Plant Health 03 (01) 2024. 87-101 DOI: 10.33687/planthealth.03.01.5128

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Available Online at EScience Press
**Plant Health**
ISSN: 2305-6835
https://esciencepress.net/journals/planthealth

# Interaction of Heavy Metals Stress (Cadmium, Copper) and Salicylic Acid on Growth, Yield and Physiological Parameters in Two Varieties of Pea

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**ARTICLE INFO**

**Article History**
Received: December 13, 2023
Revised: Februray 02, 2024
Accepted: Februray 15, 2024

**Keywords**
Antioxidant activities
Chlorophyll contents
Exogenous treatments
Heavy metal toxicity
Pea
Phytohormones

**ABSTRACT**

The goal of the current study was to evaluate the effectiveness of foliar salicylic acid spray in reducing the negative effects of heavy metal (cadmium, copper) stress on two varieties of pea (Pisum sativum L.) Meteor and Green Cross during 2020-2021. Heavy metal stress on plants (40, 60, 80 and 120 ppm) eventually caused the loss of several of their morphological traits. All morphological parameters such as root and shoot length as well as dry and fresh biomass, leaf area, number of leaves and leaf area ratio were declined with the heavy metals (Cd, Cu) stress. Antioxidant levels as well as biochemical attributes were decreased by heavy metal stress. After plants were exposed to stress, the amount of protein and carbohydrates decreased. All photosynthesis-related pigments, including chlorophyll a, b, and carotenoids, were similarly decreased. The outcomes of the experiment demonstrated that spraying plants with salicylic acid both under normal circumstances and when they were exposed to heavy metal stress was beneficial. The negative effects of heavy metal stress on plants were reduced with the use of foliar sprays containing salicylic acid. The Meteor variety of pea produced the best effects across the board, according to overall data. The study found that foliar treatments of 30 and 40 ppm of SA could help plants under heavy metal stress by enhancing morphological, physiological, biochemical, antioxidant activity, and yield catalogues in pea plants. Salicylic acid, when used to relieve heavy metal stress, can therefore be advantageous in this regard. This can be done to increase crop output, despite the fact that salicylic acid application has also proved successful. According to our research, oxidative stress is the main contributor to the phytotoxicity of heavy metals (Cd and Cu), and salicylic acid is a key component of pea plants' defence mechanisms against Cd and Cu exposure.

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## INTRODUCTION

Pea (*Pisum sativum* L.) is the major leguminous yield which is grown up for numerous purposes and it may consume for frequent benefits on all over the world (Pandey *et al*., 2021). Pea and chickpea are two examples of legumes that were initially cultivated throughout the world. The primary uses of field peas are as a source of both human food and animal feed (Shreyas *et al.,* 2022). It comprises 21- 25% protein and is used as alternate of soybean in Europe (Lu *et al.,* 2020). It contains huge amount of carbohydrates contents as well as 86-87% other digestible nutrients and the reason is that pea is an excellent feed for livestock (Nazeer *et al.,* 2020).

Heavy metal poisoning is a problem that is becoming more important for ecological, evolutionary, nutritional and environmental reasons. Heavy metals were substantial environmental contaminants (Sana *et al.,* 2020). Heavy metal contaminants, like cadmium (Cd) and copper (Cu), are challenging to remove from soil and water because they are difficult to break down chemically into benign elements (Venkatachalam *et al.,* 2017). The Pea crop recovered a significant amount of heavy metals after being submerged in contaminated water, creating a number of dangers to human health (Kishor *et al.,* 2020). Due to their accumulation in agricultural goods, several dense metals across the food web were transported to various media. Numerous dense metals' bio concentration factors (BCF) in the crop-soil interface have been identified, primarily in the basic, globally important crops (Yilkal *et al.,* 2021).

The severely harmful effects of Cd on a variety of plant metabolic activities, such as photosynthesis, respiration, nitrogen metabolism, transpiration, and food intake, have been shown in several scientific investigations (Usman *et al*., 2021). Cu is hazardous to plants because it participates in Fenton reactions, which promote the production of reactive oxygen species. ROS can harm cells by interfering with vital physiological functions including protein synthesis and enzyme activity (Swati *et al.,* 2021).

Plant hormones are active and involved in several growth processes at very low doses (Butto *et al.,* 2020). PGRs increases crop yield due to embryogenesis and vegetative growth (Ghasem *et al.,* 2022). The phenolic molecule phytohormone salicylic acid (SA) controls a number of growth as well as developmental processes like germination of seeds, photosynthesis, respiration, blooming, and senescence (Yamshi *et al.,* 2020). Additionally, when biotic and abiotic stressors such high temperatures, ozone pollution, UV radiation, heavy metals, drought, and salinity are present in plants, SA acts as a potent signaling molecule to activate their defensive mechanisms (Parankusam *et al.,* 2020).

Exogenous SA treatment has been shown in numerous studies to lessen the adverse impacts of heavy metals on plants (Sharma *et al.,* 2020). Although the participation of SA in stress adaption processes has been reported in numerous research, the effect of SA remains unclear (Muhammad *et al*., 2021). Salicylic acid is an innate signal that mediates plants' defensive reactions to different stresses, like trace metal toxicity, and to varying degrees reduces the toxicity of these stresses (Abdullah *et al*., 2020). It is thought that this hormone aids in the regulation and correction of plants' biochemical and physiological properties (El Dakak and Hassan, 2020). The current study assesses how these heavy metals (Cu, Cd) affect growth, physiological traits, seed yield and grain protein when applied independently and in combination with foliar sprays of salicylic acid.

## MATERIALS AND METHODS

### Experimental Design

Pot study experiments were conducted in the research area, University of Gujrat, Gujrat-Pakistan during 2020-2021. Three replications of the experiment were carried out using a completely random design (CRD). 27 Earthen plastic pots of each variety were used having 30cm in diameter and pots fixed polythene sacks were filled up with 8-10 kg of sandy loam soil. Pea seeds were purchased from the Gujranwala branch of the Punjab Agriculture Department. Sowing of seeds was done in November 2020 and eight seeds were sown at the depth of 2-3 cm in each pot. Tap water was utilized for water system at whatever point required. After the 10$^{th}$ day of germination thinning was done to keep 4-5 plants per pot at equal distance and same height.

### Treatments

Following were the treatments:

1. $T_0$ = Control
2. $T_1$ = 40 ppm Cd
3. $T_2$ = 60 ppm Cd
4. $T_3$ = 80 ppm Cu
5. $T_4$ = 120 ppm Cu
6. $T_5$= 40 ppm Cd + 30 ppm SA
7. $T_6$= 60 ppm Cd + 40 ppm SA
8. $T_7$= 80 ppm Cu + 30 ppm SA
9. $T_8$= 120 ppm Cu + 40 ppm SA

After 2 weeks Cadmium (Cadmium chloride) and copper (copper sulphate) were applied to plants in 2 different levels. The treatments levels were i.e., 0 (control), 40, 60, 80 and 120 ppm, by rooting medium of plants. Daily water deliveries were made to maintain equilibrium. After a few days of the Cadmium and copper stress, Salicylic Acid was applied to plants by foliar method. Salicylic acid spray was done early in the morning using a manual sprayer. The experiment was conducted in the fully randomized design (CRD).

Data on morphological, biochemical, and gaseous exchange characteristics, including leaf area, number of leaves, chlorophyll content and gas exchange were collected throughout the vegetative stage. The number of leaves on each plant was counted first, and then the mean value was determined. Chlorophyll *a, b* and carotenoid contents were calculated by Arnon procedure as described by Nazeer *et al.* (2020). Superoxide dismutase (SOD) activity was detected according to Giannopolitis and Ries method as used by Ullah *et al.* (2015). Catalase (CAT) was determined by Aebi method as used by Aziz *et al*. (2014). Peroxidase (POD) activity was estimated as described by Hussain *et al.* (2020) using the Chance and Maehly procedure. Total soluble proteins were calculated by Lowery method as described by Shen *et al.* (2013). Carbohydrates contents were recorded by following Krishnaveni procedure described by Nazeer *et al*. (2020). Bates *et al.* (1973) method was used to measure the proline contents of leaf.

**Statistical Analysis**

Data were presented as mean ± SE of three replicates and subjected to two-way analysis of variance (ANOVA) technique using Ministate-C software and mean separation was done at P ≤ 0.05 by using Tukey's HSD test, with a confidence interval of 95% (Silverman, 2018). Data was presented graphically by using MS-Excel.

## RESULTS

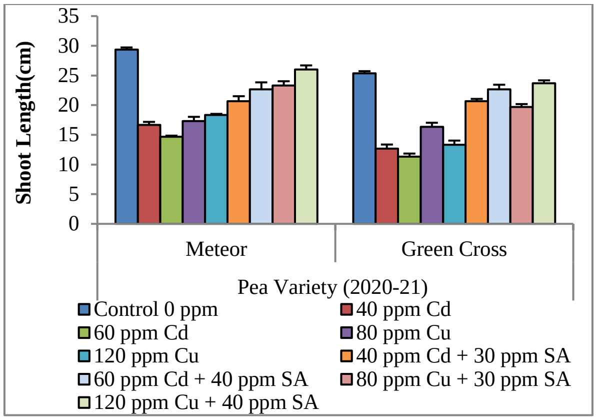
### Morphological Parameters

ANOVA from results showed highly positive response of varieties and treatments on plants and was highly significant (P ≤ 0.001) at both varieties of Pea on shoot root lengths, dry shoot weight. However, interaction of both varieties and treatments on plants gave non-significant (P>0.05) results (Table 1). ANOVA for biomass of shoot and root and dry weight of root indicated that response of varieties was non-significant (P>0.05), while effect of treatment was highly significant (P ≤ 0.001) among both varieties. Foliar application of growth regulator (Salicylic acid) on stress affected plants increased shoot length. Therefore, salicylic acid showed best results of shoot length at both varieties of pea over stress condition (Figure 1A). Tukey test from graphs clearly elaborated that heavy metals stressed plant gave less length of root at both varities as compare to those plants which were foliar sprayed with different concentration of salicylic acid (Figure 1B). The foliar application of salicylic acid on pea proved useful against heavy metals stress as well as being helpful to increase the fresh weight of shoot in normal conditions (Figure 1C). Maximum root weight was noted at 80 ppm Cu + 30 ppm SA; however, less weight was detected at 60 ppm Cd at both varieties of pea (Figure 1D). Heavy metals stress caused reduction of dry shoot weight of plant. From the results of the comparison between both the varieties it was shown that heavy metals stress affects both varieties equally (Figure 1E). However, the highest (0.266, 2) result of biomass of dry root was clearly noted at 120 ppm Cu + 40 ppm SA at both varieties of pea (Figure 1F). For the total number of leaves indicated the impacts of heavy metals and salicylic acid on all pea varieties by ANOVA Table 2. However, its interaction also showed non-significant results (P>0.05). Results revealed that heavy metals stress caused negative impact on the number of leaves/plant and leaf area. A reduction in the no. of leaves and leaf area was noticed after the treatment of heavy metal stress (Figure 1G). Maximum leaf area was recorded in Meteor when PGPR was applied. The leaf area of both pea varieties was improved after treatment of salicylic acid (80 ppm Cu + 30 ppm SA) (Figure 1H). Figure 1I showed that maximum increase in leaf area Ratio was found in Meteor at control and minimum leaf area in Green Cross at 60 ppm Cd whereas Meteor showed more leaf area ratio values as compare to other Green Cross.

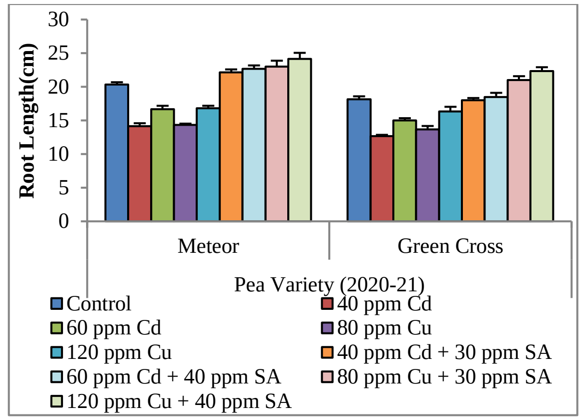
Table 1. Analysis of Variance (ANOVA) of Two Pea Varieties for Morphological Attributes with Foliar Application of Salicylic Acid under Heavy Metals (Cd and Cu) Toxicity.

| Occurrence of Variance | df | MS of Shoot Length (cm) | MS of Root Length (cm) | MS of Shoot Fresh Weight (g) | MS of Root Fresh Weight (g) | MS of Shoot Dry Weight (g) | MS of Root Dry Weight (g) |
|---|---|---|---|---|---|---|---|
| Main effects Variety | 1 | 90.741 *** | 58.074*** | 0.7211ns | 0.001678ns | 0.09160*** | 0.001975ns |
| Treatments | 8 | 143.241*** | 74.135*** | 2.9131*** | 0.148653*** | 0.21176*** | 0.002839ns |

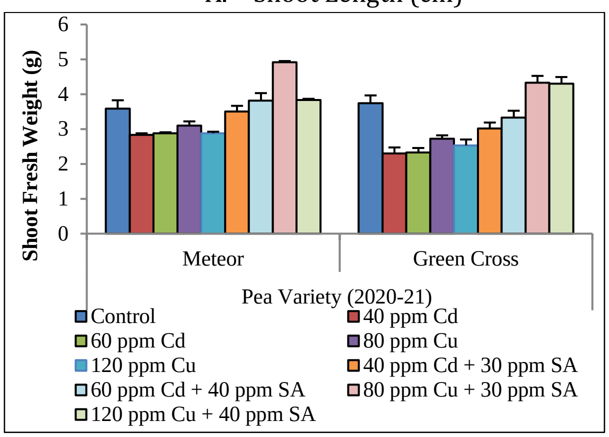
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Interactions Variety × Treatments | 8 | 5.157ns | 2.584ns | 0.2052ns | 0.014792ns | 0.01193ns | 0.000954ns |
| Error | 36 | 4.426 | 2.088 | 0.2506 | 0.019936 | 0.01022 | 0.001355 |
| Total | 53 | 1437.26 | 747.00 | 34.6892 | 2.02692 | 2.24909 | 0.081107 |



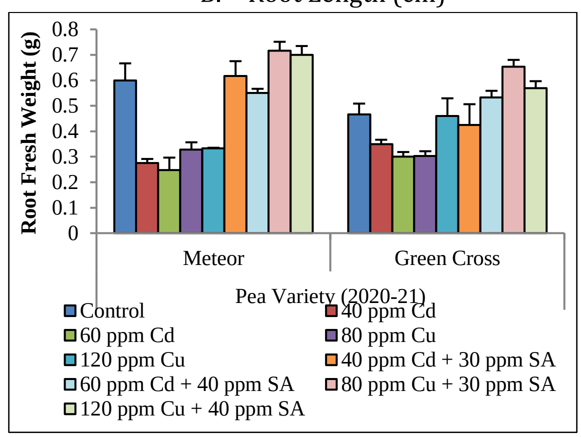

A. Shoot Length (cm)



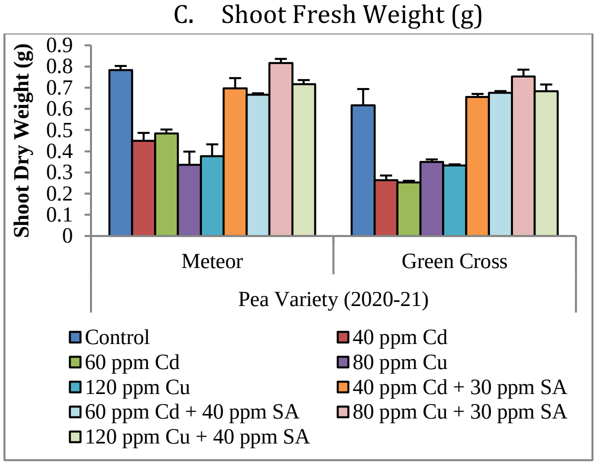

B. Root Length (cm)



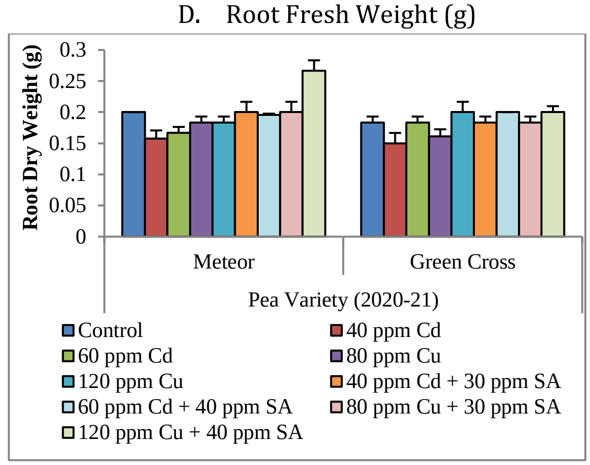

C. Shoot Fresh Weight (g)




D. Root Fresh Weight (g)

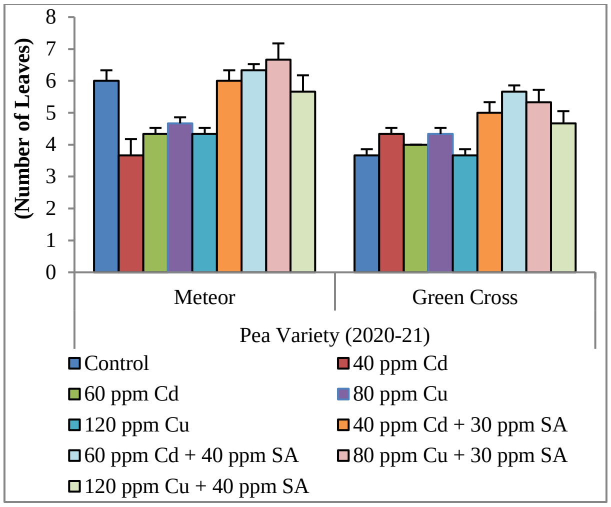



E. Shoot Dry Weight (g)

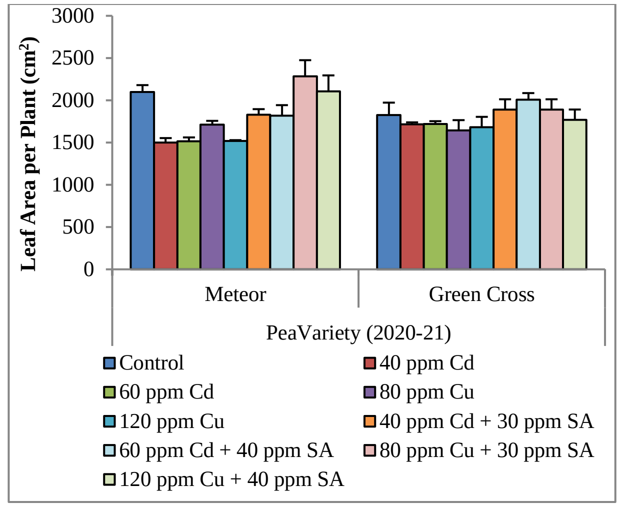



F. Root Dry Weight (g)

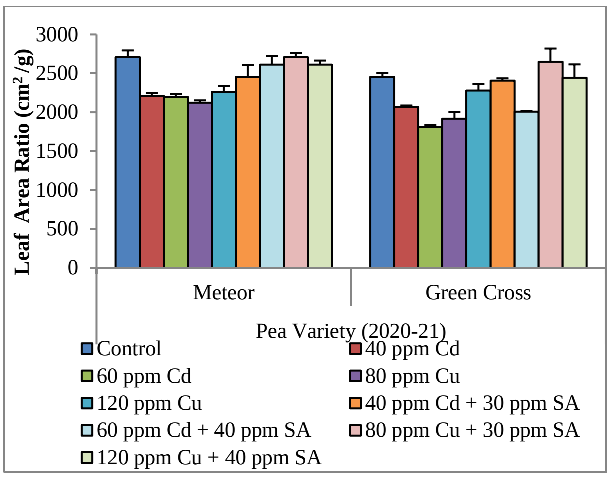
Figure 1 (a). Effective Role of Salicylic Acid (SA) of two Varieties of Pea (*Pisum sativum*) on Shoot Length (A), Root Length (B), Shoot Fresh Weight (C), Root Fresh Weight (D), Shoot Dry Weight (E), Root Dry Weight (F).




G. Number of Leaves




H. Leaf Area/ Plant (cm²)




I. Leaf Area Ratio (cm²/g)

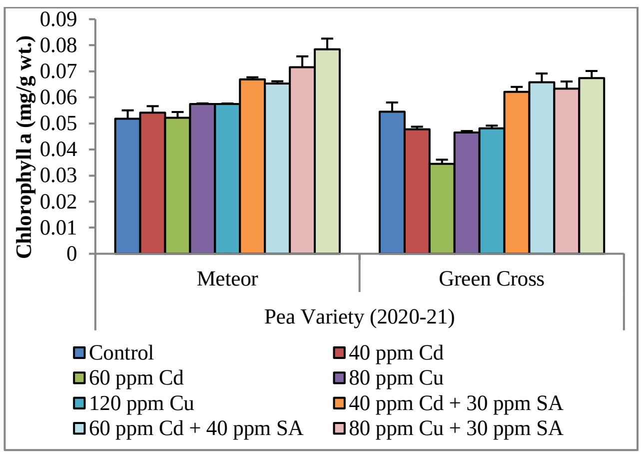
Figure 1 (b). Effective Role of Salicylic Acid (SA) of two Varieties of Pea (*Pisum sativum*) on Number of Leaves (G), Leaf Area/ Plant (H), Leaf Area Ratio (I) under Heavy Metals (Cd and Cu) Stress.

**Photosynthetic Pigments**

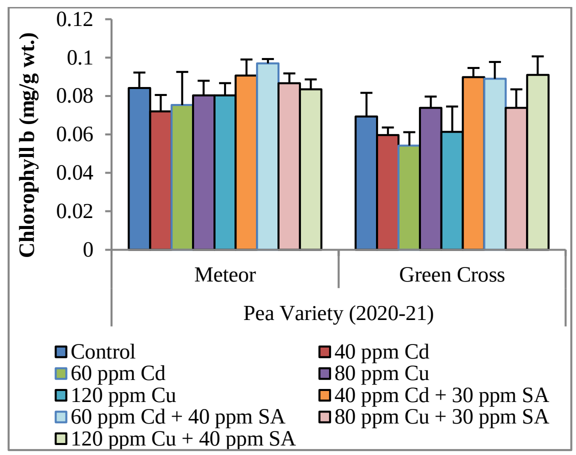
ANOVA Table 2 showed that the impact of heavy metals and salicylic acid on chlorophyll a, total chlorophyll and carotenoids contents on pea were highly significant ($P \leq 0.001$). However, its interaction were also highly significant. On the other hand, Table 2 also verified that the main effect of heavy metals and salicylic acid on chlorophyll b on two varieties of pea was non-significant ($P>0.05$). However, the varietal response was also showed non-significant ($P>0.05$) results with its interaction at both varieties. Tukey test ANOVA clearly demonstrated that foliar sprayed on heavy metals stressed plants gave best results as compared to stressed affected plants. Salicylic acid treatment proved favorable for the pea under heavy metals stress. From the results, it was demonstrated that heavy metals stress reduced concentration of Chlorophyll 'a'. This reduction was observed more pronounced in the Green Cross compared to Meteor (Figure 2A). It was clearly illustrated from Figure that, maximum content of chlorophyll "a" was observed at 120 ppm Cu + 40 ppm SA and minimum content was observed at control at both varieties. The content of chlorophyll 'b' was reduced after the treatment of plants with heavy metals stress. Both varieties effected with heavy metals stress, but the reduction was more pronounced in Green Cross as compared with Meteor (Figure 2B). It was illustrated from Figure 2C that Meteor showed maximum contents of total chlorophyll on all treatments including control as compared to Green cross. Maximum amount of carotenoids contents was more prominent at 80 ppm Cu + 30 ppm SA at both pea varieties as compared to control which showed less amount of carotenoids contents (Figure 2D).

Table 2. Analysis of Variance (ANOVA) of Two Pea (*Pisum sativum* L.) Varieties for Morphological and Photosynthetic Attributes with Foliar Application of Salicylic Acid under Heavy Metals (Cd and Cu) Toxicity.

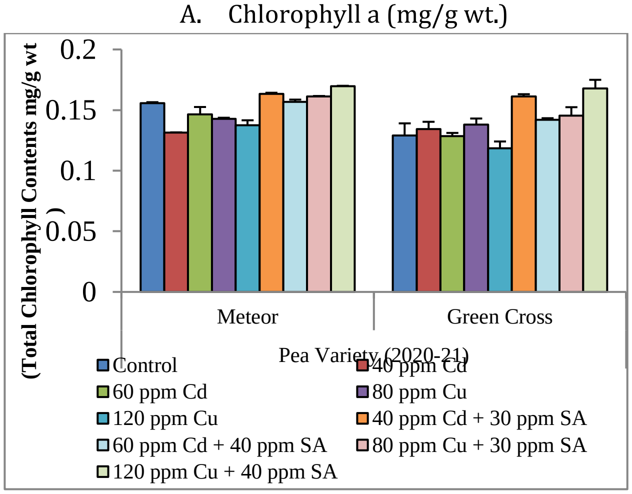
| Source of Variance | df | MS of Number of Leaves | MS of Leaf Area/Plant ($cm^2$) | MS of Leaf Area Ratio ($cm^2$/g) | MS of Chlorophyll a (mg/g F.wt.) | MS of Chlorophyll b (mg/g F.wt.) | MS of Total Chlorophyll (mg/g F.wt.) | MS of Carotenoids Contents (mg/g F.wt.) |
|---|---|---|---|---|---|---|---|---|
| Main effects | 1 | 4.167ns | 10251ns | 564471*** | 0.853526*** | 0.002754* | 0.001648*** | 0.007590*** |
| Treatments | 8 | 163.72*** | 199830* | 357134*** | 0.177861*** | 0.001095ns | 0.001102*** | 0.002601*** |
| Interactions Var. × Trt. | 8 | 5.042ns | 91886ns | 55429ns | 0.142589*** | 0.000227ns | 0.000149ns | 0.001755*** |
| Error | 36 | 2.407 | 92337 | 59383 | 0.000641 | 0.000597 | 0.000162 | 0.000023 |
| Total | 53 | 1440.98 | 5668120 | 6002749 | 3.44020 | 0.034824 | 0.017485 | 0.043255 |



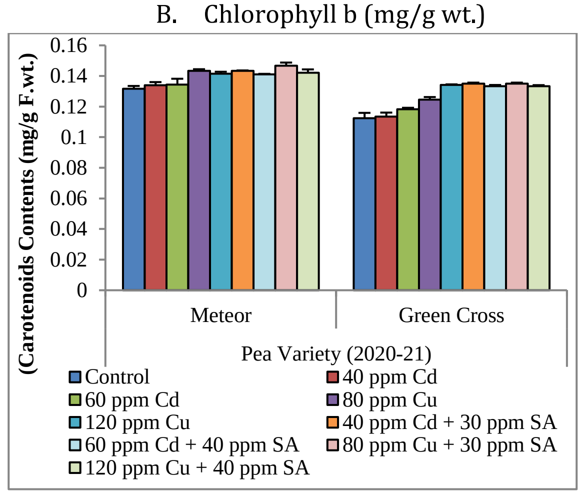

A. Chlorophyll a (mg/g wt.)




B. Chlorophyll b (mg/g wt.)




C. Total Chlorophyll Contents (mg/g wt.)




D. Carotenoids Contents (mg/g wt.)

Figure 2. Effective Role of Salicylic Acid (SA) of two Varieties of Pea (*Pisum sativum*) on Chlorophyll a (A), Chlorophyll b (B), Total Chlorophyll Contents (C), Carotenoids Contents (D) under Heavy Metals (Cd and Cu) Stress.

**Antioxidant Activities**

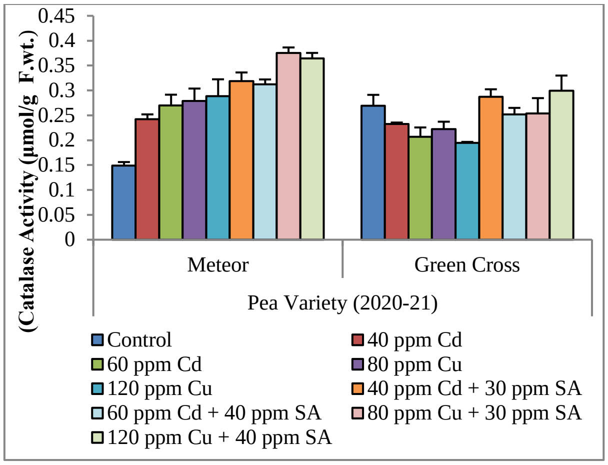
ANOVA Table 3 demonstrated that the effect of hormone and heavy metals stress on Antioxidant activities were significant. However, varietal response was also significant. While its interaction showed non-significant (P>0.05) results at both varieties. From our results Figure 3A, it was observed that catalase enzymes production reduced in the pea plant after treatment with heavy metals stress. This reduced value of enzymes was almost more prominently observed in Green Cross as compared to Meteor. From the data of our experiment, it was proved that POD concentration elevated after treatment of plants with heavy metals stress and this elevation was observed in both varieties but in Green Cross this effect was more pronounced as compared to Meteor Figure 3B. It was also clearly reported from graphs that application of salicylic acid on stressed plants gave best results of SOD as compared to control. Foliar treatment of salicylic acid proved useful for reducing the SOD concentration of wheat under heavy metals stress environment Figure 3C.

**Biochemical Constituents**

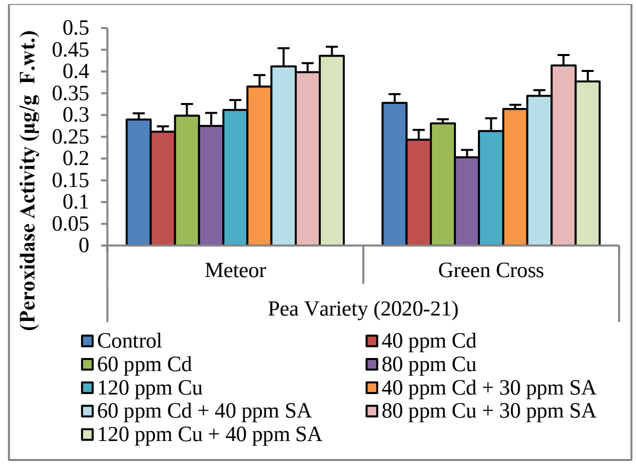
As shown in ANOVA Table 3 the impact of hormone and heavy metals stress on biochemical constituents of pea were highly significant (P ≤ 0.001). However, its interaction also showed significant results. From Figure 4A, it was demonstrated that maximum concentration of protein was clearly observed at 40 ppm Cd + 30 ppm SA and less concentration was observed in Meteor at 40 ppm Cd. It was observed from Figure 4B that Meteor showed incredible increase in carbohydrate concentration at foliar application of salicylic acid on heavy metals stressed plants. Results clearly observed that effect of heavy metals and salicylic acid on proline content of leaf was highly significant with highly significant response of varieties as well as treatments Figure 4C. Overall results also narrated that salicylic acid application under stressed plants gave best response on proline contents of both varieties of pea.

Table 3. Analysis of Variance (ANOVA) of Two Pea (*Pisum sativum* L.) Varieties for Antioxidants Activities and Biochemical Parameters with Foliar Application of Salicylic Acid under Heavy Metals (Cd and Cu) Toxicity.

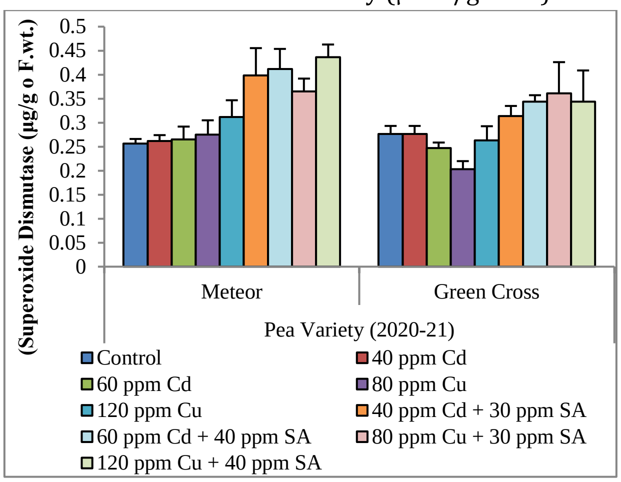
| Source of Variance | df | MS of Catalase Activity (µmol/g of F.wt.) | MS of Peroxidase Activity (µg/g of F.wt.) | MS of Superoxide Dismutase Activity (µg/g of F.wt.) | MS of Total Soluble Protein (mg/ml) | MS of Carbohydrates Contents (mg/ml) | MS of Leaf Proline Content (µmol/g f. wt.) |
|---|---|---|---|---|---|---|---|
| Main effects Variety | 1 | 0.024321** | 0.024236* | 0.039258* | 0.015167*** | 0.207576*** | 0.051646*** |
| Treatments | 8 | 0.010517** | 0.031846*** | 0.029924*** | 0.015896*** | 0.096482*** | 0.013701*** |
| Interactions Variety x Treatments | 8 | 0.007144ns | 0.004923ns | 0.005852ns | 0.002490*** | 0.065929*** | 0.004991ns |
| Error | 36 | 0.003437 | 0.005962 | 0.009291 | 0.001969 | 0.004949 | 0.004096 |
| Total | 53 | 0.28936 | 0.53302 | 0.65993 | 0.23313 | 1.6850 | 0.34865 |




A. Catalase Activity (µmol/g F. wt)

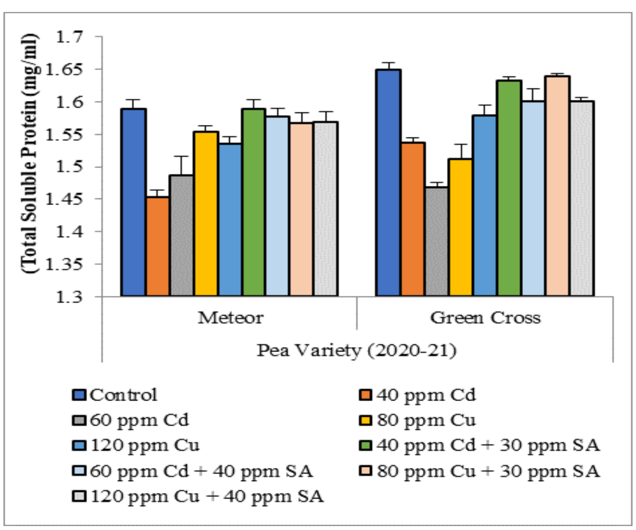


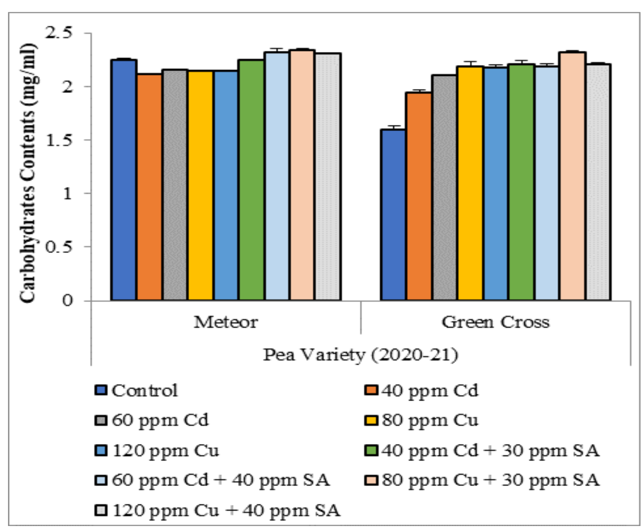

B. Peroxidase Activity (µg/g F.wt)



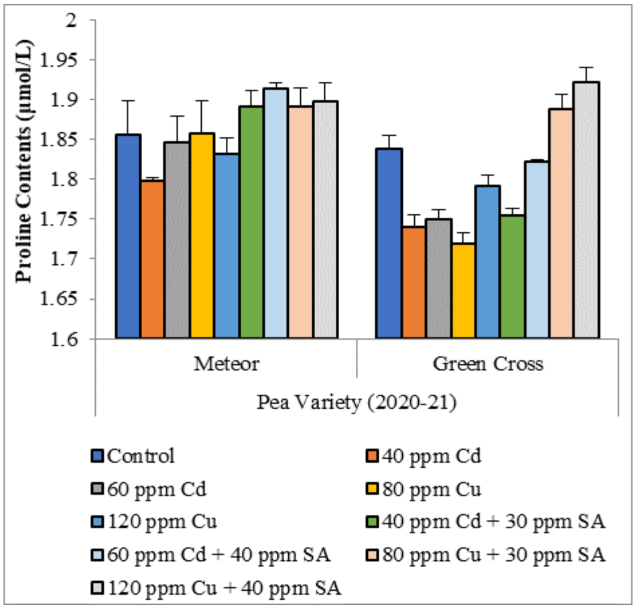

C. Superoxide Dismutase (µg/g o F.wt.)

Figure 3. Effective Role of Salicylic Acid (SA) of two Varieties of Pea on Catalase Activity (A), Peroxidase Activity (B), Superoxide Dismutase (C) under Heavy Metals (Cd and Cu) Stress.




A. Total Soluble Protein (mg/ml) B. Carbohydrates Contents (mg/ml)




C. Proline Contents (µmol/L)

Figure 4. Effective Role of Salicylic Acid (SA) of two Varieties of Pea on Total soluble protein (A), Carbohydrates contents (B), Proline contents (C) under Heavy Metals (Cd and Cu) Stress.

**Yield Parameters**

ANOVA described in Table 4 showed the effect of growth hormone and heavy metals stress on no of legumes/plant, number of seeds and total yield of two varieties of pea was more significant ($P \leq 0.001$) whereas the varietal response also showed highly significant ($P \leq 0.001$). However, the varietal response also showed significant results. While its interaction showed non-significant ($P>0.05$) response. It was also narrated that 30, 40ppm SA showed highly significant production of legumes on heavy metals affected plants on both varieties of pea. Meteor showed significant outcomes in the production of seed/plant at all treatments in which foliar spray of salicylic acid was applied. Figure 5, it was observed that Meteor showed high production of total yield of seeds at all treatments including control because there were no variations among them.

Table 4. Analysis of Variance (ANOVA) of Two Pea Varieties for Yield Attributes with Foliar Application of Salicylic Acid under Heavy Metals (Cd and Cu) Toxicity.

| Source of Variance | Df | MS of Number of Legumes/ Plant | MS of Number of Seeds/ Plant | MS of Total Yield (g) |
|---|---|---|---|---|
| Main effects Variety | 1 | 8.1667*** | 6.0000** | 5.4353*** |
| Treatments | 8 | 3.8796*** | 5.3935*** | 0.8353* |
| Interactions Variety × Treatments | 8 | 1.0000ns | 1.0417ns | 0.3296ns |
| Error | 36 | 0.7037 | 0.8704 | 0.3865 |
| Total | 53 | 72.537 | 88.815 | 28.667 |

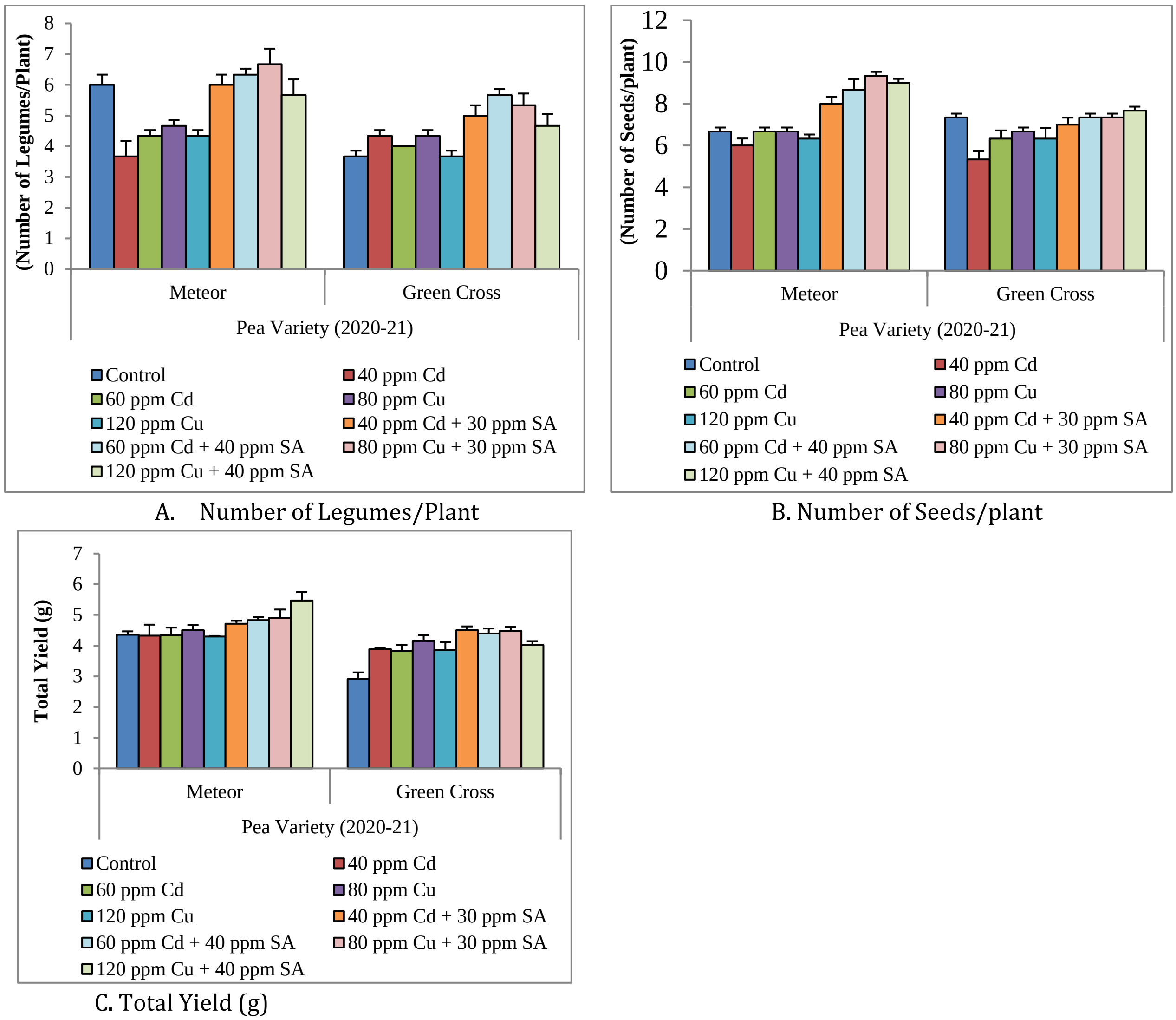



Figure 5. Effective Role of Salicylic Acid (SA) of two Varieties of Pea on Number of Legumes/Plant (A), Number of Seeds/plant (B), Total Yield (g) (C) under Heavy Metals (Cd and Cu) Stress.

## DISCUSSION

### Morphological Attributes

The findings of the present experiments study showed that morphological, physiological, antioxidants, biochemical parameters of two pea varieties were significantly decreased at heavy metals (Cadmium and Copper) stress. However, application of salicylic acid increased the values of different attributes under heavy metals stresses. Plant hormones are associated with changing various growth as well as developmental processes and are dynamic in at very minor amount (Pal *et al.,* 2021). According to the current findings, heavy metals stress had a stronger inhibitory effect on growth of plants, shoot and root length and biomass and others morphological attributes. The results reported here for morphological parameters indicated an increase in foliar spray of salicylic acid on heavy metals treated plants under heavy metals stress. Cd is often kept in the roots and only a very small amount is transported to the shoots (Hassan *et al.,* 2019). The Cd concentration in SA non-treated roots was higher than that in SA primed roots, which is in line with what Bortolin *et al.* (2020) discovered. SA reduced the detrimental effects of Cd on the growth of barley, rice and salt-stressed tomatoes (Alam *et al.,* 2021). Numerous research has reported on the significance of SA in reducing the toxicity of heavy metals in plants. According to the study, Cu stress inhibited the growth of bean seedlings' roots and stems (Moravcova *et al.,* 2018). Rooting was improved by lower SA concentrations. These results closely matched the observations provided by Bril *et al.* (2019), who found that foliar SA treatment significantly lengthened soybean roots. In the current investigation, the dry and fresh weight of leaves as well as root tissues was shown to have decreased. SA inclusion considerably lessened this decline. The dry weight of *Leucaena leucocephala* seedlings gradually decreased as the concentration of Cd increased (Wu *et al.,* 2021). According to Kamran *et al.* (2020), *Brassica napus* dry weight of the root and shoot reduced after exposure to nickel. Cu exposure causes *Helianthus annulus* to lose weight in diverse regions, both fresh and dry, whereas SA application stimulates root, stem, and leaf growth in both control and Cu stressed plants (Es-sbihi *et al.,* 2020).

**Photosynthetic Attributes**

Current results also elaborated an increase of heavy metals stress on photosynthetic pigments which decrease the concentration of different contents. The growth inhibition brought on by Cd may be at least in part attributed to its effect on the rate of photosynthesis (Aqeel *et al.,* 2021). The rate of $CO_2$ assimilation decreased, photosynthetic efficiency decreased, and chlorophyll degraded, and their biosynthesis was inhibited as a result of Cd stress. These impacts might have impacted PS 1 and PS 2's electron transport rates, which would have led to the generation of oxygen free radicals (Al-Mamary & Moussa, 2021). The assimilation of $CO_2$, the activity of photosynthetic enzymes, and the amount of chlorophyll increased significantly after SA pretreatment of plants, which was consistent with (Sharma *et al.,* 2020). According to Yordanova *et al*. (2006) both carboxylating enzymes (RuBPC and PEPC) had significantly decreased activities at all Cd concentrations used. However, pretreating wheat plants with SA prior to exposure to Cd reduced the inhibitory effect of Cd on these enzymes' activity.

In contrast, larger doses of SA had no discernable influence on the pigment content of wheat seedlings produced from grains pre-treated with a lesser quantity (10-5 M), according to Bozso & Barna, (2021). Due to oxidative stress, nutritional imbalance, and SA's encouragement of leaf senescence, total chlorophyll levels may have fallen. Plants under salt or heavy metal stress showed an increase in total chlorophyll caused by salicylic acid (Naeem *et al.,* 2020). According to the current study, SA's inhibitory effects on chlorophyll in plants exposed to copper may be caused by enhanced nutritional balance and decreased oxidative stress. Higher plants' photosynthetic systems are protected by carotenoids from oxidative damage and too many photons (Desoky *et al.,* 2020). Moustafa-Farag *et al.* (2020) discovered that SA dramatically raised both Cu stress and the total carotenoid content in the leaves of sunflower plants. According to Surowka *et al.* (2021), SA increased the rate of de-epoxidation, triggered the production of carotenoids and xanthophylls, and decreased the amount of pigmented chlorophyll and the chlorophyll a/b ratio in wheat.

**Antioxidant and Biochemical Attributes**

Similar to this, Moussa & El-Gamal, (2010) looked into how SA could reduce oxidative stress brought on by cadmium in plant roots (*Oryza sativa* L.). According to Shakir *et al.* (2018), SA reduced the toxicity caused by Hg and shielded the roots of *M. sativa* from artificially induced oxidative damage. According to reports, plants treated with Cd create more $H_2O_2$ than usual. Additionally, it was shown that SA pretreatment reduced MDA accumulation brought on by Cd, confirming its effectiveness against oxidation damage (Altaf *et al.,* 2022). The obtained results suggested that the reduction of membrane injury under Cd stress may be due to increased ABA content caused by the administration of SA prior to Cd stress (Arif *et al.,* 2020). The buildup of free proline seemed to be a reliable sign of heavy metal stress. Proline levels in plants grew from seeds pretreatment with SA showed a reduction, indicating partial recovery from Cd stress (Malik *et al.,* 2022). Additionally, proline functions as a direct antioxidant to defend the cell against the effects of free radicals and to create a more reducing environment that is conducive to phytochelation production and Cd sequestration (Kumar *et al.,* 2021).

In the current study, it was discovered that a rise in proline accumulation in the roots and leaves of bean seedlings was connected to excess Cu. Proline accumulation may be involved in the mechanism of osmoregulation. Proline may have a significant role in plants that are vulnerable to heavy metal poisoning because of its antioxidative capabilities (Yang *et al.,* 2011). Many investigators have reported the accumulation of proline in response to Cu toxicity (Zehra *et al.,* 2020). Additionally, it has been shown that exogenously adding proline to the growth medium can reduce the harmful effects of copper (Wang *et al.,* 2022). Salicylic acid caused the level of protein fractions in numerous organs of control and Cu stressed plants to significantly rise. This might be a result of the interaction between SA and heavy metals. It is generally known that stress can stimulate the accumulation of proteins (Rai *et al.,* 2021).

**Yield Attributes**

All pea plant parameters were negatively impacted by heavy metal stress, and these factors all directly decreased crop production. It was shown that heavy metals stress decreased all yield indices, including seed weight and number of pea crop legumes. Salicylic acid, however, increases pea crop output and helps to reduce the effects of heavy metal stress when applied topically. These findings of our experiment are in consistence with the results of Rashid *et al.* (2019). They determined that heavy metals stress declined the weight of gain and No. of grains in Wheat. Muhammad *et al.* (2022) also noticed declined No. of spikes, No. of spikelet, grain weight and grain number in Wheat after heavy metals treatment.

## CONCLUSION

It was concluded from results that heavy metals stress caused a reduction in almost all parameters of growth. The study came to the conclusion that plants exposed to heavy metal stress could benefit from foliar applications of 30 and 40 ppm of SA to improve morphological, biochemical, and yield parameters. Studies have supported hypotheses that salicylic acid functions as a signaling molecule that influences a number of physiological and biochemical processes under various abiotic stress conditions. According to the results of the current study, cadmium toxicity is more severe than copper toxicity, but the combination of the two led to greater losses in plant biomass, photosynthetic pigments, and altered metabolism in pea plants. Contrarily, salicylic acid treatment confers tolerance against the harmful effects of cadmium and copper stress through improved antioxidant systems, proline buildup, reduced reactive oxygen species, and also stabilizes membrane stability. According to the findings, salicylic acid applied to heavy metals stressed plants may be beneficial for pea varieties' morphological, biochemical, and yield parameters. It may also improve peas' nutritional value. This could be used as a sustainable agricultural strategy to reduce plant stress from cadmium and copper while increasing biological production. Therefore, a thorough examination of the molecular processes underlying SA-mediated tolerance is required. Since SA's effects are concentration-dependent, it would be beneficial to look into the potential causes of the diverse ways that plant tissues respond to salicylic acid.

## CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

## REFERENCES

Abdullah Al Mamun, S.M., M. Tahjib-Ul-Arif, S. Afrin, M.K. Khan, M.A. Hannan, M. Skalicky and Y. Murata. 2020. Insights into nitric oxide-mediated water balance, antioxidant defence and mineral homeostasis in rice (*Oryza sativa* L.) under chilling stress. Nitric Oxide, 100, 7-16.

Alam, P., T.H. Balawi, F.H. Altalayan, A.A. Hatamleh, M. Ashraf and P. Ahmad. 2021. Silicon attenuates the negative effects of chromium stress in tomato plants by modifying antioxidant enzyme activities, ascorbate–glutathione cycle and glyoxalase system. Acta Physiologiae Plantarum, 43(7): 1-17.

Al-Mamary, M. A. and Z. Moussa. 2021. Antioxidant activity: The presence and impact of hydroxyl groups in small molecules of natural and synthetic origin. Antioxidants Benefits, Sources, Mechanism of Action, 318-377.

Altaf, M. M., X.P. Diao, H. Wang, L.U. Khan, A. Shakoor, M.A. Altaf and T.H. Farooq. 2022. Salicylic Acid Induces Vanadium Stress Tolerance in Rice by Regulating the AsA-GSH Cycle and Glyoxalase System. Journal of Soil Science and Plant Nutrition, 22(2): 1-17

Aqeel, M., N. Khalid, A. Tufail, R.Z. Ahmad, M.S. Akhter, M. Luqman and A. Noman. 2021. Elucidating the distinct interactive impact of cadmium and nickel on growth, photosynthesis, metal-homeostasis, and yield responses of mung bean (*Vigna radiata* L.) varieties. Environmental Science and Pollution Research, 28(21): 27376-27390.

Arif, Y., F. Sami, H. Siddiqui, A. Bajguz and S. Hayat. 2020. Salicylic acid in relation to other phytohormones in plant: A study towards physiology and signal transduction under challenging environment. Environmental and Experimental Botany, 175, 104040.

Aziz, A., A. Wahid and M. Farooq. 2014. Leaf age and seasonality determines the extent of oxidative stress and induction of antioxidants in lemongrass. Pakistan Journal of Agricultural Sciences, 51(3).

Bates, L. S., R.P. Waldren, and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. Pakistan Journal of Agricultural Sciences, 39(1): 205-207.

Bortolin, G. S., S.B.Teixeira, R. de Mesquita Pinheiro, G.E. Avila, F.S. Carlos, C.E. da Silva Pedroso and S. Deuner. 2020. Seed priming with salicylic acid minimizes oxidative effects of aluminum on Trifolium seedlings. Journal of Soil Science and Plant Nutrition, 20(4): 2502-2511.

Bozso, Z. and B. Barna, 2021. Diverse effect of two cytokinins, kinetin and benzyladenine, on plant development, biotic stress tolerance, and gene expression. Life, 11(12): 1404.

Bril, F., D.M. Biernacki, S. Kalavalapalli, R. Lomonaco, S.K. Subbarayan, J. Lai and K. Cusi. 2019. Role of vitamin E for nonalcoholic steatohepatitis in patients with type 2 diabetes: a randomized controlled trial. Diabetes care, 42(8): 1481-1488.

Butto, V., A. Deslauriers, S. Rossi, P. Rozenberg, V. Shisho and H. Morin. 2020. The role of plant hormones in tree-ring formation. Tree, 34(2): 315-335.

Desoky, E. S. M., A.M. Saad, M.T. El-Saadony, A.R.M. Merwad, and M.M. Rady. 2020. Plant growth-promoting rhizobacteria: Potential improvement in antioxidant defense system and suppression of oxidative stress for alleviating salinity stress in *Triticum aestivum* (L.) plants. Biocatalysis and Agricultural Biotechnology, 30, 101878.

El Dakak, R. A. and I.A. Hassan. 2020. The alleviative effects of salicylic acid on physiological indices and defense mechanisms of maize (*Zea Mays* L.) stressed with cadmium. Environmental Processes, 7(3): 873-884.

Es-sbihi, F. Z., Z. Hazzoumi, R. Benhima and K. Amrani Joutei. 2020. Effects of salicylic acid on growth, mineral nutrition, glandular hairs distribution and essential oil composition in *Salvia officinalis* L. grown under copper stress. Environmental Sustainability, 3(2): 199-208.

Ghasem, P., A. Ebadi, M. Hashemi, B. Hawrylak-Nowak, C. Baskin and S. Jahanbakhsh, 2022. Plant Growth Regulators Improve Grain Production and Water Use Efficiency of *Foeniculum vulgare* Mill. under Water Stress. Plant, 11(13): 1718.

Hassan, M. U., M.U. Chattha, I. Khan, M.B. Chattha, M. Aamer, M. Nawaz and T.A. Khan, 2019. Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities—a review. Environmental Science and Pollution Research, 26(13): 12673-12688.

Hussain, K., S. Anwer, K. Nawaz, M.F. Malik, N. Zainab, A. Nazeer and A. Majeed, 2020. Effect of foliar applications of IAA and $Ga_3$ on growth, yield and quality of Pea (*Pisum sativum* L.). Pakistan Journal of Botany, 52(2): 447-460.

Kamran, M., Z. Malik, A. Parveen, L. Huang, M. Riaz, S. Bashir and U. Ali, 2020. Ameliorative effects of biochar on rapeseed *(Brassica napus* L.) growth and heavy metal immobilization in soil irrigated with untreated wastewater. Journal of Plant Growth Regulation, 39(1): 266-281.

Kishor, D. R., S. Moses and M. Kumar, 2020. Study of Important Insecticide Samples. Agriculture & Food: e-Newsletter, 5.

Kumar, N., V. Kumar, B. Bose and R.K. Singhal. 2021. Cadmium toxicity in plants and alleviation through seed priming approach. Plant Physiology Reports, 26(4): 647-660.

Lu, Z. X., J. F. He, Y. C. Zhang and D. J. Bing. 2020. Composition, physicochemical properties of pea protein and its application in functional foods. Critical Review of Food Science and Nutrition, 60(15): 2593-2605.

Malik, A., P. Yadav and S. Singh. 2022. Role of polyamines in heavy metal stressed plants. Plant Physiology Reports, 27(4): 680-694.

Moravcova, S., J. Tuma, Z.K. Ducaiova, P. Waligorski, M. Kula, D. Saja and M. Libik-Konieczny. 2018. Influence of salicylic acid pretreatment on seeds germination and some defence mechanisms of *Zea mays* plants under copper stress. Plant Physiology and Biochemistry, 122, 19-30.

Moussa, H. R. and S.M. El-Gamal, 2010. Effect of salicylic acid pretreatment on cadmium toxicity in wheat. Biologia Plantarum, 54(2): 315-320.

Moustafa-Farag, M., H.I. Mohamed, A. Mahmoud, A. Elkelish, A.N. Misra, K.M. Guy and M. Zhang. 2020. Salicylic acid stimulates antioxidant defense and osmolyte metabolism to alleviate oxidative stress in watermelons under excess boron. Plants, 9(6): 724.

Muhammad, A.S., T. Yasmeen, Z. Abbas, S. Ali, M. Rizwan, N.H. Aljarba and M.M. Abdel-Daim. 2021. Role of exogenous and endogenous hydrogen sulfide ($H_2S$) on functional traits of plants under heavy metal stresses: a recent perspective. Frontiers in Plant Science, 11, 545453.

Muhammad, F., M.A.S. Raza, R. Iqbal, F. Zulfiqar, M.U. Aslam, J.W.H. Yong and M.A. Ibrahim, 2022. Ameliorating Drought Effects in Wheat Using an Exclusive or Co-Applied Rhizobacteria and ZnO Nanoparticles. Biology, 11(11): 1564.

Naeem, M., Y. Sadiq, A. Jahan, A. Nabi, T. Aftab and M.M.A. Khan. 2020. Salicylic acid restrains arsenic induced oxidative burst in two varieties of Artemisia annua L. by modulating antioxidant defence system and artemisinin production. Ecotoxicology and Environmental Safety, 202, 110851.

Nazeer, A., K. Hussain, A. Hassain, K. Nawaz, Z. Bashir, S.S. Ali, N. Zainab, M. Qurban, and G. Yasin 2020. Influence of Foliar Applications of IAA, NAA and $GA_3$ on Growth, Yield and Quality of Pea (*Pisum sativum* L.). Indian Journal of Agricultural Research, 54(6): 699-707.

Pal, M., G. Szalai, O.K. Gondor and T. Janda, 2021. Unfinished story of polyamines: Role of conjugation, transport and light-related regulation in the polyamine metabolism in plants. Plant Science, 308, 110923.

Pandey, A. K., D. Rubiales, Y. Wang, P. Fang, T. Sun, N. Liu and P. Xu, 2021. Omics resources and omics-enabled approaches for achieving high productivity and improved quality in pea (*Pisum sativum* L.). Theoretical and Applied Genetics., 134(3): 755-776.

Parankusam, S., L.C.L. Jalli, P. Bhatnagar-Mathur, and K.K. Sharma. 2020. Emerging roles of salicylic acid and jasmonates in plant abiotic stress responses. Protective chemical agents in the amelioration of plant abiotic stress: Biochemical and Molecular Perspectives, 342-373.

Rai, K. K., N. Pandey, R.P. Meena and S.P. Rai. 2021. Biotechnological stand Molecularhancing heavy metal tolerance in neglected and underutilized legume crops: A comprehensive review. Ecotoxicology and. Environmental Safety, 208, 111750.

Rashid, M. U., N. Muhammad, H. Naeemi, F.A. Khan, M. Hassan, S. Faisal and U. Hamann, 2019. Spectrum and prevalence of BRCA1/2 germline mutations in Pakistani breast cancer patients: results from a large comprehensive study. Hereditary Cancer in Clinical Practice, 17(1): 1-13.

Sana, J. S., Q. uz Zaman, K. Sultan, U. Riaz, A. Aslam, N.E. Saba Sharif and S. Ibraheem. 2020. Heavy metals stress, mechanism and remediation techniques in rice (*Oryza sativa* L.): A review. Pure and Applied Biology (PAB), 9(1): 403-426.

Shakir, S. K., S. Irfan, B. Akhtar, M.K. Daud, N. Taimur and A. Azizullah, 2018. Pesticide-induced oxidative stress and antioxidant responses in tomato (*Solanum lycopersicum*) seedlings. Ecotoxicology, 27(7): 919-935.

Sharma, A., G.P.S. Sidhu, F. Araniti, A.S. Bali, B. Shahzad, D.K. Tripathi and M. Landi, 2020. The role of salicylic acid in plants exposed to heavy metals. Molecules, 25(3): 540.

Shen, Y. X., K. Xiao, P. Liang, Y.W. Ma and X. Huang. 2013. Improvement on the modified Lowry method against interference of divalent cations in soluble protein measurement. Applied Microbiology and Biotechnology, 97(9): 4167-4178.

Shreyas, E.M, D. Shakappa and Z. Rengel. 2022. A review of the nutritional and antinutritional constituents of chickpea (*Cicer arietinum*) and its health benefits. Crop and Pasture Science, 73(4): 401-414.

Silverman, B. W. 2018. Density estimation for statistics and data analysis. CRC Press, Taylor & Francis group, Routledge UK.

Surowka, E., D. Latowski, M. Dziurka, M. Rys, A. Maksymowicz, I. Żur and Z. Miszalski. 2021. ROS-scavengers, osmoprotectants and violaxanthin de-epoxidation in salt-stressed Arabidopsis thaliana with different tocopherol composition. *Internation.* Journal of Molecular Sciences, 22(21): 11370.

Swati S., S.A. Ansari, I.M. Ansari, M. Fujita, and M. Hasanuzzaman, 2021. Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. Antioxidants., 10(2): 277.

Ullah, N., I.U. Haq, N. Safdar and B. Mirza, 2015. Physiological and biochemical mechanisms of allelopathy mediated by the allelochemical extracts of *Phytolacca latbenia* (Moq.) H. Walter. Toxicology and Industrial Health, 31(10): 931-937.

Usman, Z., A. Ayub, S. Hussain, E.A. Waraich, M.A. El-

Esawi, M. Ishfaq, M. Ahmad, N. Ali and M. F. Maqsood. 2021. Cadmium toxicity in plants: Recent progress on morpho-physiological effects and remediation strategies. Journal of Soil Science and Plant Nutrition, 1-58.

Venkatachalam, P., M. Jayaraj, R. Manikandan, N. Geetha, Eldon R. Rene, N. C. Sharma and S. V. Sahi. 2017. Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in *Leucaena leucocephala* seedlings: a physiochemical analysis. Plant Physiology and Biochemistry, 110, 59-69.

Wang, J., L. Song, X. Gong, J. Xu and M. Li. 2020. Functions of jasmonic acid in plant regulation and response to abiotic stress. International Journal of Molecular Sciences, 21(4): 1446.

Wu, Z. Z., Y.X. Zhang, J.Y. Yang, Y. Zhou and C.Q. Wang. 2021. Effect of vanadium on testa, seed germination, and subsequent seedling growth of alfalfa (*Medicago sativa* L.). Journal of Plant Growth and Regulation, 40(4): 1566 -1578.

Yamshi, A., F. Sami, H. Siddiqui, A. Bajguz and S. Hayat. 2020. Salicylic acid in relation to other phytohormones in plant: A study towards physiology and signal transduction under challenging environment. Environmental and Experimental Botany, 175, 104040.

Yang, Y., Y. Zhang, X. Wei, J. You, W. Wang, J. Lu and R. Shi. 2011. Comparative antioxidative responses and proline metabolism in two wheat cultivars under short term lead stress. Ecotoxicology and Environmental Safety, 74(4): 733-740.

Yilkal. B., M. Taye and A. Kumar. 2021. Recent advancement in phytoremediation for removal of toxic compounds. In Nanobiotechnology for Green Environment. CRC Press. pp. 195-228.

Yordanova, R., V. Baydanova and V. Peeva. 2017. Nitric oxide mediates the stress response induced by cadmium in maize plants. Genetics and Plant Physiology, 7(3-4): 121-134.

Zehra, A., S. Choudhary, K.I. Wani, M. Naeem, M.M.A. Khan and T. Aftab. 2020. Exogenous abscisic acid mediates ROS homeostasis and maintains glandular trichome to enhance artemisinin biosynthesis in Artemisia annua under copper toxicity. Plant Physiology and Biochemistry, 156, 125-134.