



EFFECT OF DIFFERENT LEAD CONCENTRATIONS ON GROWTH AND HEAVY METAL ACCUMULATION IN RADISH PLANT (*RAPHANUS SATIVUS* L.)

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ABSTRACT

This investigation evaluated the ecotoxicological impacts of escalating soil lead (Pb) concentrations on the morphological, physiological, biomass, and bioaccumulative attributes of radish (*Raphanus sativus* L.). A completely randomized design (CRD) was executed under controlled glasshouse conditions comprising five soil treatments of lead nitrate [Pb(NO₃)₂]: control (0 mg/kg), 50, 100, 200, and 400 mg/kg dry soil, replicated five times. Substrate lead exposure incited significant ($P < 0.05$), concentration-dependent suppressions in all vegetative and subterranean growth parameters. Relative to controls, the 400 mg/kg regime triggered dramatic decrements in shoot length (52.4%), taproot length (57.3%), and storage root diameter (61.2%). Biomass accumulation suffered concordant losses, with shoot and root dry weights diminishing by 63.3% and 64.3%, respectively. Physiologically, lead phytotoxicity dismantled photosynthetic capacity, inducing severe losses of chlorophyll a (55.8%), chlorophyll b (61.8%), and total carotenoids (55.1%). Conversely, cellular distress was marked by a 284% surge in free proline and a 258% escalation in electrolyte leakage, revealing profound membrane peroxidative injury. Metal partitioning was characterized by radical root compartmentalization (268.4 mg/kg dry weight at 400 mg/kg soil) over aerial foliar tissues (51.2 mg/kg dry weight). Translocation factor (TF) values consistently remained below unity ($TF < 0.28$), denoting strong subterranean sequestration and highlighting major food security hazards associated with consuming root crops grown in lead-polluted lands.

Keywords: Lead; Radish (*Raphanus sativus*); Bioaccumulation; Oxidative stress; Translocation factor.

1 INTRODUCTION

The non-essential and harmful trace levels of metals in pedospheric environment has become the most critical ecological and agricultural issue, which negatively affects the productivity of the crops, equilibrium of ecosystems and biosecurity of food chain [1]. The intensive industrial growth, smelting of metal, fossil fuel combustion by vehicles, uncontrolled dumping of municipal solid waste, repeated use of low-quality fertilizers (phosphatic), and irrigating for a long period with untreated wastewater have gradually increased the potential load of hazardous elements in productive soils [2]. Lead (Pb) is considered as a toxic contaminant of high priority in the context of inorganic

environmental toxins because of its high chemical stability, lack of biological degradation and high phytotoxicity pathway throughout agroecosystems [3]. Due to the wide dispersion of heavy metals in the soil caused by biogeochemical processes within and around mining and industrial areas, there have been instances of multi-elemental soil hazards, which have had a significant impact on soil fertility and potential human health risk through dietary transfer [4].

Arable soil ecosystems are primary sinks for atmospheric deposition and anthropogenic pollutant discharges and often have surface soil concentrations of trace metals that greatly exceed regulatory limits for safe concentrations [5]. Bioavailable lead ions when dissolved in the interstitial water of the soil directly contact the root cell borders, thus causing rapid phytotoxic disruptions [6]. The ability to detoxify the mobile heavy metal ions is only present in a few metallophytes, while most traditional agricultural varieties are highly sensitive to micromolar concentrations of mobile heavy metal ions [7]. Intensified monitoring, elucidation of mechanisms and sustainable remediation technologies are needed to maintain arable land and avoid public health problems that can be caused by the build-up of toxic trace elements in agricultural landscapes [8]. The establishment of efficient bio countermeasures and screening the crop varieties for their ability to accumulate metals are indispensable measures in green agro-environmental technology [9].

Substrate lead has a significant inhibitory effect on the germination of the seeds, vigour of the seedlings and elongation of primary axis by disrupting the fundamental mechanism associated with cell functioning [10]. The soluble lead cations are absorbed to the root epidermal tissues mostly through passive apoplastic flow and secondary active transport, in competition with the essential divalent cations calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}) and zinc (Zn^{2+}) leading to the loss of the homeostatic mineral balances [11]. Once in the plasma membrane, lead will bind avidly to nucleophilic cellular targets with the main binding sites being carboxyl, hydroxyl and sulfhydryl of structural and functional proteins. Such non-selective coordination results in changes in the quaternary structure of the protein, in the inactivation of important metabolic enzymes and in the extensive disorganisation of the cytoskeleton, that slows down the kinetics of the spindle fibers and delays the onset of mitosis in root and shoot meristems [6].

At the same time, the photosynthetic apparatus is strongly affected by heavy metal toxicity as well, which involves changes in chloroplastic ultrastructure, disintegration of the stacking of thylakoid membranes and a decrease in light-harvesting efficiency [12]. When taken up by plants, lead ion displaces the central magnesium ion of tetrapyrrole porphyrin, a process that affects the chlorophyll molecules, and chelates and inhibits essential biosynthetic enzymes, such as delta-aminolevulinic acid dehydratase and protochlorophyllide reductase [10]. These interruptions lead to a reduction in photon absorption, a slowing of the photosynthetic electron flow through Photosystem II (PSII), and an induction of genotoxic stress in plants' nuclei [13]. This results in a blockage of energy use, separating the photosynthetic electron transport chains from the terminal electron acceptors, leading to the overproduction of the reactive oxygen species (ROS) inside the cells such as singlet oxygen, superoxide radicals, hydroxyl radicals and hydrogen peroxide [14].

The uncontrolled excessive production of ROS causes a lipid peroxidation attack of membrane bilayers, attacks against structural nucleic acids, and a leaky and toxic genome in the whole cell [15]. Plants respond to such oxidative stress by making complex changes in their metabolism, with the speedy production and build-up of low molecular weight osmoprotectants, mainly free proline [14]. Elucidation of intracellular role of proline revealed that it plays important function as a compatible osmolyte maintaining turgidity of cells, as efficient molecular chaperone, as temporary depot of reducing equivalents and as a chemical reagent of destructive hydroxyl radicals protecting fragile macromolecules from denaturation by metals [11].

The radish (*Raphanus sativus* L.) is a highly economically significant member of Brassicaceae family, which is widely cultivated in various climatic regions due to its short maturation period, nutritional value and large, crisp, enlarged storage hypocotyl/taproot complex [2]. The Brassicaceae are a group of plants that have been genetically engineered to accumulate high levels of metals, and they are known to have strong capacities for metal uptake, making them good candidates for studies of metal uptake and phytoremediation [3,9]. The radish has a direct pathway for the entry of toxic trace elements into human diets as it forms an edible storage organ in direct physical and chemical contact with the contaminated soil substrates [5]. Ingestion of lead through contaminated root vegetables can cause chronic human health problems such as systemic nephrotoxicity, neurocognitive decline, cardiovascular damage and disruption of heme synthesis [4].

Although considerable advances have been made in the understanding of plant-metal interactions, the morphological limits, oxidative state and internal partitioning of a metal that occur when plants are exposed to sharp gradients of internal lead exposure in the case of *Raphanus sativus* have not yet been properly quantified. Understanding if radish is an excluder or an accumulator plant under different lead pressures and/or root volumes is essential for developing agronomic management practices and determining soil limits for both the plant and the diet, as well as protecting dietary health. For this reason, it was decided that the following aims of the study would be set:

- To characterize the morphometric and biomass responses of *Raphanus sativus* exposed to increasing concentrations of substrate lead nitrate (0 to 400 mg/kg soil).
- To quantify stress-induced alterations in photosynthetic pigments, membrane structural integrity (electrolyte leakage), and osmoprotective free proline accumulation.
- To determine the tissue-specific distribution of lead between subterranean and aerial organs, calculating bioaccumulation factors (BAF) and translocation factors (TF) to delineate the ecophysiological profile of radish under heavy metal stress.

2 MATERIALS AND METHODS

2.1 Experimental Environment and Plant Material

Pot experiments were carried out in the programmatic environmental regulation in an environmentally controlled research greenhouse. The daytime ambient temperature was kept between 23 ± 2 °C and night time temperature was kept between 17 ± 2 °C with a relative humidity of 60% to 70%. A high pressure sodium lamp was used to provide a 14 hour photoperiod in addition to the natural photoperiod, which resulted in an intensity of 350 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ photosynthetically active radiation (PAR).

Earl's-type winter) and turnip (*T. napus* L. cv.-turnip) were sown by hand into the soil on April 24, 1999, and April 25, 1999, respectively. All Baladi and other certified agricultural suppliers were tapped for these. Surface-sterilization of the intact seeds of uniform sizes and weight was performed, by immersing them in 2% (v/v) sodium hypochlorite (NaOCl) solution for 5 minutes with gentle agitation during the process, in order to remove seed-borne pathogens. The seeds were then washed 5 times with DDD water to wash the disinfectant off and blotted dry on sterile Whatman No 1 filter paper before sowing.

2.2 Soil Characterization and Experimental Treatments

A sandy loam soil from the surface layer of the 0 to 20 cm of an uncontaminated field with no previous history of industrial sludge deposition or heavy use of metal agrochemicals was used. Macroscopic debris, plant roots and stones were removed by air drying the soil at ambient room temperature, then pulverizing and sieving the soil through a 2.0 mm stainless-steel mesh. The physical and chemical properties were measured following the soil analyses procedure.

Table 1: Physico-chemical properties of the experimental soil prior to treatment.

Soil Property	Unit	Value
Textural Class	-	Sandy loam
Sand	%	62.40
Silt	%	21.30
Clay	%	16.30
pH (1:2.5 H ₂ O)	-	7.35
Electrical Conductivity (EC 1:2.5)	dS/m	1.18
Soil Organic Matter	%	1.42
Cation Exchange Capacity (CEC)	cmol(+)/kg	14.80
Available Nitrogen (N)	mg/kg	38.50
Available Phosphorus (P)	mg/kg	14.20
Exchangeable Potassium (K)	mg/kg	145.00
Baseline Lead (Pb) Content	mg/kg	4.82

The soil pH and EC were determined by electrometric measurement in 1:2.5 (w/v) soil-to-water ratio with digital pH and EC meters. The soil mechanical analysis was carried out by the hydrometer method and found the soil texture to be sandy loam. Walkley-Black wet oxidation method was used to determine soil organic matter (SOM) content. The cation exchange capacity (CEC) was estimated by the neutral 1.0 M displacement of NH₄⁺ AC. The alkaline permanganate distillation method was used to determine available nitrogen, the extraction with 0.5 M NaHCO₃ (Olsen method) was used to determine available phosphorus and flame photometry was used to determine exchangeable potassium. Total

lead was measured by flame atomic absorption spectrometry after aqua regia digestion using microwave. The physical and chemical characteristics of the experimental soil are presented in Table 1.

Substrate lead contamination was established using analytical-grade lead nitrate [Pb(NO₃)₂, purity $\geq$ 99.0%, Sigma-Aldrich]. Calculated quantities of Pb(NO₃)₂ were completely dissolved in deionized water and incrementally atomized onto thin, uniform layers of air-dried soil to produce five nominal lead levels: 0 (unspiked control), 50, 100, 200, and 400 mg Pb/kg dry soil. Moisture content was adjusted to approximately 60% of the soil water holding capacity. To establish chemical equilibrium and simulate natural chemical sorption, precipitation, and complexation of Pb²⁺ across clay-humic surfaces, the spiked soils were aged and incubated in the greenhouse for 30 days under periodic gravimetric moisture adjustments with deionized water.

2.3 Experimental Design and Agronomic Management

The trial was organized according to a Completely Randomized Design (CRD) comprising five lead treatments, each replicated five times, generating 25 experimental units. High-density polyethylene pots (height: 20 cm, diameter: 18 cm) were lined with inert polyethylene sheets to prevent solute leaching and filled with 4.0 kg of the prepared soil.

Table 2: Experimental design and lead treatment specifications.

Treatment Code	Target Pb (mg/kg soil)	Added Pb(NO ₃) ₂ (mg/kg soil)	Number of Replicates	Total Plants Harvested
T0 (Control)	0	0.00	5	10
T1	50	79.93	5	10
T2	100	159.86	5	10
T3	200	319.72	5	10
T4	400	639.44	5	10

The seeds were surface-sterilized and then planted individually 6 seeds/pot at 1.5 cm of the soil surface. After 7 days after germination (DAG) the plants were manually thinned to 2 uniform and vigorous seedlings/pot. The soil moisture was gravimetrically kept at 65-70% of maximum field capacity for 45 days of cultivation, adding daily water losses due to transpiration and evaporation to the soil with deionized water. Chemical fertilizers and synthetic pesticides were not used throughout the growing cycle to prevent the introduction of competing complexing agents and counter-ions.

2.4 Morphological Growth and Biomass Measurements

The plants were cut 45 days after sowing, which corresponds to commercial maturity of the taproot. Care was taken to carefully remove the whole plants from the pots to maintain the delicate lateral root system. Root systems were removed from soil in the adhering rhizosphere after washing with a gentle stream of running tap water and then three times with cold deionized water. Cleaned plants were then blotted dry with a lint-free laboratory towel and divided at the point where the taproot or hypocotyl emerged from the soil and the shoot from the seedling to give the two parts of the plant: underground (taproot/hypocotyl) and aboveground (shoots and leaves).

The length of shoots was determined from the base of the hypocotyl to the tip of the longest leaf blade with the help of a precision ruler to within $\pm$ 0.1 cm. The length of the primary root was determined from the crown to the distal root tip. A Vernier electronic digital caliper ($\pm$ 0.01 mm) was used to measure the diameter of the storage roots at the widest transverse diameter at the base of the hypocotyl. The number of leaves per plant was counted by hand and the total surface area of the leaves per plant was measured with an area meter (LI-COR LI-3100C).

Shoots and roots fresh biomass was measured on analytical balance ($\pm$ 0.001 g). Dry biomass yields were quantified by placing separated tissues in perforated paper envelopes which were desiccated in a forced-air convection oven at 70 °C until the tissues were dry and weights were stable (72 hours).

2.5 Physiological and Biochemical Analyses

Chill acetone (80% v/v) was used for the extraction and quantification of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. Leaves were fully developed, collected at the median nodes and homogenized in 10 mL of 80% aqueous acetone with 0.1 g of calcium carbonate to neutralize the plant acids (0.5 g leaf). Homogenate was centrifuged for 10 minutes at 4 °C, 4000 x g. The clear supernatant was taken out and transferred to volumetric flasks and made up to 25 mL with 80% acetone. The optical densities were measured in UV-Vis spectrophotometer (Shimadzu UV-1800) using

80 % acetone as a blank at 663, 645 and 470 nm. The amount of pigments (mg/g fresh weight) was obtained from the Lichtenthaler and Wellburn equations:

- Chlorophyll a = $[12.7 \times A_{663} - 2.69 \times A_{645}] \times V / (1000 \times W)$
- Chlorophyll b = $[22.9 \times A_{645} - 4.68 \times A_{663}] \times V / (1000 \times W)$
- Total Chlorophyll = Chlorophyll a + Chlorophyll b
- Carotenoids = $[1000 \times A_{470} - 1.82 \times \text{Chl a} - 85.02 \times \text{Chl b}] / 198 \times V / (1000 \times W)$

where V represents final extract volume (mL), and W is the fresh leaf tissue mass (g).

Electrolyte leakage (EL) was used to measure cellular functional integrity of the cell membrane. Fresh leaf blades were punched into uniform leaf discs (8 mm diameter), washed in deionized water for a few seconds to wash away solutes from cut margins and then blotted dry. Discs were each placed in a test tube, and 20 mL of ultra-pure deionized water ($EC \sim 0.05 \mu\text{S}/\text{cm}$) was added to the tubes. Tubes were placed in water bath at 32 °C for 2 hours and the EC of the bathing medium was measured in the initial stage (EC1) on a digital conductivity meter. Tubes were then boiled in water for 30 minutes at 100 degrees celsius which resulted in complete removal of the membranes and discharge of all the solutes from the tubes. After cooling to 25 °C, the final electrical conductivity (EC2) was measured. The electrolyte leakage was determined by the following equation:

- $EL (\%) = (EC1 / EC2) \times 100$

The amount of free proline was determined by the acid ninhydrin colorimetric method. Leaf material (0.5g) was ground in 10mL of 3% (W/V) aqueous sulfosalicylic acid and centrifuged for 10 minutes at 5,000 x g. The clear filtrate (2 mL) was mixed with 2 mL of freshly made up acid ninhydrin reagent (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M orthophosphoric acid) and 2 mL of glacial acetic acid in glass test tubes. The reaction mixture was then heated up to 100 °C in a boiling water bath for 60 minutes. The reaction was stopped by placing the tubes into an ice-bath. After cooling, 4.0 mL of the analytical grade toluene was added to each tube and the tubes were then vigorously vortexed for 30 seconds. The toluene phase (with chromophore) was aspirated, and absorbance was determined at 520 nm using a toluene blank. The concentration of proline ($\mu\text{mol}/\text{g}$ fresh weight) was determined by drawing conclusions from a standard curve plotted from a known concentration of L-proline.

2.6 Lead Measurement and bioaccumulation Indices

The root and shoot tissues were oven dried, ground in an electric ball mill and then passed through a 0.5 mm sieve. The amount of 0.500 g of dried sample was taken in acid-washed polytetrafluoroethylene (PTFE) vessels and digested with an acid mixture of concentrated nitric acid (HNO_3 , 65%) and perchloric acid (HClO_4 , 70%) in the proportion of 4:1 (v/v). The digestion vessels were pre-digested cold over a 12 hour period, then digestion was completed on a programmable hot plate block followed by an increase in temperature from 80 °C to 180 °C until the digestion was complete and a clear, colorless digest was obtained, at which time dense white perchloric fumes would no longer be produced.

After cooling, the digested solutions were filtered through ashless Whatman No. 42 filter paper and brought to an exact volume of 50 mL in volumetric flasks using 1% (v/v) HNO_3 . Total lead concentrations were quantified using a Flame Atomic Absorption Spectrophotometer (FAAS, Shimadzu AA-7000). Analytical quality control was maintained using standard reference materials, procedural blanks, and periodic checks with certified multi-element calibration standards.

To evaluate lead absorption and internal transport efficiency, the Bioaccumulation Factor for roots (BAFroot), Bioaccumulation Factor for shoots (BAFshoot), and Translocation Factor (TF) were calculated as follows:

- $BAF_{\text{root}} = \frac{[Pb]_{\text{root}}}{[Pb]_{\text{soil}}}$
- $BAF_{\text{shoot}} = \frac{[Pb]_{\text{shoot}}}{[Pb]_{\text{soil}}}$
- $TF = \frac{[Pb]_{\text{shoot}}}{[Pb]_{\text{root}}}$

where [Pb]root and [Pb]shoot represent lead concentrations in dry plant organs (mg/kg DW), and [Pb]soil is the lead concentration in the corresponding soil treatment (mg/kg).

2.7 Statistical Analysis

The normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) were evaluated of data. The SPSS software (Version 26.0) was used to conduct One-way Analysis of Variance (ANOVA). The differences between the treatment means were determined by Tukey's Honest Significant Difference (HSD) test at 5% significance. Five biological replicates ($n = 5$) were performed independently and results are shown as the arithmetic mean $\pm$ SE.

3 RESULTS

3.1 Morphological Growth Attributes

The increases in soil lead concentrations caused significant ($P < 0.05$) decreases in all the morphological growth traits for the radish plants (Table 3). Shoot length exhibited an inverse relationship with substrate lead levels, decreasing from 26.82 cm in the control (T0) to 23.44 cm at 50 mg/kg (T1), 19.16 cm at 100 mg/kg (T2), 15.30 cm at 200 mg/kg (T3), and reaching 12.76 cm at 400 mg/kg (T4), representing an overall 52.4% reduction. Primary taproot elongation was also limited accordingly, which decreased from 18.64 cm in the control to 7.96 cm in the 400 mg/kg, a 57.3% decrease.

Tuberization and storage root radial expansion were the most sensitive to lead stress. Storage root diameter fell from 28.42 mm in control plants to 22.18 mm in T1, 16.54 mm in T2, 13.10 mm in T3, and reached a minimum of 11.02 mm in T4, an overall 61.2% decrease. This was followed by a decrease in foliar architecture with an average number of leaves per plant from 9.40 for controls to 5.20 for T4 (a 44.7% reduction). In the unspiked treatment, the CSA was 248.60 cm², but it decreased by over a third (64.4%) in the 400 mg/kg lead treatment to 88.40 cm². Symptoms of lead toxicity such as foliar stunting, marginal chlorosis, leaf curling and necrotic leaf margins became visible with growth at 200 and 400 mg/kg lead.

Table 3: Morphometric parameters of radish plants grown under different soil lead levels.

Treatment Code (mg/kg)	Shoot Length (cm)	Taproot Length (cm)	Storage Root Diameter (mm)	Leaf Number (per plant)	Leaf Surface Area (cm ² /plant)
T0 (0)	26.82 $\pm$ 0.68 a	18.64 $\pm$ 0.52 a	28.42 $\pm$ 0.74 a	9.40 $\pm$ 0.40 a	248.60 $\pm$ 6.12 a
T1 (50)	23.44 $\pm$ 0.55 b	15.32 $\pm$ 0.44 b	22.18 $\pm$ 0.61 b	8.20 $\pm$ 0.37 b	204.30 $\pm$ 5.40 b
T2 (100)	19.16 $\pm$ 0.48 c	12.80 $\pm$ 0.39 c	16.54 $\pm$ 0.53 c	7.00 $\pm$ 0.32 c	156.80 $\pm$ 4.25 c
T3 (200)	15.30 $\pm$ 0.42 d	10.14 $\pm$ 0.35 d	13.10 $\pm$ 0.48 d	6.00 $\pm$ 0.32 cd	118.50 $\pm$ 3.80 d
T4 (400)	12.76 $\pm$ 0.36 e	7.96 $\pm$ 0.31 e	11.02 $\pm$ 0.39 e	5.20 $\pm$ 0.20 d	88.40 $\pm$ 3.15 e

Values represent the mean $\pm$ SE ($n = 5$). Distinct letters within a column designate statistically significant differences according to Tukey's HSD test ($P < 0.05$).

3.2 Biomass Production and Partitioning

Reduction in biomass was consistent with reduction in morphological traits and was seen as a gradual dose-dependent reduction in fresh mass and dry mass (Table 4). Shoot fresh weight decreased from 39.44 g/plant in the control to 31.42 g in T1 (a 20.3% loss) and fell further to 24.18 g, 18.62 g, and 13.75 g in T2, T3, and T4, respectively, an overall 65.1% decline. A similar reduction was observed in root fresh weight from an average of 45.12 g/plant in control plants to 15.84 g/plant at 400 mg/kg lead, which is a biomass loss of 64.9%.

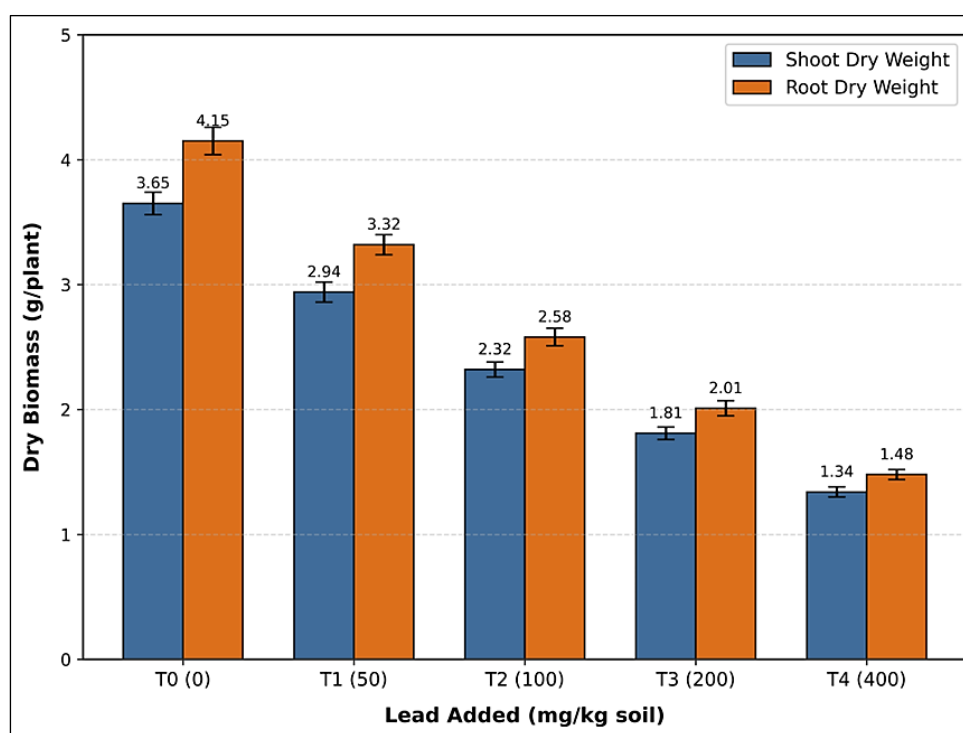
This downward trend continued for tissue dry matter production. The loss in dry weight of shoots in the T4 treatment was 63.3% (from 3.65 g/plant in controls to 1.34 g/plant). The reduction in storage organ dry weight in the subterranean parts was 64.3% when the highest level of lead was applied, which was 4.15 g/plant under control conditions to 1.48 g/plant. The dry biomass/fresh biomass ratio remained fairly constant (9.22% to 9.65 %) suggesting that growth inhibition was a co-ordinated inhibition of water uptake as well as of dry matter assimilation.

Table 4: Biomass yield of radish plants exposed to increasing concentrations of lead.

Treatment Code (mg/kg)	Shoot Fresh Weight (g/plant)	Root Fresh Weight (g/plant)	Shoot Dry Weight (g/plant)	Root Dry Weight (g/plant)	Total Dry/Fresh Ratio (%)
T0 (0)	39.44 ± 0.85 a	45.12 ± 0.98 a	3.65 ± 0.09 a	4.15 ± 0.11 a	9.22 ± 0.14 c
T1 (50)	31.42 ± 0.72 b	35.80 ± 0.81 b	2.94 ± 0.08 b	3.32 ± 0.08 b	9.31 ± 0.12 c
T2 (100)	24.18 ± 0.63 c	27.35 ± 0.69 c	2.32 ± 0.06 c	2.58 ± 0.07 c	9.51 ± 0.15 bc
T3 (200)	18.62 ± 0.51 d	20.94 ± 0.58 d	1.81 ± 0.05 d	2.01 ± 0.06 d	9.65 ± 0.18 ab
T4 (400)	13.75 ± 0.44 e	15.84 ± 0.46 e	1.34 ± 0.04 e	1.48 ± 0.04 e	9.53 ± 0.16 bc

Data points indicate the mean ± SE (n = 5). Columns sharing different superscripts denote significant divergence ($P < 0.05$) based on Tukey's test.

Figure 1 illustrates the parallel decline in root and shoot dry biomass across increasing lead concentrations, demonstrating that both organ systems were equally impaired by heavy metal stress.

**Figure 1: Progressive decline in shoot and root dry biomass yields of *Raphanus sativus* under increasing soil lead concentrations.**

3.3 Photosynthetic Pigment Dynamics

This study shows that leaf pigment content was significantly reduced as a result of soil lead exposure to all treatments (Table 5). The concentration of chlorophyll a decreased from 1.385 mg/g fresh weight in the control leaves to 1.182 mg/g in T1, 0.965 mg/g in T2, 0.774 mg/g in T3 and 0.612 mg/g in T4 with a total reduction of 55.8%. The level of chlorophyll b was even more sensitive to lead toxicity, dropping from 0.642 mg/g in the control plants to 0.245 mg/g when plants had 400 mg/kg of lead. This increase in the chlorophyll a/b ratio was caused by the increased percent removal of chlorophyll b, thereby altering the ratio, from 2.16 in control to 2.50 in T4.

The total amount of chlorophyll decreased by 57.7%, from 2.027 mg/g (control) to 0.857 mg/g (T4). Similarly, accessory carotenoids decreased with a decrease from 0.485 mg/g in controls to 0.218 mg/g in T4, a 55.1% reduction overall.

Table 5: Foliar photosynthetic pigment content of radish plants subjected to lead stress.

Treatment Code (mg/kg)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Total Chlorophyll (mg/g FW)	Carotenoids (mg/g FW)	Chl a/b Ratio
T0 (0)	1.385 ± 0.034 a	0.642 ± 0.018 a	2.027 ± 0.051 a	0.485 ± 0.012 a	2.16 ± 0.04 c
T1 (50)	1.182 ± 0.028 b	0.528 ± 0.015 b	1.710 ± 0.042 b	0.415 ± 0.010 b	2.24 ± 0.05 bc
T2 (100)	0.965 ± 0.025 c	0.410 ± 0.012 c	1.375 ± 0.036 c	0.342 ± 0.009 c	2.35 ± 0.06 b
T3 (200)	0.774 ± 0.021 d	0.318 ± 0.010 d	1.092 ± 0.030 d	0.274 ± 0.008 d	2.43 ± 0.06 ab
T4 (400)	0.612 ± 0.016 e	0.245 ± 0.008 e	0.857 ± 0.023 e	0.218 ± 0.006 e	2.50 ± 0.07 a

Means ± SE (n = 5). Different lower-case letters within each column indicate significant variations at the $P < 0.05$ probability level (Tukey's HSD).

Figure 2 highlights the comparative degradation rates of chlorophyll a, chlorophyll b, and carotenoids, illustrating the consistent decline across all three photosynthetic pigments under increasing lead stress.

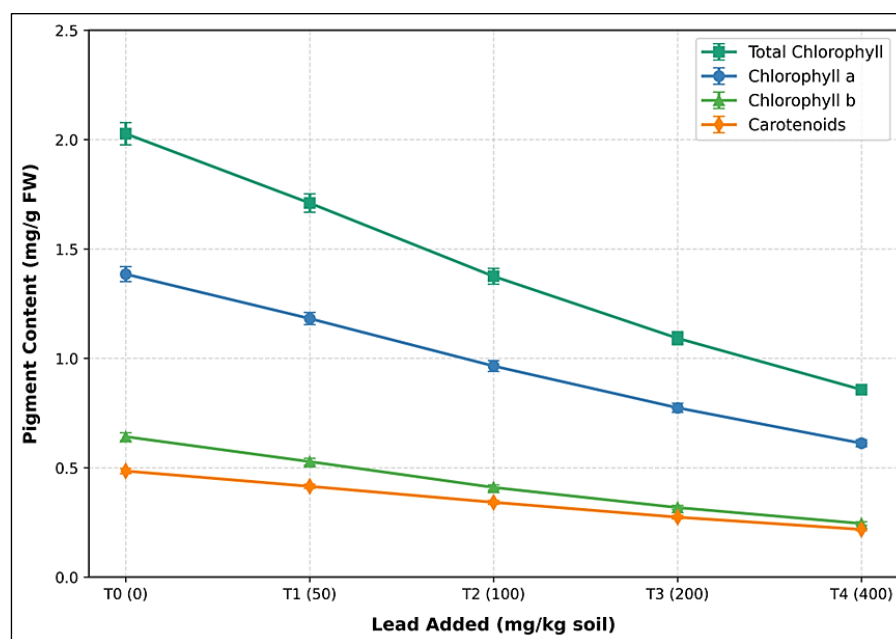


Figure 2: Degradation profiles of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in *Raphanus sativus* under increasing soil lead concentrations.

3.4 Oxidative Stress Biomarkers (Proline and Membrane Leakage)

Cellular stress biomarkers increased significantly with higher soil lead concentrations, whereas growth and pigment levels decreased with increasing soil concentration (Table 6). Free proline accumulated markedly in leaf tissues, rising from 4.25 $\mu\text{mol/g}$ fresh weight in control plants to 6.88 $\mu\text{mol/g}$ in T1 (+61.9%), 9.94 $\mu\text{mol/g}$ in T2 (+133.9%), 13.45 $\mu\text{mol/g}$ in T3 (+216.5%), and reaching 16.32 $\mu\text{mol/g}$ in T4, a 284.0% increase relative to the control.

This accumulation of proline was accompanied by a significant amount of damage to the membrane as determined by electrolyte leakage (EL). Membrane leakage rose from an initial 15.62% in unspiked controls to 23.45% in T1 (+50.1%), 32.80% in T2 (+110.0%), 43.25% in T3 (+176.9%), and peaked at 55.92% in T4, a 258.0% increase over control values.

Table 6: Free proline accumulation and electrolyte leakage percentages in radish foliage under lead exposure.

Treatment Code (mg/kg)	Free Proline ($\mu\text{mol/g FW}$)	Proline Increase Over Control (%)	Electrolyte Leakage - EL (%)	EL Increase Over Control (%)
T0 (0)	4.25 ± 0.12 e	-	15.62 ± 0.45 e	-
T1 (50)	6.88 ± 0.18 d	+61.88%	23.45 ± 0.62 d	+50.13%
T2 (100)	9.94 ± 0.25 c	+133.88%	32.80 ± 0.84 c	+109.99%
T3 (200)	13.45 ± 0.32 b	+216.47%	43.25 ± 1.10 b	+176.89%
T4 (400)	16.32 ± 0.38 a	+284.00%	55.92 ± 1.25 a	+258.00%

Data are means $\pm$ SE (n = 5). Dissimilar letters in a given column reflect significant statistical divergence ($P < 0.05$) via Tukey's test.

Figure 3 illustrates the positive relationship between free proline accumulation and electrolyte leakage across the lead treatment gradient.

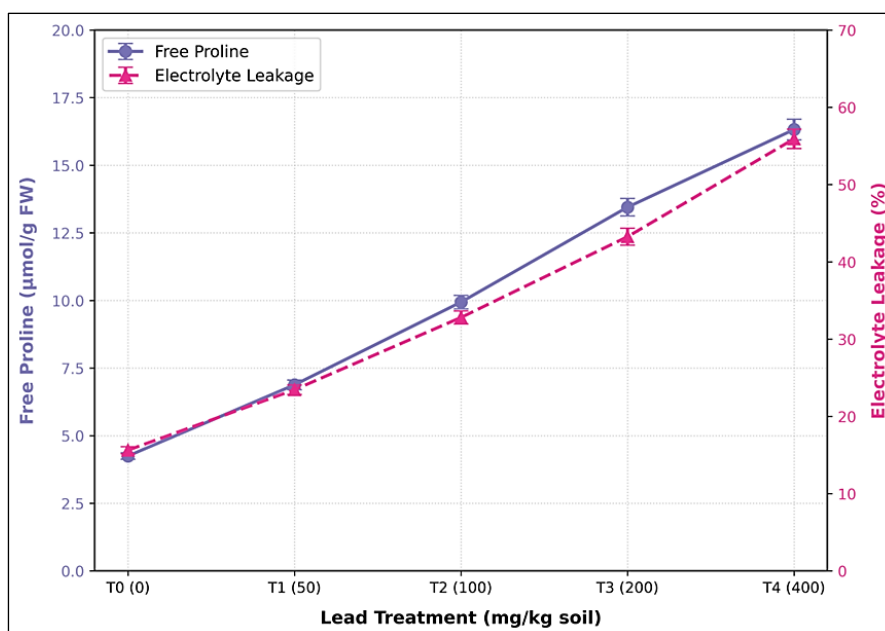


Figure 3: Concurrent increases in foliar free proline and electrolyte leakage in *Raphanus sativus* under increasing soil lead concentrations.

3.5 Bioaccumulation and Translocation Kinetics

Increased soil contamination resulted in higher levels of lead in both root and shoot tissues; the majority of the absorbed lead was in the roots (Table 7). Root lead content rose from 1.84 mg/kg dry weight in control plants to 46.50 mg/kg in T1, 92.40 mg/kg in T2, 165.20 mg/kg in T3, and reached 268.40 mg/kg in T4. Shoot tissues accumulated substantially less lead, measuring 0.52 mg/kg in controls, 12.80 mg/kg in T1, 22.45 mg/kg in T2, 36.10 mg/kg in T3, and 51.20 mg/kg in T4.

Translocation Factor (TF) values (0.19 to 0.28) were consistently less than 1 for all spiked treatments indicating that lead was not translocated to the aerial parts of the plants. The root bioaccumulation factor (Bioaccumulation Factor for roots BAF_{root}) varied between 0.67 and 0.93 in the lead treated soils, and the bioaccumulation factor for shoots (BAF_{shoot}) ranged from 0.13 to 0.26.

Table 7: Lead accumulation, bioaccumulation factors (BAF), and translocation factors (TF) in radish tissues.

Treatment Code (mg/kg)	Root Pb Concentration (mg/kg DW)	Shoot Pb Concentration (mg/kg DW)	Root Bioaccumulation Factor (BAF _{root})	Shoot Bioaccumulation Factor (BAF _{shoot})	Translocation Factor (TF)
T0 (0)	1.84 ± 0.08 e	0.52 ± 0.03 e	0.38 ± 0.02 d	0.11 ± 0.01 d	0.28 ± 0.02 a
T1 (50)	46.50 ± 1.25 d	12.80 ± 0.42 d	0.93 ± 0.03 a	0.26 ± 0.01 a	0.28 ± 0.01 a
T2 (100)	92.40 ± 2.40 c	22.45 ± 0.65 c	0.92 ± 0.02 a	0.22 ± 0.01 b	0.24 ± 0.01 b
T3 (200)	165.20 ± 4.10 b	36.10 ± 0.95 b	0.83 ± 0.02 b	0.18 ± 0.01 c	0.22 ± 0.01 bc
T4 (400)	268.40 ± 6.50 a	51.20 ± 1.30 a	0.67 ± 0.02 c	0.13 ± 0.01 cd	0.19 ± 0.01 c

Values represent means ± SE (n = 5). Different letters within an individual column indicate statistically significant differences (P < 0.05) based on Tukey's test.

Figure 4 illustrates this differential accumulation pattern between root and shoot tissues across all treatments.

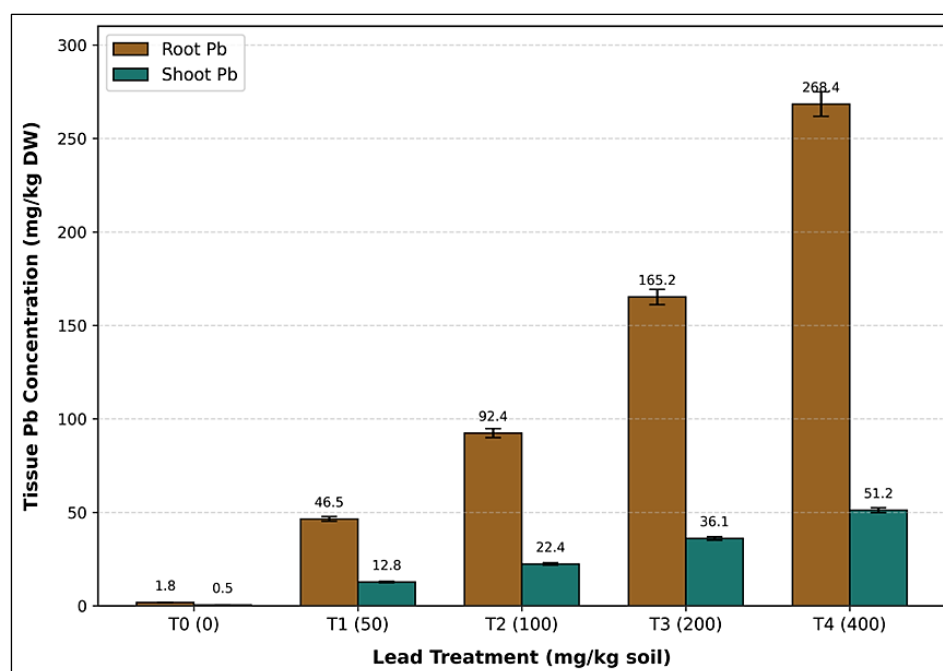


Figure 4: Differential partitioning of lead between subterranean roots and aerial shoots in *Raphanus sativus*, demonstrating root-dominated retention across all contamination levels.

4 DISCUSSION

The results obtained in this study indicate that the various parameters of *Raphanus sativus*, such as morphological, physiological and biomass characteristics, are significantly affected by the increase in soil lead content. The decreases in elongation of roots and shoots, storage organ diameter and total dry matter were similar in the observed reduction as seen in other vegetable crops under metal stress. Then Pinto et al. [16] noted the same trends in spinach plants subjected to heavy metal treatments and stated that the stunting is a result of impaired meristematic cell division and cell expansion due to interactions between the metals and the primary cell wall. The increase in storage root diameter of radish caused by increasing concentrations of lead was inversely related to the percentage change in diameter (PCD) of the storage root in the same plant. The PCD of the storage root of radish decreased as the concentration of lead in the plant increased, indicating that the higher the concentration of lead, the more it inhibited the secondary growth needed to expand the storage root diameter.

This structural inhibition is closely related with the mineral nutrition disruption. In *Arabidopsis thaliana*, the root cation transporters were shown to be disrupted by heavy metals, as was shown by Berthet et al. [17], which affected the iron and phosphate as well as the calcium homeostatic balance. Lead interferes with the divalent cation absorption sites and thus reduces the absorption of essential divalent cations, which leads to nutrient deficiencies that reduce biomass

production. Lai et al. [18] also reported similar morphological injury in sweet potato, where the concentration of toxic metals in the xylem tissues inhibited xylem transport and the growth vigour of root and shoot tissues.

The interactions of metals in soil and their bioavailability also regulate uptake and toxicity. The availability of divalent heavy metals in soil solution determines their root uptake, and the non-essential metal ions such as Pb^{2+} compete with the essential metal ions to enter the apoplast, as outlined by Zhao et al. [19]. In the case of radish, excess Pb^{2+} accumulation in the root apoplast occurs due to high soil lead levels which result in systemic metabolic stress. This energy loss leads to a decrease in dry matter accumulation, because metabolic resources are being used to detoxify the cell instead of building dry matter.

The reduction in chlorophyll a, chlorophyll b, and carotenoids is consistent with the result of Vanhoudt et al. [20] that the ultrastructure of the chloroplasts is impaired upon exposure to heavy metals and that photosynthetic pigments are downregulated in *Arabidopsis thaliana*. The effect of lead on photosynthesis occurs at several different levels: it chelates the magnesium from the porphyrin ring of chlorophyll; inhibits aminolevulinic acid dehydratase, and affects the light-harvesting complex of thylakoid membranes. In *Vicia faba*, the toxicity in heavy metal stress was confirmed by Zhang et al. [21], who observed that stress by heavy metals reduced photosynthetic pigments and impaired respiratory electron chains, causing a reduction in carbon fixation.

This functional breakdown is further reflected by the preferential loss of chlorophyll b seen in our study which led to a rise in the chlorophyll a/b ratio from 2.16 to 2.50. In *Oenanthе javanica*, Zhou et al. [22] noted a comparable effect, which has been suggested to be due to the sensitivity of outer peripheral antenna complexes against metal toxicity. In pea, Todorenko et al. [23] demonstrated that toxic metal ions affect the oxygen-evolving complex and reduce the electron transfer in PSII reaction centers. Absorbed light energy, which is not used for photochemical reactions, produces an excess of reactive oxygen species (ROS) that lead to foliar chlorosis and to the degradation of carotenoids.

Excessive amounts of ROS are harmful to cell membranes through the process of lipid peroxidation. Heavy metal stress has been reported to induce oxidation of membranes in *Arabidopsis*, because it surpasses the basal antioxidant defense thereby inducing lipid breakdown [24]. This was also observed with electrolyte leakage, which had increased 258% over controls at 400 mg/kg lead. According to Chen et al. [25], toxic metals cause imbalance in metabolic activities of roots and cause damage to the cell membrane which results in uncontrolled loss of ions and change in osmotic regulation.

In response to such oxidative stress, the plant of radish accumulated significant amounts of proline, and the proline level in the leaves rose by 284% at the highest level of lead. Under toxic metal stress, Li et al. [26] also found a similar increase in proline production in *Rohdea japonica*, which is considered to be an adaptive response to maintain the osmotic potential of the cells while protecting functional enzymes. Proline is a non-enzymatic ROS scavenger which neutralizes hydroxyl radicals, stabilizes polyribosomes and protects from the integrity of the membrane under metal induced oxidative stress.

The tissue analyses showed that the radish roots had up to 268.4 mg kg⁻¹ of lead, while the shoots had only 51.2 mg kg⁻¹, with translocation factors not exceeding unity (0.19 to 0.28). This root sequestration is analogous to that outlined by Liu et al. [27] whereby plants regulate the activity of metal transporters to restrict the movement of toxic elements to aerial photosynthetic organs by restricting their transport to the cell wall and vacuole. Heavy metals can penetrate into the root via calcium permeable cation channels, but cannot move into the plant from the root via precipitation and binding to cell wall components, as shown by Sarthou et al. [28].

By confirming structural defense barriers, such as cell wall lignification and vacuolar sequestration in the root, that limit the entry of heavy metals into the xylem, Chen et al. [29] demonstrated that these mechanisms are deployed by radish roots. The root endodermis is composed of a Casparian strip to block apoplastic flow of bulk lead into the stele. This excluder approach protects foliar photosynthetic machinery, but results in high levels of lead in the storage root. As warned by Tońska et al. [30], root vegetables growing in metal-contaminated soil present a high dietary risk since the main edible organ is the region where the metal is mainly accumulated, even if foliar symptoms are not very apparent, resulting in a high risk for food safety.

5 CONCLUSION

This work shows that the morphological, physiological and biomass parameters of *R. sativus* are strongly affected by soil lead contamination. Shoots, taproot length, storage root diameter and fresh and dry biomass were progressively reduced with increasing levels of lead in the substrates. These changes in the morphology were paralleled by the loss of photosynthetic pigments, including significant reductions in chlorophyll a, chlorophyll b and carotenoids. At the same time, the lead poisoning caused such a high degree of oxidative stress that caused a lot of the electrolytes to leak the cell

membranes in a peroxidation process, and a compensatory buildup of free proline occurred, which acts as an osmoprotectant and radical scavenger.

Elemental analysis revealed that the root zone of radish functioned mainly as an excluder, with the majority of the absorbed Lead (Pb) remaining in the root portion of the plant and translocation factors below unity in all the contamination levels. The endodermal Casparian strip and vacuolar sequestration mechanisms at the root effectively limit the movement of lead to the canopy above ground where foliar photosynthetic processes take place. But the sequestration phenomenon is a major food safety issue here as the storage root is the main edible part of this crop. Radish plants grown in lead-contaminated soils accumulate metal concentrations in their edible tissues that far exceed international food safety standards, even while the foliage shows only modest accumulation. Consequently, cultivating root vegetables in soils subject to heavy metal deposition or wastewater irrigation poses direct human health risks, underscoring the urgent need for stringent soil quality monitoring and remediative management to safeguard food security.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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