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Research Article

Effect of cadmium metal in germinating seedlings of *Pisum sativum* L.: Modeling of morpho physiological traits

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ABSTRACT

India produces a significant amount of crops. Heavy metals, especially cadmium, in the soil have a negative impact on the crops. Increased cadmium levels in soil and water are a severe issue on a global scale. Due to its biological half-life and ease of uptake by plants, cadmium (Cd) is undoubtedly a harmful metal to many plant species, affecting a number of metabolic pathways. The current study therefore sought to evaluate the effects of irrigation water with cadmium contamination levels of 1.0, 2.0, 4.0, 8.0, and 16.0 ppm on the pea plant. With an increase in cadmium concentration in irrigation water, growth and biomass characteristics (% germination, growth characteristics, shoot-root length, and fresh-dry mass) reduced. As cadmium content increased, there was a reduction in radical length and seed germination (1.0–16.0 ppm). According to the results of our research, irrigation water contaminated with cadmium has a negative impact on pea plants' photosynthetic, biochemical, and biomass processes as well as their growth and development.

Keywords: *Pisum sativum* L., growth and development, photosynthetic pigments, cadmium toxicity

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INTRODUCTION

The rising modernization of the agriculture sector has resulted in increased environmental contamination and ecological harm. One of the most significant environmental issues in the world today is heavy metal poisoning of the soil. Silver-white cadmium (Cd) is a hazardous metal element. Among the top 20 poisons, Cd, an extremely hazardous trace element, is listed seventh (Abraham et al., 2013). The absorption and buildup of Cd can hinder plants' ability to grow normally. The water solubility, fluidity, and toxicity of Cd make it simple for plant roots to absorb it, and it can change the structural and functional characteristics of plants. Cd prevents root growth and seed germination. The plant life cycle's most important activity is seed germination, which is followed by the seed emerging from dormancy (Ismael et al., 2018; Huybrechts et al., 2019). Auxin, abscisic acid, and gibberellic acid are phytohormones that regulate seed germination (Huybrechts et al., 2019). Plant genotype, bio-availability, and speciation all influence cadmium accumulation and transport (Li et al., 2018; Haider et al., 2021). Cd is transferred in the following order to the plant's reproductive, vegetative, and aforementioned sections: Roots trump leaves trump fruits trump grains (Kubier et al., 2019). According to Pinto et al. (2004), the transportation of Cd into fruits and grains varies depending on crop and cultivar.

Reduced resource storage potential (i.e., nutrition and water in the plant) is indicated by decreases in root length, surface area, and root tip number under extremely high

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levels of Cd stress (Lu et al., 2013). Long-term exposure to Cd also results in the root becoming necrotic, decaying, and mucilaginous, which reduces the length of plant roots and shoots and causes chlorosis and leaf rolling (Abbas et al., 2017). Cd can disrupt physiological activities such as photosynthesis, respiration, gas exchange, and water transport, which can degrade plant metabolism and lower the production and quality of agriculture (Rizwan et al., 2016). Plant biomass output and photosynthetic rate decrease in response to cadmium stress (Raza et al., 2020; Zhao, 2021). Cd has also been shown to interfere with plant uptake, transport, and utilisation of a variety of elements and water (Ca, Fe, Mg, P, K, Mn, Cu, and Zn). The photosynthetic apparatus and its pigments, according to Rafiq *et al*. (2014), as well as the synthesis of carotenoids and chlorophyll, are the primary sites of action for cadmium. The interaction between Cd and chlorophyll biosynthesis reduces chloroplast density. Vassilev et al. (2005) discovered cadmium to be an effective photosynthesis inhibitor.

A linear relationship between photosynthesis inhibition and transpiration was observed in oilseed, legume, and cereal crops, indicating that Cd accumulation in leaves inhibits stomatal opening (Younis et al., 2016; Zhang et al., 2019). Cd toxicity harms the photosynthetic apparatus, particularly the light-harvesting complex and photosystems I and II (Hasan et al., 2009). Iron (Fe) helps to increase chlorophyll content and the synthesis of other pigments that are directly involved in photosynthesis light harvesting (Akinola & Ekiyoyo, 2006). Cd-induced inhibition of iron (Fe^{3+}) reductase results in iron (Fe^{2+}) deficiency, which has a negative impact on the photosynthesis process and its apparatus, according to Hasan et al. (2009).

Excessive oxidation caused by Cd is prevented by complex enzymatic and non-enzymatic mechanisms that protect plant cells from oxidative damage and restore cell redox balance (Gratao et al., 2015; Alves et al., 2020). As a result, by disrupting chloroplasts in leaves, Cd may indirectly contribute to ROS production. By increasing the production of reactive oxygen species (ROS), Cd has the potential to weaken the antioxidant defence system (Gallego et al., 2012). According to Pirselova & Ondruskova (2021), chloride is a major osmotically active solute in the vacuole and plays a role in plant turgor and osmoregulation. Cl- fluxes are involved in membrane potential stabilization, pH gradient regulation, and electrical excitability. The pea (*Pisum sativum* L.) is a self-pollinating Leguminaceae family leguminous seed-pod crop. Pea seeds have a high protein and carbohydrate content (18-20% dry matter). The purpose of a recent study was to evaluate the plant growth, physiological, and biochemical responses of pea plants to cadmium-contaminated water.

MATERIALS AND METHODS

Seeds collection and preparation

Certified hybrid seeds of the pea variety Arkel were provided by the Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. The experiment was carried out at India's University of Lucknow, Department of Botany. To allow water absorption, the seeds were surface sterilized with $HgCl_2$ (0.1%) before being immersed in running water overnight.

Treatment of seeds and germination

For two weeks, seeds were treated twice a week with Cd solutions containing 0, 1.0, 2.0, 4.0, 8.0, and 16.0 ppm (prepared from $CdCl_2$) (room temperature 25 °C, photon flux density 400 mol m^2 s^1, photoperiod 12 h). The control seeds were grown in the same manner as the experimental seeds, with regular water. Each treatment received ten seeds per filter paper petriplate and three independent replications. To avoid dehydration of the seeds required for germination, the moisture level of the filter paper was kept constant throughout. The

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seeds were thought to have germinated with the emergence of the radical. A modified Timson's index of germination rate was used to calculate the germination rate (%), (Khan & Ungar 1997; Luo et al., 2019, 2020).

Biomass index

Growth and biomass were observed 15 days after the application of Cd-contaminated water. Growth parameters such as radical length, leaf number, shoot-root length, number of lateral roots, root-shoot fresh and dry weight (g) of pea were measured after treatment. The fresh and dry weights (oven-dried at 65 5 °C for 15 days) were recorded using an electric balance.

Assessment of photosynthetic pigments

Photosynthetic pigments were spectrophotometrically measured in acetone (80%) (Arnon, 1949). After 15 days of Cd treatment, the plant's leaves and roots were collected. They were cut into small pieces, ground into powder with liquid nitrogen, lyophilized, and stored for enzymatic activity in a deep freezer (-80 °C). Lowry et al. (1951) calculated the soluble protein content. Catalase activity was measured using Euller and Josephson's (1927) method, and the result was expressed in mol H_2O_2 g^{-1} FM. The Luck method was used to determine peroxidase activity (1963). The peroxidase activity was measured in terms of OD/gm fresh tissue weight. Heath and Packer's method was used to determine lipid peroxidase (1968). Lipid peroxidase activity was measured in mol MDA g^{-1} fresh tissue.

Statistical analyses

Data were processed using the statistical package of MS Excel. The significance of differences was analyzed by using a Student's t-test and $p < 0.05$ was considered as statistical significantly. Pearson's correlation coefficient was calculated between tested parameters.

RESULTS

Seed germination and growth index

The phytotoxicity of cadmium on plant growth, physiological, biochemical, and biomass characteristics was investigated in *Pisum sativum*. For 15 days, the seeds were exposed to varying cadmium concentrations in petriplates to reveal rapid responses of pea seedlings in relation to their growth characteristics. After 15 days of exposure to various levels of cadmium contamination,

Figure 1: Effect of different levels of cadmium, i.e. 1, 2, 4 8 and 16 ppm on seed germination % shown by *Pisum sativum* var. Arkel. Measurements were taken till 15 days. Values are mean (n=3) with S.E. (±).

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germination (%) was measured (0, 1.0, 2.0, 4.0, 8.0, and 16.0 ppm). It was discovered ($p < 0.05$) that the control germination rate was approximately 83%, while the 1.0 ppm cadmium germination rate was approximately 75%. When 1.0 to 16.0 ppm cadmium was used, the germination percentage dropped from 83 to 28%. The extremely high cadmium level (16.0 ppm) resulted in no seed germination (Figure 1).

Phytotoxicity evaluation

The biodynamic of germinating seeds were studied in relation to the appearance of radical length, which was influenced by cadmium concentrations. The data revealed that seedlings unaffected by cadmium (control) had a maximum radical length of 6 cm throughout (till 15 days after germination). Cadmium-affected seedlings (p 0.05) had radical lengths of nearly 2.4, 1.7, 1.5, 1.0, and 0.6 cm when cadmium was applied at 1.0, 2.0, 4.0, 8.0, and 16.0 ppm, respectively. The higher the cadmium level (16.0 ppm), the more difficult it was to acquire radical length (Figure 2B).

Cadmium phytotoxicity was found to reduce shoot length (Figure 2A). Seeds exposed to 1.0 ppm cadmium for 15 days could lose nearly 31% of their shoot length. Shoot

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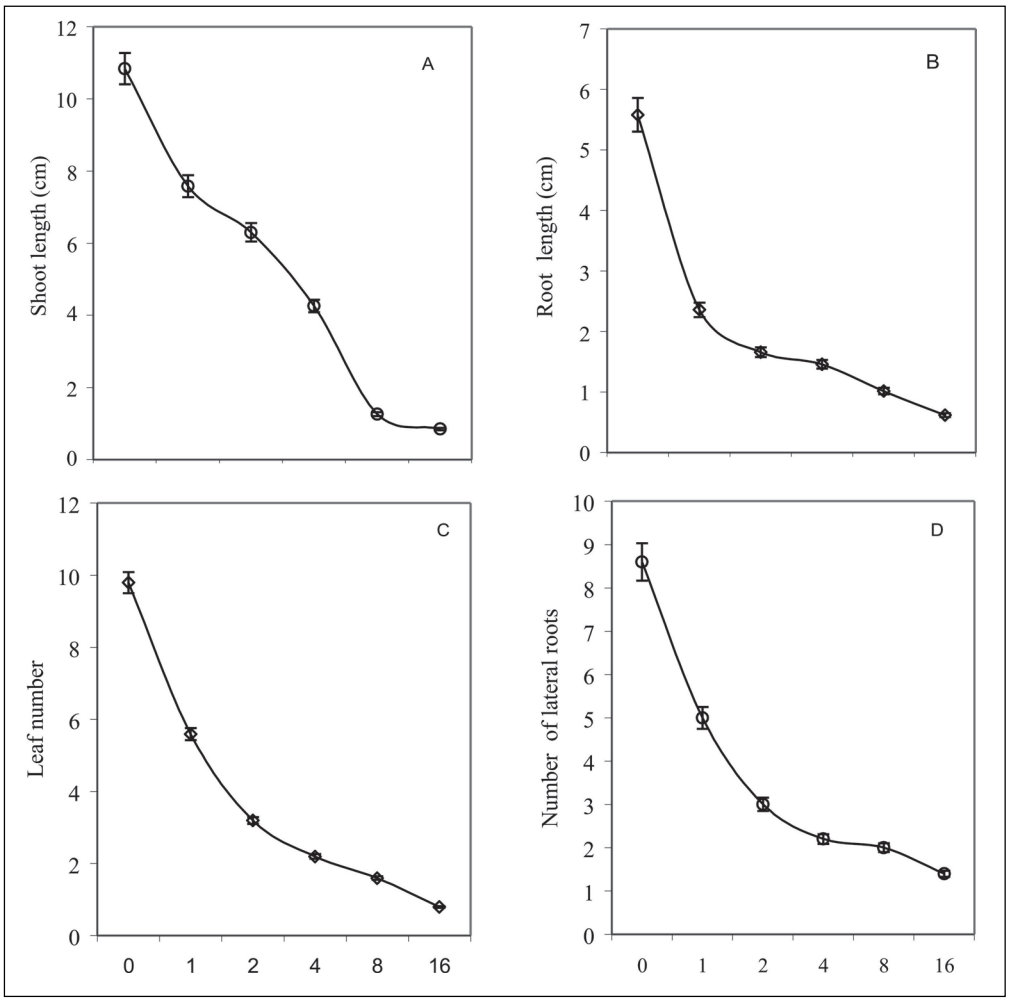

Figure 2: Effect of cadmium on shoot length (A), root length (B), number of leaves (C) and number of lateral roots (D) in *Pisum sativum* var. Arkel. The seedlings were allow to grow till 15 days after application of various concentrations (1, 2, 4, 8 and 16 ppm). Values are mean (n=3) with S.E. (±).

length was reduced by nearly 43, 56, 78, and 89% in irrigation water contaminated with 2.0, 4.0, 8.0, and 16.0 ppm cadmium. The acquisition of leaf number has also been found to be affected, as evidenced by cadmium-treated seedlings. The control could grow 10 leaves, whereas the cadmium treatment reduced this to 6, 3, 2, 2, and 1 in cases where the cadmium concentration remained at 2.0, 4.0, 8.0, and 16.0 ppm until 15 days after treatment began. When 1.0 ppm cadmium was applied to pea seedlings, the green area was reduced by nearly 21%. It has decreased further to approximately 26 to 79% in cases where treatment levels have been increased to 2.0 - 16.0 ppm (Figure 2C). With different cadmium concentrations, the trends in the differential loss of green area/leaf number began at the beginning (5 days) (1.0, 2.0, 4.0, 8.0 and 16.0 ppm). As shown in Figure 2B, D, root length and lateral root development were observed. Cadmium levels (1.0, 2.0, 4.0, 8.0, and 16.0 ppm) reduced root lengths by approximately 58,70,74,82, and 88%, respectively, for 15 days after stress. Similar trends in the number of lateral roots were observed, which were influenced by treatment levels and durations (Figure 2D). The data in Figure 3B, A represented the amount of root biomass acquired as a result of different treatment schedules and durations. As shown in Figure 3A-B, the higher the cadmium levels, the greater the down-regulation of root/shoot biomass. As a result, after 15 days of continuous exposure to a 1.0 ppm cadmium solution, shoot and root biomass were reduced by approximately 41% and 27%, respectively. In Figure 3C, the moisture content has similarly decreased from near 90 to 89%.

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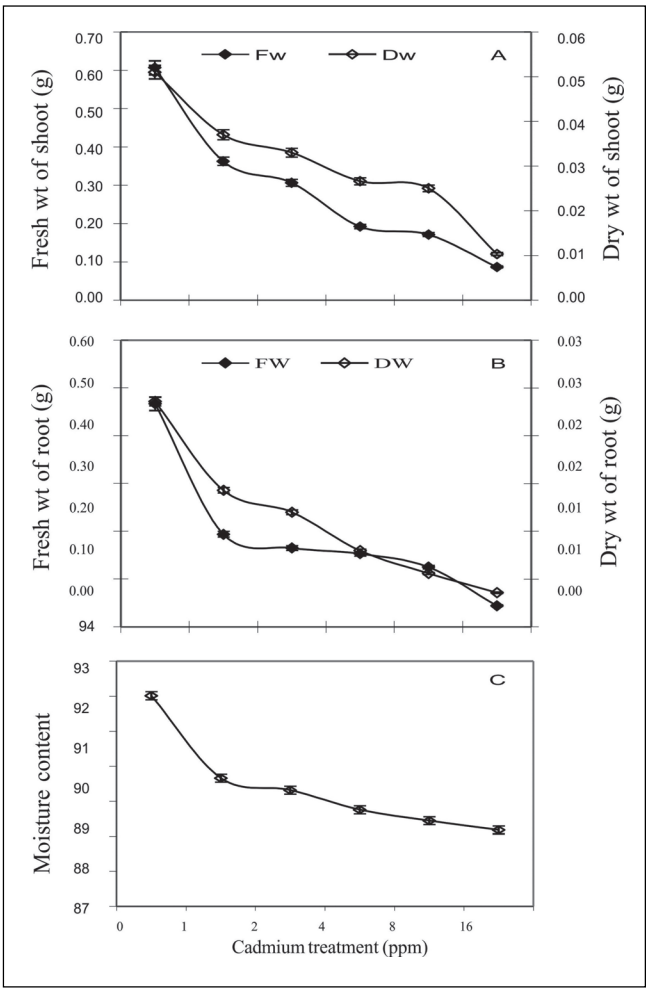

Figure 3: Effect of different cadmium concentrations (1, 2, 4, 8 and 16 ppm) of shoot fresh and dry weight (A), root fresh and dry weight (B) and (%) moisture content (C) in *Pisum sativum* var. Arkel. The seedlings were allowed to grow till 15 days. Values are mean (n=3- 5) with S.E. (±).

Pigments analysis

Chlorophylls a and b, which are photosynthetic pigments, are affected, as shown in Figure 4 A-D. Chl b was found to be more significantly affected than Chlorophyll a. Total chlorophyll values were also found to be nearly 79% degraded if pea seedlings were exposed to cadmium at 16.0 ppm for 15 days. Higher treatment levels may result in greater (p 0.05) loss of photosynthetic pigments (chl. a 31-76%, chl. b 46-93%, chl. a + b 33-79%) and carotenoid (29-65%). The effect of cadmium treatment on pea seedlings was also linked to stress-inducible enzymes like peroxidase and catalase. These enzyme genes are generally activated by adverse plant experiences, as shown in Figure 5 A, B, which clearly show increasing trends in pea seedlings. Almost all intrinsic abilities are related to an increase in peroxidase (24-76%) and catalase (18-100%) levels, which are correlated with cadmium (1 ppm-16 ppm) levels. Because

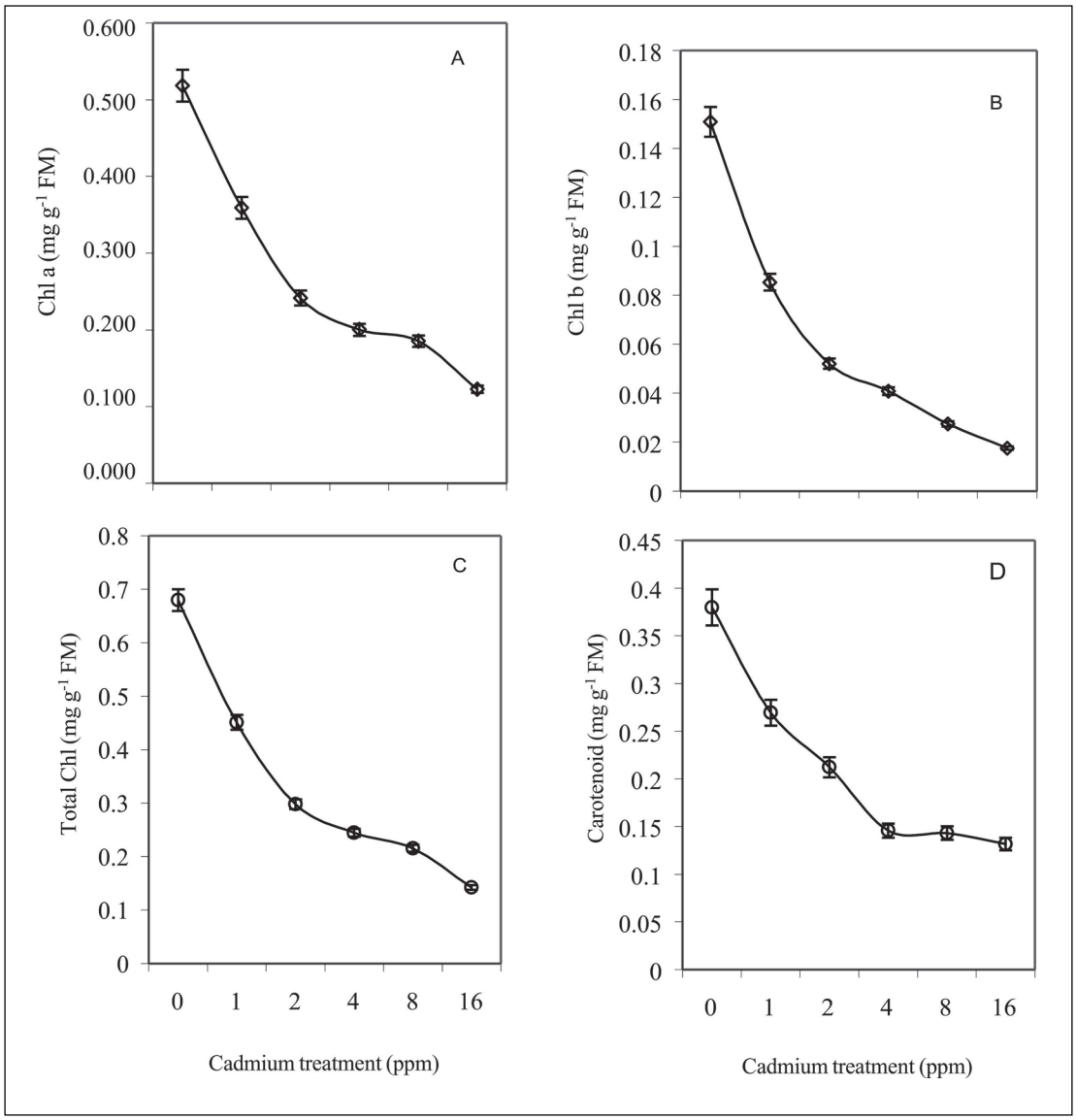

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Figure 4: Effect of different cadmium concentrations (1, 2, 4, 8 and 16 ppm) on photosynthetic pigments in *Pisum sativum* var. Arkel. Chl a (A), chl b (B), total chl (C) and carotenoid (D). Cadmium treatment maintained for a period of 15 days. Values are mean (n=3) with S.E. (±).

both of these enzymes are stress-reducing biomolecules, they have behaved biologically in support of the biological system. Similarly, lipid peroxidase activity could be increased by approximately 258% in the case of leave in case treated with 16 ppm cadmium contaminated water for 15 days in Figure 5C. Indeed, all of these stress-related enzymes have been found to be up-regulated/over-expressed when the biological system is subjected to biotic or abiotic stress.

DISCUSSION

Plant seed germination is reduced due to the accelerated breakdown of stored nutrients in the seed and changes in the selection permeability properties of the cell membrane caused by heavy metal toxicity. According to Bahmani et al. (2012), Cd can significantly reduce the percentage of seed germination in *Leucaena leucocephala* and bean (*Phaseolus vulgaris* L.). In

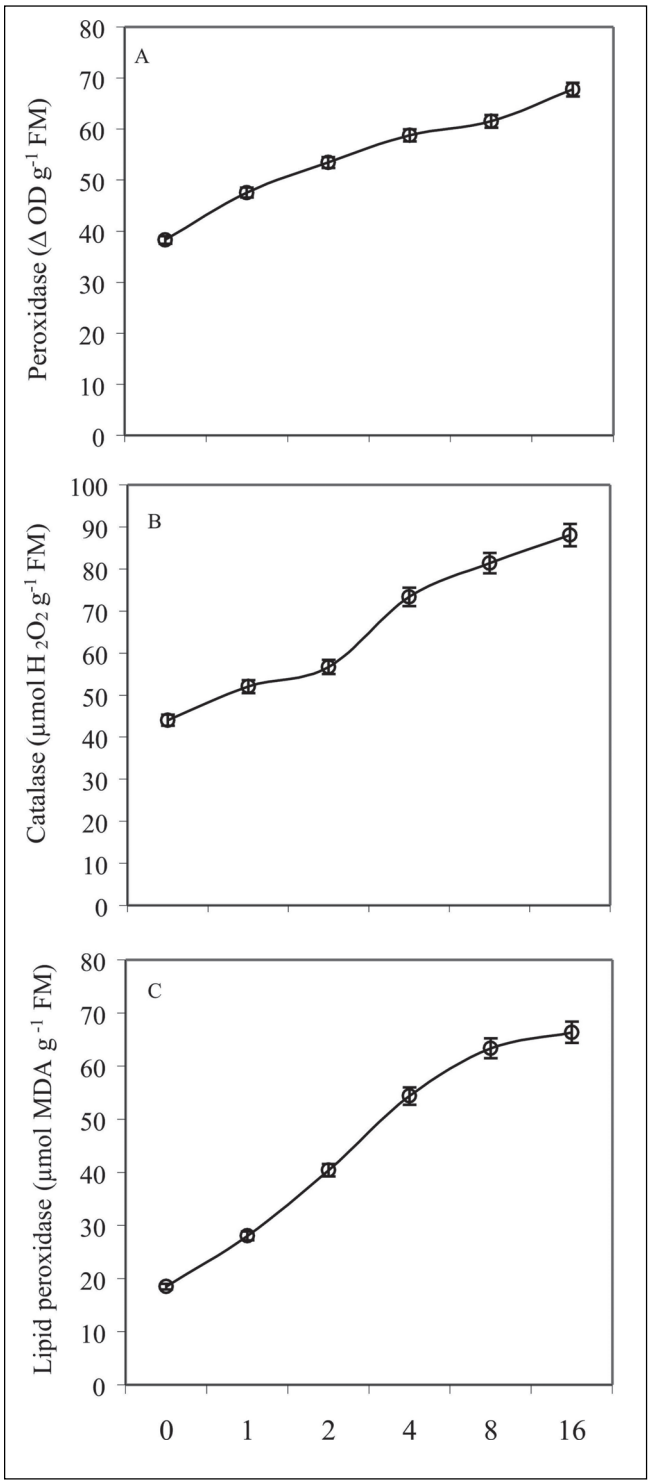

Figure 5: Effect of cadmium contaminated water on enzymes activities of peroxidase (A) catalase (B) and lipid peroxidase (C) in seedlings growth. *Pisum sativum* var. Arkel seedlings were exposed to 0, 1, 2, 4, 8 and 16 ppm Cd for a period of 15 days. Values are mean (n=3) with S.E. (±).

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the current study, cadmium exposure significantly altered the seed germination ratio, biomass content (moisture content and dry weight), root and shoot length of both cultivars. Seeds grown in various cadmium concentrations exhibited stunted growth and the formation of short lateral roots (Shekhawat et al., 2010). The germination of seedlings of soybean, lettuce, and sugar beet (*Beta vulgaris* L.) was reduced by 5 mg/L Cd exposure (Haider et al., 2021). Cadmium accumulation in plant roots reduces water and mineral uptake and, as illustrated in Figure 1, affects plant metabolism in Figure 2A, B.

As shown in Figure 2, higher Cd concentrations significantly reduced the number of leaves in pea, similar to chickpea (Faizan et al., 2011). Cd toxicity prevents the formation of lateral roots and causes the main root to become rigid, twisted, and brown in colour (Krantev et al., 2008; Haider et al., 2021). Cd toxicity has a negative effect on plant growth and can reduce root dry weight, root diameter, and the number of lateral roots in tomato plants, according to Alves et al., (2020).

Chlorophyll is the primary pigment responsible for photosynthesis in plants. Plants' photosynthesis is inhibited when they are stressed, and chlorophyll concentrations can directly

indicate the extent of stress-induced damage in plants (Lin et al., 2012). Cd treatment reduced the amount of chlorophyll a, chlorophyll b, and carotenoids in *Lemna minor*, according to Hou et al. (2007) and Katazyna & Smolik (2011). As shown in Figure 3A-C, Erdei et al. (2002) discovered a greater decrease in chlorophyll content in barley after cadmium application.

Heavy metals either directly or indirectly cause oxidative damage to plants through the formation of ROS, which causes additional severe oxidative damage to various cell organelles and biomolecules (Radotic et al., 2000). Plants have a well-organized antioxidative defence

system that includes both enzymatic and non-enzymatic antioxidants (e.g., SOD, POD, GR, GPX, and CAT) (GSH, NP-SH, PCs). These antioxidants' cooperative function is critical in scavenging ROS and maintaining the physiological redox status of organisms (Cho & Seo, 2005; Zou et al., 2012).

Cadmium chloride treatments increased the activity of the peroxidase enzyme (POD) in *B. rapa* leaves significantly, as shown in Figure 5A. For long-term exposure, CAT activity increased with increasing heavy metal concentrations and decreased with increasing concentrations (Arleta et al., 2001; Salama et al., 2009; Liu et al., 2011). The increase in CAT activity following Cd treatments could be attributed to CAT's role as an H_2O_2 scavenger, which could be mitigated by induction of specific enzymes such as CAT (Elstner et al., 1988). As shown in Fig. 5B, CAT is a major antioxidant enzyme that converts hydrogen peroxide into oxygen and water. According to Hegedus et al. (2001), Cd toxicity increased the MDA content of barley plants. Haider et al., 2021 discovered an increase in malondialdehyde (MDA) content in Cd-stressed pea (*Pisum sativum* L.) embryos due to Cd-induced membrane lipid peroxidation in Figure 5C.

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CONCLUSION

Crop production in India is substantial. Crops were negatively impacted by the presence of heavy metals in the soil, especially cadmium. On a global basis, elevated cadmium levels in both soil and water constitute a serious problem. Cadmium (Cd) is clearly a detrimental metal to many plant species, disrupting a number of metabolic pathways, due to its biological half-life and ease of uptake by plants. Determining the effects of irrigation water with cadmium contamination levels of 1.0, 2.0, 4.0, 8.0, and 16.0 ppm on the pea plant was the goal of the current study. Growth and biomass parameters (% germination, growth traits, shoot-root length, and fresh-dry mass) decreased with an increase in cadmium concentration in irrigation water. Radical length and seed germination decreased when the cadmium concentration rose (1.0–16.0 ppm). According to the findings of our study, cadmium-contaminated irrigation water adversely affects the photosynthetic, biochemical, and biomass processes as well as the growth and development of pea plants.

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