Research article

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Bioremediation potential of *Pseudomonas putida* and Selenium Nanoparticles on the Physiology of *Zea mays* L. Grown in Cd Contaminated Water from Factory

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Abstract

This study was intended to assess the role of plant growth-promoting rhizobacteria (PGPR) and Se-nanoparticles on Maize (*Zea mays* L.) grown in Pakistan Ordinance Factory (POF) produced water and tube well water installed in the vicinity of the POF. Seeds of *Golden Bantam Corn* were inoculated with plant growth-promoting rhizobacteria (PGPR), *Pseudomonas putida* (KX574857), which were obtained from Molecular Plant Pathology Lab, Quaid-e-Azam University, Islamabad, Pakistan) @ Of 10^8 cells per ml before sowing. Selenium nanoparticles (30ppm) were synthesized by green synthesis on sodium selenite reduction with *Withania somnifera* L. extract. The foliar application (5ml in 30ppm) of Se NPswas conducted on leaves in the early vegetative phase, 12d after sowing. Plants were irrigated with POF water, while tap water irrigation to plants was treated as a control. Cd metal was found above permissible limits in POF effluent water. Plants irrigated with effluent water showed a decline in development and grain production. The PGPR and Se-nanoparticles reduce water samples BOD and raise the pH of acidic water (POF) towards neutrality. The PGPR alleviated the inhibitory effects of POF-discharged water with CAT enzyme activity. The PGPR enhanced sugar content (40%) over control in POF water. It is inferred that the Cd toxicity resulted in a decline in maize growth while PGPR and Se-nanoparticles minimize ROS production CAT enzyme activity. *Pseudomonas putida* and Se-nanoparticles work synergistically to alleviate oxidative stresses.

Keywords: PGPR, green synthesize Se-nanoparticles nanoparticles, bioremediation, *Pseudomonas putida*, POF,

Highlights

- *Pseudomonas putida* is very effective for Cd tolerance in Maize.
- Foliar application of Se-nanoparticles results decline in the BOD of industrial effluent and enhances CAT enzyme activity in treated plants.

1. Introduction

Heavy metal pollution is a harmful stress that disturbs plant growth, yields, food quality, and the prevailing environment. Rapid industrialization, urbanization, synthetic chemicals application, mining activities and improper waste disposal are major sources of metal pollution (Rajput et al., 2021). The origin of heavy metals consists of both human activities and natural processes. Recently, heavy metals from human-caused sources have been settled into the ecosystem and increasingly gathered to hypothetically harmful soil levels. Furthermore, numerous human activities (pesticides, urban wastes, metal mining and chemical fertilizers) have directed to metal contamination in agricultural sites (Shi, 2018; Sun 2020).

The plant growth-promoting rhizobacteria (PGPR) improves plant growth by producing 1-aminocyclopropane-1-carboxylate deaminase (ACCD), phytohormones, siderophores, nitrogen fixation and solubilizing phosphates. PGPR increases Cd bioremediation through diverse mechanisms like biosorption, chelation, complexation and sequestration. The inoculation of Cd-resistant PGPR to reduce Cd stress in plants has a stimulating prospect, and findings showed promise for increasing food security in contaminated soil (Ghosh et al., 2022).

Nanotechnology has become a remarkable innovative approach to accomplish numerous environmental issues by providing advanced and operative results. Cadmium toxicity results decline in chlorophyll content. Moreover, Cd-stressed plants showed modulations in proline content, leaf osmotic potential and decreased water potential. Se NPs increased growth attributes (including chlorophyll, sugars, gas exchange parameters and leaf relative water content) in treated plants lessened by Cd toxicity. Application of Se NPs increases antioxidant response by increasing peroxidase (POX, and catalase (CAT) activity and preserving cellular structures through foraging reactive oxygen species and free radicals.

Additionally, Cd-stressed plants showed a higher level of MDA, whereas Se NPs enhanced membrane integrity through hydrogen peroxide detoxification. Also, Se NPs improved metal tolerance index and nutrient contents and lessened Cd level in plants, follow-on better growth of affected plants (Bello-Pérez 2021). The exclusive characteristics of NPs improve seed germination, crop production, nutrient uptake and yield of treated plants (Gudkov et al., 2020). There are numerous ways to encompass Se NPs into farming: cultivating plants on an aeroponic nutrient medium containing Se NPs, soaking seeds in Se NPs solution, foliar application on leaves and adding Se to the soil (Mitra et al. 2023).

Withania somnifera L (Ashwagandha) is a shrub of Pakistan. In HIT (Hitech Industries Taxila) locality, this herb is distributed widely and commonly known among farmers because of its medicinal value in treating diabetes, asthma, and arthritic diseases.

Catalase is a vital antioxidant enzyme in plants belonging to the oxidoreductase class. During oxidative stress can oxidize various electron-donating substrates, including H_2O_2 breakdown. Catalases as enzymes for biotechnological manufacturing have gained a status for the remediation of environmental toxins (Kaushal et al., 2018). Catalases degrade toxic environmental pollutants biologically, counting synthetic textile dyes, pesticides, herbicides and phenols (pyrogallol, cresol, guaiacol). Moreover, also act as a biomarker for oxidative stress in cells succeeding exposure to xenobiotics. Catalase-immobilized micromotors upgrade the polluted water remediation rapid accession of polluting agents in contaminated sites. Plant sugars are the source of carbon skeletons, intermediate metabolites (biochemical reactions), storage substances, signals in abiotic and biotic stresses, osmolytes and the substrate of respiratory reactions to maintain plant developmental and growth processes (Fernandez et al., 2017).

Maize is a major cereal produced globally and an elementary food harvest in the human diet (Xu et al. 2022). Hereafter, to evaluate the uptake of Cd by plants, it would be appreciated to consider the mode of action and toxicity of Cd in Maize. The main goal of contemporary research is to observe how diminishing the Cd toxicity in Maize can be cooperative in dropping the threat of food chain contamination.

2. Materials and Methods

2.1. Analysis of water samples

Metal content was determined by water sample aspiration by atomic absorption spectrophotometer and then analyzed. POF water holds Cd higher than the permissible limit of WHO standards. Plants were irrigated on daily bases with 2L water. The BOD of water samples was resolute following the procedure of the United States Environmental Protection Agency, Office of Research and Development (1986).

Table 1. Treatments are provided to the plants.

Treatments	Details
Tap	Untreated water from Tap.
POF	Plants irrigated with effluent water of Pakistan Ordinance Factory.
Tap+PGPR	Seeds soaked in PGPR *Pseudomonas putida* L. inocula and plants irrigated with tap water.
POF+PGPR	Seeds soaked in PGPR *Pseudomonas putida* L. inocula and plants irrigated with Pakistan Ordinance Factory effluent water.
Tap+Se_{NP}	Plants sprayed with Se NPs-nanoparticles (25ppm) after four weeks of seed germination and plant irrigated with tap water.
POF+Se_{NP}	Plants sprayed with Se NPs (30ppm) after four weeks of seed germination and plants irrigated with effluent water from Pakistan Ordinance Factory.
Tap+PGPR+Se_{NP}	Seeds soaked in PGPR *Pseudomonas putida* L. inocula, plants sprayed with Se NPs (30ppm) after four weeks of seed germination and plants irrigated with tap water.
POF+PGPR+Se_{NP}	Seeds soaked in PGPR *Pseudomonas putida* L. inocula, and plants sprayed with Se NPs (30ppm) after four weeks of seed germination and plant irrigated with Pakistan Ordinance Factory effluent water.

2.2. Sterilization of seeds and Inoculation

The seeds of *Zea mays* L. were given by Dr. Waseem Ahmad (University of Haripur), sterilized in 90% ethanol for 10min and washed with water (deionized). The LB media was inoculated with 50h old culture of *Pseudomonas putida* and incubated for 73h in a shaking incubator at 28^0C. Seeds soaked in bacterial inocula for 2h. For uninoculated plants, seeds were soaked in LB (uninoculated) for a similar period.

2.3. Preparation and Treatment with Se NPs (30ppm)

Klaire Labs Seleno Met supplement contains 200mcg of selenium. Selenium nanoparticles were synthesized by the green synthesis method on sodium selenite reduction with *Withania somnifera* L. extract. 3mL of selenium solution (0.2 mol L^{-1}) was mixed with distilled water (20mL). Afterward, 3 mL of *Withania somnifera* L. infusion (infusion/Se in 1:1 ratio) was further supplemented. The reaction mixture contains a 2.15×10^{-3} concentration of plant extract. Nanoparticles after collection were stabilized.

The Ultra Violet–vis absorption spectra showed a range of 40-100nm and were recorded with Elmer spectrophotometer having 1.5cm cuvettes (in length). Spectra were recorded using the suitable brew as blank. The sample was diluted 60 times to take measurements. The morphological assembly of Se NPs was further explained by FT-IR and SEM/SEM-EDX analysis that reviled Se nanoparticles prepared to have a spherical shape.

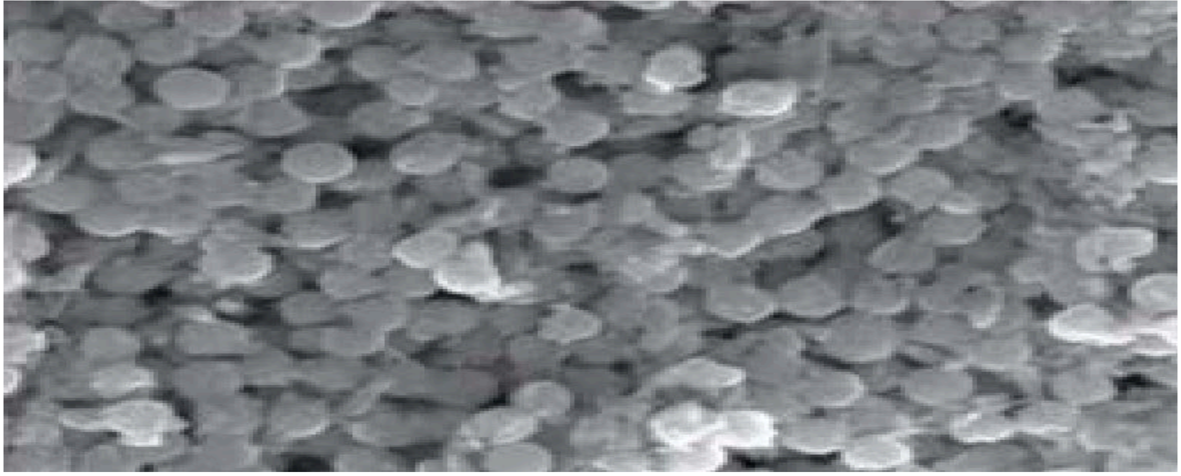

Figure 1. Image showing Se-nanoparticles (under 9500 magnification) under an electron microscope.

Results demonstrate Se-nanoparticles have higher absorbance (1.2a.u) at 580nm wavelength after this wavelength; absorbance shows a significant decline gradually.

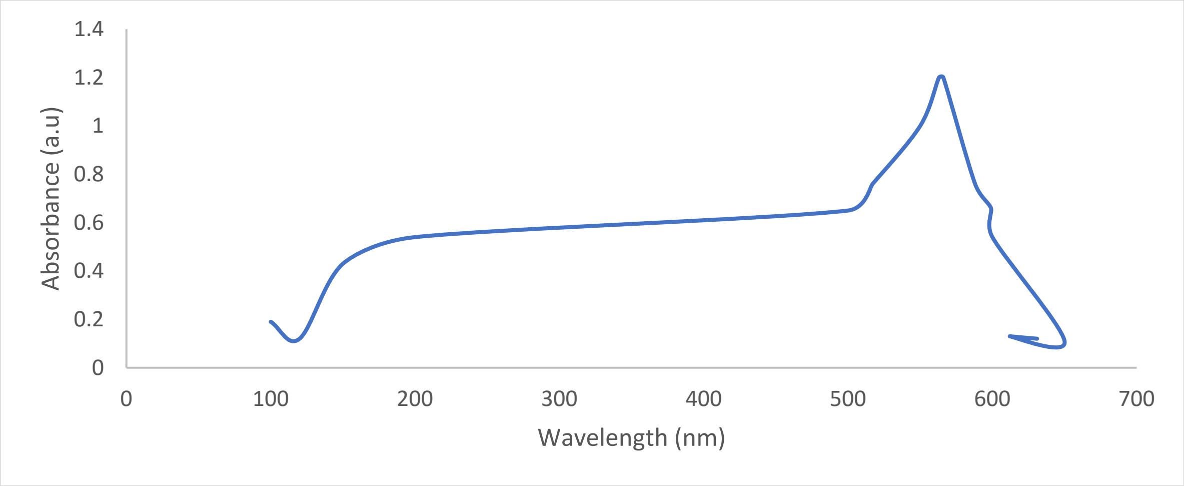

Figure 2. Image showing Se-nanoparticles absorption under various wavelengths.

2.4. Experimental Plan

Zea mays L. was grown in the greenhouse. Seeds were planted in earthen pots 23×35cm^2 in three replicates with four seeds per pot. After three days of sowing, germination was recorded.

2.5. Estimation of enzymes CAT (catalase) activity and sugar content

The CAT activity was determined through the method described by Kumar et al. (2010). The plant extract of 0.15 ml was taken to prepare the reaction mixture, 0.5M of 0.5mL potassium phosphate buffer (pH 7) and 3% of 0.1 mL H_2O_2 were added to the extract supernatant. The absorbance was noted at 240nm at 0min and 3min. Catalase activity was represented as: (units/mg protein/min = Decrease in optical density (initial reading at 0min-final reading at 3min) ÷ protein amount (mg) × 100.

The Dubois et al. (1956) method was applied to determine the sugar content with modifications by Johnson *et al.* (1966). Plant (leaves) were ground in a mortar, mixed in 10mL deionized water, and centrifuged at 250rpm for 10min. In 0.1mL of supernatant, 1mL of 0.5% (v/v) phenol was added. At 25^{0}C reaction mixture was incubated for 1h. After incubation, 5mL of sulfuric acid (80%) was added. The optical density was measured at 420nm against blank.

2.6. Statistical analyses

Software graph pad prism was used to calculate mean and standard error using three replicates per treatment. Pots were settled in a completely randomized design. The bar on the figures represents standard error. Letters represent statistical means ($p>0.05$) of Tukey's HSD tests on "Statistix 8.1".

3. Results

3.1. Water analysis

The results presented in Table 2 showed significantly higher Cd 1375% and 300% in POF water than in control (tap water). Lead (Pb) was only detected in POF water. Cd exceeded the recommended standard (World Health Organization, 1989). Treatment details are given in Table 1.

Table. 2 Metal content in water samples.

Metals	WHO recommended values	POF	TAP
Zn	3	0.177^{a} (±0.004)	0.012^{b} (±0.005)
Cr	0.05	0.004^{a} (±0.01)	0.001^{b} (±0.009)
Cd	0.02	0.90^{a} (±0.01)	0.002^{b} (±0.003)
Pb	2	0.001^{a} (±0.002)	0.000^{b} (±0.00)

Data describes the means of 3 replicates, and ± depicts their standard error values. Measurements were made after 10d of seed sowing in pots.

3.2. Analysis of pH and BOD of water samples

The results presented in Table 3 revealed that the POF water has acidic pH. The addition of PGPR, both alone and in combination with SeNPs, resulted in a significant rise in pH toward neutrality. The pH value of the POF+PGPR treatment was 6.7% higher than the control (Tap). Water from POF has a higher BOD (994%) over control. The addition of PGPR alone and combined with Se-nanoparticles resulted in a significant decline in BOD. The maximum decline in BOD resulted in the pH value of Tap+SeNPs treatment, which was 53% higher than the control (Tap). POF water showed deviation from the permissible limit of WWF and WHO standards (pH, 6.5-8.4, and BOD mgL^{-1} 10).

Table 3. Role of PGPR and Se-nanoparticles on pH and BOD of water samples under different treatments.

Sample	pH	BOD
Tap (Control)	7.16^{abc} (±0.21)	55.31^{b} (±4.61)
POF	4.87^{de} (±0.02)	602.13^{a} (±1.52)
Tap+PGPR	7.55^{ab} (±0.04)	30.46^{cd} (±1.00)
POF+PGPR	7.64^{a} (±0.06)	39.55^{c} (±1.00)
Tap+SeNPs	6.96^{abcd} (±0.20)	26.32^{de} (±1.00)
POF+SeNPs	7.17^{abc} (±0.03)	28.16^{d} (±0.33)
Tap+PGPR+SeNPs	6.58^{de} (±0.11)	31.12c^{d} (±0.52)
POF+PGPR+SeNPs	6.77^{de} (±0.02)	31.43c^{d} (±0.61)

Data describes the means of 3 replicates, and ± depicts their standard error values. Treatment details, Tap = Tap water from biosciences Lab University of Wah, POF = effluent water from POF factory, Tap+PGPR = Tap water from Biosciences Lab University of Wah with *Pseudomonas putida* incubated for 3h at 30^0C, POF+PGPR = effluent water from POF factory with *Pseudomonas putida* incubated for 3h at 30^0C, Tap+SeNPs = Tap water from Biosciences Lab University of Wah with Se NPs (5ml in 30ppm) incubated for 3h at 30^0C, POF+SeNPs = effluent water from POF factory with Se NPs (5ml in 30ppm) incubated for 3h at 30^0C, Tap+PGPR+SeNPs = Tap water from Biosciences Lab University of Wah with *Pseudomonas putida* and Se NPs (5ml in 30ppm) incubated for 3h at 30^0C, POF+PGPR+SeNPs = effluent water from POF factory with *Pseudomonas putida and* Se NPs (5ml in 30ppm) incubated for 3h at 30^0C.

3.3. Root and shoot weight, plant length and yield

The significant increase in the fresh weight of the shoot in all the treatments was noted and reported in Table (4). The maximum increase (266%) in shoot weight resulted in POF+PGPR+SeNPs, and the least increase (30%) was shown in POF over control. The maximum increase (211%) in shoot weight resulted in HIT+PGPR+SeNPs, and least increase (12%) was shown in POF. Significant increase in dry shoot weight of all the treatments except POF. The maximum increase (417%) in dry shoot weight resulted in POF+PGPR+SeNPs. The maximum increase (31%) in shoot length was in POF+PGPR, and the least increase (13%) was in POF+SeNPs. The maximum increase (91%) in root length resulted in POF+PGPR, and the least increase (16%) resulted in POF+SeNPs. All the treatments resulted in an increase in flower and yield content. The maximum increase (63%) in yield resulted in POF+PGPR+SeNPs, and the least increase (9%) resulted in POF+SeNPs over control (Tap).

Table 4. Role of PGPR and Se-nanoparticles on shoot and root weight of *Zea mays* L. under different treatments.

Treatments	Shoot FW	Root FW	Shoot DW	Root DW	Shoot length	Root length	Yield Fruit count	Flower count
	g	g	g	g	cm	cm	g	Per plant
Tap (Control)	7.59^{d} (±0.08)	1.31^{c} (±0.35)	1.28^{cd} (±0.16)	0.28^{c} (±0.41)	72.14^{c} (±3.05)	29^{d} (±0.57)	3.66^{e} (±0.33)	1.66^{f} (±0.33)
HIT	9.84^{c} (±0.22)	0.31^{c} (±0.08)	1.31^{c} (±0.34)	0.25^{c} (±0.16)	57.04^{d} (±2.51)	34.02 (±2.64)	4.00^{d} (±0.33)	1.03^{g} (±0.5)
Tap+PGPR	12.62^{ab} (±0.23)	1.45^{b} (±0.18)	3.73^{b} (±0.59)	0.46^{b} (±0.01)	94.33^{a} (±5.18)	55.66^{a} (±4.26)	4.98^{bc} (±0.33)	2.88^{cd} (±0.21)
POF+PGPR	22.87^{a} (0.34)	1.32^{b} (0.31)	1.31^{c} (±0.34)	0.25^{c} (±0.16)	57.13^{d} (±2.51)	34.00^{c} (±2.64)	4.00^{d} (±0.33)	1.66^{e} (±0.5)
Tap+SeNP	20.62^{a} (±0.23)	1.45^{b} (±0.18)	3.73^{b} (±0.59)	0.46^{b} (±0.01)	78.11^{c} (±0.33)	35.66^{c} (±0.64)	3.98^{d} (±0.43)	2.69^{c} (±0.2)
POF+SeNP	11.05^{ab} (±0.57)	1.23^{b} (±0.03)	2.46^{c} (±0.29)	0.60^{a} (±0.09)	83.21^{ab} (±0.57)	33.11^{c} (±2.08)	5.44^{b} (±0.33)	3.00^{c} (±0.57)
Tap+PGPR+SeNP	27.8^{a} (±1.55)	4.05^{a} (±0.04)	6.62^{a} (±0.18)	0.66^{a} (±0.00)	92.33^{a} (±1.23)	33.32^{c} (±2.08)	6.00^{a} (±0.33)	3.33^{b} (±0.33)
POF+PGPR+SeNP	12.7^{ab} (±1.56)	1.66^{b} (±0.29)	3.93^{b} (±0.54)	0.63^{a} (±0.03)	86.66^{ab} (±1.85)	45.66^{b} (±2.33)	4.98^{bc} (±0.88)	3.96^{a} (±0.57)

Data describes the means of 3 replicates and ± depicts their standard error values. Measurements were made in the harvesting phase after 30d of seed germination. Treatment details are given in Table 1.

3.4. Sugar content and CAT enzyme activity

The analysis of CATenzymes was conducted due to their role in the reduction of ROS under polluted water and soil, while sugar contents were analyzed as the first primary process of growth of plants. These are affected by ecological anxieties that induce the generation of reactive oxygen species (ROS), consequently reducing plant growth and development. The CAT enzyme construes the hydrogen peroxide (H_2O_2) to water (H_2O) and reduces the ROS levels to safe the plants cell death

Results showed that all treatments increased CAT activity (Fig. 3). POF showed a 48% increase in CAT activity, while PGPR resulted in a 10 and 40% increase in CAT activity. Tap+Se_{NP} and POF+Se_{NP} resulted in 76% and 98% increases in CAT activity. Combined treatment of PGPR and Se NP resulted in 66% and 88% increase in CAT activity over control (tap water). The maximum increase of 98% resulted in POF+SeNPs over control (tap water). Similarly, all treatments increased sugar except POF (Fig. 3). PGPR resulted in 40% and 33% increase in sugar contents. Tap+Se_{NP} and POF+Se_{NP} resulted in 36% and 28% increase in sugar contents. Combined treatment of PGPR and SeNP resulted from 26% and 22% increase in sugar contents over control (tap water).

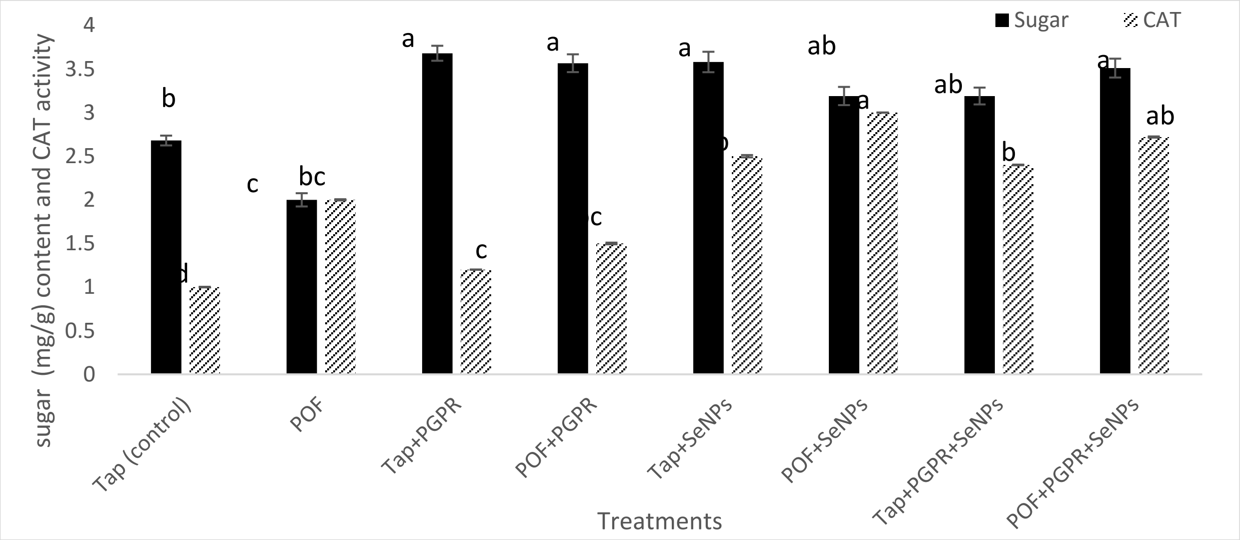

Figure 4. Effect of PGPR and Se-nanoparticles on enzyme CAT activity and sugar content in Zea mays L. (The data represent the means of 3 replicates and ± depicts their standard error values. The bar carrying similar alphabets does not differ significantly. Measurements were made in the harvesting phase after 30d of seed germination. Treatment details are given in Table 1. The CAT activity was expressed as DOD min^{-1} (mg^{-1} protein)

4. Discussion

The data indicated a significant accumulation of Cd in POF water above permissible limits, while Zn and Cu are higher that are necessary for chloroplast functions. Application of PGPR and Se-nanoparticles resulted in a reduction in the BOD of water. Polluted water (POF) irrigation resulted in a decline in maize root and shoot growth and yield. That may be due to the toxic effects of cadmium. PGPR and Se-nanoparticles reduce Cd toxicity with a significant increase in root and shoot dry weight and increased sugar production. The POF irrigated plants showed a significant increase in CAT enzyme activity that was declined with PGPR application that showed ROS scavenging with PGPR. Selenium alleviates oxidative damage by scavenging ROS through enzymatic CAT activity. Compared to untreated plants, selenium nanoparticles and PGPR-treated plants enhance the antioxidant system(Liu et al., 2021).The investigation on Se NPs and PGR suggests that the CAT enzymes control the ROS and convert H_2O_2 in H_2O as CAT deficiency showed susceptibility to stress.The current results follow the study of Gondim et al. (2012) , who also established that CAT enzymes play a crucial role in the stress condition in Maize.Similarly Shah et al. (2001) also report the CAT activity in rice seedlings at a moderate (100 µM) and a marked decline at the high dose of Cd (500 µM). Literature also reported that CAT activity reflects a variable response under different heavy metal accumulations (Ghosh & Majumdar, S. 2022; Askari & Azmat 2016).

5. Conclusion

It was concluded that the overall upsurge in CAT activity is considered an adaptation in plants under metal accumulation to help reduce in tissue damage by H_2O_2 noxiousness.

$H_2O_2 \rightarrow CATH_2O+1O_2.$

Results reviled that Se NPs may become effective for remediated Cd stress by improving CAT enzyme activity, reducing oxidative injury, and improving plant growth. This research reveals the Se NPs potential to acquire sustainable cereal production under Cd stress. Commonly, Cd toxicity adversely affects Zea mays L.

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Conflict of interest

The authors have no conflict of interest in this publication.

Author Contributions

Main idea, paper writing and experimental design with performance, ST; Data curation, AAA and FK; Funding acquisition, AAA and FK; All authors recite and whole manuscript before approval.

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