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Unveiling the Effect of EDTA and SA in Mustard under Cd and Pb Stress

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ABSTRACT

Phytoremediation, particularly phytoextraction, shows promise in addressing this issue. Phytoextraction involves plants absorbing contaminants from the soil through their roots and transferring them to their shoots and leaves. However, in polluted environments, certain metals like lead (Pb) and cadmium (Cd) may have limited bioavailability, posing significant challenges to the phytoextraction process. One way to overcome this limitation is by applying chelators. The experiment conducted in a randomized block design to investigate the effects of two chelators, EDTA and salicylic acid (SA), in enhancing the anthocyanin, flavanoid and yield attributing parameters of *Brassica juncea* L. The seeds were sown in pots and subjected to various treatments of Cd and Pb of concentrations 10 and 20 mM, either alone and in combination with 0.5 mM EDTA and SA. The toxicity induced by Pb and Cd in *B. juncea* was recorded through increased levels of flavonoid and anthocyanin content and decreased value of yield parameters. Additionally, various yield parameters including 100 seed test weight, number of flower plant^{-1}, number of pod plant^{-1}, number of seeds plant^{-1} were highly affected by stress. Conversely, the supplementation of EDTA and SA to Cd, Pb-treated plants effectively mitigated negative impact of heavy metal stress by increasing flavonoid and anthocyanin content, along with yield parameter. Notably EDTA and SA also led to a significant increase in Pb and Cd accumulation.

Key words: *Phytoextraction, Yield, Flavonoid, Anthocyanin, Heavy metal*

Introduction

Heavy metal contamination in soil poses significant risks to human and environmental health, prompting the exploration of effective remediation methods. Phytoremediation, particularly phytoextraction, shows promise in addressing this issue. Phytoextraction involves plants absorbing contaminants from the soil through their roots and transferring them to their shoots and leaves. This method is deemed effective as it diminishes metal concentration in the root zone, which is crucial for cleanup, and metal uptake can be mitigated by harvesting the metal-rich plant matter (Shah and Daverey, 2020). Phytoextraction offers a safer and cleaner alternative by eliminating the need to transport large quantities of contaminated soil, allowing plant growth to naturally thrive in the clean soil. This approach is particularly crucial when addressing heavy metal contamination near residential areas or stemming from industrial sites. Residential soil contamination commonly arises from sources like house paint or airborne metal particles from nearby industries. (Shah and Daverey, 2020). Heavy metal affects various parameters of plant like growth, biochemical and yield parameters (Saviti *et* *al*., 2017). Phytoextraction is one of the phytoremediation techniques that exploit the unique ability of some plants to accumulate unusually high amount of chemicals/toxic metals including salt ions in their harvestable plant part (Devi *et al*., 2016). Indian mustard (*Brassica juncea*) is recognized as a hyperaccumulator of heavy metals, making it a focal point in research on this topic. Its ability to endure and accumulate significant levels of specific heavy metals sets it apart, as it does not manifest the severe toxic symptoms observed in other plant species (Raj *et al*., 2020). According to Timmermans *et al.* (2016), successful Phytoremediation hinges on plants that can generate significant biomass and accumulate toxic elements without growth inhibition. Indian mustard fulfills these requirements while also offering advantages such as being a cost-effective crop with a short growth cycle.

Chelator-enhanced phytoextraction (CEP) relies on chelating agents to form soluble complexes with heavy metals, enhancing their availability in soil for plant uptake. This method suggests that plants can accumulate greater metal quantities in harvestable parts from soils with low metal availability, potentially boosting metal phytoextraction efficacy (Diarra *et al*., 2021). Chelation enhances metal uptake by boosting metal ion activity in the soil solution and creating a steeper metal concentration gradient between the soil solution and root surfaces. After absorption by the chelate and uptake into the plant, the complex can be transported to the shoots, a crucial step for efficient phytoextraction given that most plant biomass is above ground. Chelate translocation can proceed through the xylem or the symplastic pathway, with the xylem typically serving as the main route for water and nutrient transport from roots to shoots. However, chelates and their metal complexes face obstruction from reaching the xylem due to the apoplastic nature of the endodermis and exodermis, compounded by heightened radial binding of metals to root cell walls. Consequently, these metal complexes must re-enter the symplastic pathway to circumvent the barrier before resuming their journey to the xylem. Despite the slower pace of the symplastic pathway, studies have noted metal translocation from roots to shoots via both pathways (Chen *et al*., 2020).

EDTA is widely recognized as a highly effective chelating agent, extensively employed for metal chelation in soils and sediments owing to its robust ligand properties across various pH levels. The formation constant of a chelating agent denotes its capacity to create a complex with a metal ion; a higher formation constant indicates a stronger and more stable complex (Cheng *et al*., 2020). Synthetic chelators like EDTA are artificial compounds capable of forming potent complexes across a broad pH spectrum and exhibiting high water solubility (Salih *et al*., 2022).

(SA), a plant metabolite, has been demonstrated to boost the extraction of certain metals without heightening their mobility in the rhizosphere. However, the precise mechanism through which chelators elevate metal uptake in the presence of plants remains intricate. (Sharma *et al*., 2020). However, it's widely acknowledged that chelators enhance metal solubility and availability to plants by forming metal-chelator complexes, preventing metal hydrolysis and the formation of insoluble precipitates (Diarra *et al*., 2021). Studies have observed increased shoot and root biomasses in plants exposed to a Cd-containing nutrient solution when supplemented with salicylic acid. This suggests that salicylic acid doesn't just have specific functions but also exerts a protective effect against various heavy metals, reducing their toxicity (Sharma *et al*., 2020). Toxicity from Pb and Cd reduces biochemical and reproductive parameters in *B. juncea*. EDTA and SA supplements alleviate heavy metal-induced damage in treated plants (Kumar *et al*., 2024).

A rise in flavonoid content was noted in cadmium-treated plants compared to their respective controls (Sardar *et al*., 2022). This increase in flavonoid synthesis could suggest the presence of cellular damage, potentially triggered by protein damage and induction of phenolic compounds. Consequently, flavonoids might serve as a potential biomarker for metal toxicity in *Brassica juncea* (Tan *et al*., 2021). Anthocyanins, water-soluble vacuolar pigments, exhibit red, purple, or blue hues contingent upon pH levels. Classified as flavonoids, they are glycosides of anthocyanidins and are recognized for their antioxidant capabilities. These compounds are purported to shield plants from UV light, pathogens, and oxidative harm (Merecz-Sadowska *et al*., 2023). Anthocyanins serve diverse functions in plant development and defense against both biotic and abiotic stresses (Naing and Kim, 2021). Research indicates that the accumulation of anthocyanins in response to stress is governed by coordinated genetic regulation (Yan *et al*., 2021). Elevated metal concentrations were found to impede root and shoot elongation, leading to diminished plant growth. These growth alterations subsequently contributed to reduced yield parameters (Akeel and Jahan, 2020). The decline in yield parameters of *Brassica juncea* due to cadmium treatments was observed to be dependent on concentration. Across all parameters in both experiments, it is apparent that the impact of cadmium on *B. juncea* yield is more severe than that of lead (Dhaliwal *et al*., 2021). Cadmium exhibits a significantly greater toxic effect compared to lead, evident from the decline in almost all measured parameters. Lead demonstrated *Brassica juncea* potential for phytoremediation, as the plant exhibited greater tolerance to this heavy metal, with a more gradual decline in parameters (Malecka *et al*., 2021). In current investigation we assessed how flavonoid, anthocyanin and yield parameters were effected under heavy metal stress and chelators helps to improve biochemival and yield parameters of *Brassica juncea*.

Materials and Methods

The experiment was conducted at the Herbal Garden, Maharshi Dayanand University (Rohtak), India (latitude 28º 54′ 0″ N, longitude 76º 34′ 0″ E, Altitude 220m). *Brassica juncea* L. healthy seeds of genotype (RH0725) were procured from the Chaudhary Charan Singh Haryana Agriculture University (CCSHAU) Hisar, India. Six kilogram of sand was placed into the 12-inch pots, which were lined with polyethylene bags. The sand underwent cleaning with 0.1 N HCl to remove fungal contamination, followed by rinsing with DDW before being transferred into the pots. The study was designed according to a Complete Randomised Design (CRD) methodology, with three replications. The aim of this study was to examine the response of Indian mustard (*B. juncea*) to heavy metal (Pb and Cd) at 0 mM (Control), 10mM, 20mM, and Chelator application, i.e. EDTA and SA (0.5mM) to the phyto-accumulation capacity of mustard for different concentration of heavy metals (Pb and Cd). *Brassica* seeds were grown in soil that had been artificially treated with various levels of heavy metals. After an interval of thirty days, the chelator EDTA and SA (0.5mM) were added. The sampling was done after fifteen days of chelators treatments.

Treatments

The plants were supplied with Wilson and Reisenauer (1963) nutrient solution with 200 ml added per pot.

Sr. No.	Treatments	Chemical Combination
1.	T_0	Control
2.	T_1	10 mMCd
3.	T_2	20 mMCd
4.	T_3	10 mMCd + EDTA
5.	T_4	10 mMCd + SA
6.	T_5	20 mMCd + EDTA
7.	T_6	20 mMCd + SA
8.	T_7	10 mMPb
9.	T_8	20 mMPb
10.	T_9	10 mMPb + EDTA
11.	T_{10}	10 mMPb + SA
12.	T_{11}	20 mMPb + EDTA
13.	T_{12}	20 mMPb + SA

Flavonoid and anthocyanin content

The Dualex 4 Scientific (Dx4) analyzer was used to determine the flavonoid and anthocyanin content. This involved, place the leaf into the device and closing the measuring head. The Dx4 analyzer provides real-time SPAD values as well as a time value that correlates with the leaf's flavonoid and anthocyanin content.

Measurement of yield parameters

Test weight of 100 seeds

Three sets of 100 seeds each were randomly chosen from every treatment for the measurement of seed weight.

Number of flowers per plant: At 85 days after planting (DAP), the number of flowers per experimental plant was counted and recorded upon reaching the flowering stage.

Number of pods harvested per plant: At 120 days after planting (DAP), the number of pods harvested per experimental pot was counted and recorded when the plant reached maturity and was ready for harvest.

Number of seeds per plant: At 120 days after planting (DAP), the number of pods harvested per experimental pot was recorded at plant maturity. Subsequently, the number of seeds was counted from the harvested pods.

Results

Flavonoid content

The flavonoids increased along with the heavy metal concentration, indicating that the biosynthetic pathways of flavonoids were stimulated by heavy metal ions. Flavonoid content were increased with the increase of heavy metal concentration. One-way ANOVA revealed heavy metal stress and EDTA and SA had significant effects on flavanoid content ($P < 0.05$). The Flavonoid content increased with increase of heavy metal stress from 10 mM to 20 mM Lead and Cadmium concentration. The flavonoid content showed a significant rise by 51.70%, 34.69% in cadmium and lead stress as compared with the control treatments. The co-application of EDTA and SA at a concentration of 0.5 mM with cadmium and lead stress induces a significant increase in flavonoid content as compared to the application of cadmium and lead stress alone (Fig. 1). The exogenous application of EDTA was found to increase its activity by 29.15% in cadmium and 26.77% in lead treated plants, respectively. Similarly, SA increased flavonoid content by 18.39% in plants subjected to 20 mM Cd stress. In addition, it was found that an increased amount of Pb with SA led to enhanced flavonoid content by 15.15%.

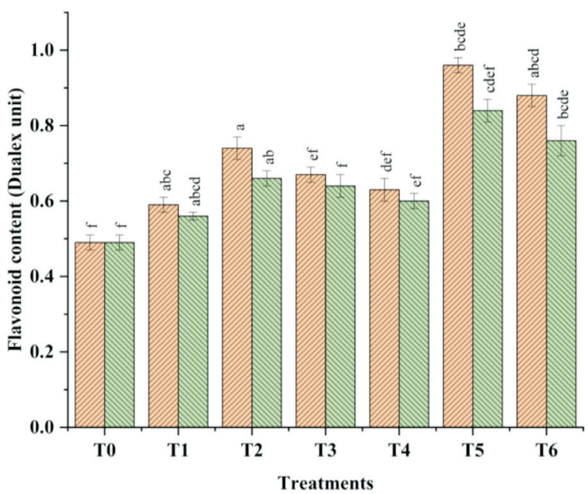

Fig. 1. The effect of EDTA and SA on Flavonoid content of *Brassica* under heavy metal stress, subjected to 0, 10, 20 mM Cd and Pb respectively. Each value represents the average of three replicates, with standard error (SE) calculated. [Control (T0),10 mM (T1), 20 mM (T2), 10 mM+EDTA (T3), 10 mM+SA (T4), 20 mM+ EDTA (T5), 20 mM+SA (T6)].

Anthocyanin content

Anthocyanin content was increased with the increase of heavy metal concentration. One-way ANOVA revealed heavy metal stress and EDTA and SA had significant effects on anthocyanin content ($P < 0.05$). The anthocyanin content increased with increase of heavy metal stress from 10 mM to 20 mM Lead and Cadmium concentration. The anthocyanin content showed a significant rise by 32.79%, 22.95% in cadmium and lead stress as compared with the control treatments. The co-application of EDTA and SA at a concentration of 0.5 mM with cadmium and lead stress induces a significant increase in anthocyanin content as compared to the application of cadmium and lead stress alone (Fig. 2). The exogenous application of EDTA was found to its increase its activity by 20.99% in cadmium and 18.67% in lead treated plants, respectively. Similarly, SA increased anthocyanin content by 14.81% in plants subjected to 20 mM Cd stress. In addition, it was found that an increased amount of Pb with SA led to raised anthocyanin content by 10.67%.

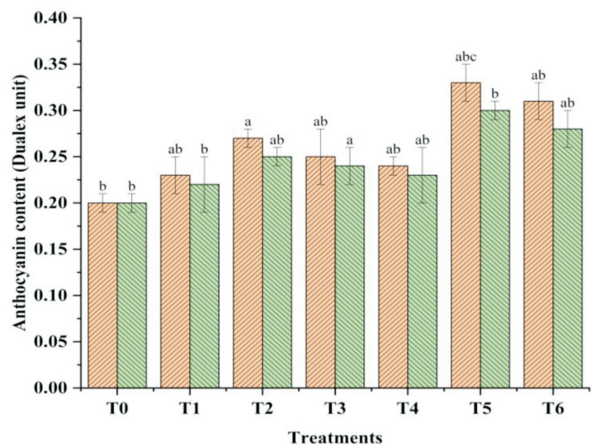

Fig. 2. The effect of EDTA and SA on Anthocyanin content, of *Brassica* under heavy metal stress, subjected to 0, 10, 20 mM Cd and Pb respectively. Each value represents the average of three replicates, with standard error (SE) calculated. [Control (T0), 10 mM (T1), 20 mM (T2), 10 mM+EDTA (T3), 10 mM+SA (T4), 20 mM+ EDTA (T5), 20 mM+SA (T6)].

Test weight of 100 seeds

Test weight of 100 seeds was decreased with the increase of heavy metal concentration. One-way ANOVA revealed heavy metal stress and EDTA and SA had significant effects on test weight of 100 seeds ($P < 0.05$). The test weight of 100 seeds decreased with increase of heavy metal stress from 10 mM to 20 mM Lead and Cadmium concentration. The test weight of 100 seeds showed a significant decrease in weight by 35.55%, 27.01% in cadmium and lead stress as compared with the control treatments. The co-application of EDTA and SA at a concentration of 0.5 mM with cadmium and lead stress induces a significant increase in test weight of 100 seeds as compared to the application of cadmium and lead stress alone (Fig. 3). The exogenous application of EDTA was found to its increase its activity by 21.96% in cadmium and 15.47% in lead treated plants, respectively. Similarly, SA increased test weight of 100 seeds by 18.69% in plants subjected to 20 mM Cd stress. In addition, it was found that an increased amount of Pb with SA led to increase in test weight of 100 seeds by 11.03%.

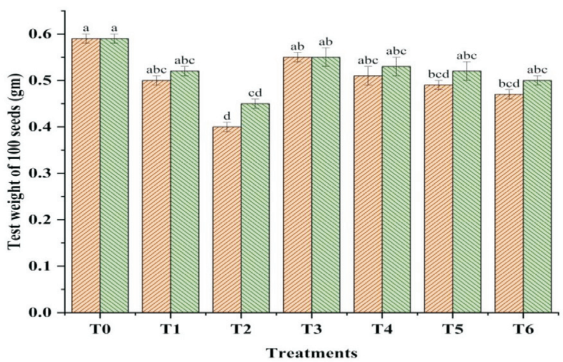

Fig. 3 The effect of EDTA and SA on test weight of 100 seeds, of *Brassica* under heavy metal stress, subjected to 0, 10, 20 mM Cd and Pb respectively. Each value represents the average of three replicates, with standard error (SE) calculated. [Control (T0),10 mM (T1), 20 mM (T2), 10 mM+EDTA (T3), 10 mM+SA (T4), 20 mM+ EDTA (T5), 20 mM+SA (T6)].

Number of flowers per plant

Numbers of flowers per plant were decreased with the increase of heavy metal concentration. One-way ANOVA revealed heavy metal stress and EDTA and SA had significant effects on number of flowers per plant ($P < 0.05$). The number of flowers per plant decreased with increase of heavy metal stress from 10 mM to 20 mM Lead and Cadmium concentration. The number of flowers per plant showed a significant decrease in numbers by 32.10%, 28.86% in cadmium and lead stress as compared with the control treatments. The co-application of EDTA and SA at a concentration of 0.5 mM with cadmium and lead stress induces a significant increase in numbers of flowers per plant as compared to the application of cadmium and lead stress alone (Fig. 4). The exogenous application of EDTA was found to its increase its activity by 24.15% in cadmium and 16.55% in lead treated plants, respectively. Similarly, SA increased numbers of flowers per plant by 18.36% in plants subjected to 20 mM Cd stress. In addition, it was found that an increased amount of Pb with SA led to increased numbers of flowers by 11.36%.

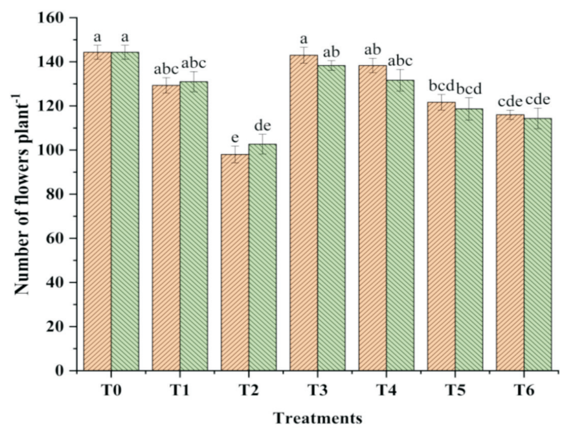

Fig. 4. The effect of EDTA and SA on number of flowers per plant content of *Brassica* under heavy metal stress, subjected to 0, 10, 20 mM Cd and Pb respectively. Each value represents the average of three replicates, with standard error (SE) calculated. [Control (T0),10 mM (T1), 20 mM (T2), 10 mM+EDTA (T3), 10 mM+SA (T4), 20 mM+ EDTA (T5), 20 mM+SA (T6)].

Number of pods per plant

Numbers of pods per plant were decreased with the increase of heavy metal concentration. One-way ANOVA revealed heavy metal stress and EDTA and SA had significant effects on number of seeds per plant ($P < 0.05$). The number of pods per plant decreased with increase of heavy metal stress from 10 mM to 20 mM Lead and Cadmium concentration. The numbers of pods per plant showed a significant decrease in numbers by 53.05%, 41.47% in cadmium and lead stress as compared with the control treatments. The co-application of EDTA and SA at a concentration of 0.5 mM with cadmium and lead stress induces a significant increase in numbers of pods per plant as compared to the application of cadmium and lead stress alone (Fig. 5). The exogenous application of EDTA was found to its increase its activity by 64.38% in cadmium and 30.76% in lead treated plants, respectively. Similarly, SA increased numbers of pods by 52.74% in plants subjected to 20 mM Cd stress. In addition, it was found that an increased amount of Pb with SA led to increased number of pods per plant by 18.13%.

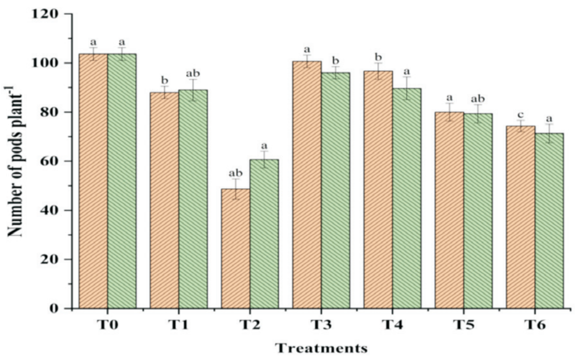

Fig. 5. The effect of EDTA and SA on number of pods per plant, of *Brassica* under heavy metal stress, subjected to 0, 10, 20 mM Cd and Pb respectively. Each value represents the average of three replicates, with standard error (SE) calculated. [Control (T0), 10 mM (T1), 20 mM (T2), 10 mM+EDTA (T3), 10 mM+SA (T4), 20 mM+ EDTA (T5), 20 mM+SA (T6)].

Number of seeds per plant

Numbers of seeds per plant were decreased with the increase of heavy metal concentration. One-way ANOVA revealed heavy metal stress and EDTA and SA had significant effects on number of seeds

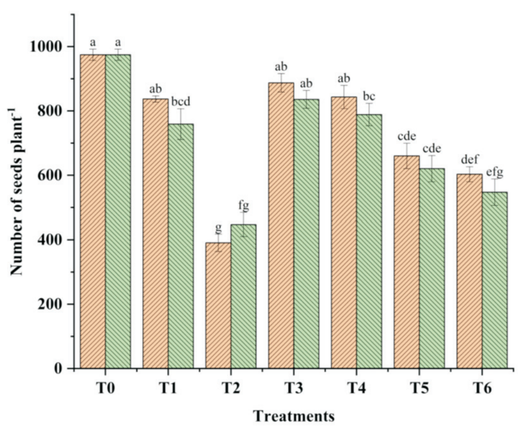

Fig. 6. The effect of EDTA and SA on number of seeds per plant, of *Brassica* under heavy metal stress, subjected to 0, 10, 20 mM Cd and Pb respectively. Each value represents the average of three replicates, with standard error (SE) calculated. [Control (T0),10 mM (T1), 20 mM (T2), 10 mM+EDTA (T3), 10 mM+SA (T4), 20 mM+ EDTA (T5), 20 mM+SA (T6)].

per plant ($P < 0.05$). The number of seeds per plant decreased with increase of heavy metal stress from 10 mM to 20 mM Lead and Cadmium concentration. The numbers of seeds per plant showed a significant decrease in numbers by 59.91%, 54.10% in cadmium and lead stress as compared with the control treatments. The co-application of EDTA and SA at a concentration of 0.5 mM with cadmium and lead stress induces a significant increase in numbers of seeds per plant as compared to the application of cadmium and lead stress alone (Fig. 6). The exogenous application of EDTA was found to its increase its activity by 68.94% in cadmium and 38.74% in lead treated plants, respectively. Similarly, SA increased numbers of seeds by 54.43% in plants subjected to 20 mM Cd stress. In addition, it was found that an increased amount of Pb with SA led to increased number of seeds by 22.35%.

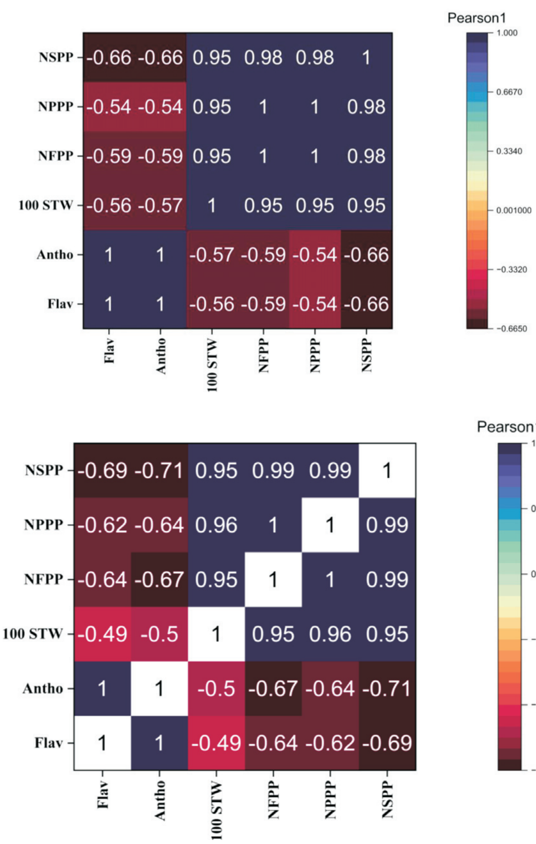

Fig. 7. A correlation graph based on Pearson's coefficient was created to analyze relationships among parameters in *Brassica juncea* under Cadmium (A) and Lead (B) stress. Mean values of variables were standardized and grouped, with color intensity indicating normalized mean values. Abbreviations such as Flav (Flavonoids), Antho (Anthocyanin),

100 STW (Test weight of 100 seeds), NFPP (Number of flowers per plant), NPPP (Number of pods per plant), and NSPP (Number of seeds per plant) are used. Lines from the center denote positive or negative correlations, with proximity indicating correlation strength.

Principal component analysis

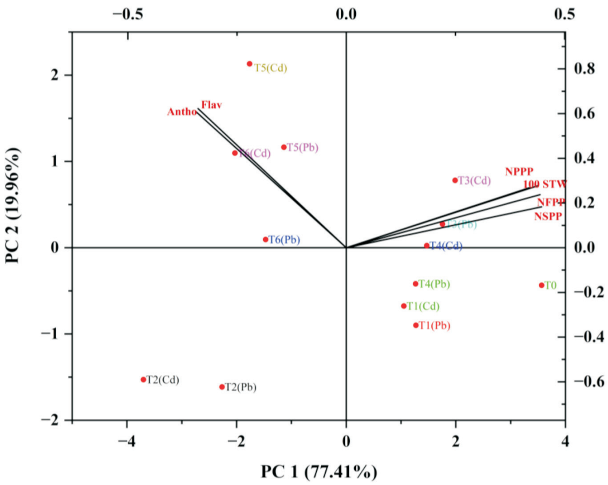

Fig. 8. Principle Component 1(PC 1) effectively segregates the treatments into two distinct categories on the biplot: the non stress (T0) conditions are positioned on the right side, while the stress (T2 Cd) and (T2 Pb) conditions are on the left side. The non stress and mild stress combinations are characterized by relatively high levels of test weight of 100 seed, numbers of flowers per plant, number of pods per plant and number of seeds per plant. Conversely, heavy metal stress treatment (T2 Cd) and (T2 Pb) exhibits relatively high levels for parameters located on the left side of the biplot, such as flavonoid and anthocyanin content and relatively low levels for those on the right side, including 100 STW, NFPP, NPPP, NSPP. This indicates that the Indian mustard under heavy metal and control conditions manifest contrasting states for those variables.

PC 2 separates the treatments under heavy metal-stress conditions. (T5, T6 Cd) and (T5, T6 Pb) are placed on the upper left of the biplot, characterized by relatively high levels of anthocyanin and flavonoid. (T2 Cd) and (T2 Pb) is placed on the lower left part of the biplot.

Discussion

Flavonoid

The present investigation revealed a significant increase in flavonoid content in leaves of *Brassica* genotypes subjected to different level of lead and cadmium heavy metal stress. The flavanoid content was enhanced owing the heavy metal stress and more raised was observed in *Brassica* genotype with different heavy metal level (10 mM, 20 mM) in comparison to non stressed plants (Fig. 1). Plant damage occurs when antioxidant mechanisms fail to counteract reactive oxygen species (ROS) synthesis. Phenolic compounds, like flavonoids, are key in plant defense. Okra plants stressed with heavy metal showed increased phenols and flavonoids, akin to responses seen in plants exposed to trace elements (González-Mendoza *et al.*, 2018). Pb toxicity increased flavonoid levels in okra, possibly due to reduced chlorophyll, enhancing stress tolerance. EDTA boosted flavonoids in Pb-stressed plants, suggesting *Brassica juncea* L. potential for phytoremediation. SA raised flavonoids and anthocyanins in salt-stressed soybeans via increased free radicals (Simaei *et al*., 2012).

Anthocyanin

Anthocyanins, water-soluble pigments derived from the flavonoid pathway, are commonly present in various plant tissues and are known for their antioxidative properties (Wang *et al*., 2019). They play a crucial role in mitigating oxidative stress, thereby enhancing plants resistance to abiotic stress (Luo *et al*., 2021). According to our observation the anthocyanin pigment were increased with increasing of heavy metal stress (Pb, Cd) upto 20 mM (Fig. 2). Under heavy metal stress, the application of chelators EDTA and SA significantly increased anthocyanin pigment levels (Fig. 2). Furthermore, anthocyanins act as chelators for metals (Ahammed and Yang, 2022) particularly aiding in enhancing plant resilience against heavy metal-induced toxicity and ROS.

Anthocyanin and xanthophylls content increased under Cd metal stress in *B. juncea*, possibly due to the activation of the antioxidative enzyme (glutathione-S-transferase) stimulating anthocyanin production (Milborrow, 2001). These findings align with previous studies (Amiri *et al*., 2012), suggesting that plants bolster anthocyanin production under stress to enhance antioxidant activities and osmotic balance regulation (Cirillo *et al*., 2021). EDTA application further boosts anthocyanin ratio in leaves, indicating increased antioxidant potential, consistent with other research (Tirani *et al*., 2022). However, plants subjected to Pb stress, with or without

SA, exhibited reduced anthocyanin compared to their control. Exogenous SA application has been shown to increase anthocyanin accumulation rice seedlings under saline stress (Khan *et al*., 2019), consistent with our findings suggesting SA role in enhancing anthocyanin content in Pb-treated plant.

Yield parameters

The present investigation revealed a significant decrease in yield attributing parameters of brassica genotypes subjected to different level of lead and cadmium heavy metal stress. Higher concentrations of heavy metal led to a notable decrease in flower numbers, numbers of pods, numbers of seeds per plant, 100 seed test weight likely due to elevated metal levels (Gothberg *et al*., 2004). Heavy metal stress can delay flowering, influenced by environmental factors (Tooke *et al*., 2005). Stress reduces the production of photosynthetic materials, shortening seed filling periods and resulting in smaller, lighter seeds (Cui *et al*., 2019; Singh *et al*., 2016). Direct leaf application of chelators enhances grain yield (Sabet and Mortazaeinezhad, 2018). Similar results with aminochelates in green bean plants (Aslani and Souri, 2018), attributed to enhanced absorption and mobility chelators, leading to improved flowering, podding duration, leaf area, and photosynthesis rate, resulting in higher pod grain numbers and grain yield (Liu and Lal, 2015).

Conclusion

Flavonoid and anthocyanin content in *Brassica juncea* increased in response to heavy metal stress. Heavy metals affects yield attributing parameters includes number of flowers per plant, numbers of pods per plant, number of seeds per plant, 100 seed test weight. EDTA and salicylic acid (SA) have been employed in numerous studies involving various species within the Brassicaceae family, serving as metal chelators to investigate a variety of biochemical and yield parameters EDTA show more effectiveness in minimizing the adverse effects in *Brassica*. The current study assessed the phytoremediation potential of EDTA and SA in reducing the negative effects of heavy metal stress on biochemical and reproductive parameters in *Brassica*. Comparison of different heavy metal treatments, either alone or in combination with EDTA and SA, revealed that combining EDTA and SA with Cd and Pb was the most effective in alleviating the adverse effects of heavy metal stress. Even under severe heavy metal stress (20 mM), synergistic effects were observed with the application of EDTA and SA, leading to improvements in biochemical and quality parameters. EDTA and SA treatment notably enhanced osmotic potential, chlorophyll content, and reproductive parameters.

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Conflict of interest

There is no conflict of interest among the authors.

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