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Comparative Study of Pb and Ni Phytoextraction Potential: in Two Raya Varieties of Brassica juncea (L.) Czern for Tolerance and Accumulation

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ABSTRACT

Heavy metal contamination poses a major threat to crop productivity and food security. The present study evaluated the comparative effects of nickel (Ni) and lead (Pb) on growth, physiology, and anatomy of two Brassica varieties, Khanpur Raya (V1) and Raya RL-18 (V2), under controlled pot experiments. Plants were exposed to 80 mg Ni kg^{-1} soil (T1) and 80 mg Pb kg^{-1} soil (T2) for 14 days. Results revealed that Ni exerted greater phytotoxic effects than Pb, significantly reducing plant height, shoot fresh and dry weight, and photosynthetic traits (net CO_2 assimilation, stomatal conductance,

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transpiration rate, and intercellular CO_2 concentration). Raya RL-18 exhibited greater sensitivity, showing higher reductions in chlorophyll a, chlorophyll b, and total chlorophyll content compared to Khanpur Raya. Anatomical observations highlighted pronounced root modifications (epidermal and cortical alterations) and decreased leaf lamina thickness in V2, whereas V1 maintained comparatively stable structures. Both metals increased water use efficiency and altered stomatal density and aperture. Collectively, the study demonstrates that Ni toxicity is more severe than Pb, with varietal differences shaping stress responses. Khanpur Raya exhibited greater tolerance, suggesting its potential for cultivation in moderately contaminated soils and for use in phytoremediation strategies.

Keywords: Heavy Metals, Brassica Varities, Toxicity, Morphology, Chlorophyll, Anatomy

Introduction

Over many years, Pb and Ni contamination has build up in the environment from mining and industrial activities (Pinto et al., 2004). Lead is one of the major pollutants in both terrestrial and aquatic ecosystems and numerous remediation researches focus on this pollutant (Paivoke, 2002). Beside natural wreathing process the main sources of lead pollution includes exhaust fumes from automobiles, chimneys of factories using Pb, effluents from storage batteries, industries, mining and smelting of Pb ores, metal planting and finishing operations, fertilizers, pesticides, and additives in pigments and gasoline (Eick *et al.*,1999).

Nickel resulting from anthropogenic activities i.e (industrial effluents , wastes, urban runoff, sewage treatment plant, boating activities , run off from agricultural fungicides, domestic garbage dumps and mining operations have detrimentally affected different ecosystems (Mcaforlance and Burchett, 2001).Toxic metal stress affects various physiological and biochemical processes through modifying many metabolic pathways (Dubey *et al.*, 2018; Fu and Xi, 2020).

Pb is not an essential element for plants but it can be easily absorbed and accumulated in different plant parts. Its exposure can occur through multiple pathways, through inhalation of air, water, soil or dust, as it is emitted in the environment through vehicles and automobiles. It can also enter in our food chain via plants (Wierzbicka and

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Antosiewiez, 1993). Lead at lower concentrations promote seedling growth but at higher concentration reduces growth and chlorophyll content, inhibits photosynthesis, upset mineral nutrient and water balance, changes hormonal and anatomical status and affects membrane structure and permeability. Either excessive lead accumulation can also cause mental retardation and behavioral disorder in human (Sharma and Rama, 2005).

Nickel (an essential element) that is toxic and possibly carcinogenic in high concentrations (ATSDR, 2003). Ni toxicity in humans usually results from repeated occupational exposure and causes dermatitis, asthma and headaches (Akeeson and Skerfing, 1985). However Ni toxicity in plants leads to Fe deficiency, foliar necrosis and also has adverse effect on plant metabolism (Hassan *et al*., 2019; Ameen *et al*.,2019).

Excess Pb and Ni in Soil cause adverse human health effects, Crops fatalities, and the disruption of the natural ecosystems.Several physicochemical techniques including stabilization (solubilization) in the soil, extraction, capping, in situ immobilization or ex situ vitrification and reclamation were tested for treating heavy metals contaminated sites. These techniques are rather expensive and could have toxic effects on biological and ecological system (Calgaro, 2020). Hence, biological treatment like phytoextraction could be as environmentally beneficial method to remediation of heavy metal contaminated soil (Sharma *et al*.,2023). Efficiency of phytoextraction is, however, reliant on mobility/bioavailability of heavy metals, heavy metal hyper accumulating plants, plant growth rate and biomass yield (Suman *et al*.,2018).

Indian mustard (fast growing crop) has been identified as a high biomass yielding crop with high heavy metal mobility/bioavailability through addition of EDTA (Huang *et al*.,2024). In this regard Raya belongs to family Brassicaceae is reported to be successful in partially removing heavy metals from soils contaminated by long term application of heavy metal ion containing sludge (Roy and Mondal, 2020).

Within this perspective, the morphological, Physio-chemical and anatomical characters of Raya (*Brassica juncea* L. Czern) plants are important. It is also important to study the structural changes in different plant parts to understand the overall process of phytoextraction. Hence in this study, we seek to assess the potential of Pb and Ni tolerance potential in two cultivars of Raya [*Brassica juncea* (L.) Czern], examine the morphogenic, biochemical, physiological and anatomical responses of two Raya varieties

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to Pb and Ni pollution, compare the Pb accumulating behavior of root and shoot of Raya plant and also investigate the effects of Pb on ionic distribution in Brassica plants.

Materials and Methods

1 Plant culture and phytoremediation experimental design

The soil used for the experimental purpose was taken from the experimental area of the Department of Botany, University of Agriculture Faisalabad Pakistan.

Chemical analysis of soil was carried out for electrical conductivity (EC) and pH according to the methods described in USDA salinity Lab Staff in Agriculture Hand Book No. 60 (1954). The soil used was sandy loam in texture and following characters of soil were determined i.e saturation percentage = 24.15%, Electrical conductivity of soil extract (ECe) = 3.78ms/cm and pH= 8.05.

Seeds of two raya cultivars i.e., Khanpur Raya and RL-18 were obtained from the oil seed section, Ayub Agriculture Research Institute (AARI), Faisalabad. Seeds were sown in plastic pots, each containing ten holes about 1 cm deep at equal distance. One seed was put in each hole which was then covered with a small amount of soil. Germination took place 7 days after sowing. Plants were thinned after 15 days of germination, to maintain five seedlings in each pot. The plants were irrigated with tap water according to their requirement.

Nickel (Ni^{2+}) and lead (Pb^{+2}) of Annular grade, were used in the form of $PbCl_2$ and $NiCl_2.6H_2O$ already available in the store of Botanical Department, University of Agriculture, Faisalabad. Experiment was carried out in the Botanical Garden, University of Agriculture, Faisalabad, during October 2005-2006 under natural conditions.

Metal treatments was applied per pot, supplied with solution of nickel chloride ($NiCl_2.H_2O$) was prepared by dissolving 1.29g (40 mg $Ni^{2+}kg^{-1}$) and 2.59g (80 mg Ni^{2+} kg^{-1}) of nickel chloride and solution of lead chloride ($PbCl_2$) was prepared by dissolving 0.429g (40mg Pb^{2+} kg^{-1}) and 0.858g (80 mg Pb^{2+} kg^{-1}) of lead chloride. Since samples were collected for microscopic study, the Pb and Ni treated groups were harvested 23 days after the start of metal treatment (varying in the levels of nickel and lead were applied)

Morphological Parameters

1. Plant Height (cm)

Three plants from each pot were randomly harvested and their roots and shoots were separated. Plant height from the stem- base to the tip of the youngest leaf of each plant

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was measured with the help of a meter rod in centimeters and the mean value of each replication was calculated.

2. Shoot Fresh Weight (g)/Plant

Shoots of the selected plants were carefully removed and dried by placing them in an oven at 75^{0}C for 72 hours. Shoots of each plant was weighed on electrical balance in grams.

3. Shoot Dry Weight(g)/Plant

Shoots of the selected plants were carefully removed and dried by placing them in an oven at 75^{0}C for 72 hours. Shoots of each plant was weighed on electrical balance in grams.

Physiochemical Parameters

Chlorophyll Concentration

The fresh leaves of 0.5g were chopped into small pieces and extracted with 80% acetone. The absorbance was measured at 645 nm and 663 nm for Chl.a and Chl.b and total chlorophyll respectively by using spectrophotometer (Hitach Model U 2001 Japan). The chlorophyll a chlorophyll b and total chlorophyll contents were determined according to Arnon when crop was 35 days old (Arnon, 1949).

Determination of Mineral Elements in Plant Tissues

Roots, shoots and leaves were analyzed for the determination of K^+, Ca^{2+}, N, P, Ni^{2+} and Pb^{2+} of 36 days old crop.The dried ground leaves, shoot and root material (0.1 g) was digested with sulphuric acid and hydrogen peroxide according tothe method of Wolf (1982). Total nitrogen contents of leaves, shoot and root were obtained by using Kjeldahl method (Bremner, 1965).

Lead and Nickel Determination

The dissolved Nickel and lead were determined through atomic absorption spectrophotometer (Hitachi AAS-Z-8200 with polarized Zemaan effect). A graded series of 10, 20, 30, 40 and 50 mg L^{-1} was run for the determination of standard curve.

Gas Exchange Parameters

These were measured when crop was 35 days old. Measurements were made on 2nd leaf from top of each plant using an open system LDA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddeson, England). Leaf surface area 6.25 cm^2, ambient CO_2 concentration(Cref) 290.1 µmol $mole^{-1}$, temperature of leaf chamber (Tch) varied from 41 to 43.8 ^{0}C, leaf chamber gas flow rate (V) 394 ml min^{-1}, leaf chamber gas

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rate (U) 256.66 µmol s^{-1}, ambient pressure (P) 98.9 k Pa, water vapor pressure (e ref) in to chamber ranged from 4.4 to 6.6 m bar, molar flow of air per unit leaf area (US) 410.6 mol m^{-2} S^{-1}. Net CO_2 assimilation rate (A) (µmol m^{-2} S^{-1}), Transpiration rate (E) (µmol m^{-2} S^{-1}), Stomatal conductance (g) (µmol m^{-2} S^{-1}), Sub stomatal CO_2 concentration (Ci) (µmol/mol), Water use efficiency (A/E) (µmol CO_2/µmol H_2O was also recorded.

Anatomical Characteristics

For the anatomy of leaf, root and stem, the material was fixed in formalin acetic alcohol (FAA) fixative for 36 hours and subsequently transferred to acetic alcohol solution for long term storage. Permanent slides of free hand cut sections were prepared by serial dehydration in ethane. Double staining procedure (Safranine and fast green) was used to study various cells tissues. Measurements were taken with a light microscope, using an ocular micrometer which was calibrated with stage micrometer. Following organs, tissues and cells at root, stem and leaf levels were studied. Area of the above studied characters was calculated by the following formula:

Area = Maximum length × Maximum breadth

Where = Constant (22/7)

Yield Components

Three plants from each treatment of a variety were randomly selected and tagged in the beginning of experiment for following yield parameters i.e Dry biomass (g) with an electrical balance,100 seed weight (g) (from each selected plant of a treatment)withan electrical balance and average was calculated, Seed yield $plant^{-1}$(g) by takingseeds from each plant treatment (after maturity) and then dried to constant weight in an oven at 75 ^{0}C for 48 hours (Average for each treatment was worked out), Total number of Number of siliqua plant was also counted from each replicate from the selected plants and mean was calculated.

Statistical Analysis

Data for various growths,physio-chemical, anatomical and yield components were statistically analyzed using two-way analysis of variance and means were compared using DMR test, by using COSTAT Computer Software.

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Results

Effect of Pb and Ni Accumulation on Plant Morphology and Anatomy

After 14 days of treatment with heavy metal (Pb & Ni), observed that treatment T1 (80 mg Ni kg-1 soil) showed relatively greater decrease in plant height, shoot fresh weight, shoot dry weight than T2 (80 mg Pb kg-1 soil). It reveals that nickel is more toxic as compared to lead. Chlorosis was visually observed during the treatment processat 80 mg Niand 80 mg Pb towards the endof the experiment for both varieties. There was gradual decrease in plant height with increasing level of nickel and lead in both varieties.

The anatomical responses of two Brassica varieties (V1 and V2) under different treatments (T1 and T2) revealed distinct varietal and treatment-dependent variations. Overall, treatment T1 promoted greater anatomical development in both varieties, with V2 consistently showing higher values across most parameters including root area, epidermal, cortical, pericycle, medullary ray, metaxylem, phloem, and pith cell areas, suggesting superior adaptability.

In contrast, treatment T2 caused a marked reduction in all anatomical traits of both varieties, reflecting cell shrinkage, impaired tissue expansion, and suppressed vascular differentiation. Between the two varieties, V2 displayed greater resilience, particularly under T1, where its cortical, vascular, and medullary tissues attained maximum cell areas compared to V1. These findings indicate that V2 possesses stronger anatomical plasticity and structural robustness, while T2 exerts a pronounced inhibitory effect on cellular and tissue growth in both Brassica varieties (Figure 1).

Effect of Pb and Ni Accumulation on Plant Physiology

Net CO_2 assimilation rate (A) stomatal conductance (g) transpiration rate (E) and sub stomatal conductance (Ci) were reduced by the application of nickel and lead in both varieties. All the above factors were severely reduced inV2(Raya RL-18) as compared toV1 (Khanpurraya). On the other hand, the water use efficiency A/E was consistently increased in both varieties by the application of nickel and lead.

The analysis of total chlorophyll concentrationsconfirmed that $V_2$was more sensitive to $PbCl_2$and $NiCl_2.6H_2O$ than V1. However,both varieties have slightly decreasing trend for chlorophyll “a”,chlorophyll “b” and total chlorophyll contents in response to increase in nickel and lead level.

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Data recording leaf and stem anatomical changes such as epidermis, cortex, metaxylem, phloem and parenchymatous cells relatively less evident in both varieties under nickel and lead application as compared to control. However, root anatomical changes, epidermis. Cortex pericycle was more pronounced under both, lead and nickel treatment in RL-18 as compare to Khanpur raya. Metals accumulations listed in Table were based on the dry weight basis of plant material. A comparison between variety means and treatment means showed that leaf lamina thickness recorded in V1 was 45.89 % greater than in V2. Hence V2 was severely effected by 80 mg Ni^{2+} kg^{-1} soil than that 80 mg Pb^{2+} kg^{-1} soil as compared to control (Figure 2).Stomata number and stomatal aperture was also found imperative anatomical features effected by metals accumulation in leaves of both varieties.

Table 1: Morphological and Physiological Analysis of both Brassica Verities at Different Treatments

	V1		V2	
	T1	**T2**	**T1**	**T2**
Morphological Parameters				
Plant Height (cm/day)	41.52	25.80	51.37	29.48
Shoot Fresh Weight (g/day)	35.54	22.81	43.08	28.24
Shoot Dry Weight(g/day)	36.51	20.20	43.29	25.61
Physiochemical Parameters				
Chl 'a' (mg/gFW)	19.23	5.76	22.45	6.12
Chl 'b' (mg/gFW)	20	10	33.3	13.33
Total Chl. Contents (mg/gFW)	22.2	6.94	27.86	8.19
Stomatal conductance ($mmolm^{-2}S^{-1}$)	61.90	23.81	72.22	27.77
Sub-stomatal conductance ($mmolm^{-2}S^{-1}$)	38.42	29.47	40.61	32.45
Stomata number (µm)	33.48	13.48	37.77	14.89
Stomatal aperture cell area	69.96	38.06	78.68	41.20
Assimilation rate ($\mu molm^{-2}S^{-1}$)	37.48	22.87	41.5	25.37
Transpiration rate	72.1	45.49	76.69	48.12

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(m molm^{-2}S^{-1})				
Water use efficiency (µmolCO_2/mmol H_2O)	56.34	28.80	60.94	31.48
Anatomical Characters				
Leaf Anatomy				
epidermal cell area(µm^2)	40.09	28.48	42.68	30.98
Parenchymatous cell area(µm^2)	55.36	23.37	64.88	29.47
Metaxylem cell area(µm^2)	67.05	37.69	71.13	48.67
phloem cell area(µm^2)	59.62	36.30	64.85	46.66
Lamina thickness (µm^2)	59.34	38.69	70.16	47.43
Stem Anatomy				
Stem area (mm^2)	50.10	21.51	54.51	32.87
Stem epidermal cell area (µm^2)	56.54	38.31	77.78	58.80
Stem cortical cell area(µm^2)	59.06	38.97	73.30	56.95
Stem cortical thickness (µm^2)	46.4	24.12	56.09	24.99
Number of vascular bundles (µm^2)	45.10	20.59	49.25	27.06
Stem metaxylem cell area (µm^2)	48.98	26.10	51.14	32.31
Stem phloem cell area (µm^2)	50.65	22.08	57.61	30.89
Stem parenchymatous cell area(µm^2)	55.85	42.55	60.01	46.79
Root Anatomy				
Root area (mm^2)	39.74	26.19	53.27	33.46
epidermal cell area (µm^2)	67.65	27.92	70.19	51.13
cortical cell area (µm^2)	44.69	33.39	57.44	39.02
pericycle cell area (µm^2)	57.29	23.37	67.66	39.63
medullary ray cell area (µm^2)	60.42	30.72	75.53	41.05
metaxylem cell area (µm^2)	43.65	28.40	51.43	30.95
phloem cell area(µm^2)	49.44	29.61	53.58	35.75
pith cell area (µm^2)	57.21	29.32	64.29	33.48

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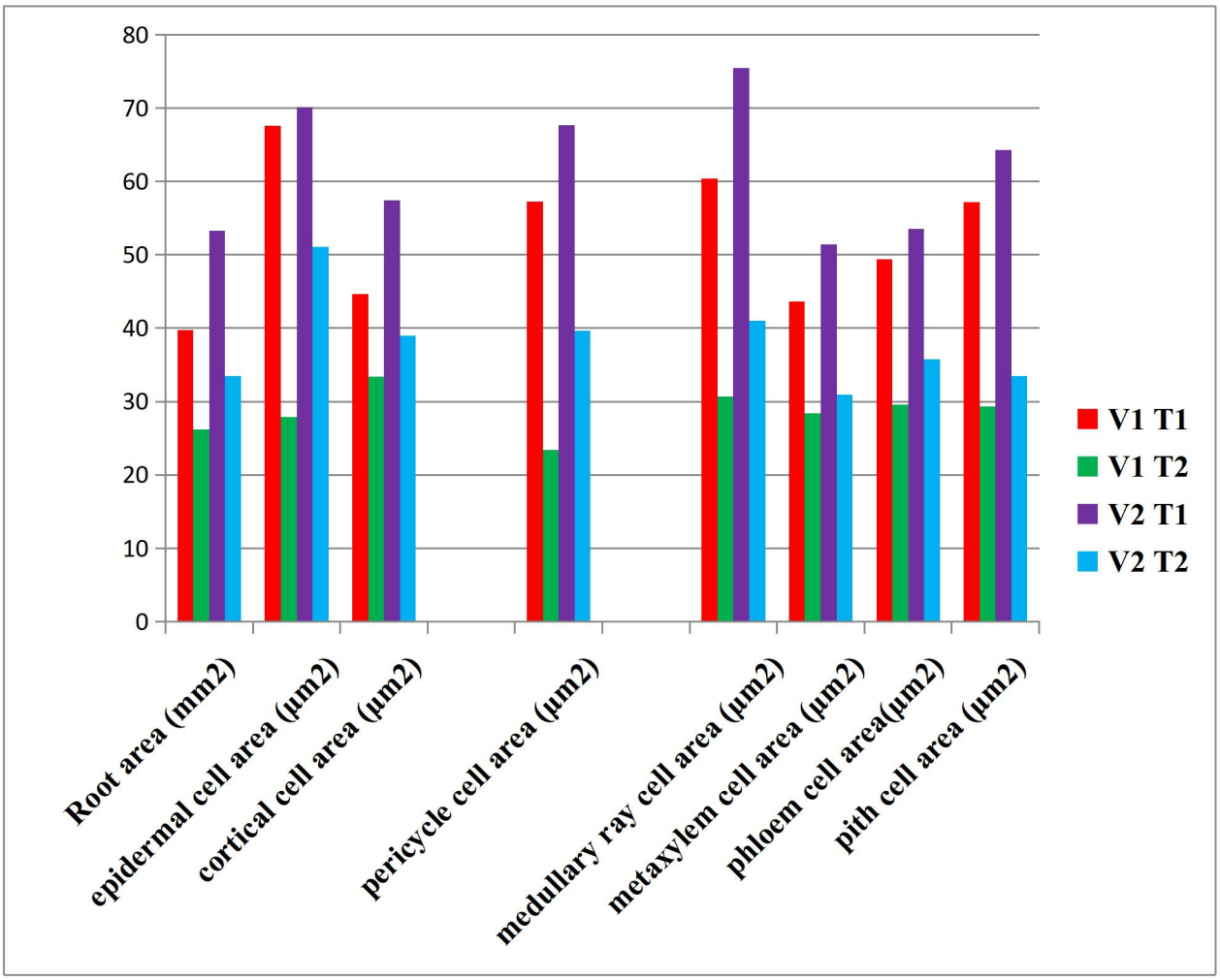


Figure 1: Effect of Pb and Ni Accumulation on Plant Morphology and Anatomy

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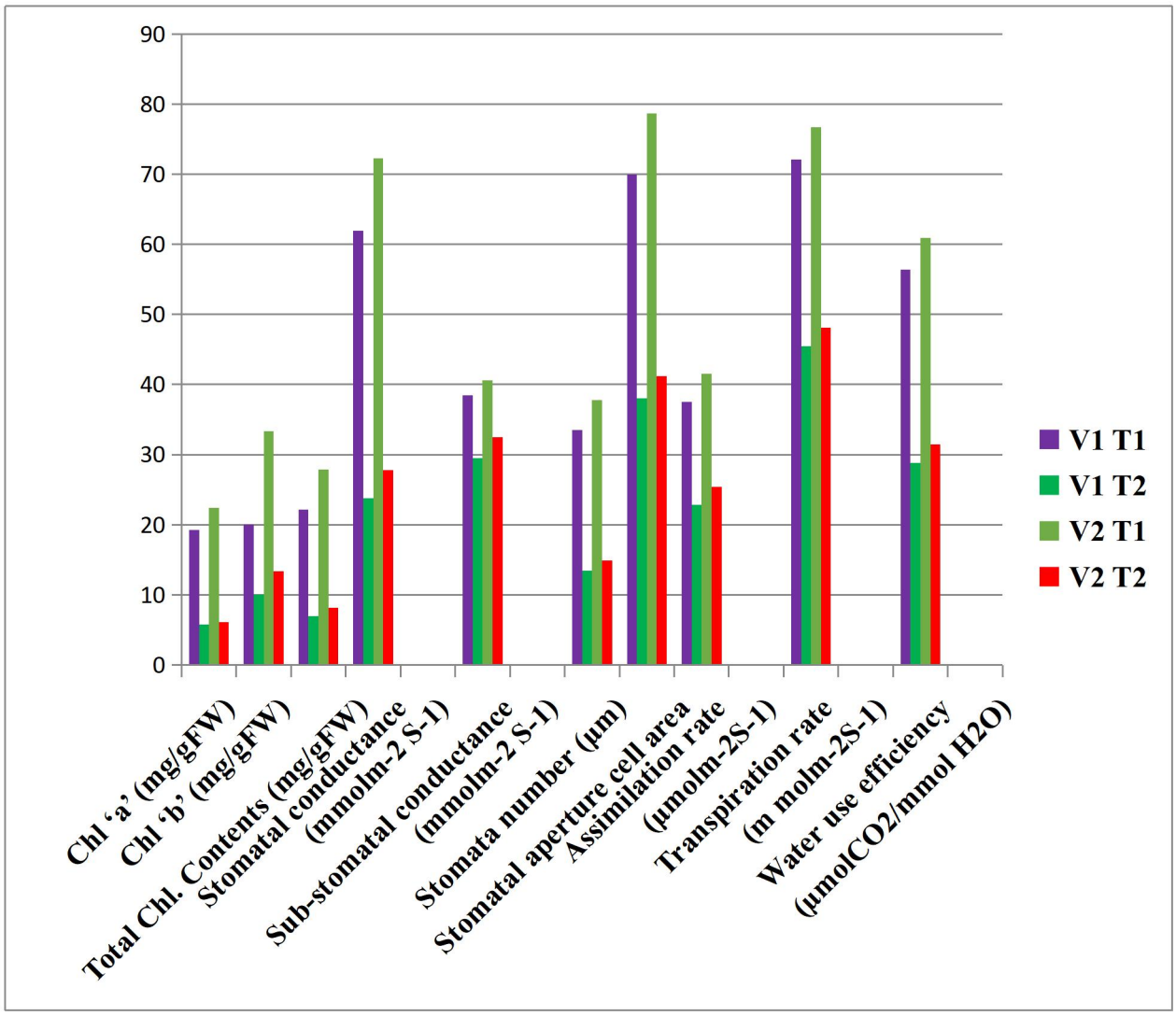


Figure 2: Effect of Pb and Ni Accumulation on Plant Physiology

Effect of Pb and Ni on Mineral Elements in Plant Tissues

After nickel and lead treatment, all the (K, Ca, N, P) nutrient decreased (Table 2). This effect becomes more evident with increasing external Pb^{2+} and Ni^{2+} concentration. The accumulation of K^{+}, Ca^{+2}, P and N was greater in leaves followed by stem and roots. In this study, V1(Khanpurraya) showed for bearing behavior as compared to V2(RL-18).

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Table 2: Different Mineral Analysis of both Brassica Varities at Different Treatments

Varieties	Treatments	K^+ Contents (mg/g) % decrease			Ca^{2+} Contents (mg/g) % decrease		
		leaves	Stem	Roots	leaves	Stem	Roots
V1	T1	24.45	21.61	36.65	37.14	59.74	67.82
	T2	6.29	21.61	10.62	7.74	23.63	30.61
V2	T1	47.08	43.45	47.29	40.59	62.96	70.89
	T2	9.15	8.58	11.96	11.19	26.16	33.61
Varieties	**Treatments**	**(N) Contents (mg/g) % decrease**			**(P) Contents (mg/g) % decrease**		
		leaves	Stem	Roots	leaves	Stem	Roots
V1	T1	37.8	35.87	38.72	43.53	44.25	43.06
	T2	7.59	16.42	21.27	15.54	17.3	9.32
V2	T1	41.68	41.52	39.74	46.54	46.63	47.06
	T2	10.15	19.35	22.65	19.94	19.25	13.35

Effect of Pb and Ni on Yield Components

Variety V1 surpassed variety V2 in forming biomass. A comparison between varieties means showed that plant biomass (15.58%), number of Siliqua/Plant (13.69%) and yield/plant (30.71%) was increased in V1 over V2 (after heavy metals treatment). While comparison among treatment means indicated that nickel caused more toxic effect and inhibited number of siliqua/plant, plant biomass and yield/plant over control. However, Variety V1 formed slightly heavier seeds than V2.The 100-seeds weight observed under nickel treatment was 0.269g with respect to 0.329g observed under lead application.

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Table 3: Plant Biomass Analysis of both Brassica Varities at Different Treatments

Varieties	Treatments	Plant Biomass (g) % decrease	Numberof Siliqua/Plant. % decrease	100 seeds weight (g) % decrease	Seed yield/plant % decrease
V1	T1	46.03	49.44	31.12	58.01
	T2	11.05	17.88	16.99	31.69
V2	T1	52.71	57.39	39.26	67.06
	T2	16.73	28.4	22.9	37.83

Discussion

Heavy metals strongly influence growth and tissue ionic concentration (Bharti and Sharma, 2022). With increase in heavy metals concentration, plants undergo a depression in visual growth and development. Dry weight shoot and root show significant reduction with increase in heavy metals stress (Bharti and Sharma, 2022).

In the present study, both Brassica varieties showed differential effects of metals (lead and nickel) stresses on relative increase in plant height, fresh and dry weights was significantly reduced. Lead stress was less damaging followed by nickel, which caused the most adverse effect onmorphological, physiochemical, anatomical and yield parameters. It was noticeable fresh and dry weights were better under control than under metal stress. This is probably due to the reason that toxicity of heavy metals significantly inhibited root vitality, preventing plant from absorbing inorganic nutrients and leading to decrease in plant growth (Angon *et al.*,2024). Plant growth inhibition by nickel and lead results from metabolic disorder and immediate inhibition of cell division (Khan *et al.*,2025).

Both varieties displaying a minimal reduction under lead stress as compared to nickel, however, nickel proved most detrimental to chlorophyll species (particularly, chlorophyll b) (Table 1). Which characteristically give rise to chlorosis because these metals inhibit the translocation of Mg and Fe from root to shoot. Iron and magnesium required for the biosynthesis of chlorophyll resulting in decreased chlorophyll concentration (Li *et al.*,2021).

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Among the various gas exchange parameters, net rate of photosynthesis(pn), transpiration rate (E), water use efficiency (WUE), stomatal conductance(gs) and substomatal CO_2 concentration(ci) are of great significant in the exhibition of dry matter and grain yields under Ni and Pb stress. The diminished rate of photosynthesis is related to disrupted chloroplast structure, blocked chlorophyll synthesis, disordered electron and inhibited activities of the Calvin cycle enzymes and CO_2 deficit caused by stomatal closure (Hameed*et al.*, 2023). High concentration of Ni and Pb in the soil environment causes imbalance of mineral nutrients in growing plants. Significant changes in nutrient contents as well as internal ratios of nutrients occur in plants under metal toxicity (Feng et al., 2023).

In the present study, Ni and Pb significantly decreased the uptake of K, Ca, N and P. Availability of nutrients in soil determines ionic composition of plants. Both heavy metal treatments indicated reduction in K^+ and Ca^{+2} in different plant parts. All macronutrients (N, P, K and Ca) are essentially required for the activities of enzymes, protein synthesis, integrity of cell wall and plasma membrane, and as component proteins, photosynthetic protein complexes, photosynthetic pigments, RNA and DNA (Dalal, 2021). Analysis of these macronutrients clearly indicated that metal stress decreased the shoot and root P, N, K^+ Ca^{+2}. However, N and P contents got reduced due to lead and nickel application (Table 2).

In the present study stem and leaf anatomy indicated that metal stress decreased the stem area, epidermal, cortical, metaxylem phloem cell area and vascular number (Table1). Similarly, leaf epidermal, parenchymatous, metaxylem, phloem cell area, lamina thickness, stomata number, stomatal aperture cell size were also decreased.

The results of our experiment revealed that application of lead and nickel in soil adversely affected the number of Siliqua/Plant, seed yield/plant, 100 seeds weight, dry biomass presented in (Table 3). These findings are supported by (Raihan *et al.*, 2022) who investigated that grain yield of wheat significantly decreased by heavy metals, *i.e.*, Pb and Ni. Same results are observed by Wu *et al.*,2023). This is probably due to reason that metal pollution may decrease the yield and its components by creating nutritional deficiency (NPK) at the stages of flower initiation, floral development and fruit setting or may be due to low rate of photosynthesis or high transpiration rate.

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Conclusion

Our results indicated that, both cultivars of Raya [*Brassica juncea* (L.) Czern] are generally sensitive to heavy metals (Pb and Ni) stress and this effect is evident on the biochemical, gas exchange, anatomical and yield attributes. Among heavy metals stress, lead was the less and nickel was the more damaging, although the effects of these stresses were individualistic. Less WUE of V_1 (Khanpurraya) than V_2 (RL-18) and other results indicated that V1 is more efficient to extract heavy metals from contaminated soil than V2.Therefore, Khanpurraya could be considered as promising crop for high yield (even under stress) and heavy metals phyto-extraction.

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