants treated with 100–400 mg kg^{-1} of Zn indicated that low concentration improved root system and helped in efficient water and nutrient absorption with corresponding growth. The result obtained here were in agreement with Reichman (2002), who demonstrated increase in growth parameter of plants subjected to moderate concentration of Zn. Whereas, lower concentrations of Zn promoted growth and biomass yield (Table 1), concentrations above 400 mg kg^{-1} adversely affected plant physiology. Reduced growth due to excess heavy metals has been established (Seyyedi *et al.*, 1999). Application of the metals at higher concentrations might inhibit growth by

interfering with enzymatic pathways and cause a shift in microbial activities in root rhizosphere resulting in reduced growth. In addition, higher concentrations of Zn in the soil could lead to Iron (Fe), Manganese (Mn) and Copper (Cu) deficiencies which hinder the transfer of these micronutrient from the root to the shoot (Ebbs and Kochian, 1997).

Furthermore, higher concentrations of lead and all concentrations of Cd adversely affected plant growth in terms of shoot and root length at all growth stages (Tables 2 and 3). High Cd in the soil could induce stress on plants, causing low water potential, reduced nutrient uptake and disturbance of macrotubules organization in the meristem region. Also, it may cause irreversible inhibition on the proton pump responsible for absorption mechanism which consequently leads to stunted growth (Eun *et al.*, 2000). Plants grown on soil with high Cd concentration reportedly exhibit retarded growth, chlorosis, browning of root cap and eventually death (Guo *et al.*, 2008).

The Leaf area of plant treated with Zinc concentrations of ≤ 100 -400 mg kg⁻¹ was higher compared with control, but 600-800 mg kg⁻¹ concentrations resulted in smaller leaf area (Fig 1). Increase in leaf area under low concentrations suggests Zn involvement in chlorophyll synthesis, cell division and meristematic activity. Thus a decrease in the leaf area at higher concentrations can be attributed to reduction in the number of cell or cell size (Meiri and Poljakoff-Mayber, 1967). Decline in leaf area was observed from 60-75 days after sowing (DAS) for all treatment concentrations (Fig 1). Compared with control, lead treated plant yielded slightly higher leaf area for 100-400 mg kg⁻¹ which declined at 600-800 mg kg⁻¹ from 60-75 DAS (Fig2). The trend was similar for Cd treated plants (Fig 3). The decline in response to metal concentrations at post flower stage (60-75 DAS) was due to shift to maturity which involves senescence accompanied with reduced nutrient uptake. This finding concurred with the previous result which observed reduction in growth and biomass yield of *B. juncea* under heavy metal stress at post fruiting stage (John *et al.*, 2009).

Although 100-400 mg kg⁻¹ concentrations enhanced total biomass yield among Zn and Pb treated plants, the highest values of total biomass yield (177.80 g of wet and 81.42 g dry weight) was obtained with Zn 400 mg kg⁻¹ at 75 DAS (Fig 4). Similarly, 400 mg kg⁻¹ of Pb yielded 165.31 g of wet and 72.46g of dry weight ahead of Control (159.55 g wet and 58.64 g dry weight). The effect of Cd on biomass yield of *B. juncea* at 75 DAS is shown in Figure 4. Reduced wet and dry weights were recorded for all the metal treatments at higher concentrations of 600-800 mg kg⁻¹ of soil (Fig 4).

2. Biochemical parameters

Figures 5-7 showed effects of Zn, Pb and Cd on total chlorophyll content of mustard at different growth stages. Lower concentrations (100-400 mg kg⁻¹) produced higher chlorophyll while concentrations of 600-800 mg kg⁻¹ resulted in a decreasing trend with respect to chlorophyll production (Fig 5-7). Total photosynthetic pigments increased from 15-45 DAS with 100-400 mg kg⁻¹ treatments but declined from 60-75 DAS. Low concentrations of Zn increased chlorophyll production because Zn was involved in synthesis or as a cofactor in chlorophyll biosynthesis pathway (Van Assche *et al.*, 1988). Higher concentration (600-800 mg kg⁻¹) however was toxic, resulting in decreased chlorophyll production. Similar decrease in chlorophyll production at higher Cd concentrations was earlier reported in almond (Eloumi *et al.*, 2007). Higher level of Zn, Pb and Cd indicated marked depression in photosynthetic pigments production of mustard plants by interfering with its biosynthesis. Since chlorophyll synthesis depends on the adequate supply of iron (Fe) and Magnesium (Mg), excess zinc would induce physiological Fe and Mg deficiencies that affect chlorophyll synthesis (Siedlecka and Krupa, 1999). On the other hand, Pb and Cd stress are capable of disrupting biosynthesis enzymes, impairment of photochlorophyll reductase and inhibition of the activity of carboxylation enzymes in pigment biosynthetic pathway (Van Assche and Clijsters, 1990). The 100-400 mg kg⁻¹ concentrations progressively increased carbohydrate production (15-45 DAS). Decline in carbohydrate was observed at 600-800 mg kg⁻¹ concentrations (Fig 8). The trend was similar for Pb and Cd, carbohydrate content was remarkably higher in all concentrations of Pb and some Cd treated plants,

while Zn treated plants had the least carbohydrates across all concentrations and days of evaluation (Fig 8). Decrease in carbohydrate from 60-75 DAS revealed that carbohydrate synthesis is directly related to photosynthetic assimilation. Previous studies have shown that carbohydrate accumulation in response to heavy metal treatment depends on concentrations of such metal (Nagajyoti *et al.*, 2010).

The results of protein content in response to metal concentrations are shown in Fig 9. The decrease in protein content at higher concentrations (600-800mgkg⁻¹) as observed in this study, in particular on Pb and Cd treated plants likely arose from protein degradation process as a result of increased protease activity which is found to increase under stress condition (Palma *et al.*, 2002). Furthermore, it is possible these heavy metals induced lipid peroxidation and fragmentation of proteins which led to reduced protein content. This is in tandem with other findings which reported decrease in total soluble protein of *B. juncea* with increase in Cd and Pb concentrations (John *et al.*, 2009). Proline production trend was similar for all the metals evaluated. At 15-30 DAS, proline content was low but increased considerably from 45-75 DAS for all the metal concentrations (Fig 10). It was demonstrated that plants accumulate considerable amount of proline and amino acid under stress condition (Ahmed *et al.*, 2008). This may contribute to osmoregulation, enzymatic methylation and reduction in macromolecules or cellular organelle stability. Accumulation of proline was favoured by heavy metal stress, which may increase due to de novo synthesis of biochemical in plants (Kasai *et al.*, 1998).

3. Heavy metal accumulation

Heavy metal accumulation at maturity (75 DAS) is shown in Table 4. Generally, accumulation of metals was higher in the root than in the shoot system and metal accumulation increased with increase in concentrations. The trend of accumulation was Cd>Pb>Zn, thus accumulation of Zn in the plant was considerably low compared with Pb and Cd. Since Zn is required by plants in little amount, its accumulation in the root was the least. Highest accumulation (85.28 mgg⁻¹dw) was recorded at 800mg kg⁻¹ in the root of Cd treated plant (Table 4). Higher metal accumulation in the root indicates the roots evolved mechanism of preventing total absorbed metal from translocation to the shoot system. This mechanism may involve chemiosmotic regulation in the root membrane which is selectively permeable. However, it was argued that translocation hindrance would affect efficiency of macronutrients movement from the root to the shoot (Nagajyoti *et al.*, 2010). High Cd accumulation in the studied plant conform to earlier studies where higher Cd accumulation in the root of plant was documented (John *et al.*, 2009). Moreover, heavy metal may be unevenly distributed in the root, therefore, different species may act differently under apoplastic and symplastic transport which makes transportation to the shoot system restricted. The accumulation of heavy metal occurs in the vacuoles of root cells thus limiting the heavy metal transportation to shoots, so heavy metals are stored predominantly in the roots:

CONCLUSION

The results of the present study showed that low concentrations of Zn may increase biomass yield, but application of Zn at higher concentration adversely affected biomass yield. However, decrease in growth and biomass yield were obtained with Pb and Cd. Total chlorophyll, carbohydrate, protein and proline increased at lower concentration which decreased at higher concentrations. Also, higher concentrations of heavy metals adversely affected biomass and biochemical yield at post flowering stage (60-75 DAS). Least metal accumulation was recorded in Zn while Cd was most accumulated followed by Pb. The study concludes that higher concentrations of heavy metal negatively affected biomass, biochemical yield and increase accumulation of heavy metal predominantly in the root of *B. juncea* and the plant may be used as a model plant in studying remediation of heavy metal contaminated soil.

Table 1: Effect of Zn concentrations on growth of *Brassica juncea* at different growth stages

Metal (mg kg ⁻¹)	Plant growth (cm)	15 days	30 days	45 days	60 days	75 day
Control	Root length	9.25±1.49	16.85±3.01	22.41±3.06	27.05±3.57	29.35±3.21
	Shoot height	27.55±1.35	33.21±2.57	44.40±4.14	52.12±4.82	56.2±4.41
100	Root length	9.31 ± 1.27	18.92±1.04	26.39±2.15	27.41±3.16	31.51±2.47
	Shoot height	28.02±0.26	34.25±3.17	46.25±3.90	55.16±4.49	58.74±3.14
200	Root length	9.37±1.32	17.45±2.22	23.69±3.23	31.39±3.42	33.82±2.09
	Shoot height	27.75±2.06	36.33±2.49	45.45±3.07	57.41±3.12	59.24±4.07
400	Root length	10.3±0.14	18.67±1.28	24.93±2.06	29.4±3.13	31.24±2.22
	Shoot height	29.34±2.14	38.41±2.11	49.55±3.07	58.77±4.24	60.81±3.13
600	Root length	8.71±1.16	11.73±0.09	16.54±2.34	19.49±1.07	25.19±3.15
	Shoot height	16.34±0.08	24.67±2.24	34.24±4.23	41.46±4.19	44.68±3.12
800	Root length	8.23±0.09	10.69±2.13	14.32±2.11	16.87±1.10	17.89±2.12
	Shoot height	15.21±2.12	19.61±2.29	26.12±2.16	34.55±3.07	37.69±3.12

Table 2: Effect of Pb concentrations on growth of *Brassica juncea* at different growth stages

Metal (mg kg ⁻¹)	Plant growth (cm)	15 days	30 days	45 days	60 days	75 days
Control	Root length	9.25±1.49	16.85±3.01	22.41±3.06	27.05±3.57	29.35±3.21
	Shoot height	27.55±1.35	33.21±2.57	44.40±4.14	52.12±4.82	56.2±4.41
100	Root length	9.01 ± 1.04	16.42±2.34	22.19±2.05	25.41±2.10	26.51±3.15
	Shoot height	22.02±2.26	26.22±2.10	36.25±3.44	49.06±4.01	51.74±3.11
200	Root length	10.37±1.32	14.47±2.46	26.05±2.24	38.22±3.12	32.12±3.62
	Shoot height	28.23±3.03	36.12±3.39	43.05±3.42	49.33±4.12	51.29±4.07
400	Root length	10.38±0.14	20.14±2.24	24.18±3.06	28.47±3.64	30.34±3.24
	Shoot height	30.11±2.06	36.41±3.41	44.57±3.01	52.32±4.04	55.81±4.31
600	Root length	8.71±1.03	9.81±0.09	14.24±2.54	17.29±1.07	22.19±2.15
	Shoot height	11.29±1.18	16.44±2.21	25.24±3.21	36.22±3.11	38.68±3.01
800	Root length	7.23±0.09	9.69±2.11	12.31±2.09	13.87±1.01	14.81±1.10
	Shoot height	11.33±2.12	15.01±2.04	21.12±1.16	24.55±2.26	27.69±3.18

Table 3: Effect of Cd concentrations on growth of *Brassica juncea* at different growth stages

Metal (mg kg ⁻¹)	Plant growth (cm)	15 days	30 days	45 days	60 days	75 days
Control	Root length	9.25±1.49	16.85±3.01	22.41±3.06	27.05±3.57	29.35±3.21
	Shoot height	27.55±1.35	33.21±2.57	44.40±4.14	52.12±4.82	56.2±4.41
100	Root length	8.41±0.98	13.56±1.01	21.36±1.15	26.73±3.22	28.51±2.08
	Shoot height	20.33±1.22	26.20±2.16	33.25±2.10	34.89±3.49	44.97±3.01
200	Root length	7.41±0.56	12.45±1.59	16.29±3.23	19.96±2.42	23.82±2.60
	Shoot height	17.75±2.34	26.37±3.06	33.45±2.07	38.78±3.56	39.24±3.14
400	Root length	8.34±0.19	14.90±2.01	19.48±2.01	26.14±3.03	28.25±2.22
	Shoot height	18.08±2.41	29.40±2.52	40.31±4.02	44.77±4.01	46.30±3.47
600	Root length	7.20±0.16	11.40±1.01	15.24±2.04	17.22±1.07	21.23±3.02
	Shoot height	10.34±1.03	17.34±2.12	23.19±3.18	26.06±3.13	28.31±3.03
800	Root length	6.21±0.04	10.04±1.15	13.81±1.11	15.87±1.10	16.91±2.14
	Shoot height	7.60±0.68	12.61±1.33	17.15±2.03	21.55±2.07	23.40±2.10

Table 4: Heavy metal accumulation in the root and shoot of *B. juncea* treated with different concentrations of Zn, Pb and Cd

Metals	Metal Accumulation (mgg ⁻¹ dw)					
	Zn		Pb		Cd	
	Shoot	Root	Shoot	Root	Shoot	Root
Conc. (mg/kg of soil)						
Control (0)	0.38±0.02	1.19±0.08	0.38±0.02	1.19±0.08	0.38±0.02	1.19±0.08
100	5.25±0.06	9.41±1.05	8.53±1.01	38.12±3.02	9.25±1.45	41.21±3.65
200	7.11±1.01	13.44±1.01	21.40±2.05	43.33±2.60	11.41±1.95	52.18±4.02
400	13.23±0.90	21.48±1.52	19.25±2.52	44.81±3.25	22.56±2.50	73.35±4.75
600	19.41±1.95	34.26±2.80	22.25±2.18	54.46±3.05	26.10±2.05	81.66±4.05
800	23.44±2.05	41.50±3.02	27.31±2.55	61.54±3.54	27.82±1.90	85.28±4.01

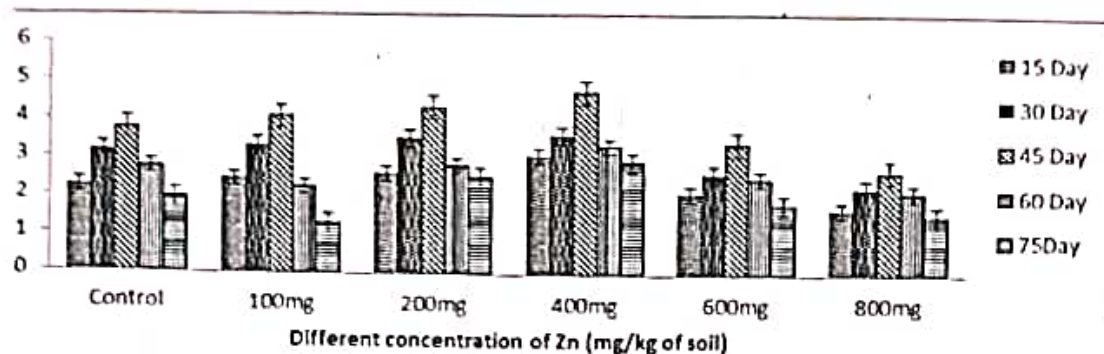


Fig 1: Effect of Zn concentration on Leaf area of *Brassica juncea* at different growth stages

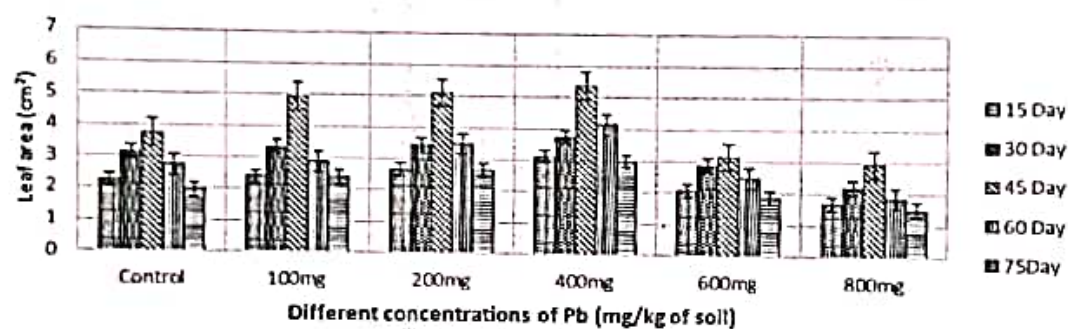


Fig 2: Effect of Pb concentration on Leaf area of *Brassica juncea* at different growth stages

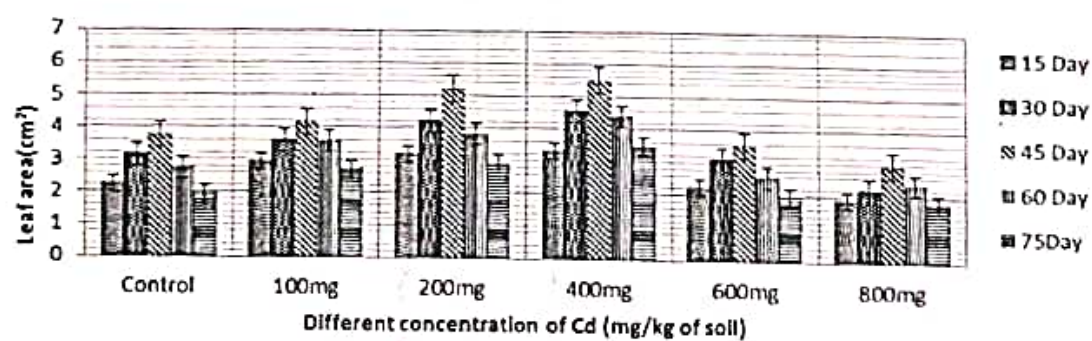


Fig 3: Effect of Cd concentration on Leaf area of *Brassica juncea* at different growth stages

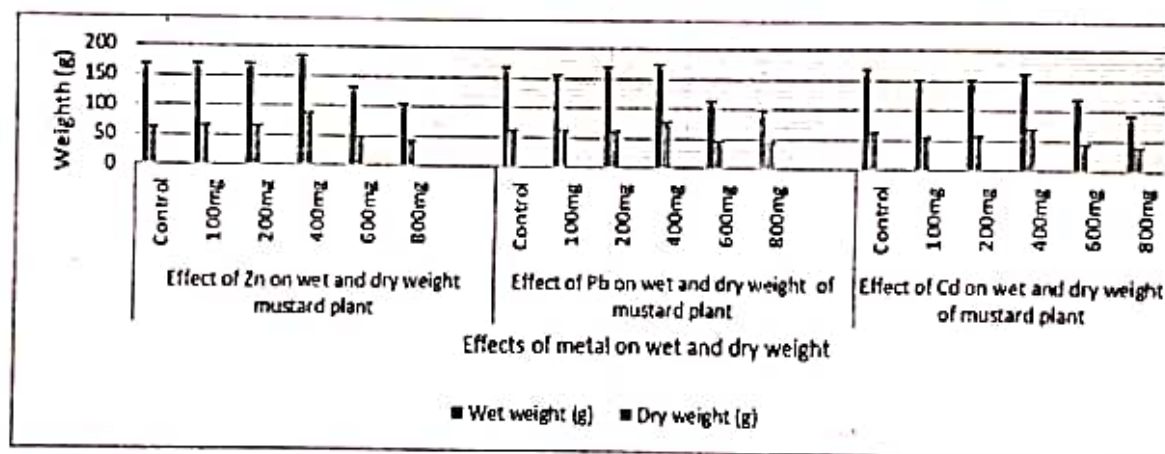


Fig 4: Effects of Zn, Pb and Cd concentrations on the wet and dry weights of *Brassica juncea*

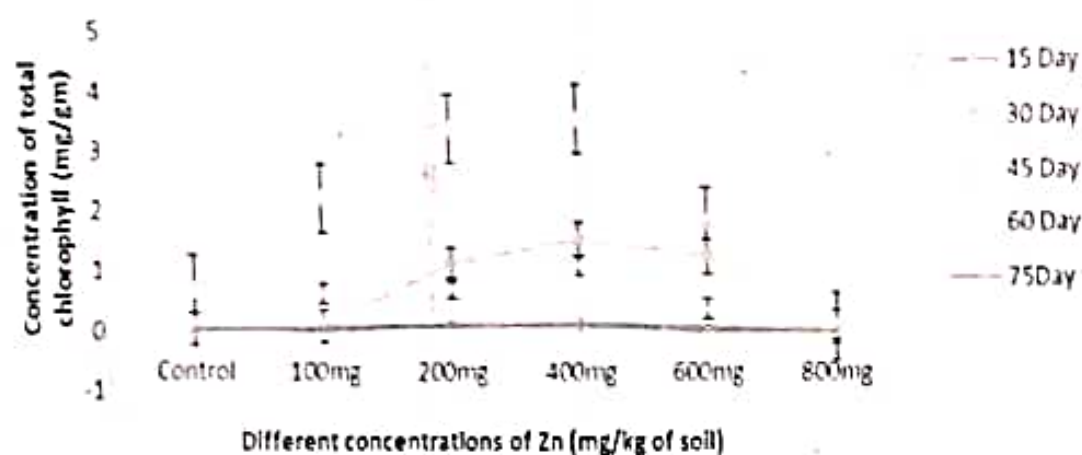


Fig 5: Effect of Zn on total Chlorophyll content of *Brassica juncea* at different growth stages

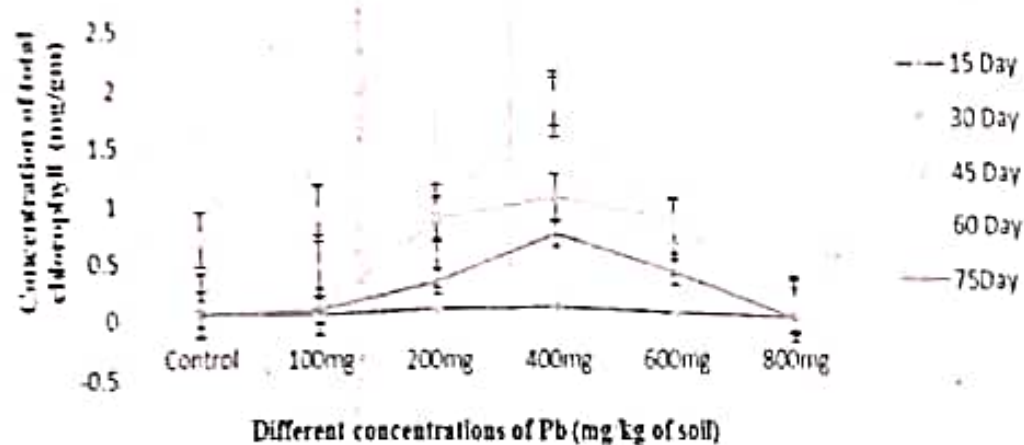


Fig 6: Effect of Pb on total Chlorophyll content of *Brassica juncea* at different growth stages

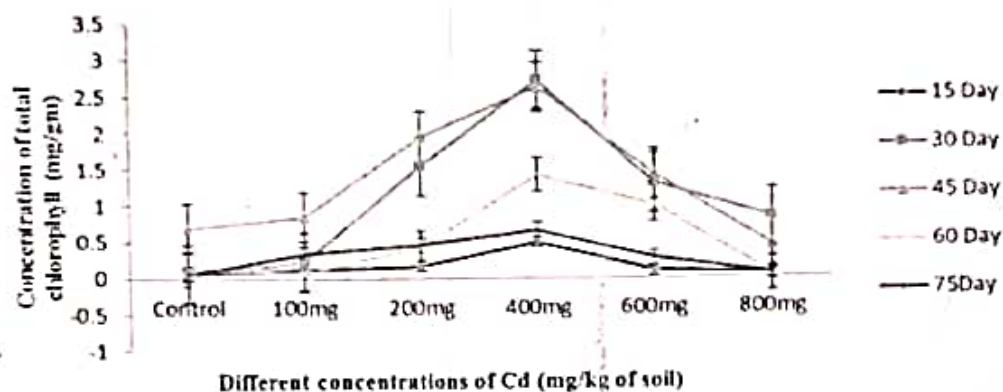


Fig 7: Effect of Cd on total Chlorophyll content of *Brassica juncea* at different growth stages

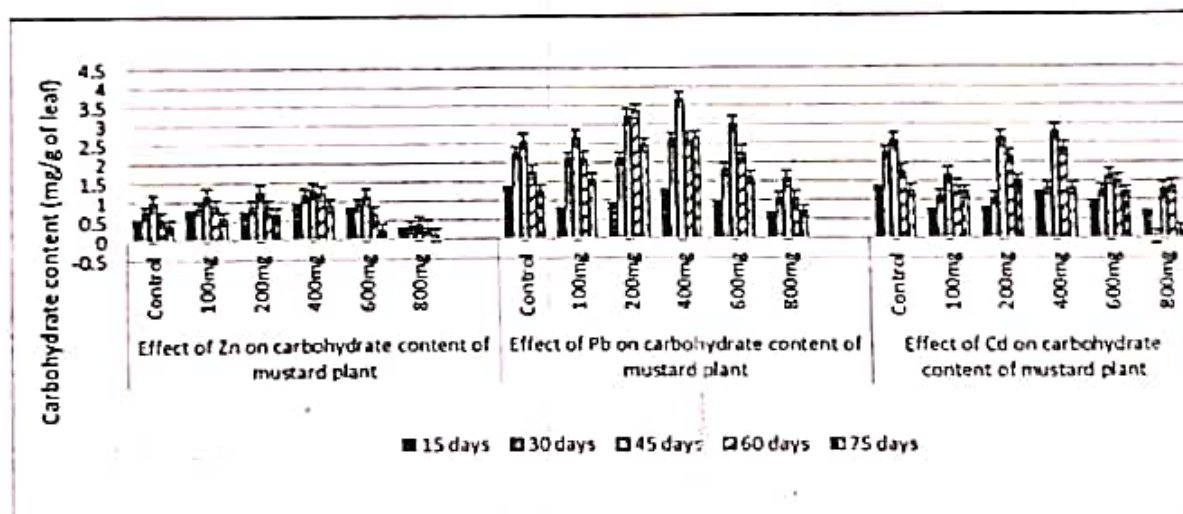


Fig 8: Effects of different concentrations of Zn, Pb and Cd on Carbohydrate content of *B. juncea* at different growth stages

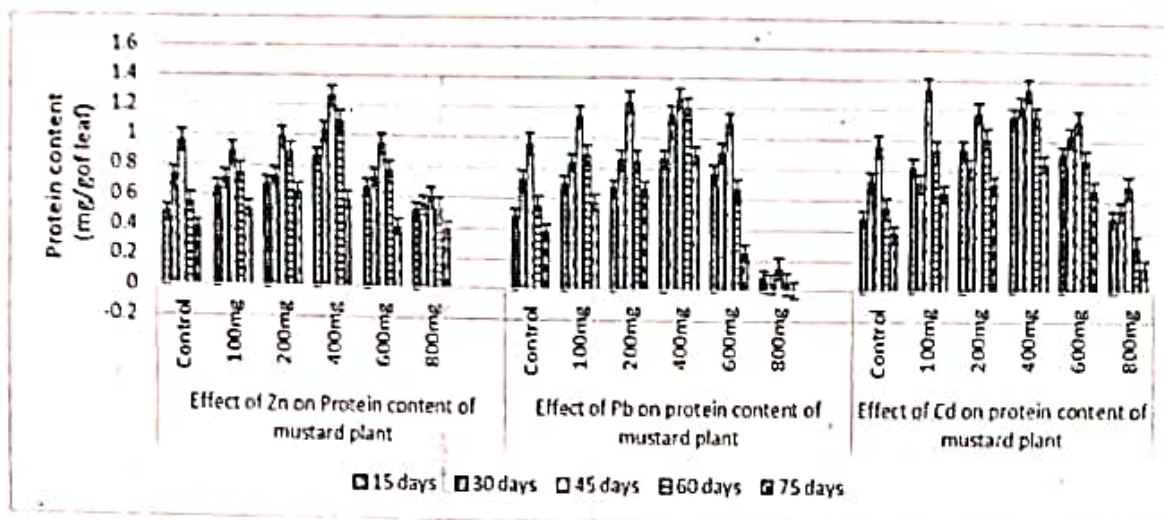


Fig 9: Effects of different concentrations of Zn, Pb and Cd on total protein content of *B. juncea* at different growth stages

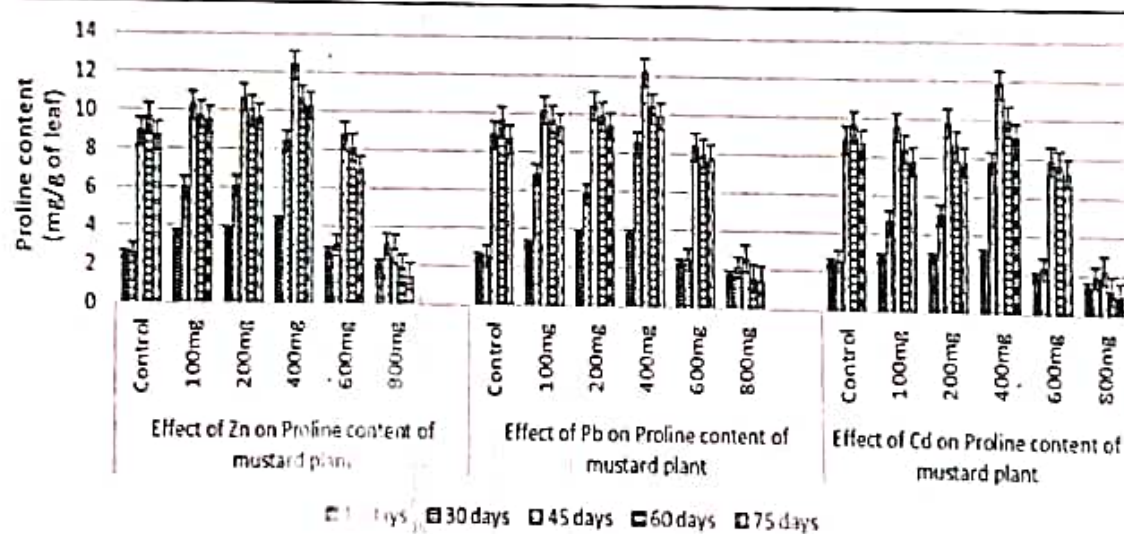


Fig 10: Effects of different concentrations of Zn, Pb and Cd on proline content of *B. juncea* at different growth stages

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