Pak. J. Bot., 54(5): 1707-1713, 2022. DOI: http://dx.doi.org/10.30848/PJB2022-5(28)

THE INFLUENCE OF SALICYLIC ACID FOLIAR SPRAY ON THE GROWTH, BIOCHEMICAL TRAITS, AND Cd-UPTAKE IN RADISH (*RAPHANUS SATIVUS* L.)

SYED HAMMAD RAZA[1], FAHAD SHAFIQ[2*] AND SUMERA ANWAR[2]

[1]*Department of Botany, Government College University Faisalabad, Pakistan*
[2]*Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Pakistan*
[*]*Corresponding author's e mail:fahadsheikh1800@gmail.com*

Abstract

Due to agricultural mismanagement, cadmium (Cd) enrichment in cultivated soils raises concern over crop growth, productivity, and edible safety. In this present field study, the influence of salicylic acid (SA) on some growth, biochemical, and ion attributes of locally cultivated radish (*Raphanus sativus* L.) variety Mino towards Cd exposure is reported. The Cd-stress (0, 0.5, and 1 mM $CdCl_2$) was applied to 20-d old plants. The foliar application of salicylic acid (0 and 300 mg L^{-1}) was performed after 10-days of Cd exposure, and data for various growth and biochemical traits were recorded after 15-days of spray treatments. The inhibition of radish root growth was recorded even at 0.5 mM $CdCl_2$, and severe inhibition was evident at 1 mM $CdCl_2$ stress. In contrast, an increase in the shoot growth characters was noticed in response to Cd and SA treatments. Furthermore, Cd-stress reduced the chlorophyll contents, and these were further reduced by SA spray. Moreover, the SA-treated plants exhibited higher Cd contents in the shoot leading to chlorosis. The shoot Cd contents ranged between 6-57 µg/g DW while, the Cd concentrations in root were below 6 µg/g DW. Based on the results, we conclude that radish concentrated a majority of Cd-fraction in shoot compared to the root with minimal values.

Key words: Cadmium; Photosynthetic pigments; Salicylic acid; Metal toxicity; Vegetables.

Introduction

Cadmium (Cd) is a persistent environmental pollutant discharged extensively into agricultural lands from anthropogenic sources (Hegedûs *et al*., 2001; Han *et al*., 2015). Some of the prime sources of Cd release include the use of Cd containing inorganic phosphate fertilizers, sewage sludge application, and metal-working industries (Raza *et al*., 2014; Anwar *et al*., 2016). The relatively high mobility of Cd metal in the rhizosphere promotes rapid uptake by roots leading to its accumulation in different plant parts (Das *et al*., 1998). Consuming contaminated food exposes people to a lot of Cd, which can disrupt the nomal body functions, resulting in short- or long-term health problems (Balali-Mood *et al*., 2021). For Instance, Cd attaches to albumin in plasma in the same way as zinc does, replaces calcium from bones and causes osteoporosis (Brzóska *et al*., 2001). In the same way it disrupts iron absorption causing anemia (Fujiwara *et al*., 2020) and altering zinc homeostasis (Urani *et al*., 2015). In addition, Cd accumulates in the kidney and liver, causing chronic nephrotoxicity and nephrocarcinogenicity (Lech & Sadlik, 2017), and designated as carcinogenic (group I) to humans by the International Agency for Research on Cancer (Anon., 1993).

When plants are exposed to Cd stress, they respond primarily by modulating biochemical and physiological processes. Once up-taken, Cd suppresses uptake of iron, zinc, and other nutrients (Xu *et al*., 2017), inhibits plant growth and carbon fixation (Drazkiewicz *et al*., 2003; Song *et al*., 2019), induces leaf chlorosis by degrading chlorophyll (Rydzyński *et al*., 2019), and disturbs photosynthetic apparatus (Perfus-Barbeoch *et al*., 2002).

Because food is the main source of Cd toxicity, there is increased concern about Cd pollution in food and associated health consequences. Vegetables are one of the largest food sources causing Cd toxicity because they are usually cultivated in sub-urban areas with wastewater (Muchuweti *et al*., 2006; Hussain *et al*., 2013; Anwar *et al*., 2016). Many researchers studied Cd uptake and translocation in different vegetables under various soil conditions, which helps understand the uptake and transport mechanism. The concentration of Cd taken up by plants varies with the consumable part, vegetable type, and stage of vegetable growth (Shentu *et al*., 2008; Lai & Chen, 2013; Huang *et al*., 2021).. In this context, Shentu *et al*., (2008) reported that radish accumulated more Cd in shoot than root compared to pakchoi and tomato.

Radish is a Brassicaceae vegetable with an edible root portion and is regarded as a model (indicator) crop for studying plant responses against various environmental pollutants, including heavy metals (Verma *et al*., 2007). However, its edible portion is directly in contact with the contaminated soil, which raises concerns over its edible safety and quality after harvest. On the other hand, salicylic acid (SA) is an important plant hormone and a signaling molecule (Guo *et al*., 2009). It has a crucial role in abiotic stress tolerance (Alvarez, 2000), including defensive mechanisms at low temperature, salt, and water stress conditions (Borsani *et al*., 2001). Exogenous application of SA on various species either through rooting medium or as foliar spray showed promising results in mitigating Cd toxicity in several plant species, including crops (Xu *et al*., 2015; Han *et al*., 2015; Sheng *et al*., 2015; Zhang *et al*., 2015; Shakirova *et al*., 2016), however, most often the studies are limited to laboratory conditions and their practical applicability is limited. Therefore, a practical approach was opted to investigate the influence of exogenously applied SA on Cd contents in radish plants under artificially Cd contaminated soils.

Materials and Methods

Experiment details: A field experiment was arranged in a split-plot design. Three main plots (4 m × 2 m) were prepared as described by Raza and Shafiq (2013a). Seeds of radish (*Raphanus sativus* L. variety Mino) were sown on both sides of the ridges. Three ridges were made in each main plot, the distance between ridges was 50 cm, and the height of each ridge was 15 cm. After 15 d of seed germination, plant to plant distance of 10 cm was maintained, and three levels of Cd, *i.e.*, 0, 0.5, and 1 mM $CdCl_2$, were applied to the main plots. Then after 10 d of Cd exposure, plants on the first and second ridges (two rows each) were sprayed with salicylic acid (SA, 300 mg L^{-1}, 0.1% tween-20 was added as a surfactant) and water sprayed, respectively, while plants on the third ridge were not sprayed (control).

Determination of growth and biochemical traits: After 15 d of SA treatments, plants were harvested, and various shoot and root lengths were measured. The shoot and root were weighed (fresh weight), dried at 70 °C, and weighed again (dry weight). Chlorophyll and carotenoid contents from the acetone (80%) extracted leaf tissues were determined as described by Arnon (1949) and Kirk & Allen (1965).

Determination of inorganic ions: Finely powdered, dried shoot and root (0.5 g) samples were used for element analysis after wet-acid digestion (Netondo *et al.*, 2002). The Cd content from the digested samples was determined by atomic absorption spectrophotometer (novAA400, Analytik Jena, Germany). Sodium (Na^+) and potassium (K^+) ions were determined using a flame photometer (Jenway PFP, UK).

Statistical analysis

Analysis of variance of the data was carried out using CoStat statistical software. Differences between means of treatments were assessed by Duncan's Multiple Range test (p<0.05) using MStat software.

Results

Radish vegetative growth in response to Cd stress and salicylic acid foliar application: The exposure of radish to cadmium (Cd) contamination resulted in a significant reduction in root length (p<0.05), fresh root weight (p<0.001), and root dry weight (p<0.01) (Table 1). The application of salicylic acid (SA) did not improve the root length of radish plants compared to control plants, although improved root fresh and dry biomass in comparison with Cd-stressed (no spray) radish plants (p<0.05). In contrast, radish plants exposed to Cd contamination exhibited significantly higher shoot length (p<0.01) and fresh and dry biomass (p<0.01) than the control. A stimulation in the leaf area of Cd-stressed radish plants (p<0.001) and leaf numbers (p<0.05) were recorded. The foliar application of SA further improved the shoot growth attributes like shoot length; shoot fresh and dry weight, respectively (p<0.05). Furthermore, SA application increased the number of leaves and leaf area per plant (p<0.001) under Cd contamination.

Table 1. The influence of salicylic acid foliar spray on various growth characteristics of radish under varying cadmium chloride levels.

Treatments		Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	No. of leaves	Leaf area (cm^2)
Cadmium (mM $CdCl_2$)	Foliar treatment								
0	No spray	$13.3^{e} \pm 1.6$	$26.6^{ab} \pm 0.7$	$16.6^{e} \pm 2.0$	$29.8^{a} \pm 2.4$	$1.85^{e} \pm 0.07$	$2.24^{a} \pm 0.27$	$14^{d} \pm 1.0$	$294.81^{c} \pm 74.8$
	Water	$14.2^{e} \pm 0.4$	$27.6^{a} \pm 0.5$	$17.1^{e} \pm 2.3$	$31.2^{a} \pm 2.5$	$1.97^{e} \pm 0.08$	$2.33^{a} \pm 0.30$	$14^{d} \pm 1.3$	$306.37^{c} \pm 79.3$
	Salicylic acid	$17.8^{d} \pm 0.5$	$22.0^{cd} \pm 1.7$	$27.9^{d} \pm 2.6$	$27.5^{ab} \pm 3.6$	$2.89^{d} \pm 0.19$	$2.07^{a} \pm 0.27$	$15^{cd} \pm 0.6$	$455.09^{c} \pm 5.6$
0.5	No spray	$19.1^{cd} \pm 0.6$	$23.5^{bcd} \pm 1.2$	$39.5^{c} \pm 0.3$	$20.5^{bc} \pm 2.2$	$3.44^{cd} \pm 0.12$	$1.21^{bc} \pm 0.19$	$17^{bc} \pm 1.3$	$686.57^{b} \pm 35.3$
	Water	$19.8^{bcd} \pm 1.5$	$24.6^{abc} \pm 0.9$	$40.9^{bc} \pm 1.1$	$21.2^{bc} \pm 2.3$	$3.52^{cd} \pm 0.12$	$1.23^{bc} \pm 0.30$	$19^{b} \pm 1.2$	$691.50^{b} \pm 36.7$
	Salicylic acid	$25.1^{a} \pm 1.0$	$21.0^{d} \pm 0.5$	$75.0^{a} \pm 1.4$	$26.6^{ab} \pm 2.8$	$6.89^{a} \pm 0.65$	$1.67^{abc} \pm 0.33$	$22^{a} \pm 1.2$	$1093.53^{a} \pm 26.7$
1	No spray	$21.7^{bc} \pm 1.5$	$22.1^{cd} \pm 1.1$	$43.7^{bc} \pm 2.6$	$15.9^{c} \pm 2.4$	$4.15^{bc} \pm 0.78$	$0.91^{c} \pm 0.20$	$15^{cd} \pm 0.3$	$690d.30^{b} \pm 131$
	Water	$22.6^{ab} \pm 2.0$	$22.6^{cd} \pm 1.3$	$45.6^{b} \pm 2.6$	$17.0^{c} \pm 2.6$	$4.35^{b} \pm 0.81$	$1.00^{bc} \pm 0.25$	$16^{cd} \pm 0.9$	$695.71^{b} \pm 130.4$
	Salicylic acid	$21.9^{bc} \pm 0.3$	$17.6^{e} \pm 1.3$	$40.3^{c} \pm 1.6$	$27.8^{ab} \pm 1.8$	$4.04^{bc} \pm 0.45$	$1.77^{ab} \pm 0.18$	$16^{cd} \pm 1.0$	$648.27^{b} \pm 28$

Salicylic acid was applied at 300 mg L^{-1}; Different letters on mean values (mean ± SE; n=3) in each column indicate significant difference

Radish chlorophyll contents in response to Cd stress and salicylic acid foliar application: Exposure to Cd markedly reduced chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total Chl contents in radish plants (Table 2). The maximum values were recorded in control, while the least was evident in plants exposed to 1 mM $CdCl_2$ contamination. The foliar application of salicylic acid improved the Chl *a* and *b* contents of control plants; however, its effect on these attributes of Cd-stressed plants was negative. We recorded significantly lower Chl *a* and *b* contents in response to SA foliar application ($p<0.01$). Therefore, SA application to Cd-stressed plants further reduced the total Chl contents of radish leaves, although it improved Chl contents of plants grown without cadmium.

Inorganic ions in radish plant parts in response to Cd stress and salicylic acid foliar application: The addition of $CdCl_2$ to the growth medium significantly enhanced plant Cd contents (Table 3). In the root, the Cd content ranged between 0.33 - 5.99 $\mu g\ g^{-1}$ DW ($p<0.05$), while in the shoot, it ranged 6.67 - 40.2 $\mu g\ g^{-1}$DW ($p<0.001$) respectively. The foliar application of SA increased the root Cd contents that ranged between 0.31 -4.90 $\mu g\ g^{-1}$DW ($p<0.05$). Similarly, radish plants treated with SA exhibited shoot Cd concentrations that ranged between 11.2-57.4 $\mu g\ g^{-1}$DW respectively ($P<0.001$). The addition of Cd to the growth medium and the foliar SA application differentially altered the Na^+ contents of radish root and shoot. However, in contrast to Na^+ ions, we recorded prominent uptake of K^+ by radish plants in both root and shoot, respectively ($p<0.001$). The application of SA as foliar spray improved the root K^+ contents ($p<0.01$); however, its effect on shoot K^+ contents was insignificant.

Correlation of Cd contents in shoot and root with different growth indices: The Cd in shoot was positively related with Cd content in root, root length, and chlorophyll content (Fig. 1). The Cd fraction in root was negatively related with the fresh and dry weight of root and chlorophyll content. Leaf area was negatively related with Chl*b* and total Chl. Carotenoid content was not related with Cd content or any growth trait.

Discussion

Cadmium (Cd) is a hazardous, non-essential element with potential toxicity and carcinogenicity. It is one of the most dangerous elements due to its mobility, tendency to accumulate in the vegetative plant's parts, and relatively less damaging effect on plants than on humans and animals. Vegetables can accumulate high levels of Cd and develop well with no visible phytotoxicity symptoms or yield decline (Wang, 2002; Liu *et al*., 2003; Shentu *et al*., 2008).

In this study, no decrease in the growth of radish aboveground plant parts was recorded even at the highest Cd application (1 mM of $CdCl_2$). The fresh weight of radish shoot showed 2.4 and 2.6- and 12.8-fold increase and dry weight were 1.85- and 2.24-fold increased at 0.5 and 1 mM of Cd, respectively than the control. Previous reports showed that Cd had no inhibitory effect on the shoot growth of radish (Shentu *et al*., 2008) or even had stimulated the growth of aboveground parts (Wu & Zhang, 2002; Ivanov *et al*., 2003; Liu *et al*., 2003; John *et al*., 2008; Januškaitienė, 2010) as found in the present study. The initial increase in growth by exposure to toxic ions might be due to hormesis (Poschenrieder *et al*., 2013), a response commonly evident in plants exposed to low concentrations of toxic ions (Calabrese, 2009).

Plants appear to be able to defend and protect their integrity against low metal stress. The low dose of Cd may serve as an activator of hormones and enzymes in cytokinin metabolism. Bruno *et al*., (2017) observed an increase in auxin level and enhancement of cytokinin signaling in Arabidopsis roots at lower Cd concentration (10 μM). Furthermore, plants cope with Cd stress by manufacturing low molecular weight Cd-binding proteins in response to low heavy metal stress, and this type of protein has been proven to play a role in metal tolerance (Epstein & Bloom, 2005).

In contrast to the aboveground parts, a dose-dependent reduction in the root growth attributes of radish plants was observed. The exposure to 0.5 mM and 1 mM Cd reduced the root fresh weight by 31 and 47% and root dry weight by 46 and 59%, respectively, as compared to control. Root is the first part of plants exposed to metal stress. The reduction in root growth can be attributed to Cd-induced morpho-anatomical modifications (Lux *et al.,* 2011). Due to its small radius, Cd could replace Ca by entering cells through a Ca channel, binding to calmodulin, and interrupting cell division in root cells (Liu *et al*., 2003). SA application under Cd stress increased the root fresh weight by 30 and 75% and root dry weight by 38 and 94% compared to 0.5 mM and 1 mM Cd alone, respectively. These results showed the anti-stress, and beneficial effect of SA as SA enhances the membrane integrity, reduces the oxidative damage produced by Cd, lowers the malondialdehyde content, and electrolyte leakage (Belkhadi *et al*., 2010; Ahmad *et al*., 2011).

The application of salicylic acid (SA) increased the shoot fresh weight and dry weight by 75% and 100% under 0.5 mM Cd. Salicylic acid application during early growth stages helps plants avoid damage from Cd toxicity (Ahmad *et al*., 2011; Raza & Shafiq, 2013a) by expressing defense-related enzymes and particular proteins that help plants adjust themselves to reduce Cd toxicity (Chen *et al*., 2007; Gondor *et al.*, 2016). Although under high Cd stress (1 mM $CdCl_2$), SA foliar reduced the shoot fresh and dry weight by 7% and 2% at 1 Cd stress. The decreased shoot biomass at high Cd might be due to the high Cd accumulation in the aboveground by SA spray.

Furthermore, an increase in the leaf area might be an adaptative response to compensate for a reduced photosynthetic rate. In this context, increased investment in the leaf is linked to carbon gain via better photosynthetic efficiency and faster growth rates to complete the life cycle rapidly (Poorter & Remkes, 1990). We integrate Cd-induced increased foliage growth with hormesis, which urges further exploration at the molecular level.

Table 2. Photosynthetic pigments of radish under varying cadmium chloride regimes in response to exogenous salicylic acid application.

Treatments		Photosynthetic pigments (mg g^{-1} FW)			
Cadmium (mM $CdCl_2$)	Foliar treatment	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll	Carotenoids
0	No spray	$2.73^{b} \pm 0.08$	$0.843^{ab} \pm 0.10$	$3.58^{bc} \pm 0.19$	$0.338^{bcd} \pm 0.04$
	Water	$2.78^{b} \pm 0.21$	$0.870^{a} \pm 0.09$	$3.65^{b} \pm 0.31$	$0.347^{abc} \pm 0.05$
	Salicylic acid	$3.18^{a} \pm 0.22$	$0.950^{a} \pm 0.10$	$4.13^{a} \pm 0.32$	$0.438^{a} \pm 0.05$
0.5	No spray	$2.41^{b} \pm 0.17$	$0.653^{cd} \pm 0.01$	$3.06^{d} \pm 0.17$	$0.389^{ab} \pm 0.01$
	Water	$2.44^{b} \pm 0.19$	$0.731^{bc} \pm 0.04$	$3.17^{cd} \pm 0.15$	$0.410^{ab} \pm 0.03$
	Salicylic acid	$1.74^{c} \pm 0.09$	$0.509^{e} \pm 0.02$	$2.25^{ef} \pm 0.12$	$0.287^{cd} \pm 0.03$
1	No spray	$1.89^{c} \pm 0.23$	$0.560^{de} \pm 0.03$	$2.45^{e} \pm 0.26$	$0.345^{abc} \pm 0.04$
	Water	$1.91^{c} \pm 0.17$	$0.583^{de} \pm 0.04$	$2.50^{e} \pm 0.21$	$0.350^{abc} \pm 0.04$
	Salicylic acid	$1.32^{d} \pm 0.18$	$0.476^{e} \pm 0.04$	$1.80^{f} \pm 0.22$	$0.242^{d} \pm 0.02$

Salicylic acid was applied at 300 mg L^{-1}; Different letters on mean values (mean ± SE; n=3) in each column indicate significant difference

Table 3. Concentration of sodium, potassium, and cadmium content in radish under varying cadmium chloride regimes in response to exogenous salicylic acid treatment.

Treatments		Sodium (mg g^{-1} DW)		Potassium (mg g^{-1} DW)		Cadmium (µg g^{-1} DW)	
Cadmium (mM $CdCl_2$)	Foliar treatment	Shoot	Root	Shoot	Root	Shoot	Root
0	No spray	$23.5^{b} \pm 3.0$	$33.9^{b} \pm 2.7$	$20.8^{b} \pm 1.7$	$47.8^{d} \pm 2.4$	$6.67^{d} \pm 1.3$	$0.33^{c} \pm 0.0$
	Water	$24.7^{b} \pm 3.2$	$35.6^{ab} \pm 2.9$	$21.9^{b} \pm 1.8$	$50.3^{d} \pm 2.6$	$7.03^{d} \pm 1.4$	$0.34^{c} \pm 0.0$
	Salicylic acid	$31.0^{a} \pm 1.4$	$43.7^{a} \pm 4.4$	$41.5^{a} \pm 5.0$	$62.1^{c} \pm 4.8$	$11.2^{d} \pm 1.3$	$0.31^{c} \pm 0.2$
0.5	No spray	$22.1^{b} \pm 2.1$	$21.9^{d} \pm 3.0$	$47.0^{a} \pm 4.5$	$60.3^{c} \pm 2.8$	$26.3^{c} \pm 0.9$	$2.30^{b} \pm 0.6$
	Water	$23.3^{b} \pm 2.3$	$23.1^{d} \pm 3.1$	$49.5^{a} \pm 4.7$	$63.5^{bc} \pm 3.0$	$27.7^{c} \pm 0.7$	$2.42^{b} \pm 0.3$
	Salicylic acid	$23.9^{b} \pm 1.4$	$25.1^{cd} \pm 2.3$	$50.0^{a} \pm 3.3$	$72.0^{a} \pm 5.1$	$32.2^{c} \pm 4.3$	$3.14^{b} \pm 0.7$
1	No spray	$32.5^{a} \pm 2.8$	$35.4^{ab} \pm 1.8$	$44.5^{a} \pm 2.9$	$67.0^{abc} \pm 5.2$	$40.2^{b} \pm 2.2$	$5.29^{a} \pm 0.1$
	Water	$34.2^{a} \pm 2.9$	$37.3^{ab} \pm 1.9$	$46.8^{a} \pm 3.1$	$70.5^{ab} \pm 5.4$	$42.3^{b} \pm 2.4$	$5.57^{a} \pm 0.1$
	Salicylic acid	$31.0^{a} \pm 2.9$	$31.8^{bc} \pm 2.4$	$46.2^{a} \pm 6.9$	$64.4^{bc} \pm 2.1$	$57.4^{a} \pm 5.9$	$4.90^{a} \pm 0.3$

Salicylic acid was applied at 300 mg L^{-1}; Different letters on mean values (mean ± SE; n=3) in each column indicate significant difference

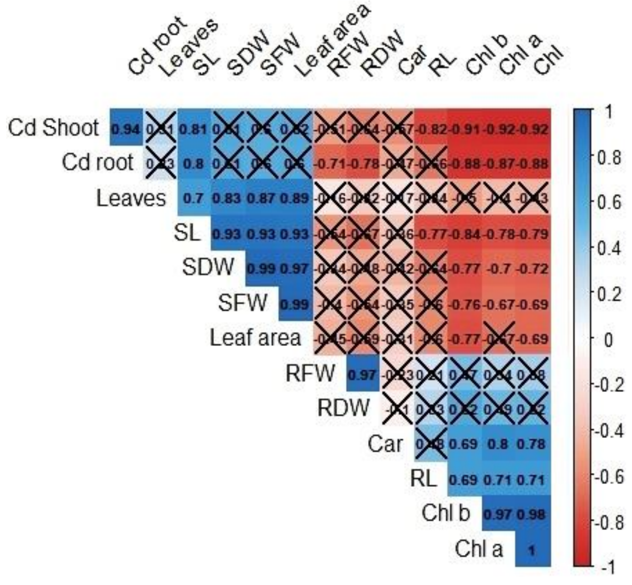

Fig. 1. Pearson's correlation co-efficient for the data of different growth and biochemical traits. The image is constructed using R-Studio (CorrPlot package) using mean values (n=3).

We also recorded a reduction in chlorophyll contents in a Cd dose-dependent manner with maximum values for control plants. Despite the decrease in Chl *a*, Chl *b*, total chlorophyll with an increase in $CdCl_2$ doses, more pigment reduction was evident in salicylic acid (SA) treated plants under Cd stress, and this is consistent with the possibility discussed above. No positive effects of SA application can be linked with osmotic water correlations (Larque-Saavedra, 1978). Still, in the present study, these adverse effects were prominently evident in $CdCl_2$ harmful levels that can be explained further. Salicylic acid is reported to improve nutrient uptake (Glass, 1974). We propose that SA application might increase the uptake of Cd, and high Cd contents in SA-treated plants were confirmed through element analysis of shoots.

The Cd stress is known to decrease photosynthetic pigments, especially the Chl reduction is most apparent (Pandey, 2000). Similar reports were documented by Abdel-Latif (2008), Pandey (2000), and Farouk *et al*., (2011). Lanaras *et al*. (1993) attributed this Cd-mediated Chl reduction to inhibition of certain enzymes like ALAdehydratase and Protochlorophyllreductase involved in Chl biosynthesis. In addition, some authors linked this reduction in pigments with the impaired uptake of iron and magnesium (Anwar *et al*., 2017). Alternatively, substituting magnesium in the Chl molecules by Cd is also responsible for this (Kupper *et al*., 1998). In addition, Anuradha *et al*., (2009) attributed these reductions in pigments to imbalanced water relations and damage to chloroplast structure.

Above all, in terms of Na^+ ions, a differential response was evident, while on the other hand, Cd enrichment increased the uptake of K^+ ions in both the root and shoot of radish plants. Contrary to these findings, a reduction in the K^+ contents of sugarcane calli exposed to 1 mM $CdCl_2$ is reported (Raza *et al*., 2013b). Generally, Cd enters through K^+ and Ca^{2+} channels and impairs these channels, which disturb ion homeostasis (Li *et al*., 2012). However, we recorded increased K^+ contents, and SA application further enhanced these ions in radish plant parts.

The addition of $CdCl_2$ to the growth medium significantly enhanced Cd contents in radish plant parts. The storage roots of radish accumulated a much lower concentration of Cd (0.33 - 5.99 μg g^{-1}DW) than in the aboveground shoot part (6.67 - 40.2 μg g^{-1} DM). Similar results were also reported by Shentu *et al*., (2008). Vegetables easily uptake and translocate heavy metals from the soil to the shoots via transpiration-based passive uptake (Marchiol *et al*., 2004). Therefore, the amount of Cd in the edible part of the vegetable is positively associated with the amount of Cd in the soil, represented by a linear or quadratic equation (Shentu *et al*., 2008). Cd is very mobile, and therefore it enters plants from the roots and is then transported through the symplastic pathway in the ionic form into the xylem via ascent of sap and/or transporters through shoots (Dong *et al*., 2019) or enters into the xylem through the apoplastic pathway (Kuriakose & Prasad, 2008).

The foliar application of SA increased shoot Cd contents that ranged between 11.2-57.4 μg g^{-1} DW, respectively. At 1 mM of Cd, the foliar spray of SA increased the Cd content in shoot by 43% as compared to no spray, while the root Cd was unaffected. Similar results were also reported by Gondor *et al*., (2016). The high uptake of Cd by shoot might be due to the high synthesis of phytochelatins in the roots and their sequestration with Cd and transportation to the shoots (Gondor *et al.,* 2016). These results indicate that radish has a high tolerance and accumulating potential of Cd and therefore accumulate a higher Cd concentration in the aboveground part compared to roots. Foliar application of SA improved the aboveground biomass and Cd uptake by shoot, but chlorophyll content, the biomass of storage root, and Cd uptake by root remained unaffected.

References

Abdel-Latif, A. 2008. Cadmium induced changes in pigment content, ion uptake, proline content and phosphoenolpyruvate carboxylase activity in *Triticum aestivum* seedlings. *Aust. J. Basic Appl. Sci.*, 2: 57-62.

Ahmad, P., G. Nabi, and M. Ashraf. 2011. Cadmium-induced oxidative damage in mustard [*Brassica juncea* (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. *S. Afr. J. Bot*., 77: 36-44.

Alvarez, M.E. 2000. Salicylic acid in the machinery of hypersensitive cell death and disease resistance. *Plant Mol. Biol.*, 44: 429-442.

Anonymous. 1993. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Cadmium and cadmium compounds. In Beryllium, Cadmium, Mercury, and Exposures in the Glass Manufacturing Industry. International Agency for Research on Cancer. Volume 58, p. 119.

Anuradha, S. and S.S.R. Rao. 2009.Effect of 24-epibrassinolide on the photosynthetic activity of radish plants under cadmium stress. *Photosynthetica*, 4: 317-320.

Anwar, S., M.F. Nawaz, S. Gul, M. Rizwan, S. Ali and A. Kareem. 2016. Uptake and distribution of minerals and heavy metals in commonly grown leafy vegetable species irrigated with sewage water. *Environ. Monitor. Assess.*, 188(9): 1-9.

Anwar, S., S. Khan, M.Y. Ashraf, A. Noman, S. Zafar, L. Liu, S. Ullah and S. Fahad. 2017. Impact of chelator-induced phytoextraction of cadmium on yield and ionic uptake of maize. *Int. J. Phytorem*., 19(6): 505-513.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts.Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.*, 24: 1-15.

Balali-Mood, M., K. Naseri, Z. Tahergorabi, M.R. Khazdair and M. Sadeghi. 2021. Toxic mechanisms of five heavy metals: Mercury, lead, chromium, cadmium, and arsenic. *Front. Pharmacol.*, 12. https://doi.org/10.3389/fphar.2021.643972.

Belkhadi, A., H. Hediji, Z. Abbes, I. Nouairi, Z. Barhoumi, M. Zarrouk, W. Chaïbi and W. Djebali. 2010. Effects of exogenous salicylic acid pre-treatment on cadmium toxicity and leaf lipid content in *Linum usitatissimum* L. *Ecotoxicol. Environ. Saf*., 73: 1004-1011.

Borsani, O., V. Valpuesta and M.A. Botella. 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiol*., 126: 1024-1030.

Bruno, L., M. Pacenza, I. Forgione, L.R. Lamerton, M. Greco, A. Chiappetta and M.B. Bitonti. 2017. In *Arabidopsis thaliana* cadmium impact on the growth of primary root by altering SCR expression and auxin-cytokinin cross-talk. *Front. Plant Sci*., 8, p. 1323.

Brzóska, M.M., J. Moniuszko-Jakoniuk, M. Jurczuk, M. Gałażyn-Sidorczuk and J. Rogalska. 2001. The effect of zinc supply on cadmium-induced changes in the tibia of rats. *Food Chemical Toxicol.*, 39(7): 729-737.

Calabrese, E.J. 2009. Getting the dose–response wrong: why hormesis became marginalized and the threshold model accepted. *Arch. Toxicol.*, 83(3): 227-247.

Chen, J., C. Zhu, L.P. Li, Z.Y. Sun and X.B. Pan. 2007. Effects of exogenous salicylic acid on growth and H_2O_2 metabolizing enzymes in rice seedlings under lead stress. *J. Environ. Sci*., 19: 44-49.

Das, P., S. Samantaray and R. Rout. 1998. Studies on cadmium toxicity in plants: A review. *Environ. Pollut*., 98: 29-36.

Dong, Q., J. Fang, F. Huang and K. Cai. 2019. Silicon amendment reduces soil Cd availability and Cd uptake of two pennisetum species. *Int. J. Environ. Res. Public Health*, p. 1624

Drazkiewicz, M., A. Tukendorf and T. Baszynski. 2003. Age-dependent response of maize leaf segments to cadmium treatment: Effect on chlorophyll fluorescence and phytochelatin accumulation. *J. Plant Physiol*., 160: 247-254.

Epstein, E. and A.J. Bloom. 2005. Mineral Nutrition of Plant Principles and Perspective (2nd ed.), Sinauer Associates, Inc. Publishers, Massachusetts.

Farouk, S., A.A. Mosa, A.A. Taha, H.M. Ibrahim and A.M. EL-Gahmery. 2011. Protective effect of humic acid and chitosan on radish (*Raphanus sativus* L. var. *sativus*) plants subjected to cadmium stress. *J. Stress Physiol. Biochem*., 7: 99-116.

Fujiwara, Y., J.Y. Lee, H. Banno, S. Imai, M. Tokumoto, T. Hasegawa, Y. Seko, H. Nagase and M. Satoh. 2020. Cadmium induces iron deficiency anemia through the suppression of iron transport in the duodenum. *Toxicol. Lett*., 332: 130-139.

Glass, A.D.M. 1974. Influence of phenolic acid on ion uptake. III. Inhibition of potassium absorption. *J. Exp. Bot.*, 25: 1104-1113.

Gondor, O.K., M. Pál, E. Darkó, T. Janda and G. Szalai. 2016. Salicylic acid and sodium salicylate alleviate cadmium toxicity to different extents in maize (*Zea mays* L.). *PLoS One*, 11(8), p.e0160157.

Guo, B., Y. Liang and Y. Zhu. 2009. Does salicylic acid regulate antioxidant defence system, cell death, cadmium uptake and partitioning to acquire cadmium tolerance in rice? *J. Plant Physiol.*, 166: 20-31.

Han, Y., G. Chen, Y. Chen and Z. Shen. 2015. Cadmium toxicity and alleviating effects of exogenous salicylic acid in *Iris hexagona*. *Bullet. Environ. Contam. Toxicol.*, 95(6): 796-802.

Hegedûs, A., S. Erdei and G. Horváth. 2001. Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. *Plant Sci.*, 160: 1085-1093.

Huang, W.L., W.H. Chang, S.F. Cheng, H.Y. Li and H.L. Chen. 2021. Potential risk of consuming vegetables planted in soil with lead and cadmium and the influence on vegetable antioxidant activity. *Appl. Sci*., 11(9): p. 3761.

Hussain, A., S. Alamzeb and S. Begum. 2013. Accumulation of heavy metals in edible parts of vegetables irrigated with waste water and their daily intake to adults and children, District Mardan, Pakistan. *Food Chem.*, 136: 1515-1523.

Ivanov, V.B., E.I. Bystrova and I.V. Seregin. 2003. Comparative impacts of heavy metals on root growth as related to their specificity and selectivity. *Russ. J. Plant Physiol.*, 50: 398-406.

Januškaitienė, I. 2010. Impact of low concentration of cadmium on photosynthesis and growth of pea and barley. *Environ. Res. Engi. Manag.*, 3: 24-29.

John, R., P. Ahmad, K. Gadgil and K. Sharma. 2008. Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. *Plant Soil Environ*., 54: 262-270.

Kirk, J.T.O. and R.L. Allen. 1965. Dependence of chloroplast pigment synthesis on protein synthesis: Effect of actidione. *Biochem. Biophys. Res. Commun*., 21: 523-530.

Kupper, H., F. Kupper and M. Spiller. 1998. *In situ* detection of heavy metal substituted chlorophylls in water plants. *Photosynth. Res*. 58: 123-133.

Kuriakose, S.V. and M.N.V. Prasad. 2008. Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor* (L.) Moench by changing the activities of hydrolyzing enzymes. *Plant Growth Regul*., 54: 143-156.

Lai, H.Y. and B.C. Chen. 2013. The dynamic growth exhibition and accumulation of cadmium of pakchoi (*Brassica campestris* L. ssp. chinensis) grown in contaminated soils. *Int. J. Environ. Res. Public Health*, 10: 5284-5298.

Lanaras, T., M. Moustakas, L. Symeonidis, S. Diomantoglou and S. Karataglis. 1993. Plant metal content, growth responses and some photosynthetic measurements of field-cultivated wheat growing on ore bodies enriched in Cu. *Physiol. Plant*., 88: 307-314.

Larque-Saavedra, A. 1978. The antitranspirant effect of acetylsalicylic acid on *Phaseolus vulgaris* L. *Physiol. Plant*. 43: 126-128.

Lech, T. and J.K. Sadlik. 2017. Cadmium concentration in human autopsy tissues. *Biol. Trace Elem. Res.*, 179(2): 172-177.

Li, S., J. Yu, M. Zhu, F. Zhao and S. Luan. 2012. Cadmium impairs ion homeostasis by altering K^+ and Ca^{2+} channel activities in rice root hair cells. *Plant Cell Environ*., 35(11): 1998-2013.

Liu, D., W. Jiang and X. Gao. 2003. Effects of cadmium on root growth, cell division and nucleoli in root tip cells of garlic. *Biol. Plant.*, 47(1): 79-83.

Lux, A., M. Martinka, M. Vaculík and P.J. White. 2011. Root responses to cadmium in the rhizosphere: A review. *J. Exp. Bot.*, 62(1): 21-37.

Marchiol, L., P. Sacco, S. Assolari and G. Zerbi. 2004. Reclamation of polluted soil: phytoremediation potential of crop-related *Brassica* species. *Water Air Soil Pollut*., 158: 345-356.

Muchuweti, M., J.W. Birkett, E. Chinyanga, R. Zvauya, M.D. Scrimshaw and J.N. Lester. 2006. Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: implications for human health. *Agric. Ecosys. Environ*., 112(1): 41-48.

Netondo, G.W., J.C. Onyango and E. Beck. 2004. Sorghum and salinity: II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Sci.*, 44(3): 806-811.

Pandey, D.M., C.L. Goswami, B. Kumar and S. Jain. 2000. Hormonal regulation of photosynthetic enzymes in cotton under water stress. *Photosynthetica*, 38: 403-407.

Perfus–Barbeoch, L., N. Leonhardt, A. Vavasseur and C. Forestier. 2002. Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *Plant J.*, 32: 539-48.

Poorter, H. and C. Remkes. 1990. Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia*, 83: 553-559.

Poschenrieder, C., C. Cabot, S. Martos, B. Gallego and J. Barceló. 2013. Do toxic ions induce hormesis in plants?. *Plant Science*, 212: 15-25.

Raza, S.H. and F. Shafiq. 2013a. Exploring the role of salicylic acid to attenuate cadmium accumulation in radish (*Raphanus sativus*). *Intl. J. Agric. Biol.*, 15(3): 547-552.

Raza, S.H., F. Shafiq and M. Tahir. 2013b. Screening of cadmium tolerance in sugarcane using antioxidative enzymes as a selection criteria. *Pak. J. Life Social Sci.*, 11: 8-13.

Raza, S.H., F. Shafiq, U. Rashid, M. Ibrahim and M. Adrees. 2014. Remediation of Cd-Contaminated Soils: Perspectives and Advancements. *In: Soil Remediation and Plants*. *Elsevier*, pp. 571-597.

Rydzyński, D., A.I. Piotrowicz-Cieślak, H. Grajek and D.J. Michalczyk. 2019. Chlorophyll degradation by tetracycline and cadmium in spinach (*Spinacia oleracea* L.) leaves. *Int. J. Environ. Sci. Technol*., 16(10): 6301-6314.

Shakirova, F.M. C.R. Allagulova, D.R. Maslennikova, E.O. Klyuchnikova, A.M. Avalbaev and M.V. Bezrukova. 2016. Salicylic acid-induced protection against cadmium toxicity in wheat plants. *Environ. Exp. Bot*., 122: 19-28.

Sheng, H., J. Zeng, F. Yan, X. Wang, Y. Wang, H. Kang and Y. Zhou. 2015. Effect of exogenous salicylic acid on manganese toxicity, mineral nutrients translocation and antioxidative system in polish wheat (*Triticum polonicum* L.). *Acta Physiol. Plant.*, 37(2): 1-11.

Shentu, J., Z. He, X.E. Yang and T. Li. 2008. Accumulation properties of cadmium in a selected vegetable-rotation system of Southeastern China. *J. Agric. Food Chem*., 56(15): 6382-6388.

Song, X., X. Yue, W. Chen, H. Jiang, Y. Han and X. Li. 2019. Detection of cadmium risk to the photosynthetic performance of Hybrid Pennisetum.*Front. Plant Sci*., 10, p.798.

Urani, C., P. Melchioretto, M. Bruschi, M. Fabbri, M. M.G. Sacco and L. Gribaldo. 2015. Impact of cadmium on intracellular zinc levels in HepG2 cells: quantitative evaluations and molecular effects. *BioMed Res. Int.*, 2015: 949514.

Verma, P., K.V. George, H.V. Singh and R.N. Singh. 2007. Modeling cadmium accumulation in radish, carrot, spinach and cabbage. *Appl. Math. Model*., 31(8): 1652-1661.

Wang, K.R. 2002. Tolerance of cultivated plants to cadmium and their utilization in polluted farmland soils. *Acta Biotechnol.*, 22: 189-198.

Wu, F.B. and G.P. Zhang. 2002. Genotypic differences in effect of Cd on growth and mineral concentrations in barley seedling. *Bull. Env. Contam. Toxicol*., 69: 219-227.

Xu, L.L., Z.Y. Fan, Y.J. Dong, J. Kong and X.Y. Bai. 2015. Effects of exogenous salicylic acid and nitric oxide on physiological characteristics of two peanut cultivars under cadmium stress. *Biol. Plant.*, 59(1): 171-182.

Xu, Z.M., Q.S. Li, P. Yang, H.J. Ye, Z.S. Chen, S.H. Guo, L.L. Wang, B.Y. He and E.Y. Zeng. 2017. Impact of osmoregulation on the differences in Cd accumulation between two contrasting edible amaranth cultivars grown on Cd-polluted saline soils. *Environ. Poll*., 224: 89-97.

Zhang, Y., S. Xu, S. Yang and Y. Chen. 2015. Salicylic acid alleviates cadmium-induced inhibition of growth and photosynthesis through up-regulating antioxidant defense system in two melon cultivars (*Cucumis melo* L.). *Protoplasma*, 252(3): 911-924.

(Received for publication 12 June 2021)