ONLINE ISSN :3007-309X
PRINT ISSN :3007-3081

Journal of Medical & Health Sciences Review

ALLEVIATION OF MERCURY HEAVY METAL STRESS BY THE APPLICATION OF SALICYLIC ACID IN ERUCA SATIVA L

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ARTICLE INFO

Keywords: *Eruca sativa*, Salicylic acid, Mercury Chloride, Heavy metal stress, Chlorophyll, Carotenoids, Plant Growth Regulator, Abiotic Stress, Morpho-Physiological Traits, Foliar Applications.

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ABSTRACT

Environmental pollution is one of the most pressing global concerns, primarily driven by rapid urbanization and industrialization. Among various pollutants, mercury (Hg) stands out as one of the most toxic heavy metals, posing serious threats to human, animal, and plant health. This study investigates the potential role of salicylic acid (SA), a plant growth regulator known for its stress-alleviating properties, in mitigating mercury-induced stress in *Eruca sativa* (arugula), a member of the Brassicaceae family recognized for its nutritional and medicinal benefits. The experiment involved foliar application of salicylic acid at two concentrations (0.5 mM and 2.5 mM) under varying levels of mercury chloride stress (0.5 mM, 1 mM, 2 mM, and 3 mM). Results indicated that mercury stress significantly reduced morphological parameters such as root length, shoot length, leaf length, fresh weight, and dry weight. Additionally, physiological traits including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were adversely affected. However, the application of salicylic acid ameliorated these negative effects, suggesting its effectiveness in enhancing plant tolerance to heavy metal stress. This study concludes that exogenous salicylic acid application is a promising approach for mitigating mercury toxicity in *Eruca sativa*.

1. Introduction

Environmental change, driven by climate variability and unsustainable resource use, is increasingly undermining freshwater availability for agriculture. Extended droughts, over-extraction of surface and groundwater, and the diversion of clean water supplies to urban and industrial centers all exacerbate irrigation deficits (Ensink, Mahmood et al. 2004).In many Pakistani peri-urban and metropolitan areas, untreated municipal and industrial effluents have become the de facto water source for farmers, supplying valuable dissolved nutrients and organic matter that can improve soil fertility and structure(Mushtaq and Khan 2010). Yet, this practice also introduces a suite of inorganic contaminants, especially heavy metals, that jeopardize both soil health and crop productivity. Heavy metals such as nickel (Ni), manganese (Mn), and zinc (Zn) are micronutrients essential for microbial enzyme function and structural stability within cells, but when present in excess, they become phytotoxic(Babalola, Fadiji et al. 2020). Other metals—including lead (Pb), arsenic (As), mercury (Hg), cadmium (Cd), and silver (Ag)—have no known biological role and pose considerable ecological and human health risks even at low concentrations(Mapanda, Mangwayana et al. 2007). Continuous application of contaminated wastewater and intensive agrochemical use can drive these elements to accumulate in soils to levels that inhibit plant growth, disrupt physiological processes, and enter the food chain (Gao, Kamran et al. 2020). In soils, heavy metals exert toxicity through multiple mechanisms: they compete with essential nutrients for uptake sites, bind to and inactivate sulfhydryl groups on proteins, catalyze the overproduction of reactive oxygen species (ROS), and disrupt membrane integrity(Ghosh, Chowdhury et al. 2022). For example, excess Cd impairs photosynthesis and nutrient uptake, induces chlorosis, and stunts growth(Ghosh, Chowdhury et al. 2022), while

elevated Zn can inhibit root and shoot development and cause premature leaf senescence(Mirshekali, Hadi et al. 2012). Copper (Cu), although necessary for CO_2 assimilation and ATP synthesis, becomes harmful at high concentrations, inducing oxidative stress and metabolic disruption (Mwamba, Islam et al. 2020). Chromium (Cr) from tanning and industrial effluents can retard germination, deform root tips, and reduce biomass accumulation (Ertani, Francioso et al. 2018). Lead contamination arises from municipal sludge, paint, and gasoline residues, and is known to inhibit key enzymatic processes, interfere with water relations, and damage photosynthetic apparatus (Yaashikaa, Kumar et al. 2022). Arsenate, mimicking phosphate, hijacks phosphate transporters in roots, disrupting energy metabolism and nutrient homeostasis (Armendariz, Talano et al. 2019).

Mercury deserves special attention due to its persistence and mobility. Globally, natural and anthropogenic sources release approximately 10,000 Mg $year^{-1}$ of Hg into the environment(Cui, Zhou et al. 2014), with gold mining and ore processing contributing heavily to atmospheric and soil contamination (Niane 2023).In soils, Hg binds strongly to clay minerals, organic matter, and sulfides, remaining bioavailable to plants and microorganisms. Once absorbed, Hg disrupts mitochondrial function, triggers ROS formation, and compromises membrane lipids and cellular metabolism (Bala, Mondal et al. 2017). Through bioaccumulation and biomagnification, mercury enters the human food chain, posing long-term health hazards (Liu, Wang et al. 2017). Plants deploy a range of adaptive and intrinsic mechanisms to mitigate heavy metal stress. At the cellular level, phytochelatins and metallothioneins—cysteine-rich peptides—sequester metal ions in vacuoles, reducing their cytosolic toxicity (Ghori, Ghori et al. 2019).Exclusion and efflux systems in the plasma membrane limit metal uptake and may actively transport excess ions back into the apoplast or soil(Kumar and Gupta 2010). Changes in cell-wall composition and the release of organic chelators in root exudates also immobilize metals at the root–soil interface (Sharma, Kumar et al. 2020).Additionally, symbiotic associations with mycorrhizal fungi—such as Glomus species—bind heavy metals in fungal biomass or alter soil pH to reduce metal bioavailability (Dhalaria, Kumar et al. 2020). At the biochemical and molecular levels, heavy metal exposure elicits complex signal transduction cascades. Plants sense stress through membrane-localized receptors, transmit signals via secondary messengers, and modulate gene expression to produce detoxification proteins, antioxidant enzymes, and stress-responsive metabolites (Etesami 2024).Enzymatic antioxidants—such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX)—scavenge ROS, while non-enzymatic antioxidants like glutathione, ascorbic acid, and proline protect cellular structures and maintain redox homeostasis(Dixit and Kumar 2024).Phytohormones are central to integrating these defense responses. Compounds such as auxins, cytokinins, gibberellins, abscisic acid, jasmonic acid, ethylene, brassinosteroids, and notably salicylic acid (SA), regulate growth, development, and stress adaptation. Exogenous application of these hormones—or "priming"—can pre-condition plants to withstand heavy metal stress by enhancing antioxidant capacity, modulating nutrient uptake, and regulating stress-related gene networks(Dixit and Kumar 2024). Among them, SA has attracted particular interest as a "quasi-essential" signal molecule that bolsters both biotic and abiotic stress defenses (Chandwani and Amaresan 2022). Extensive research demonstrates that foliar or soil application of SA ameliorates heavy metal toxicity across diverse species. In rice, 0.1 mM SA reduced Cd accumulation and oxidative damage by down-regulating malondialdehyde (MDA) and H_2O_2 levels and modulating gene expression of miR166, HD-Zip, and OsLCD (Wang, Huo et al. 2016).In Phaseolus vulgaris, SA (0.1 mM), alone or combined with calcium and kinetin, enhanced growth, carbohydrate metabolism, and antioxidant enzyme activities under Ni and Pb stress (Chmielewska, Barratt et al. 2021).In maize, the co-application of SA with spermidine improved chlorophyll content and reduced Cr uptake,

thereby mitigating oxidative injury (Naz et al., 2021). However, supra-optimal SA concentrations may paradoxically elevate ROS production and cause tissue damage, underscoring the need to optimize dosage (Janda, Szalai et al. 2007).*Eruca sativa* (rocket, arugula), a Brassicaceae member, is valued for its nutritional profile—rich in vitamins A, C, K, carotenoids, tocopherols, and folates—and its resilience to environmental stresses such as salinity and disease(Grami, de Marco et al. 2022). Native to Pakistan's semi-arid regions, *E. sativa* thrives on marginal lands unsuitable for staple crops, offering potential as both a leafy vegetable and biodiesel feedstock (Chakrabarti and Ahmad 2009)Despite its adaptability, the responses of rockets to heavy metal stress, particularly mercury, and the protective role of SA remain poorly characterized.

2. Materials and Methods

2.1 Experimental Site and Overview: A greenhouse-pot trial was carried out at the Botanical Garden of the Islamia University of Bahawalpur to evaluate the effects of mercury chloride ($HgCl_2$) stress and the potential ameliorative role of exogenously applied salicylic acid (SA) on the morpho-physiological performance of *Eruca sativa*. All biochemical and physiological analyses were performed in the Botany Department laboratories under controlled conditions.

2.2 Soil Preparation and Potting: Topsoil was collected from adjacent agricultural fields, air-dried, and sieved to remove stones and plant debris. Uniform 5-kg quantities were dispensed into plastic pots (20 cm diameter). Three days before sowing, each pot was irrigated to field capacity to ensure consistent moisture for seed germination.

2.3 Seed Procurement and Sowing: Certified *Eruca sativa* seeds were obtained from the Ayub Agricultural Research Institute (Faisalabad). In September, 10–15 seeds were sown per pot at a depth of approximately 1 cm. After germination, pots were thinned to five uniform seedlings each.

2.4 Heavy Metal Treatments: Mercury stress was imposed using mercury chloride ($HgCl_2$) at five levels: 0 mM (control), 0.5 mM, 1 mM, 2 mM, and 3 mM. Stock solutions were prepared by dissolving 0.1357 g, 0.2714 g, 0.5431 g, and 0.8114 g of $HgCl_2$, respectively, in 1 L of distilled water. Treatments were administered as follows:

First application: 40 days after sowing, when seedlings were well-established, 10 mL of the appropriate $HgCl_2$ solution was added to the soil around each plant's root zone.

Subsequent applications: Two further doses were applied at 10-day intervals, following the same procedure.

2.5 Salicylic Acid Treatments: Salicylic acid (SA) was tested at two concentrations—0.5 mM and 2.5 mM—to assess its potential to mitigate Hg-induced stress. Stock solutions were prepared by dissolving 1.7612 g (for 0.5 mM) and 3.5224 g (for 2.5 mM) of SA in 1 L of distilled water.

Foliar Application Protocol: SA treatments were applied by foliar spray at the same three time points as the $HgCl_2$ applications.

For each treatment, 10 mL of SA solution was gently misted onto the foliage using a handheld sprayer. A polythene barrier was placed around each pot during spraying to prevent cross-contamination.

Treatment groups included:

Control (no Hg, no SA)

Hg only (four stress levels)

Pre-treatment with SA (each SA concentration applied before each Hg dose)

Post-treatment with SA (each SA concentration applied after each Hg dose)

2.6 Sampling and Data Collection: Ten days after the third Hg/SA application, plants were carefully harvested for analysis. Samples were divided for morphological measurements,

pigment assays, biochemical analyses, and heavy metal quantification in roots, shoots, and whole plants.

2.7 Morphological Measurements: Root and Shoot Length: Measured from the base to the tip using a metric ruler (cm).Fresh Weight: Recorded immediately after harvest using an electronic balance (g).Dry Weight: Samples were oven-dried at 70 °C for 72 h, and then weighed to constant mass (g).Leaf Count: Total number of fully expanded leaves per plant was noted.

2.8 Photosynthetic Pigment Analysis: Approximately 0.1 g of fresh leaf tissue was homogenized in 6 mL of 80% (v/v) ethanol. The homogenate was centrifuged at 5,000 ×g for 10 min, and the supernatant collected for spectrophotometric analysis. Absorbance readings were taken at 663 nm, 645 nm, and 470 nm to estimate chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents using the following equations (Ali et al., 2021):

Chlorophyll a = (12.7 × A663) – (2.49 × A645)

Chlorophyll b = (12.9 × A645) – (4.7 × A663)

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Carotenoids = OD × 4

3. Results

3.1 Morphological Parameters

3.1.1 Plant Fresh Weight

The effects of varying concentrations of mercury chloride ($HgCl_2$), salicylic acid (SA), and their combinations—applied at different timings—on plant fresh weight are summarized in Figures 3.1. Under control conditions (T0), average fresh weight was 5.31 g. Application of SA alone led to a concentration-dependent increase: 0.5 mM SA (T1) yielded 6.27 g, while 2.5 mM SA (T2) reached 8.83 g. Conversely, $HgCl_2$ stress alone caused a progressive decline in fresh weight with increasing dose: 0.5 mM (T3) at 5.21 g, 1 mM (T4) at 4.37 g, 2 mM (T5) at 3.91 g, and 3 mM (T6) at only 1.81 g (Figure 3.1).

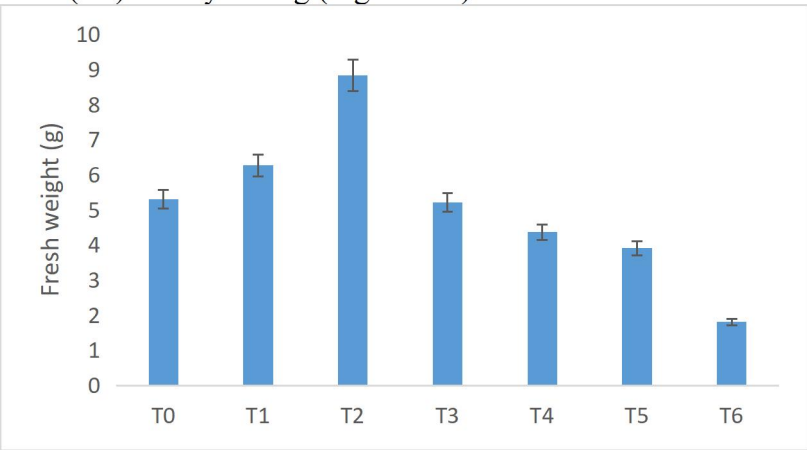


Figure 3.1: The effects of varying concentrations of mercury chloride ($HgCl_2$), salicylic acid (SA), and their combinations applied at different timings on plant fresh weight.

Co-application of SA and $HgCl_2$ mitigated metal toxicity to varying degrees depending on timing and concentration. For the lowest $HgCl_2$ dose (0.5 mM, Figure 3.2), simultaneous treatment with 0.5 mM SA (T7) and 2.5 mM SA (T11) raised fresh weights to 5.37 g and 5.56 g, respectively. Pre-treatment with $HgCl_2$ followed one day later by SA (T15 and T19) further improved weights to 5.46 g and 5.71 g, respectively, as did the reverse sequence (SA→$HgCl_2$; T23, T27). The greatest recovery occurred when SA was applied one day after metal exposure, regardless of SA concentration.

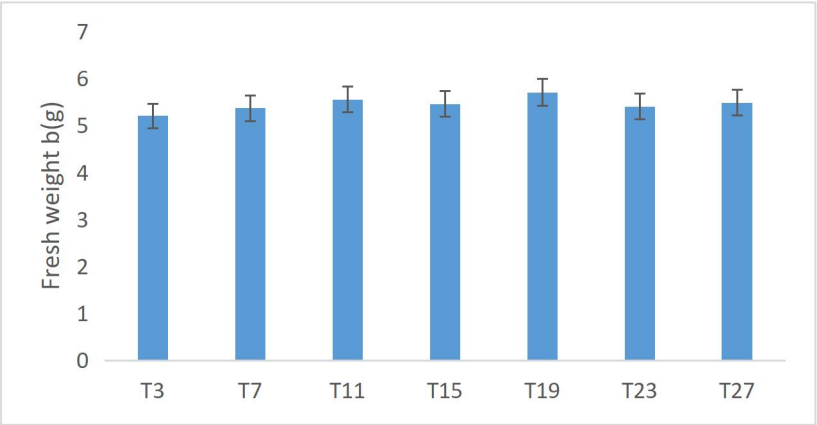


Figure 3.2. *Comparison of Plant fresh weight (g) between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different time. T3(0.5mM $HgCl_2$), T7(0.5mM $HgCl_2$ + 0.5mM SA), T11(0.5mM $HgCl_2$+2.5mM SA), T15(pre 0.5mM $HgCl_2$+ post 0.5mM SA), T19(pre 0.5mM $HgCl_2$+ post 2.5mM SA), T23(post 0.5mM $HgCl_2$+ pre 0.5mM SA), T27(post 0.5mM $HgCl_2$+ pre 2.5mM SA).*

At 1 mM $HgCl_2$ (Figure 3.3), pure metal treatment led to 4.37 g (T4). Addition of SA (0.5 mM and 2.5 mM) during or after metal stress (T8–T28) uniformly increased fresh weight by approximately 0.2–0.6 g above T4, with post-stress SA applications (T24, T28) yielding 4.54 g and 4.69 g, respectively.

Under moderate $HgCl_2$ stress (2 mM; Figure 3.4), control T5 plants weighed 3.91 g. Sequential SA treatments again enhanced weight, with post-treatment SA (T17–T29) producing fresh weights between 3.26 g and 3.47 g. The greatest improvement (3.47 g) was observed for pre-metal SA (2.5 mM) followed by $HgCl_2$ (T21).

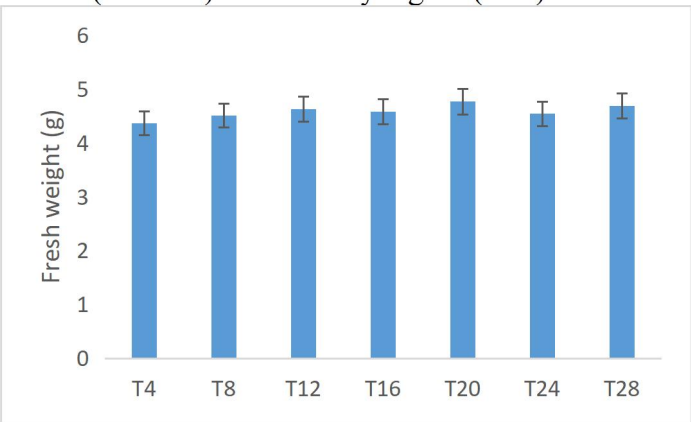


Figure 3.3: Comparison of Plant fresh weight (g) between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different time. T4(1mM $HgCl_2$), T8(1mM $HgCl_2$+ 0.5mM SA), T12(1mM $HgCl_2$+ 2.5mM SA), T16(pre 1mM $HgCl_2$+ post 0.5mM SA), T20(pre 1mM $HgCl_2$+post 2.5mM SA), T24(post 1mM $HgCl_2$+ pre 0.5mM SA), T28(post 1mM $HgCl_2$+ pre 2.5mM SA).

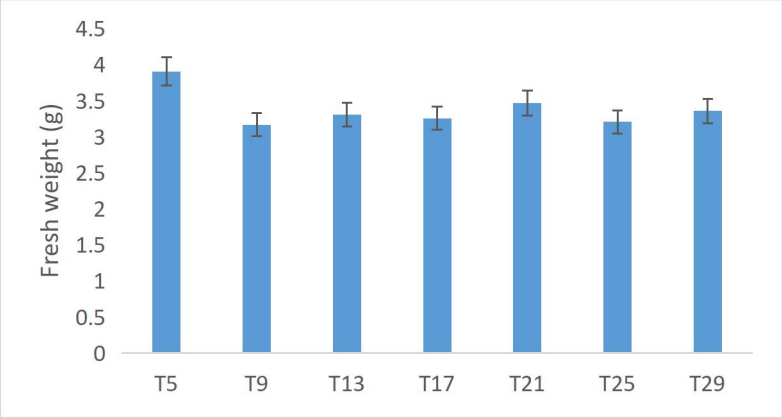
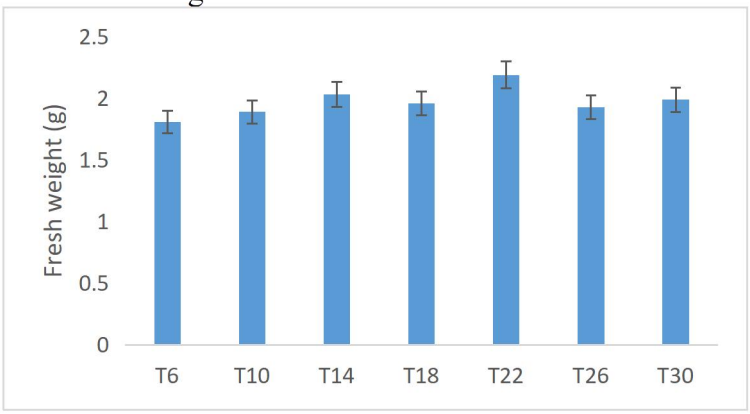


Figure 3.4. Comparison of Plant fresh weight (g) between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different time. T5(2mM $HgCl_2$), T9(2mM $HgCl_2$+ 0.5mM SA), T13(2mM $HgCl_2$+ 2.5mM SA), T17(pre 2mM $HgCl_2$+ post 0.5mM SA), T21(pre 2mM $HgCl_2$+ post 2.5mM SA), T25(post 2mM $HgCl_2$+ pre 0.5mM SA), T29(post 2mM $HgCl_2$+ pre 2.5mM SA).

Finally, at the highest metal concentration (3 mM; Figure 3.5), $HgCl_2$ alone (T6) resulted in the lowest fresh weight (1.81 g). Combined treatments with SA (0.5 mM or 2.5 mM), whether simultaneous or staggered by one day, marginally increased fresh weight up to 2.19 g (T22), with the strongest effect again seen when SA followed $HgCl_2$ treatment by one day. Overall, SA alone maximized fresh weight, while SA application—particularly 24 h after $HgCl_2$ exposure—significantly ameliorated metal-induced biomass reduction, most effectively at low to moderate $HgCl_2$ concentrations.

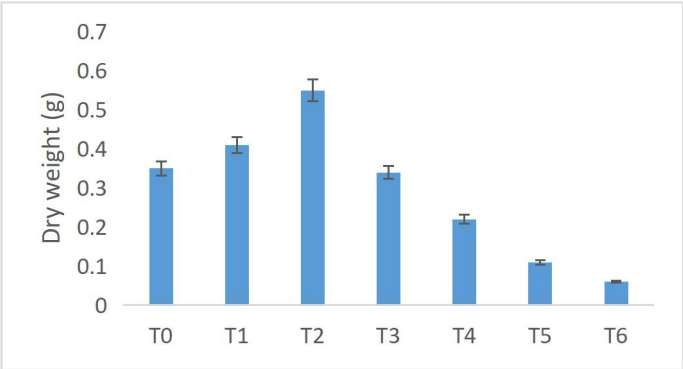


3.5: Comparison of Plant fresh weight (g) between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different time

T6(3mM $HgCl_2$), T10(3mM $HgCl_2$+ 0.5mM SA), T14(3mM $HgCl_2$+ 2.5mM SA), T18(pre 3mM $HgCl_2$+ post 0.5mM SA), T22(pre 3mM $HgCl_2$+ post 2.5mM SA), T26(post 3mM $HgCl_2$+ pre 0.5mM SA), T30(post 3mM $HgCl_2$+ pre 2.5mM SA).

3.1.2 Plant Dry Weight

Dry weight measurements (Figures 3.6–3.10) mirrored trends observed in fresh weight. Control plants (T0) exhibited 0.35 g dry weight. SA treatments alone increased dry biomass to 0.41 g (T1, 0.5 mM SA) and 0.55 g (T2, 2.5 mM SA). $HgCl_2$ stress alone reduced dry weight progressively from 0.34 g (T3, 0.5 mM) to 0.22 g (T4, 1 mM), 0.11 g (T5, 2 mM), and 0.06 g (T6, 3 mM) (Figure 3.6).



3.6: Comparison of Plant dry weight (g) between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different time
T0 (Control), T1(0.5mM SA), T2(2.5mM SA), T3(0.5mM $HgCl_2$), T4(1mM $HgCl_2$), T5(2mM $HgCl_2$), T6(3mM $HgCl_2$).

Under 0.5 mM $HgCl_2$ stress (Figure 3.7), simultaneous SA application (T7, T11) raised dry weight to 0.37 g and 0.43 g, respectively. Staggered treatments, where SA was applied either one day before (T15, T19) or after (T23, T27) $HgCl_2$, yielded further improvements up to 0.49 g. The largest recovery again occurred with post-stress SA.

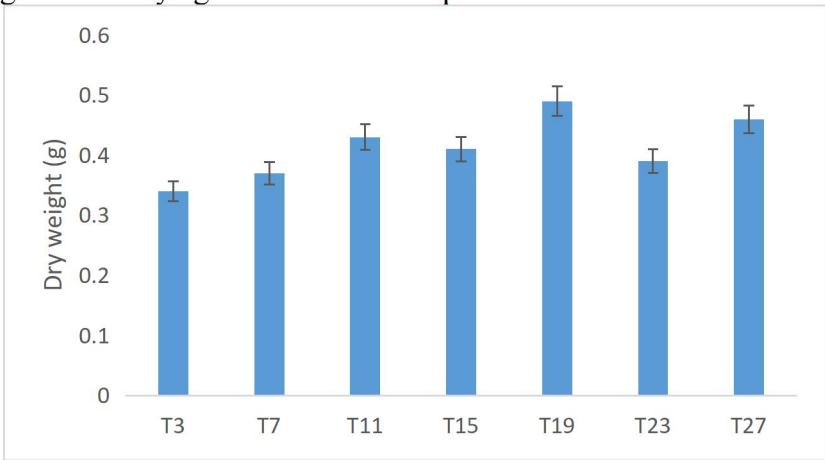


Figure 3.7: *Comparison of Plant dry weight (g) between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different time.*
T3(0.5mM $HgCl_2$), T7(0.5mM $HgCl_2$ + 0.5mM SA), T11(0.5mM $HgCl_2$+ 2.5mM SA), T15(pre 0.5mM $HgCl_2$+ post 0.5mM SA), T19(pre 0.5mM $HgCl_2$+ post 2.5mM SA), T23(post 0.5mM $HgCl_2$+ pre 0.5mM SA), T27(post 0.5mM $HgCl_2$+ pre 2.5mM SA).
At 1 mM $HgCl_2$ (Figure 3.8), T4 dry weight of 0.22 g increased to between 0.27 g and 0.36 g with combined SA treatments, peaking in post-stress applications (T12, T20, T28).

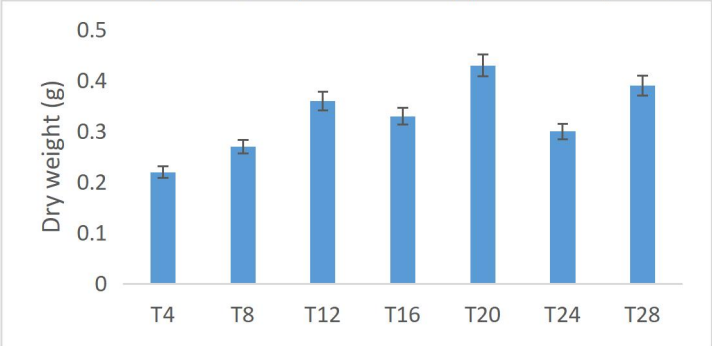


Figure 3.8: *Comparison of Plant dry weight (g) between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different time*

T4(1mM $HgCl_2$), T8(1mM $HgCl_2$+ 0.5mM SA), T12(1mM $HgCl_2$+ 2.5mM SA), T16(pre 1mM $HgCl_2$+ post 0.5mM SA), T20(pre 1mM $HgCl_2$+post 2.5mM SA), T24(post 1mM $HgCl_2$+ pre 0.5mM SA), T28(post 1mM $HgCl_2$+ pre 2.5mM SA).
For 2 mM $HgCl_2$ (Figure 3.9), dry biomass rose from 0.11 g (T5) to between 0.14 g and 0.26 g across combined treatments, with the greatest enhancement (0.26 g, T21) achieved when 2.5 mM SA followed metal exposure by one day.

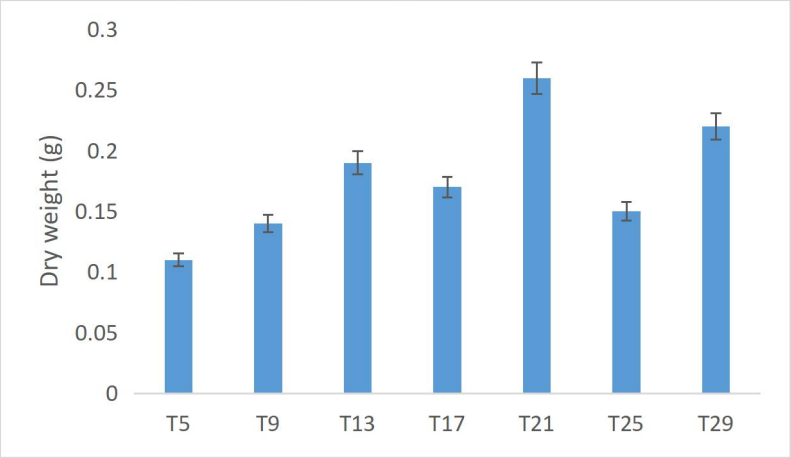


Figure3.9: *Comparison of Plant dry weight (g) between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different time.*
T5(2mM $HgCl_2$), T9(2mM $HgCl_2$+ 0.5mM SA), T13(2mM $HgCl_2$+ 2.5mM SA), T17(pre 2mM $HgCl_2$+ post 0.5mM SA), T21(pre 2mM $HgCl_2$+ post 2.5mM SA), T25(post 2mM $HgCl_2$+ pre 0.5mM SA), T29(post 2mM $HgCl_2$+ pre 2.5mM SA).

At the highest stress level (3 mM; Figure 3.10), the minimal dry weight (0.06 g, T6) was modestly elevated by SA co-treatments to as much as 0.20 g (T22), again most effectively when SA was applied post-stress.

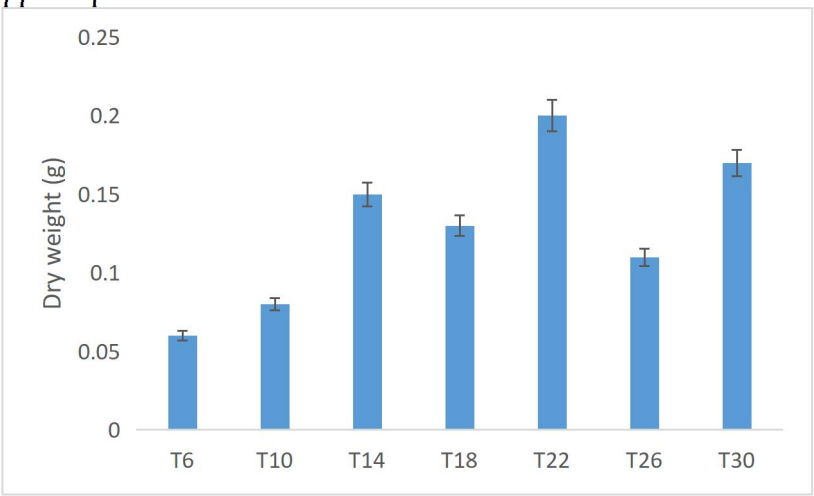


Figure 3.10: *Comparison of Plant dry weight (g) between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different time*
T6(3mM $HgCl_2$), T10(3mM $HgCl_2$+ 0.5mM SA), T14(3mM $HgCl_2$+ 2.5mM SA), T18(pre 3mM $HgCl_2$+ post 0.5mM SA), T22(pre 3mM $HgCl_2$+ post 2.5mM SA), T26(post 3mM $HgCl_2$+ pre 0.5mM SA), T30(post 3mM $HgCl_2$+ pre 2.5mM SA).

3.1.3 Plant Shoot Length

The impact of $HgCl_2$ stress, SA regulation, and their combined application (simultaneous or staggered by one day) on shoot elongation is illustrated in Figures 3.11–3.15. Under control conditions (T0), mean shoot length measured 21.7 cm. Exogenous SA alone promoted

extension in a dose-dependent manner: 0.5 mM SA (T1) increased length to 24.3 cm, while 2.5 mM SA (T2) achieved the maximum of 28.5 cm. Conversely, $HgCl_2$ treatment alone progressively curtailed shoot growth, with lengths of 21.1 cm (0.5 mM, T3), 18.3 cm (1 mM, T4), 15.6 cm (2 mM, T5), and 12.1 cm (3 mM, T6).

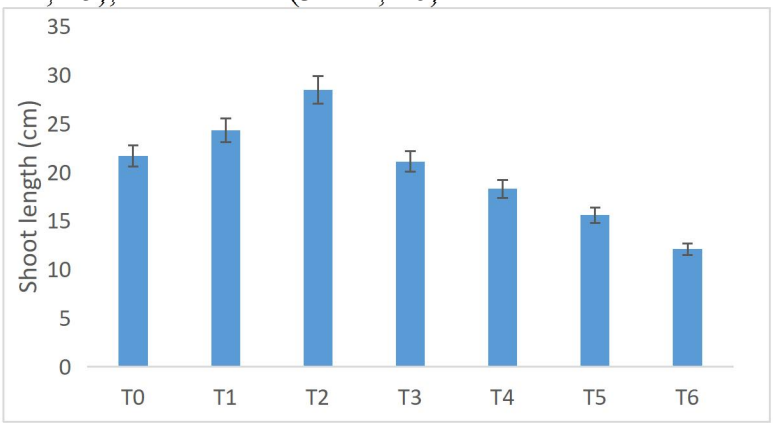


Figure 3.11: *Comparison of Plant shoot length (cm) between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different time.*
T0 (Control), T1(0.5mM SA), T2(2.5mM SA), T3(0.5mM $HgCl_2$), T4(1mM $HgCl_2$), T5(2mM $HgCl_2$), T6(3mM $HgCl_2$).

When 0.5 mM $HgCl_2$ was combined with SA (Figure 3.12), simultaneous co-application of 0.5 mM (T7) and 2.5 mM SA (T11) modestly increased shoot length to 21.4 cm and 22.3 cm, respectively. Pre-treatment with $HgCl_2$ followed by SA one day later (T15, T19) further improved shoot lengths to 21.9 cm and 23.6 cm, while reversing the sequence (SA then $HgCl_2$; T23, T27) yielded 21.6 cm and 22.8 cm. The greatest recovery in this low-stress scenario was observed when SA was applied 24 h post-$HgCl_2$ exposure.

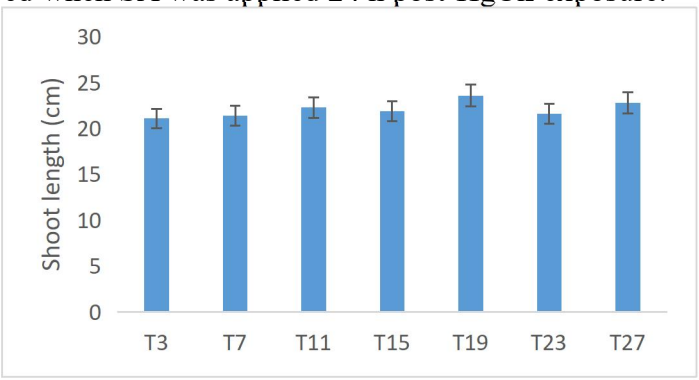


Figure 3.12: *Comparison of Shoot length (cm) between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different time.*
T3(0.5mM $HgCl_2$), T7(0.5mM $HgCl_2$ + 0.5mM SA), T11(0.5mM $HgCl_2$+ 2.5mM SA), T15(pre 0.5mM $HgCl_2$+ post 0.5mM SA), T19(pre 0.5mM $HgCl_2$+ post 2.5mM SA), T23(post 0.5mM $HgCl_2$+ pre 0.5mM SA), T27(post 0.5mM $HgCl_2$+ pre 2.5mM SA).
At 1 mM $HgCl_2$ (Figure 3.13), untreated plants (T4) averaged 18.3 cm. Adding SA (0.5 mM or 2.5 mM), whether concurrently or staggered, consistently raised shoot length by approximately 0.4–1.5 cm. Most notably, post-stress SA treatments (T24, T28) achieved lengths of 19.0 cm and 20.3 cm, indicating that delayed SA application better counteracts moderate metal toxicity.

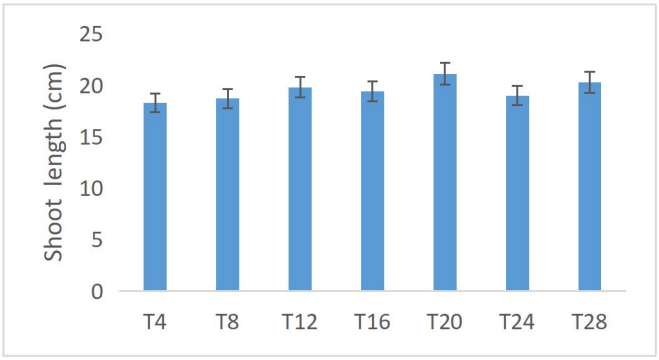


***Figure 3.13**: Comparison of Shoot length (cm) between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different times.*
T4(1mM $HgCl_2$), T8(1mM $HgCl_2$+ 0.5mM SA), T12(1mM $HgCl_2$+ 2.5mM SA), T16(pre 1mM $HgCl_2$+ post 0.5mM SA), T20(pre 1mM $HgCl_2$+post 2.5mM SA), T24(post 1mM $HgCl_2$+ pre 0.5mM SA), T28(post 1mM $HgCl_2$+ pre 2.5mM SA).

Under stronger stress (2 mM $HgCl_2$; Figure 3.14), shoot length in T5 was reduced to 15.6 cm. Co-treatments with SA produced incremental gains of 0.3–2.3 cm. Pre-SA followed by $HgCl_2$ (T17, T21) extended shoots to 16.5 cm and 17.9 cm, while the post-SA treatments (T25, T29) delivered 16.2 cm and 17.2 cm, again highlighting the benefit of SA application after metal stress.

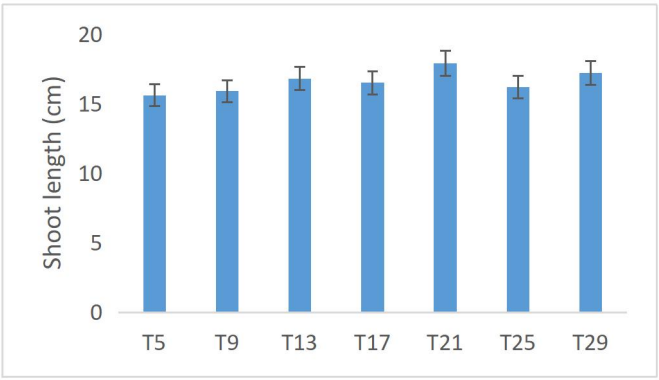


***Figure 3.14:** Comparison of Shoot length (g) between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different times.*
T5(2mM $HgCl_2$), T9(2mM $HgCl_2$+ 0.5mM SA), T13(2mM $HgCl_2$+ 2.5mM SA), T17(pre 2mM $HgCl_2$+ post 0.5mM SA), T21(pre 2mM $HgCl_2$+ post 2.5mM SA), T25(post 2mM $HgCl_2$+ pre 0.5mM SA), T29(post 2mM $HgCl_2$+ pre 2.5mM SA).

At the highest $HgCl_2$ level (3 mM; Figure 3.15), shoots were severely stunted (12.1 cm, T6). Simultaneous SA co-application (T10, T14) and staggered treatments (T18–T30) yielded only slight improvements (up to 14.9 cm, T22), but once more, SA applied one day following $HgCl_2$ (T22) provided the greatest amelioration.

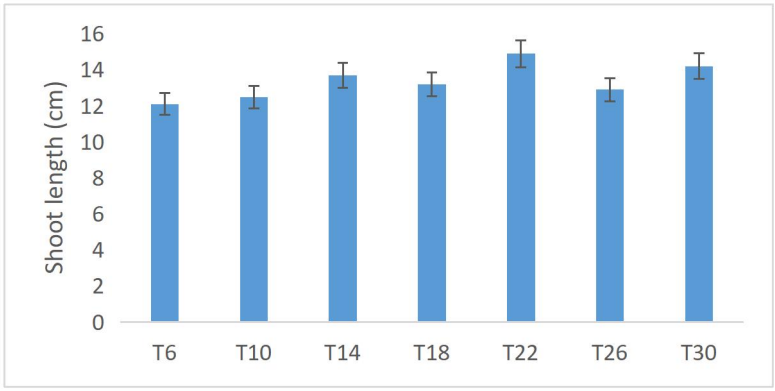


Figure 3.15: *Comparison of Shoot length (cm) between different levels of Mercury chloride, Salicylic acid, and Mercury chloride + Salicylic acid when applied at different times.*
T6(3mM $HgCl_2$), T10(3mM $HgCl_2$+ 0.5mM SA), T14(3mM $HgCl_2$+ 2.5mM SA), T18(pre 3mM $HgCl_2$+ post 0.5mM SA), T22(pre 3mM $HgCl_2$+ post 2.5mM SA), T26(post 3mM $HgCl_2$+ pre 0.5mM SA), T30(post 3mM $HgCl_2$+ pre 2.5mM SA).
In summary, SA alone maximizes shoot length, while sequential application, particularly administering SA 24 h after $HgCl_2$ exposure, most effectively mitigates metal-induced inhibition of shoot growth, with the most pronounced benefits under low to moderate $HgCl_2$ concentrations.

3.1.4 Plant Root Length

Root elongation responses under the various treatments are presented in Figures 3.16–3.20. Control plants (T0) exhibited an average root length of 5.66 cm. SA alone enhanced rooting: 0.5 mM (T1) yielded 6.78 cm, and 2.5 mM (T2) reached 8.16 cm. Treatment with $HgCl_2$ alone progressively reduced root length: 5.60 cm (0.5 mM, T3), 4.70 cm (1 mM, T4), 3.91 cm (2 mM, T5), and 3.11 cm (3 mM, T6).

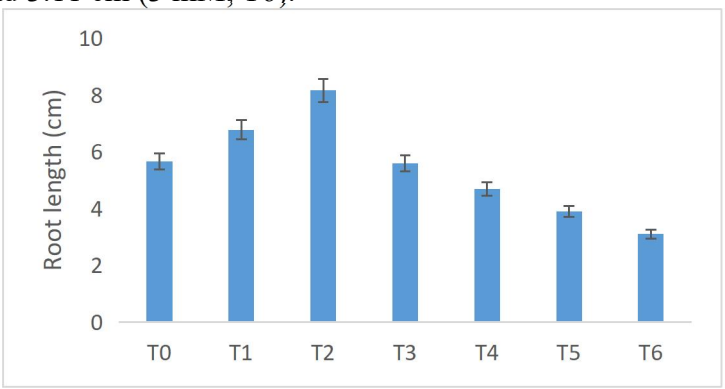


Figure 3.16: *Comparison of Root length (cm) between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different times.*
T0 (Control), T1(0.5mM SA), T2(2.5mM SA), T3(0.5mM $HgCl_2$), T4(1mM $HgCl_2$), T5(2mM $HgCl_2$), T6(3mM $HgCl_2$).

Under mild metal stress (0.5 mM; Figure 3.17), combining 0.5 mM and 2.5 mM SA simultaneously with $HgCl_2$ (T7, T11) slightly increased root length to 5.64 cm and 5.77 cm. Applying SA one day after $HgCl_2$ (T23, T27) raised lengths to 5.69 cm and 5.83 cm, respectively, while pre-SA followed by $HgCl_2$ (T15, T19) produced 5.73 cm and 5.93 cm. As with shoots, post-stress SA afforded the greatest recovery.

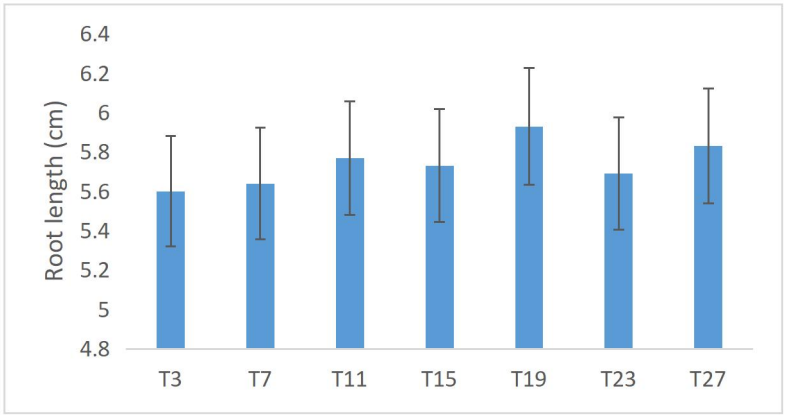


Figure 3.17: Comparison of Root length (cm) between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different times.
T3(0.5mM $HgCl_2$), T7(0.5mM $HgCl_2$ + 0.5mM SA), T11(0.5mM HgCl2+ 2.5mM SA), T15(pre 0.5mM $HgCl_2$+ post 0.5mM SA), T19(pre 0.5mM $HgCl_2$+ post 2.5mM SA), T23(post 0.5mM $HgCl_2$+ pre 0.5mM SA), T27(post 0.5mM $HgCl_2$+ pre 2.5mM SA).

In the 1 mM $HgCl_2$ group (Figure 3.18), control roots (T4) measured 4.70 cm. Addition of SA at either concentration—concurrent or delayed—increased length by up to 0.43 cm, with T24 and T28 (post-SA) reaching 4.85 cm and 4.98 cm, respectively.

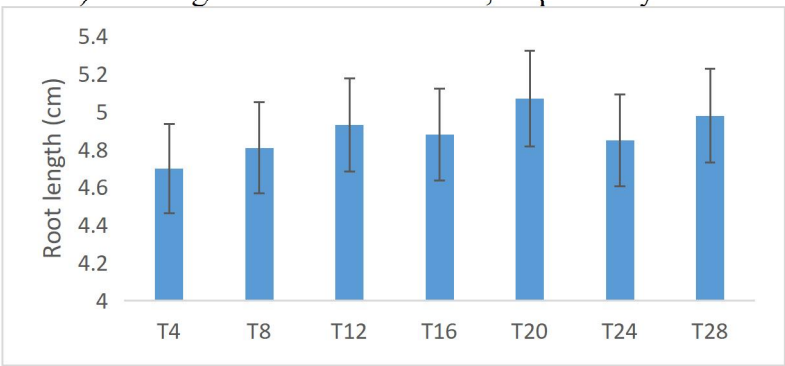


Figure 3.18: *Comparison of Root length (cm) between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different times.*
T4(1mM $HgCl_2$), T8(1mM $HgCl_2$+ 0.5mM SA), T12(1mM $HgCl_2$+ 2.5mM SA), T16(pre 1mM $HgCl_2$+ post 0.5mM SA), T20(pre 1mM $HgCl_2$+post 2.5mM SA), T24(post 1mM $HgCl_2$+ pre 0.5mM SA), T28(post 1mM $HgCl_2$+ pre 2.5mM SA).

Under 2 mM $HgCl_2$ (Figure 3.19), roots in T5 were curtailed to 3.91 cm. Co-applications with SA elevated length across treatments: simultaneous (T9, T13) to 3.99 cm and 4.12 cm; pre then post (T17, T21) to 4.08 cm and 4.23 cm; and post then pre (T25, T29) to 4.03 cm and 4.18 cm. The highest enhancement (4.23 cm) occurred when SA (2.5 mM) was applied one day before $HgCl_2$.

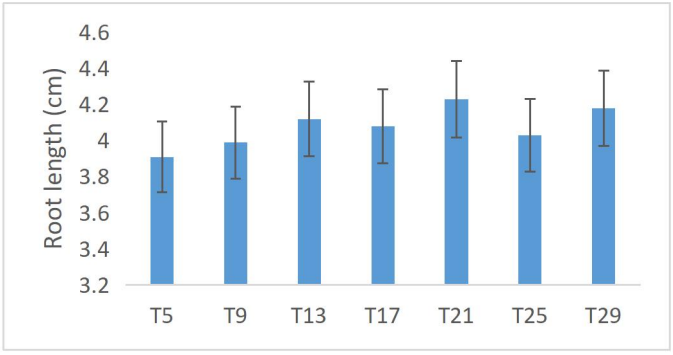


Figure 3.19: *Comparison of Root length (cm) between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different times.*
T5(2mM $HgCl_2$), T9(2mM $HgCl_2$+ 0.5mM SA), T13(2mM $HgCl_2$+ 2.5mM SA), T17(pre 2mM $HgCl_2$+ post 0.5mM SA), T21(pre 2mM $HgCl_2$+ post 2.5mM SA), T25(post 2mM $HgCl_2$+ pre 0.5mM SA), T29(post 2mM $HgCl_2$+ pre 2.5mM SA).

At the highest stress level (3 mM; Figure 3.20), $HgCl_2$ alone (T6) limited roots to 3.11 cm. SA co-treatments provided marginal improvement (up to 3.34 cm in T22), with the largest gain when SA was applied one day before $HgCl_2$ (T22). However, the overall trend persists: SA administered 24 h around $HgCl_2$ exposure alleviates root growth inhibition, especially at lower to moderate metal concentrations.

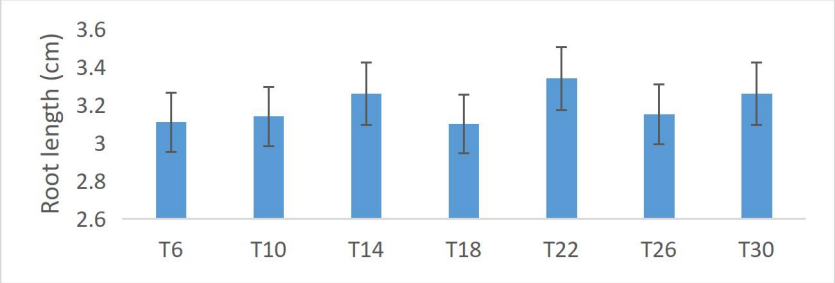


Figure 3.20: *Comparison of Root length (cm) between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different time*
T6(3mM $HgCl_2$), T10(3mM $HgCl_2$+ 0.5mM SA), T14(3mM $HgCl_2$+ 2.5mM SA), T18(pre 3mM $HgCl_2$+ post 0.5mM SA), T22(pre 3mM $HgCl_2$+ post 2.5mM SA), T26(post 3mM $HgCl_2$+ pre 0.5mM SA), T30(post 3mM $HgCl_2$+ pre 2.5mM SA).
Collectively, this study demonstrates that exogenous SA, particularly when applied one day after $HgCl_2$ exposure, substantially mitigates mercury-induced root growth suppression, with the extent of recovery depending on both metal and SA concentrations as well as treatment timing.

3.2 Physiological Parameters

3.2.1 Chlorophyll a

The concentration of chlorophyll a varied markedly among treatments (Figure 3.21). In the absence of $HgCl_2$ stress (T0), chlorophyll a averaged 1.675 mg g^{-1} FW. Exogenous application of salicylic acid (SA) alone elevated pigment levels in a dose-dependent manner: 0.5 mM SA (T1) increased chlorophyll a to 2.135 mg g^{-1}, while 2.5 mM SA (T2) yielded the highest content of 3.409 mg g^{-1}. Conversely, $HgCl_2$ stress alone induced a progressive decline in chlorophyll a, with values of 1.672 mg g^{-1} at 0.5 mM $HgCl_2$ (T3), 1.153 mg g^{-1} at 1.0 mM (T4), 0.232 mg g^{-1} at 2.0 mM (T5), and a minimum of 0.084 mg g^{-1} at 3.0 mM (T6).

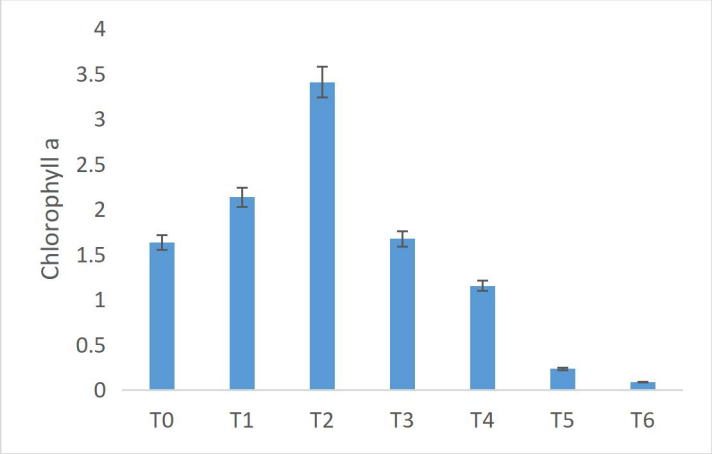


Figure 3.21: *Comparison of chlorophyll a content between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different times.*
T0 (Control), T1(0.5mM SA), T2(2.5mM SA), T3(0.5mM $HgCl_2$), T4(1mM $HgCl_2$), T5(2mM $HgCl_2$), T6(3mM $HgCl_2$). When SA and $HgCl_2$ were combined, timing and concentration of SA markedly influenced chlorophyll a restoration (Figure 2). Under mild $HgCl_2$ stress (0.5 mM; T3), simultaneous application of 0.5 mM and 2.5 mM SA (T7, T11) raised chlorophyll a to 1.751 and 1.837 mg g^{-1}, respectively. Pre-treatment with SA one day before $HgCl_2$ (T15, T19) further increased levels to 1.795 and 1.986 mg g^{-1}, while post-treatment (SA applied one day after $HgCl_2$; T23, T27) produced similar gains (1.772 and 1.896 mg g^{-1}). Under moderate $HgCl_2$ stress (1.0 mM; T4), simultaneous SA applications (T8, T12) elevated chlorophyll a to 1.189 and 1.276 mg g^{-1}; pre- and post-treatments (T24, T28) achieved values of 1.237 and 1.318 mg g^{-1}. In higher-stress scenarios (2.0 mM $HgCl_2$; T5), SA supplementation also recovered pigment content: simultaneous applications (T9, T13) yielded 0.284 and 0.396 mg g^{-1}; sequential treatments before and after $HgCl_2$ (T17, T21; T25, T29) reached 0.367/0.468 and 0.317/0.421 mg g^{-1}, respectively. Even at the highest stress (3.0 mM; T6), minimal improvements were observed with SA: simultaneous SA co-application (T10, T14) increased chlorophyll a slightly to 0.087 and 0.098 mg g^{-1}; pre- and post-treatments (T18, T22; T26, T30) achieved up to 0.117 mg g^{-1}(Figure 3.22).

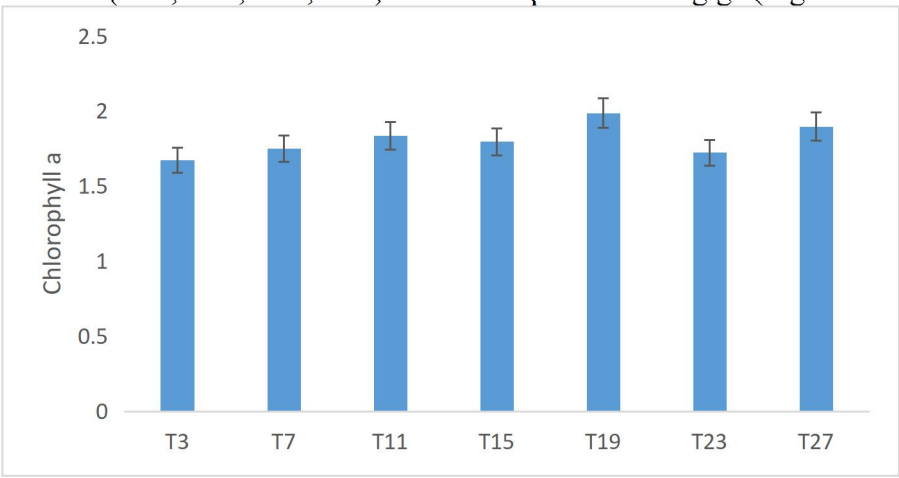


Figure 3.22: *Comparison of chlorophyll a content between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different times.*
T3(0.5mM $HgCl_2$), T7(0.5mM $HgCl_2$ + 0.5mM SA), T11(0.5mM $HgCl_2$+ 2.5mM SA), T15(pre 0.5mM $HgCl_2$+ post 0.5mM SA), T19(pre 0.5mM $HgCl_2$+ post 2.5mM SA), T23(post 0.5mM $HgCl_2$+ pre 0.5mM SA), T27(post 0.5mM $HgCl_2$+ pre 2.5mM SA).

3.2.2 Chlorophyll b

The changes in chlorophyll b mirrored those of chlorophyll a, though absolute values differed (Figure 3.23). Without stress, control plants (T0) contained 1.181 mg g^{-1}. SA alone enhanced chlorophyll b to 2.887 mg g^{-1} at 0.5 mM (T1) and to 2.819 mg g^{-1} at 2.5 mM (T2). Increasing $HgCl_2$ concentrations alone led to declines from 1.187 mg g^{-1} at 0.5 mM (T3) down to 0.081 mg g^{-1} at 3.0 mM (T6).

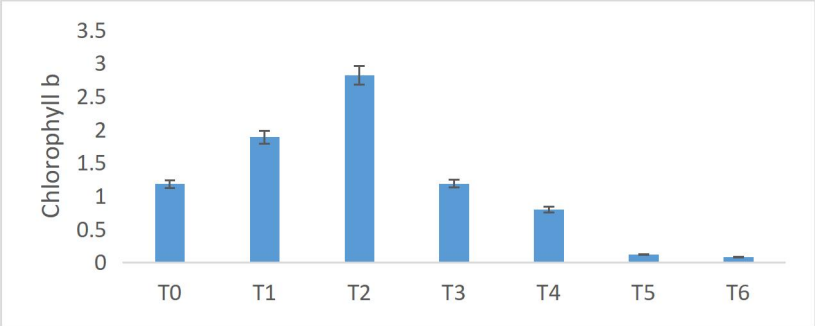


Figure 3.23: *Comparison of chlorophyll b content between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different times.* T0 (Control), T1(0.5mM SA), T2(2.5mM SA), T3(0.5mM $HgCl_2$), T4(1mM $HgCl_2$), T5(2mM $HgCl_2$), T6(3mM $HgCl_2$).

Under combined treatments (Figure 3.24), SA ameliorated $HgCl_2$-induced losses of chlorophyll b. At 0.5 mM $HgCl_2$, simultaneous SA co-application (T7, T11) raised levels to 1.386 and 1.516 mg g^{-1}; pre- and post-treatments (T15, T19; T23, T27) brought values to 1.485/1.674 and 1.423/1.588 mg g^{-1}, respectively. With 1.0 mM $HgCl_2$, simultaneous SA (T8, T12) improved chlorophyll b from 0.979 mg g^{-1} (T4) to 0.884 and 0.917 mg g^{-1}; sequential SA applications (T24, T28) further increased it to 0.897 and 0.945 mg g^{-1}. Under 2.0 mM stress, SA co-application (T9, T13) elevated chlorophyll b from 0.117 mg g^{-1} to 0.195 and 0.221 mg g^{-1}, and sequential treatments (T17, T21; T25, T29) yielded 0.215/0.291 and 0.205/0.246 mg g^{-1}, respectively. Even at 3.0 mM $HgCl_2$, SA provided modest recovery: co-applications (T10, T14) achieved 0.085 and 0.097 mg g^{-1}; sequential additions (T18, T22; T26, T30) reached up to 0.108 mg g^{-1}.

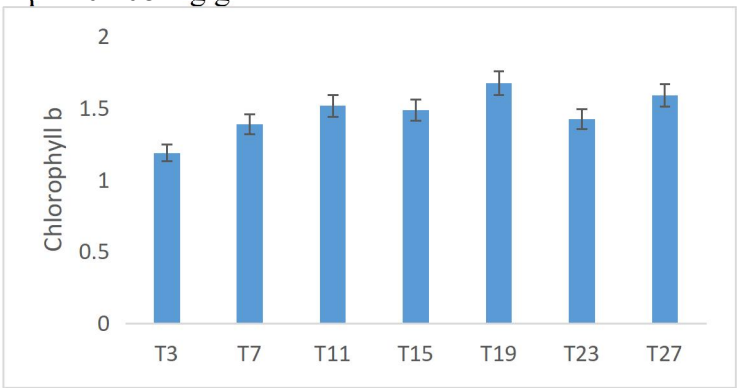


Figure 3.24: *Comparison of chlorophyll b content between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different times.*
T3(0.5mM $HgCl_2$), T7(0.5mM $HgCl_2$ + 0.5mM SA), T11(0.5mM $HgCl_2$+ 2.5mM SA), T15(pre 0.5mM $HgCl_2$+ post 0.5mM SA), T19(pre 0.5mM $HgCl_2$+ post 2.5mM SA), T23(post 0.5mM $HgCl_2$+ pre 0.5mM SA), T27(post 0.5mM $HgCl_2$+ pre 2.5mM SA).

3.2.3 Carotenoids

The carotenoid profile also reflected significant interactions between $HgCl_2$ stress and SA treatment (Figure 3.25). Control plants (T0) contained 0.861 mg g^{-1}, which increased to 0.972 mg g^{-1} with 0.5 mM SA (T1) and to 1.183 mg g^{-1} with 2.5 mM SA (T2). $HgCl_2$ alone reduced carotenoids from 0.863 mg g^{-1} at 0.5 mM (T3) to 0.051 mg g^{-1} at 3.0 mM (T6).

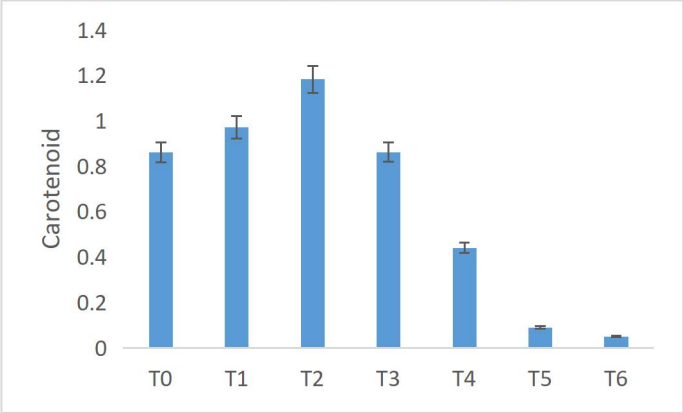


Figure 3.25: *Comparison of carotenoid content between different levels of Mercury chloride, Salicylic acid and Mercury chloride + Salicylic acid when applied at different times.*
T0 (Control), T1(0.5mM SA), T2(2.5mM SA), T3(0.5mM $HgCl_2$), T4(1mM $HgCl_2$), T5(2mM $HgCl_2$), T6(3mM $HgCl_2$).

When SA was combined with 0.5 mM $HgCl_2$, protective effects were evident: simultaneous treatments (T7, T11) increased carotenoids to 0.887 and 0.917 mg g^{-1}; pre- and post-treatments (T15, T19; T23, T27) raised levels to 0.904/0.937 and 0.896/0.927 mg g^{-1}, respectively. Under 1.0 mM $HgCl_2$, co-application (T8, T12) recovered carotenoids from 0.441 mg g^{-1} (T4) to 0.451 and 0.466 mg g^{-1}; sequential SA treatments (T24, T28) further improved pigment levels to 0.456 and 0.673 mg g^{-1}. In 2.0 mM $HgCl_2$-stressed plants (T5, 0.091 mg g^{-1}), SA co-application (T9, T13) restored carotenoids to 0.097 and 0.109 mg g^{-1}, while sequential SA (T17, T21; T25, T29) produced 0.105/0.117 and 0.101/0.113 mg g^{-1}. Even at the highest $HgCl_2$ level (T6), SA treatments provided slight improvements, with co-applications (T10, T14) reaching 0.056 and 0.069 mg g^{-1} and sequential additions (T18, T22; T26, T30) up to 0.084 mg g^{-1}(Figure 3.26).

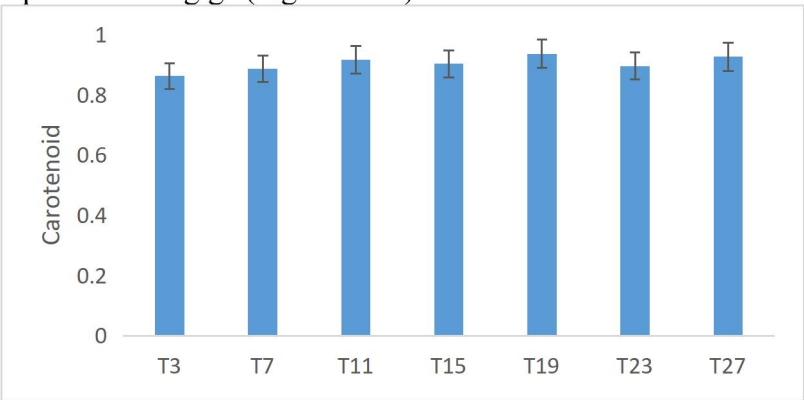


Figure 3.26: *Comparison of carotenoid content between different levels of Mercury Chloride, Salicylic acid and Mercury Chloride + Salicylic acid when applied at different times.*

T3(0.5mM $HgCl_2$), T7(0.5mM $HgCl_2$ + 0.5mM SA), T11(0.5mM $HgCl_2$+ 2.5mM SA), T15(pre 0.5mM $HgCl_2$+ post 0.5mM SA), T19(pre 0.5mM $HgCl_2$+ post 2.5mM SA), T23(post 0.5mM $HgCl_2$+ pre 0.5mM SA), T27(post 0.5mM $HgCl_2$+ pre 2.5mM SA).

Overall, salicylic acid markedly mitigated $HgCl_2$-induced declines in chlorophyll a, chlorophyll b, and carotenoids. Both simultaneous and sequential applications of SA restored pigment contents in a dose- and timing-dependent manner, with the greatest protective effects observed under mild to moderate $HgCl_2$ stress. These findings underscore the potential of SA as an effective regulator of photosynthetic pigments under heavy metal stress.

4. Discussion

4.1 Morphological Responses

Environmental pollution particularly heavy-metal contamination, poses a growing threat to ecosystems and human health, especially in developing countries where industrial and agricultural activities are intensifying (Shah, Khan et al. 2018).Heavy metals, defined by densities exceeding 5 g cm^{-3}(Zhang, Li et al. 2014), include both essential micronutrients (Fe, Mn, Cu, Zn, Mo) and highly toxic elements (As, Hg, Pb, Cd). While micronutrients support normal metabolism at low concentrations, excess levels can be phytotoxic; non-essential metals like mercury inflict damage even at trace amounts.

In our study, elevated $HgCl_2$ concentrations significantly impaired *Eruca sativa* growth and biomass accumulation. Treatments exceeding 3 mM $HgCl_2$ caused dramatic reductions in shoot and root parameters, consistent with previous reports linking mercury stress to disrupted water uptake, nutrient deficiency, and oxidative stress (Asema and Adejumo 2022).The most severe growth inhibition occurred in roots, reflecting their direct exposure to soil contaminants and heightened vulnerability to metal-induced mitotic disruption (Pant, Tripathi et al. 2011).Root meristem arrest during metaphase likely underpins the observed shorter root length and reduced biomass(Karray, Elloumi et al. 2022). Above-ground tissues likewise exhibited chlorosis and stunted leaf development, hallmarks of impaired cellular metabolism under heavy-metal stress (Millhollen, Gustin et al. 2006).

Exogenous salicylic acid (SA) application ameliorated many of these adverse effects. Foliar sprays of SA, particularly when applied one day after $HgCl_2$ exposure, significantly restored shoot and root growth metrics relative to metal-only controls. These findings support SA's role in enhancing stress tolerance by modulating antioxidant defenses, stabilizing membranes, and improving nutrient assimilation (Gontia-Mishra et al. 2016). Our results align with Verma and Dubey (2003) and Sengar et al. (2010), who documented SA-mediated mitigation of mercury's inhibitory impact on plant development. The timing of SA application proved critical: post-treatment consistently outperformed simultaneous or pre-treatment regimes, suggesting that inducing SA-dependent protective pathways after initial metal uptake may optimize recovery.

4.2 Physiological Adaptations

Heavy-metal stress not only alters morphology but also disrupts key physiological processes. Under increasing $HgCl_2$ levels, total soluble sugar contents rose—an osmoprotective response reported by Aldoobie and Beltagi (2013). Concurrently, overall protein levels declined, reflecting the selective induction of stress proteins and downregulation of general protein synthesis under abiotic stress (Janmohammadi, Bihamta et al. 2013).

Photosynthetic pigment concentrations were particularly sensitive to $HgCl_2$ toxicity. Chlorophyll a, chlorophyll b, and total chlorophyll content decreased sharply with rising

mercury stress, corroborating earlier findings in *Vigna radiata* and wheat (Liu, Asseng et al. 2016).Mercury's interference with magnesium and iron uptake—essential cofactors for chlorophyll biosynthesis—along with its affinity for protein thiol groups, likely disrupts photosynthetic complexes(Romanis, Pearson et al. 2021). Carotenoid levels, vital for photoprotection and light harvesting, were similarly diminished (Kumar, Gandhi et al. 2020),reflecting compromised antioxidant capacity and heightened photodamage under metal stress (Puzon et al. 2014).

SA treatments counteracted these declines: low-dose SA elevated chlorophyll a and b synthesis, enhancing photosynthetic performance and stress resilience (Gupta and Seth 2023).Moreover, SA's upregulation of carotenoid biosynthetic pathways bolstered light capture and scavenging of reactive oxygen species(Xiang, Liu et al. 2019). Again, applying SA one day after $HgCl_2$ stress yielded the most pronounced recovery in pigment content, underscoring the importance of application timing to maximize SA's regulatory effects on photosynthetic machinery.

5. Conclusion

Heavy-metal pollution, notably mercury contamination, severely compromises plant growth and physiological function by disrupting root development, photosynthetic pigment synthesis, and overall biomass accumulation. In *Eruca sativa*, increasing $HgCl_2$ concentrations induced dose-dependent reductions in morphological and biochemical parameters, with roots exhibiting the greatest vulnerability. Exogenous foliar application of salicylic acid effectively mitigated these deleterious effects by enhancing antioxidant defenses, stabilizing chloroplast structure, and promoting osmolyte accumulation.

Our randomized block experiment comprising 31 distinct treatment combinations of $HgCl_2$ and SA at varying timings demonstrated that a post-stress SA spray (applied one day after $HgCl_2$ exposure) consistently delivered the greatest improvements in fresh and dry weight, shoot and root lengths, chlorophyll a and b, and carotenoid content. These findings affirm SA's potential as a practical and economical strategy to bolster crop resilience against heavy-metal stress.

Implementing SA treatments in contaminated agroecosystems could safeguard yield and food quality, particularly in semi-arid regions where heavy-metal accumulation poses an escalating threat. Future research should explore the molecular mechanisms underlying SA-mediated detoxification pathways and evaluate field-scale applications across diverse crop species and soil conditions

7. References

Armendariz, A. L., et al. (2019). "Impact of double inoculation with Bradyrhizobium japonicum E109 and Azospirillum brasilense Az39 on soybean plants grown under arsenic stress." Plant Physiology and Biochemistry **138**: 26-35.

Asema, J. and B. Adejumo (2022). QUALITY ENHANCEMENT OF MASA: A REVIEW. Maiden International Conference.

Babalola, O. O., et al. (2020). "The nexus between plant and plant microbiome: revelation of the networking strategies." Frontiers in Microbiology **11**: 548037.

Bala, A., et al. (2017). "Oxidative stress in inflammatory cells of patient with rheumatoid arthritis: clinical efficacy of dietary antioxidants." Inflammopharmacology **25**: 595-607.

Chakrabarti, M. H. and R. Ahmad (2009). "Investigating possibility of using least desirable edible oil of Eruca sativa L., in biodiesel production." Pakistan Journal of Botany **41**(1): 481-487.

Chandwani, S. and N. Amaresan (2022). "Role of ACC deaminase producing bacteria for abiotic stress management and sustainable agriculture production." Environmental Science and Pollution Research **29**(16): 22843-22859.

Chmielewska, B., et al. (2021). "Effects of the COVID-19 pandemic on maternal and perinatal outcomes: a systematic review and meta-analysis." The Lancet Global Health **9**(6): e759-e772.

Cui, J., et al. (2014). "Atmospheric wet deposition of nitrogen and sulfur in the agroecosystem in developing and developed areas of Southeastern China." Atmospheric Environment **89**: 102-108.

Dhalaria, R., et al. (2020). "Arbuscular mycorrhizal fungi as potential agents in ameliorating heavy metal stress in plants." Agronomy **10**(6): 815.

Dixit, M. and P. P. Kumar (2024). "Embodied impacts of buildings from energy-carbon-water nexus perspective: A case study of university buildings." Cleaner energy systems **7**: 100108.

Ensink, J. H., et al. (2004). "A nationwide assessment of wastewater use in Pakistan: An obscure activity or a vitally important one?" Water policy **6**(3): 197-206.

Ertani, A., et al. (2018). "Evaluation of seaweed extracts from Laminaria and Ascophyllum nodosum spp. as biostimulants in Zea mays L. using a combination of chemical, biochemical and morphological approaches." Frontiers in Plant Science **9**: 428.

Etesami, H. (2024). "Enhancing soil microbiome resilience: the mitigating role of silicon against environmental stresses." Frontiers in Agronomy **6**: 1465165.

Gao, N., et al. (2020). "Thermochemical conversion of sewage sludge: A critical review." Progress in Energy and Combustion Science **79**: 100843.

Ghori, N.-H., et al. (2019). "Heavy metal stress and responses in plants." International journal of environmental science and technology **16**: 1807-1828.

Ghosh, P., et al. (2022). "Evaluation of recombinase-based isothermal amplification assays for point-of-need detection of SARS-CoV-2 in resource-limited settings." International Journal of Infectious Diseases **114**: 105-111.

Grami, F., et al. (2022). "Cerebellar transcranial direct current stimulation reconfigures brain networks involved in motor execution and mental imagery." The Cerebellum **21**(4): 665-680.

Gupta, P. and C. S. Seth (2023). "24-epibrassinolide regulates functional components of nitric oxide signalling and antioxidant defense pathways to alleviate salinity stress in Brassica juncea L. cv. Varuna." Journal of Plant Growth Regulation **42**(7): 4207-4222.

Janda, T., et al. (2007). "Factors contributing to enhanced freezing tolerance in wheat during frost hardening in the light." Phytochemistry **68**(12): 1674-1682.

Janmohammadi, M., et al. (2013). "Influence of rhizobacteria inoculation and lead stress on the physiological and biochemical attributes of wheat genotypes."

Karray, R., et al. (2022). "A novel bioprocess combining anaerobic co-digestion followed by ultra-filtration and microalgae culture for optimal olive mill wastewater treatment." Journal of environmental management **303**: 114188.

Kumar, D., et al. (2020). "Prevalence and correlation of dental caries with its specific risk factors in 5–15-year-old school-going children in urban population of Ghaziabad." International Journal of Clinical Pediatric Dentistry **13**(1): 72.

Kumar, K. and B. Gupta (2010). "Synthesis, characterization, CV, and X-ray structures of aryl cobaloximes." Journal of Organometallic Chemistry **695**(19-20): 2233-2239.

Liu, B., et al. (2016). "Similar estimates of temperature impacts on global wheat yield by three independent methods." Nature Climate Change **6**(12): 1130-1136.

Liu, S., et al. (2017). "Towards better analysis of machine learning models: A visual analytics perspective." Visual Informatics **1**(1): 48-56.

Mapanda, F., et al. (2007). "Uptake of heavy metals by vegetables irrigated using wastewater and the subsequent risks in Harare, Zimbabwe." Physics and Chemistry of the Earth, Parts A/B/C **32**(15-18): 1399-1405.

Millhollen, A. G., et al. (2006). "Foliar mercury accumulation and exchange for three tree species." Environmental science & technology **40**(19): 6001-6006.

Mirshekali, H., et al. (2012). "Effect of zinc toxicity on plant productivity, chlorophyll and Zn contents of sorghum (Sorghum bicolor) and common lambsquarter (Chenopodium album)."

Mushtaq, N. and K. S. Khan (2010). "Heavy metals contamination of soils in response to wastewater irrigation in Rawalpindi region." Pak. J. Agri. Sci **47**(3): 215-224.

Mwamba, T. M., et al. (2020). "Comparative metabolomic responses of low-and high-cadmium accumulating genotypes reveal the cadmium adaptive mechanism in Brassica napus." Chemosphere **250**: 126308.

Niane, P. M. (2023). Modélisation de la méningite bactérienne dans l'interface Environnement-Climat-Société par approche multi-agents: cas d'application au Sénégal, Sorbonne Université; Université Cheikh Anta Diop (Dakar, Sénégal; 1957-....).

Pant, P. P., et al. (2011). "Effect of heavy metals on some biochemical parameters of sal (Shorea robusta) seedling at nursery level, Doon Valley, India." Journal of Agricultural Sciences **2**(1): 45-51.

Romanis, C. S., et al. (2021). "Cyanobacterial blooms in wastewater treatment facilities: Significance and emerging monitoring strategies." Journal of Microbiological Methods **180**: 106123.

Shah, L. A., et al. (2018). "Superabsorbent polymer hydrogels with good thermal and mechanical properties for removal of selected heavy metal ions." Journal of Cleaner Production **201**: 78-87.

Sharma, A., et al. (2020). "Photosynthetic response of plants under different abiotic stresses: a review." Journal of Plant Growth Regulation **39**: 509-531.

Wang, Q., et al. (2016). "Impact of saline water irrigation on water use efficiency and soil salt accumulation for spring maize in arid regions of China." Agricultural water management **163**: 125-138.

Xiang, J., et al. (2019). "The fluorescence interference in Raman spectrum of raw coals and its application for evaluating coal property and combustion characteristics." Proceedings of the Combustion Institute **37**(3): 3053-3060.

Yaashikaa, P., et al. (2022). "A review on bioremediation approach for heavy metal detoxification and accumulation in plants." Environmental Pollution **301**: 119035.

Zhang, J.-c., et al. (2014). "R (−)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine." Pharmacology Biochemistry and Behavior **116**: 137-141.