International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 15 Issue 6, June-2026

Impact of ZnO NPs on Growth, Antioxidant System and Accumulation Efficiency of *Helianthus annuus* for Pb and Cd

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ABSTRACT

Heavy metal contamination is a serious global concern that interrupts the food chain and jeopardizes human health. This study has been undertaken to evaluate the impacts of heavy metals (Pb & Cd) and ZnO NPs on the growth and antioxidant defense system of *Helianthus annuus* plants. The ZnO NPs-assisted phytoremediation efficiency of *Helianthus annuus* for Pb and Cd was also evaluated. Experimental findings revealed a concentration-dependent decrease in growth parameters like seed germination, seedling height, fresh and dry weight under the influence of various doses of Pb (2.7, 3.7, and 4.7 mg/L) and Cd (0.1, 0.2, and 0.3 mg/L). The maximum inhibition was observed at the highest levels of Pb (4.7 mg/L) and Cd (0.3 mg/L). In contrast to heavy metals, a concentration-dependent increase was noticed with all the doses of ZnO NPs (25, 50 & 75 mg/L), but the maximum increase was noticed with the highest dose of ZnO NPs (75 mg/L). In the case of antioxidant enzyme activities (SOD, CAT, and APX activity), a significant, dose-dependent increase was noticed with all the doses of heavy metals (Pb & Cd). Under the influence of various doses of ZnO NPs (25, 50, and 75 mg/L) a significant reduction in antioxidant enzyme activities was clearly noticed. In combined treatments of heavy metals with ZnO NPs, the mitigation of metal toxicity was clearly noticed in terms of an increase in growth parameters. The accumulation of heavy metals (Pb & Cd) through *Helianthus annuus* was increased with all the doses of ZnO NPs (25, 50 & 75 mg/L). Thus, it is clear from the present investigation that ZnO NPs efficiently enhanced the accumulation potential of *Helianthus annuus* to Pb and Cd.

Keywords-, Accumulation, , Cadmium, Heavy metals, Lead ,Phytoremediation, , Zinc oxide nanoparticles.

INTRODUCTION

Heavy metal contamination in soil remains a serious long-term concern in environmental studies because these elements cannot be broken down by biological processes and may remain in soil for many years. However, at higher concentrations, they can reduce soil fertility, decrease microbial activity, restrict plant growth, lower food quality, and pose serious risks to human health (Briffa et al., 2020; Jaishankar et al., 2014; Tchounwou et al., 2012).

Lead (Pb), known for centuries, is a ubiquitous trace contaminant that can be an accumulative metabolic poison. Lead has been a majorly concerning, xenobiotic metal owing to its severe bioaccumulation, properties, complete lack of biological utility, and profound organ-specific toxicity (Tahir & Alkheraije, 2023). Humans have been using lead for the last 5000 years, and it is one of the earliest discovered heavy metals. Lead has a wide range of applications owing to its exceptional properties of ductility, low melting point, resistance to corrosion, and softness. This non-biodegradable metal continues to persist heavily in ambient soil, air, and global water sources, resulting in ongoing entry into ecological food chains (Xu et al., 2024).

Cadmium (Cd) is a non-essential, highly toxic heavy metal and is placed in seventh position in the top ten priorities hazardous substances list by the Agency for Toxic Substances and Disease Registry (ATSDR) and therefore is considered one of the major environmental pollutants and produces toxic effects in living organisms (Kamnev and Lelie, 2000; Shah et al., 2001; Rascio et al., 2008). Cd pollution is detrimental to health because of its non-biodegradability, biological magnification, and long persistence in the environment. The most common toxicity of Cd is to cause oxidative stress

through the assembly of reactive oxygen species (ROS) (Benavides et al. 2005; Shah et al 2001). In human beings, Cd toxicity causes harmful effects on the kidneys, liver, lungs, and bone (ASTDR, 2011).

Phytoremediation is the use of plants, together with microorganisms present around their roots, to remove, sequester, transform, or detoxify pollutants in soil and water. The main mechanisms of phytoremediation include Phytoextraction, Phytostabilization, Rhizofiltration, Phytovolatilization, Phytodegradation, Rhizodegradation, and Phytodesalination. Among these methods, phytoextraction and phytostabilization are especially important for heavy metal remediation because metals cannot be mineralized or broken down like many organic pollutants (Pilon-Smits, 2005; An et al., 2020). The plant vacuole system serves as a storage compartment for all kinds of toxic compounds (Eapen and D'Souza, 2004; Rascio and Navari-Izzo, 2010).

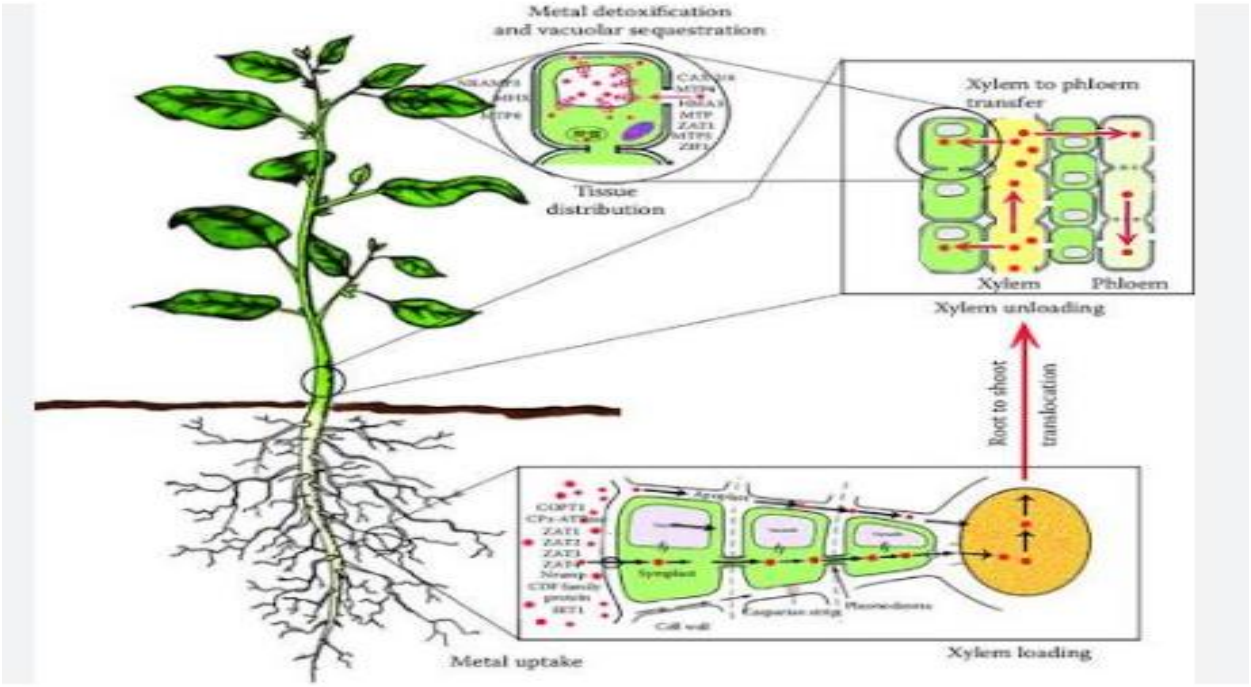


Fig.1: Mechanism of the major process involved in a heavy metal hyperaccumulator plant

Nanotechnology has introduced new possibilities for environmental remediation because nanoparticles possess high surface area, small size, tunable surface chemistry, and distinctive reactivity compared with their bulk counterparts. Nanomaterials are generally understood as engineered or natural materials with at least one dimension at or below approximately 100 nm. However, the same properties that make nanoparticles technologically valuable may also create phytotoxic and ecotoxic risks if their concentration, size, dissolution, aggregation, or surface chemistry are not carefully controlled (Nel et al., 2006; Rastogi et al., 2017; Rajput et al., 2022). Zinc oxide nanoparticles (ZnO NPs) are among the most widely studied metal oxide nanoparticles in plant science and environmental applications. At excessive concentrations, however, they may inhibit germination, reduce chlorophyll and carotenoid content, trigger reactive oxygen species formation, and cause oxidative damage (Zafar et al., 2016; Rastogi et al., 2017; Zhou et al., 2020). (Rajput et al., 2022) noted that combining nanotechnology with biological remediation can open new pathways for restoring contaminated soils, while (Rastogi et al., 2017) emphasized that nanoparticle effects on plants depend strongly on particle size, concentration, exposure route, plant species, and environmental conditions.

MATERIALS & METHODS

The seeds of *Helianthus annuus* were purchased from G.B. Pant University of Agriculture and Technology, Pantnagar.



Pot preparation & Seed Showned



Examination of Plants

Growth Conditions:
The pot experiment was designed in an experimental site situated in the Botanical Garden, Plant Science Department, M.J.P. Rohilkhand University to evaluate the impacts of heavy metals (Pb & Cd) and ZnO NPs on different parameters. Plants were treated with different doses of heavy metals Pb (2.7 mg/L,3.7 mg/L & 4.7 mg/L) & Cd (0.1 mg/L,0.2 mg/L& 0.3 mg/L) and ZnO NPs (25 mg/L,50 mg/L & 75 mg/L) at 10, 20 & 30 days of old plant after emergence of plants. Samples were collected at 10, 20, & 30-days old plant after the treatments.

Growth Parameters
Seed Germination:
For seed germination analysis, another set of seeds with similar treatments was placed on moist filter paper in a Petri dish to count germination. The germination frequency was noticed at regular intervals of 3rd, 5th and 7th days for critical analysis impact of heavy metals (Pb and Cd) and ZnO NPs solutions on seed germination.

Seedling Height (Plant Height):
Five plants were randomly selected from each treatment. The plant height was measured in 10-, 20-, and 30-day-old plants in centimeters**.**

Fresh weight: The fresh weight was measured in grams (per plant) and observed in 10, 20 days and 30 days old plants.
Dry weight: The plants were dried in an oven at 60 °C for 48 hours. The dry weight of 10-, 20-, and 30-day-old plantlets was measured in grams.

Antioxidant Defense System
Fresh leaves were collected from both control and treated plants and homogenized in enzyme-specific extraction buffer using a mortar and pestle under ice-cold conditions. All enzymatic assays were performed at **25 °C**.

Superoxide dismutase (SOD)
Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed by using photochemical NBT (nitro blue tetrazolium) reduction method as directed by Giannopolitis and Reis (1977).

Catalase
Catalase (CAT; EC 1.11.3.6) activity was determined in terms of decrease in absorbance at 240 nm spectrophotometrically according to the method of Aebi (1984).

Ascorbate Peroxidase
Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined spectrophotometrically by following the method of Nakano and Asada (1981).

Heavy Metal Accumulation
Sample of plants were oven dried at 60^{o}C for 48 hours. Dried plant samples (250 mg) were weighted in 50 ml of conical flask, 3 ml HNO_3 was added and kept over night. The plant samples were heated in conical flask at 70^{0}C on hot plate till it

dried. After drying 1 ml of Perchloric acid ($HClO_3$) was added till a clear solution was obtained. Again samples were heated till it become dry at 70^0C. After completion of digestion 2-3 ml of Triple Distilled Water (TDW) was added. It was left for 24 hours. The solution was filtered through Whatman filter paper. Finally volume was make up to 15 ml by using milli-Q-water. The sample was analysed by using Atomic Absorption Spectrophotometer (G.B.C Avanta).

Statistical data

Results were statistically analysed by analysis of variance (ANOVA) using SPPP-10 software. Duncan's multiple range test was applied for mean separation for significant differences among treatments at the $P<0.05$ level. The results presented are the means of three independent experiments.

RESULTS

Seed Germination

In the present investigation, the individual and combined impacts of heavy metals (Pb, Cd & ZnO NPs) and nanoparticles (ZnO NPs) were analyzed in 10-, 20-, and 30-day-old plants of *Helianthus annuus*. The dose-dependent decrease in seed germination percentage was noticed with all doses of heavy metals. However, the maximum reductions were noticed at the highest doses of Pb 4.7 mg/L (58, 68 & 77% seed germination, respectively, after 3, 5, & 7 days of sowing) and Cd 0.3 mg/L (56, 65 & 74% seed germination, respectively, after 3, 5, & 7 days of sowing). Among the heavy metals, Cd was found to be more toxic than Pb. ZnO NPs treatments increased seed germination with all concentrations (25,50 & 75 mg/L), but maximum increase (75, 81 & 88 %, respectively, after 3, 5 & 7 days of sowing) was noticed with the highest dose of ZnO NPs (75 mg/L) as compared to the higher doses of heavy metals (Pb & Cd).

In combined treatments of various doses of heavy metals (Pb & Cd) with ZnO NPs, the mitigation of the toxic effects of heavy metals was noticed as compared to heavy metal treatments. The maximum alleviation of metal toxicity (70, 79, & 87 %) was noticed in the combined treatment of the lowest doses of Pb with the highest dose of ZnO NPs (2.7 mg/L Pb + 75 mg/L ZnO NPs) as compared to the various doses of heavy metal treatments and control plants. Similar results were also noticed with the lowest dose of Cd and the highest dose of ZnO NPs, where the seed germination percentage was 66, 77, & 85%, respectively, after 3, 5, & 7 days of sowing (Table 1).

Seedling Height

The seedling height was measured in centimeters in 10, 20, & 30 days old plants of *Helianthus annuus*. The results of the present investigation clearly indicate that different concentrations of the heavy metals Pb (2.7, 3.7 & 4.7 mg/l) and Cd (0.1, 0.2 & 0.3 mg/l) significantly reduced the seedling height of Sunflower compared to control plants. However, the maximum reductions were noticed at the highest doses of Pb 4.7 mg/L (2.55, 7.50, & 16.40 cm, respectively, in 10, 20, & 30 days old plants) and Cd 0.3 mg/L (2.51, 7.31, & 16.00 cm, respectively, in 10, 20, & 30 days old plants). Among the heavy metals, Cd was found to be more toxic than Pb. However, ZnO NPs enhanced the seedling height at different concentrations (25, 50 &75 mg/L). The maximum increase in seedling height (3.87, 12.00, & 26.80 cm, respectively, in 10, 20, & 30 days old plants) was noticed with the highest dose (75 mg/L) of ZnO NPs as compared to lower doses (25 & 50 mg/L).

Table 1- Impact of Heavy metals (Pb & Cd) and ZnO NPs on seed germination of *Helianthus annuus.* *Significant at 5% level

Treatments (mg/L)	Seed Germination Percentage		
	After 3 days (% Seed germinated)	After 5 days (% Seed germinated)	After 7 days (% Seed germinated)
CONTROL	**76**	**85**	**94**
Pb 2.7	64	73	82
Pb 3.7	61	70	79
Pb 4.7	**58**	**68**	**77**
Cd 0.1	63	71	81
Cd 0.2	59	69	77
Cd 0.3	**56**	**65**	**74**
ZnO 25	70	78	84
ZnO 50	72	80	86

ZnO 75	**75**	**81**	**88**
Pb 2.7 +ZnO 25	65	74	83
Pb 2.7 +ZnO 50	67	76	85
Pb 2.7 +ZnO 75	**70**	**79**	**87**
Pb 3.7+ ZnO 25	63	71	80
Pb 3.7+ ZnO 50	66	73	83
Pb 3.7+ ZnO 75	68	75	85
Pb 4.7+ ZnO 25	61	69	79
Pb 4.7+ ZnO 50	64	71	81
Pb 4.7+ ZnO 75	66	74	84
Cd 0.1+ ZnO 25	61	72	81
Cd 0.1+ ZnO 50	64	74	82
Cd 0.1+ ZnO 75	**66**	**77**	**85**
Cd 0.2+ ZnO 25	60	70	79
Cd 0.2 +ZnO 50	64	72	81
Cd 0.2+ ZnO 75	67	73	83
Cd 0.3+ ZnO 25	58	68	78
Cd 0.3+ ZnO 50	60	70	80
Cd 0.3+ ZnO 75	63	72	82

In the combined treatments of heavy metals (Pb & Cd) with ZnO NPs, the mitigation of metal toxicity in terms of a significant increase in seedling height was clearly noticed. The maximum increase in seedling height in combined treatments (3.15, 9.30, & 30.50 cm, respectively, in 10, 20, & 30-day-old plants) was also noticed with the lowest doses of Pb and the highest dose of ZnO NPs (2.7 mg/L Pb + 75 mg/L ZnO NPs). Similarly, the maximum increase in seedling height in combined (3.08, 9.10, & 20.04 cm, respectively, in 10, 20, & 30-day-old plants) was also noticed with the lowest doses of Cd and the highest dose of ZnO NPs (0.1 mg/L Cd + 75 mg/L ZnO NPs). (Table 2).

Fresh Weight of Plant

The fresh weight of plants was measured in grams in 10, 20, & 30-day old plants of *Helianthus annuus*. The results of the present investigation clearly revealed that different concentrations of the heavy metals Pb (2.7, 3.7 & 4.7 mg/L) and Cd (0.1, 0.2 & 0.3 mg/L) significantly reduced the fresh weight of Sunflower as compared to control plants. However, the maximum reductions were noticed at the highest doses of Pb 4.7 mg/L (0.94, 12.70, & 66.50 g, respectively, in 10, 20, & 30-day-old plants) and Cd 0.3 mg/L (0.92, 12.50, & 65.01 g, respectively, in 10, 20, & 30-day-old plants). Among the heavy metals, Cd was found to be more toxic than Pb. However, ZnO NPs enhanced the fresh weight at different concentrations (25, 50 &75 mg/L). The maximum increase in fresh weight (1.36, 18.88, & 100.98 g, respectively, in 10, 20, & 30-day-old plants) was noticed with the highest dose (75 mg/L) of ZnO NPs as compared to lower doses (25 & 50 mg/L) and control plants.

In the combined treatments of heavy metals (Pb & Cd) with ZnO NPs, the mitigation of metal toxicity in terms of a significant increase in fresh weight was clearly observed. The maximum increase in fresh weight in combined treatments (1.26, 17.48, & 93.98 g, respectively, in 10, 20, & 30-day-old plants) was also noticed with the lowest doses of Pb and the highest dose of ZnO NPs (2.7 mg/L Pb + 75 mg/L ZnO NPs). Similarly, the maximum increase in fresh weight in combined (1.20, 16.79, & 91.87 g, respectively, in 10, 20, & 30-day-old plants) was also noticed with the lowest doses of Cd and the highest dose of ZnO NPs (0.1 mg/L Cd + 75 mg/L ZnO NPs). **(Table 3)**

Dry Weight of Plants

The dry weight of plants was measured in grams in 10, 20, & 30-day-old plants of *Helianthus annuus*. The results of the present investigation clearly indicated that different concentrations of the heavy metals Pb (2.7, 3.7 & 4.7 mg/L) and Cd (0.1, 0.2 & 0.3 mg/L) significantly reduced the dry weight of Sunflower as compared to control plants. However, the maximum reductions in dry weight were noticed with the highest doses of Pb, 4.7 mg/L (0.110, 1.77, & 10.20 g, respectively, in 10, 20, & 30-day-old plants), and Cd 0.3 mg/L (0.109, 1.71, & 9.80 g, respectively, in 10, 20, & 30-day-old plants). Among the heavy metals, Cd was found

Table 2- Impacts of Heavy metals (Pb & Cd) and ZnO NPs on seedling height of *Helianthus annuus.* *Significant at 5% level

, Treatments mg/L	Seedling Height of Plants (in cm.)					
	10 DAYS		20 DAYS		30 DAYS	
	Mean + SE	% to control	Mean + SE	% to control	Mean +SE	% to control
CONTROL	**3.00+0.053**		**9.00 + 0.021**		**20.00 + 0.021**	
Pb 2.7	2.71*+ 0.068	90.33	8.00*+ 0.075	89.88	17.77*+ 0.077	88.50
Pb 3.7	2.60*+ 0.077	89.00	7.85*+ 0.085	87.22	17.35*+ 0.097	86.75
Pb 4.7	**2.55*+ 0.095**	**85.00**	**7.50*+ 0.060**	**83.33**	**16.40*+ 0.083**	**82.00**
Cd 0.1	2.68*+ 0.074	89.33	7.81*+ 0.084	86.77	17.00*+ 0.081	85.00
Cd 0.2	2.65*+ 0.055	88.33	7.70*+ 0.077	85.55	16.88*+ 0.074	84.40
Cd 0.3	**2.51*+ 0.083**	**83.66**	**7.31*+ 0.068**	**81.22**	**16.00*+ 0.075**	**80.00**
ZnO 25	3.22 *+ 0.076	107.33	9.98 *+ 0.087	109.77	22.50 *+ 0.076	111.25
ZnO 50	3.52 *+ 0.087	117.33	10.89 *+ 0.098	120.66	24.50 *+ 0.072	122.25
ZnO 75	**3.87 *+ 0.081**	**129.00**	**12.00 *+ 0.091**	**133.00**	**26.80 *+ 0.089**	**134.00**
Pb 2.7 +ZnO 25	3.02 *+ 0.076	100.66	9.02 *+ 0.072	100.22	19.90 *+ 0.079	99.50
Pb 2.7 +ZnO 50	3.06 *+ 0.086	102.00	9.14 *+ 0.087	101.55	20.00 *+ 0.087	100.00
Pb 2.7 +ZnO 75	**3.15 *+ 0.077**	**105.00**	**9.30 *+ 0.051**	**103.33**	**20.50 *+ 0.072**	**102.50**
Pb 3.7+ ZnO 25	3.00 *+ 0.079	100.00	9.00 *+ 0.073	100.00	19.80 *+ 0.057	99.00
Pb 3.7+ ZnO 50	3.09 *+ 0.090	103.00	9.09 *+ 0.069	101.00	20.08 *+ 0.061	100.40
Pb 3.7+ ZnO 75	3.14 *+ 0.085	104.66	9.21 *+ 0.071	102.33	20.20 *+ 0.085	101.00
Pb 4.7+ ZnO 25	2.99 *+ 0.092	99.66	8.90 *+ 0.085	98.88	19.70 *+ 0.071	98.50
Pb 4.7+ ZnO 50	3.02 *+ 0.089	100.66	9.03 *+ 0.070	100.33	19.81 *+ 0.090	99.05
Pb 4.7+ ZnO 75	3.08 *+ 0.060	102.66	9.18 *+ 0.088	102.00	20.00 *+ 0.062	100.00
Cd 0.1+ ZnO 25	3.00 *+ 0.071	100.00	8.90 *+ 0.081	99.88	19.71 *+ 0.081	98.55
Cd 0.1+ ZnO 50	3.05 *+ 0.070	101.66	9.00 *+ 0.024	100.00	19.89 *+ 0.073	99.45
Cd 0.1+ ZnO 75	**3.08 *+ 0.077**	**102.66**	**9.10 *+ 0.075**	**101.11**	**20.04 *+ 0.068**	**100.25**
Cd 0.2+ ZnO 25	2.99 *+ 0.078	99.66	8.90 *+ 0.089	98.88	19.70 *+ 0.087	98.50
Cd 0.2 +ZnO 50	3.01 *+ 0.066	100.33	8.95 *+ 0.076	99.44	19.85 *+ 0.090	99.25
Cd 0.2+ ZnO 75	3.09 *+ 0.083	103.00	9.09 *+ 0.066	101.00	20.07 *+ 0.070	100.35
Cd 0.3+ ZnO 25	2.97 *+ 0.067	99.00	8.90 *+ 0.096	98.88	19.50 *+ 0.077	97.50
Cd 0.3+ ZnO 50	3.00 *+ 0.059	100.00	8.99 *+ 0.086	99.88	19.78 *+ 0.060	98.90
Cd 0.3+ ZnO 75	3.07 *+ 0.030	102.33	9.10 *+ 0.086	101.11	19.90*+ 0.097	99.5

Table 3- Impacts of Heavy metals (Pb & Cd) and ZnO NPs on fresh weight of *Helianthus annuus.* *Significant at 5% level

Treatments mg/L	Fresh Weight of Plants (in grams)					
	10 DAYS		20 DAYS		30 DAYS	
	Mean + SE	% to control	Mean + SE	% to control	Mean +SE	% to control
CONTROL	**1.10 + 0.027**		**15.17 + 0.37**		**80.71 + 1.30**	
Pb 2.7	1.00*+ 0.031	90.90	13.60 *+0.97	89.65	71.80 .*+2.75	88.96
Pb 3.7	0.98*+ 0.052	89.09	13.30*+0.98	87.67	70.00 *+2.71	86.73
Pb 4.7	**0.94 *+ 0.033**	**85.45**	**12.70 *+ 0.81**	**83.71**	**66.50 *+ 1.99**	**82.39**
Cd 0.1	0.97 *+ 0.038	88.18	13.10*+1.00	86.35	69.00.*+1.19	85.49
Cd 0.2	0.95*+ 0.037	86.33	13.01 *+1.11	85.76	68.00 *+1.19	84.25
Cd 0.3	**0.92 *+ 0.050**	**83.66**	**12.40 *+ 0.97**	**81.74**	**65.01 *+ 1.93**	**80.54**
ZnO 25	1.19 *+ 0.051	108.18	16.47*+1.01	108.56	88.71*+1.85	109.91
ZnO 50	1.28 *+ 0.063	116.36	17.87*+0.99	117.79	95.71*+2.16	118.58
ZnO 75	**1.36 *+ 0.081**	**123.63**	**18.88*+ 0.95**	**124.45**	**100.98*+ 2.01**	**125.11**
Pb 2.7 +ZnO 25	1.11* + 0.072	100.90	16.08*+1.11	105.72	85.98*+1.98	106.52
Pb 2.7 +ZnO 50	1.14 *+ 0.096	103.18	16.68*+1.01	111.27	89.98*+2.12	111.48
Pb 2.7 +ZnO 75	**1.26 *+ 0.081**	**114.54**	**17.48*+ 1.21**	**115.33**	**93.98*+ 2.31**	**116.49**
Pb 3.7+ ZnO 25	1.09 *+ 0.087	99.09	15.85*+1.42	104.36	84.98*+2.17	105.29
Pb 3.7+ ZnO 50	1.15 *+ 0.054	104.54	16.01*+0.97	108.83	87.98*+2.31	109.00
Pb 3.7+ ZnO 75	1.19 *+ 0.051	108.18	16.81*+0.81	110.81	89.98*+3.37	111.48
Pb 4.7+ ZnO 25	1.08 *+ 0.067	98.18	15.64*+0.93	103.09	84.88*+1.90	105.16
Pb 4.7+ ZnO 50	1.14 *+ 0.081	103.63	16.39*+0.74	108.04	87.98*+2.11	109.00
Pb 4.7+ ZnO 75	1.17 *+ 0.065	106.36	16.71*+0.79	110.15	89.88*+1.77	111.36
Cd 0.1+ ZnO 25	1.10 *+ 0.071	100.00	15.91*+0.88	104.87	84.88*+2.65	105.16
Cd 0.1+ ZnO 50	1.17 *+ 0.088	106.36	16.48*+0.94	108.63	88.84*+1.96	110.07
Cd 0.1+ ZnO 75	**1.20 *+ 0.077**	**109.09**	**16.79*+ 1.05**	**111.99**	**91.87*+ 2.69**	**113.82**
Cd 0.2+ ZnO 25	1..08 *+ 0.066	98.18	16.09*+1.13	106.06	86.99*+3.01	107.78
Cd 0.2 +ZnO 50	1.12 *+ 0.069	101.81	16.93*+1.14	111.60	89.48*+1.11	110.10
Cd 0.2+ ZnO 75	1.16 *+ 0.055	105.45	17.69*+1.06	114.23	93.48*+2.18	115.82
Cd 0.3+ ZnO 25	1.07 *+ 0.069	97.27	15.69*+0.98	103.42	84.78*+0.97	105.04
Cd 0.3+ ZnO 50	1.11 *+ 0.069	100.90	16.30*+1.08	107.44	86.81*+1.78	107.55
Cd 0.3+ ZnO 75	1.14 *+ 0.074	103.00	16.59*+1.07	109.36	87.99*+1.81	109.01

Table 4- Impacts of Heavy metals (Pb & Cd) and ZnO NPs on dry weight of *Helianthus annuus.* *Significant at 5% level

Treatments (mg/L)	Dry Weight of Plants					
	10 DAYS		20 DAYS		30 DAYS	
	Mean + SE	% to control	Mean + SE	% to control	Mean + SE	% to control
CONTROL	**0.129 + 0.001**	-	**2.09 + 0.031**	-	**12.17+0.98**	-
Pb 2.7	0.117 *+ 0.006	90.69	1.87 *+ 0.04	89.47	10.80.*+ 0.92	88.74
Pb 3.7	0.114 *+ 0.001	88.37	1.84 *+ 0.071	88.03	10.60 *+1.06	87.0
Pb 4.7	**0.110 *+ 0.008**	**85.27**	**1.77 *+ 0.081**	**84.68**	**10.20 *+1.16**	**83.81**
Cd 0.1	0.115 *+ 0.090	89.14	1.80 *+ 0.069	86.12	10.40 *+1.18	85.46
Cd 0.2	0.122 *+ 0.080	87.59	1.76 *+ 0.056	84.21	10.00 *+0.92	82.16
Cd 0.3	**0.109 *+ 0.070**	**84.65**	**1.71 *+ 0.048**	**81.81**	**09.80 *+0.88**	**80.52**
ZnO 25	0.139 *+ 0.063	107.75	2.25 *+ 0.048	107.65	13.11 *+1.03	107.72
ZnO 50	0.149 *+ 0.019	115.50	2.45 *+ 0.033	117.22	14.41*+0.95	118.40
ZnO 75	**0.159 *+ 0.018**	**123.25**	**2.60 *+ 0.081**	**124.40**	**15.31 *+1.11**	**125.80**
Pb 2.7 +ZnO 25	0.130 *+0.014	100.77	2.12*+ 0.066	101.43	12.38 *+0.81	101.72
Pb 2.7 +ZnO 50	0.135* + 0.019	104.30	2.23 *+ 0.088	106.69	12.99 *+1.04	106.73
Pb 2.7 +ZnO 75	**0.148 *+ 0.039**	**114.72**	**2.40 *+ 0.057**	**114.83**	**13.99 *+1.19**	**114.95**
Pb 3.7+ ZnO 25	0.128 *+ 0.056	99.22	2.10 *+ 0.077	100.47	12.39 *+0.92	101.80
Pb 3.7+ ZnO 50	0.135 *+ 0.066	104.65	2.21 *+ 0.074	105.74	12.89 *+0.97	105.91
Pb 3.7+ ZnO 75	0.140 *+ 0.061	108.52	2.30 *+ 0.089	110.04	13.59 *+1.06	111.66
Pb 4.7+ ZnO 25	0.127 *+ 0.073	98.44	2.09 *+ 0.075	100.00	12.37 *+0.88	101.64
Pb 4.7+ ZnO 50	0.134 *+ 0.079	103.87	2.16 *+ 0.067	103.34	12.77 *+0.87	104.93
Pb 4.7+ ZnO 75	0.139 *+ 0.071	107.75	2.25 *+ 0.047	107.65	13.11 *+0.91	107.72
Cd 0.1+ ZnO 25	0.129 *+ 0.071	100.00	2.10 *+ 0.039	100.47	12.28 *+0.92	100.98
Cd 0.1+ ZnO 50	0.139 *+ 0.061	107.75	2.31 *+ 0.068	110.52	13.50 *+0.19	110.92
Cd 0.1+ ZnO 75	**0.146 *+ 0.083**	**113.17**	**2.39 *+ 0.051**	**114.35**	**13.99 *+0.93**	**114.95**
Cd 0.2+ ZnO 25	0.127 *+ 0.054	98.49	2.06 *+ 0.068	98.56	12.06 *+0.91	99.09
Cd 0.2 +ZnO 50	0.134 *+ 0.058	103.67	2.16 *+ 0.073	103.34	12.69 *+0.81	104.27

Cd 0.2+ ZnO 75	0.138 *+ 0.036	106.97	2.23 *+ 0.091	106.69	12.99 *+0.77	100.72
Cd 0.3+ ZnO 25	0.126 *+ 0.038	97.67	2.04 *+ 0.069	97.60	11.96 *+0.56	98.27
Cd 0.3+ ZnO 50	0.131 *+ 0.090	101.55	2.11 *+ 0.066	100.95	12.39 *+0.66	101.80
Cd 0.3+ ZnO 75	0.137 *+ 0.088	106.20	2.19 *+ 0.047	104.78	12.86 *+0.69	105.66

to be more toxic than Pb. However, ZnO NPs enhanced the dry weight at different concentrations (25, 50 &75 mg/L). The maximum increase in dry weight (1.59, 2.60, & 15.31 g, respectively, in 10, 20, & 30-day-old plants) was noticed with the highest dose (75 mg/L) of ZnO NPs as compared to lower doses (25 & 50 mg/L) and control plants.

In the combined treatments of heavy metals (Pb & Cd) with ZnO NPs, the mitigation of metal toxicity in terms of a significant increase in dry weight was clearly observed. The maximum increase in dry weight in combined treatments (0.148, 2.40, & 13.99 g, respectively, in 10, 20, & 30-day-old plants) was noticed with the highest dose of ZnO NPs (2.7 mg/L Pb + 75 mg/L ZnO NPs). Similarly, the maximum increase in dry weight in combined treatments (0.146, 2.39, & 13.99 g, respectively, in 10, 20, & 30-day-old plants) was also noticed with the lowest doses of Cd and the highest dose of ZnO NPs (0.1 mg/L Cd + 75 mg/L ZnO NPs) **(Table 4).**

Antioxidant enzyme activities

The data for the analysis of the effect of Pb, Cd, and ZnO NPs with different concentrations on the antioxidant system of *Helianthus annuus* in terms of Superoxide dismutase (SOD), Catalase (CAT), and Ascorbate Peroxidase (APX) enzyme activities have been presented in **Figs. 2, 3, & 4.**

SOD (Superoxide Dismutase) activity

The results of the present investigation clearly indicate that different doses of Pb (2.7, 3.7 & 4.7 mg/L) and Cd (0.1, 0.2 & 0.3 mg/L) minutely affected the SOD activity. The maximum increase in SOD activity was noticed with the highest dose of Pb (4.7 mg/l) as 12.31, 15.76 & 17.80, unit min^{-1} mg^{-1} of protein in 10,20 & 30 days old plants, respectively, as compared to their respective controls. Similar results were also noticed with the highest of Cd (0.3 mg/L) as 12.32, 15.78 & 17.89 units min^{-1} mg^{-1} of protein. In the case of ZnO NPS, the reductions in SOD activities were noticed as compared to heavy metal treatments and their respective controls in 10,20, & 30-day-old plants. The maximum reductions in SOD activities were noticed with the highest dose (75 mg/L) of ZnO NPs as 09.00, 12.00, & 13.60 units min^{-1} mg^{-1} of protein as compared to lower doses (25 & 50 mg/L) and their respective controls as 11.07,14.18 and 16.11 units min^{-1} mg^{-1} of protein in 10,20 & 30-day-old plants.

In the case of combined treatment of Pb & Cd with ZnO nanoparticles, some reductions were noticed with the lowest dose of Pb (2.7mg/L) & Cd (0.1 mg/L) and highest dose of ZnO NPs (75mg/l) as 10.58, 13.50 and 15.31 unit min^{-1} mg^{-1} of protein in Pb and 10.80, 13.80 & 15.67unit min^{-1} mg^{-1} of protein in Cd in 10,20 and 30-days old plants. (Fig.2)

CAT(Catalase) Activity

The results of the present study in the case of CAT activity were also similar as obtained in the case of SOD activity. The different doses of Pb (2.7, 3.7 &4.7 mg/L) and Cd (0.1, 0.2 & 0.3 mg/L) minutely affected the CAT activity. The maximum increase in CAT activity was obtained with the highest doses of Pb (4.7 mg/l) & Cd (0.3mg/L) as 37.09, 38.34, and 44.11& 39.31, 38.92, & 44.37 units min-1 mg-1protein10,20 & 30-day-old plants, respectively, as compared to their respective controls. The catalase activity was decreased with different doses of ZnO NPs (25, 50 & 75 mg/L) as compared to the control. The maximum decrease in CAT activities (43.82, 48.34 & 55.28 units min^{-1} $mg^{-1}$of protein in 10, 20 & 30-day-old plants)was obtained with the highest dose of ZnO nanoparticles (75 mg/l) as compared to control plants.

In combined treatments, a slight decrease in CAT activity was also noticed with the lowest dose of Pb (2.7mg/L) and Cd(0.1 mg/L), and the highest dose ZnO NPs (75 mg/L), as,32.29,36.02&42.21 and 31.71, 35.02& 39.37, respectively, as compared to their respective controls (31.72,34.69 &39.71) in 10, 20, & 30-days plants. (Fig.3)

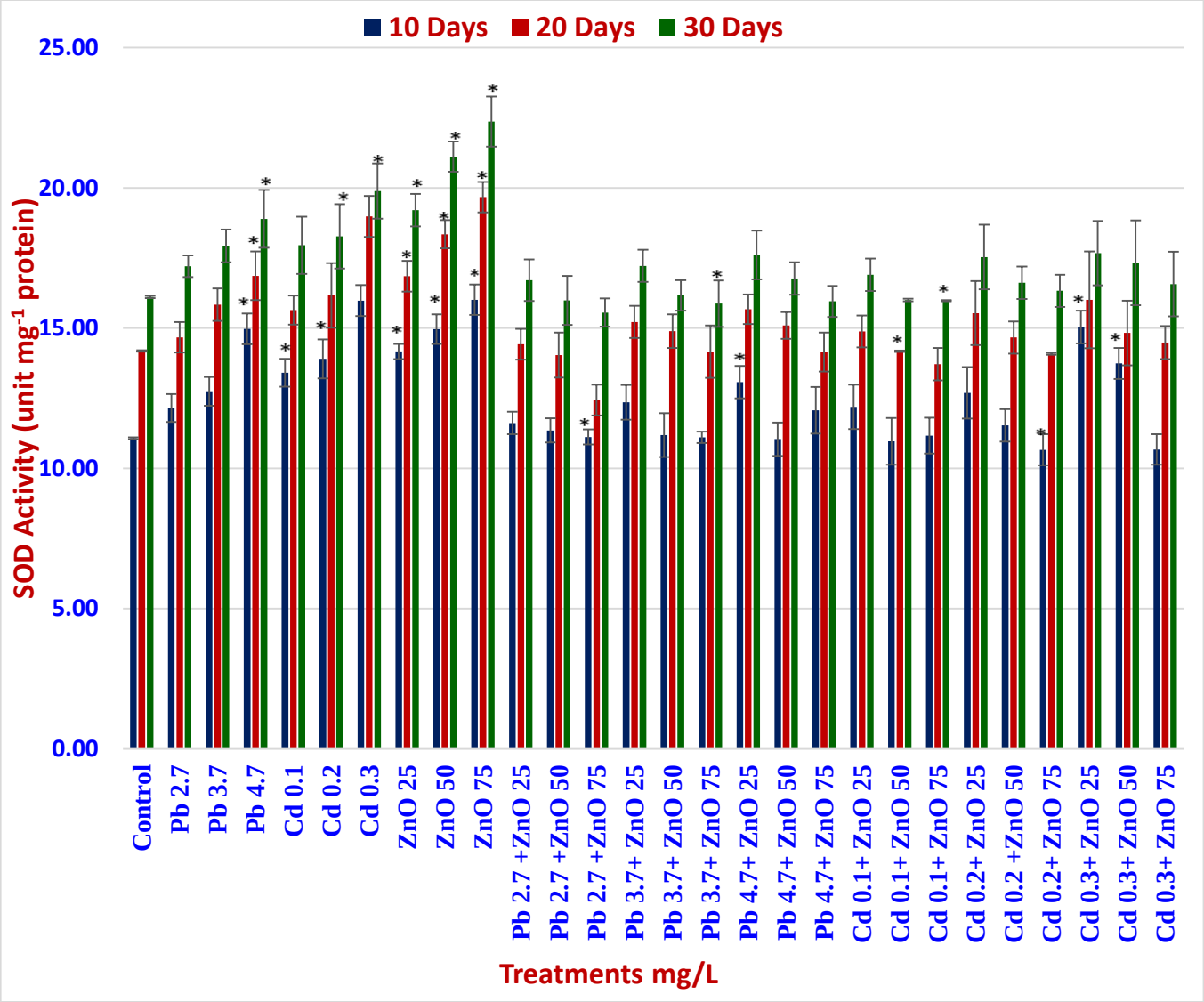


Fig. 2 Impact of Pb and Cd, alone and in combination with ZnO nanoparticles, on SOD Activity in *Helianthus annuus*.

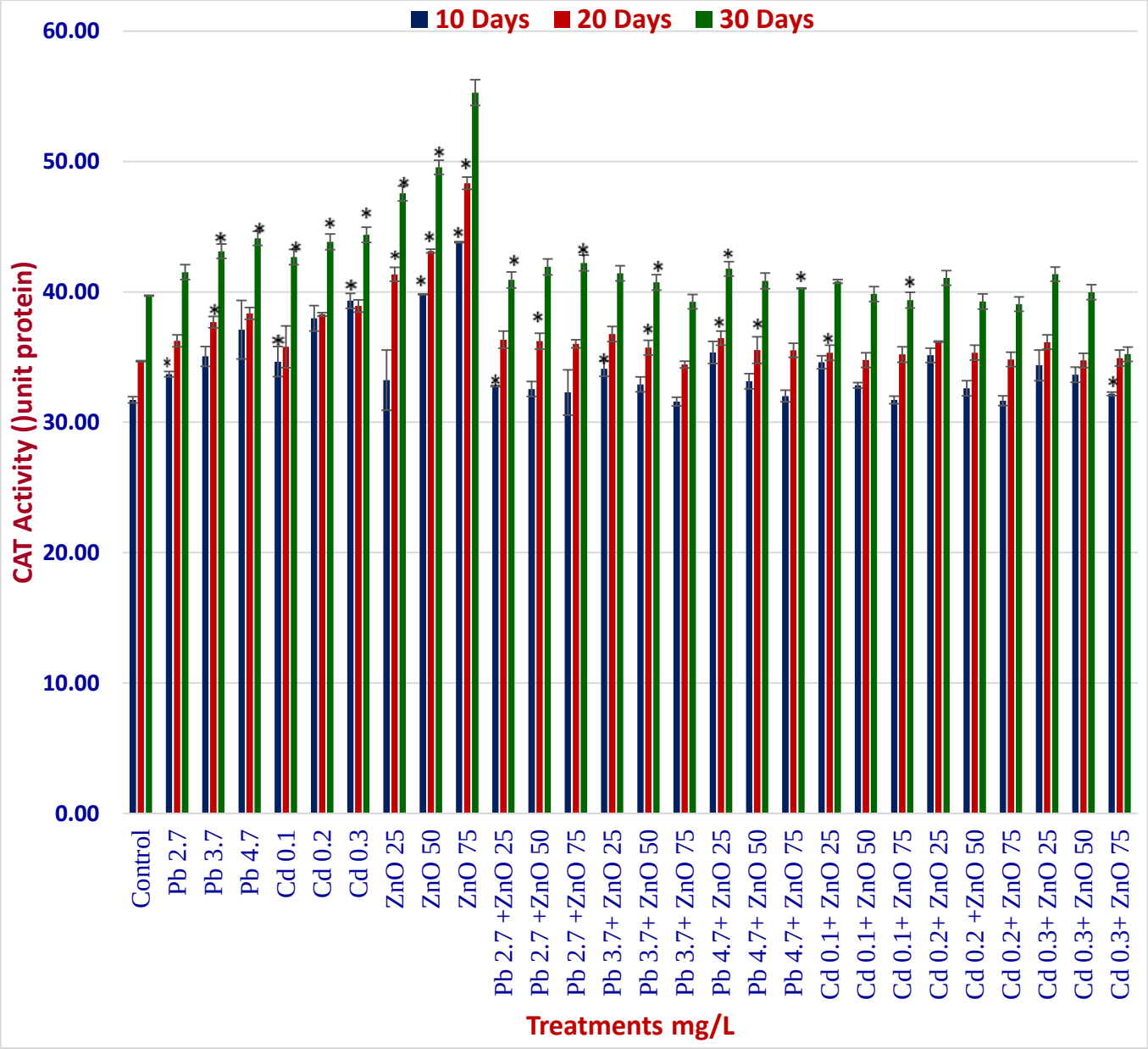


Fig. 3 Impact of Pb and Cd, alone and in combination with ZnO nanoparticles, on CAT Activity in *Helianthus annuus*

APX Activity

The results of the present study in the case of APX activity were also similar as obtained in the case of CAT activity. Different doses of Pb (2.7, 3.7 & 4.7 mg/L) and Cd (0.1, 0.2 & 0.3 mg/L) minutely affected APX activity. The maximum increase in APX activity was obtained with the highest doses of Pb (4.7 mg/L) & Cd (0.3mg/L) as 01.12, 01.52, & 01.79 and 01.13, 01.57, & 01.81units min^{-1} mg^{-1}protein10,20 & 30-day-old plants, respectively, as compared to their respective controls. The ascorbate peroxidase activity was decreased with different doses of ZnO NPs (25,50 & 75 mg/L) as compared to the control. The maximum decrease in APX(1.35, 1.85, & 2.21 units min^{-1} $mg^{-1}$of protein in 10, 20 & 30-day-old plants)was obtained with the highest dose of ZnO nanoparticles (75 mg/l) as compared to control plants.

In combined treatments, slight decrease in APX activity was also noticed with the lowest dose of Pb (2.7mg/L) and Cd(0.1 mg/L) and highest dose ZnO NPs (75 mg/L) as, 1.01, 1.41, 1.56 and 1.00, 1.39&1.60, respectively, as compared to their respective controls (31.72,34.69&39.71) in 10, 20 & 30-days-plants **(Fig.4).**

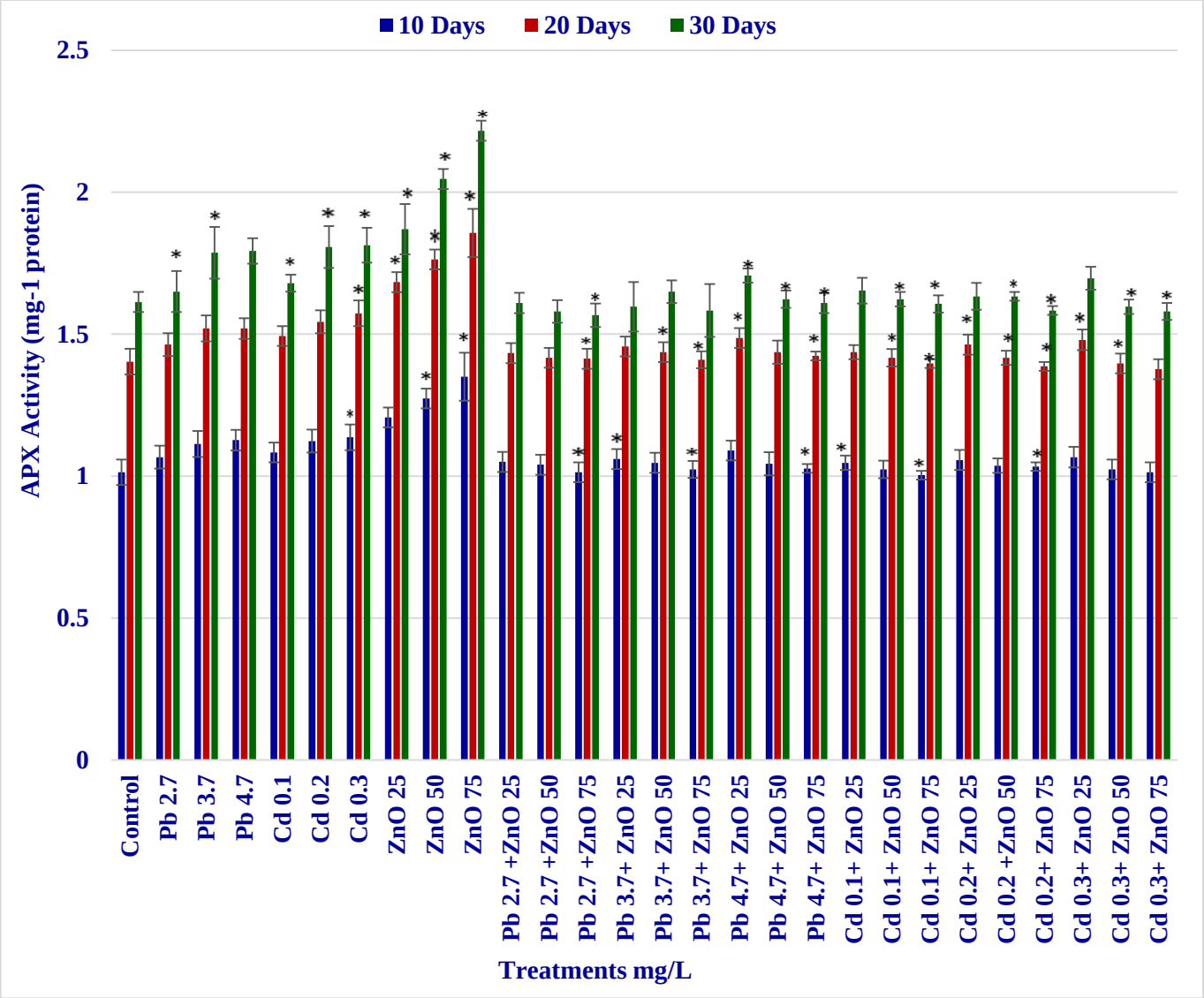


Fig. 4 Impact of Pb and Cd, alone and in combination with ZnO nanoparticles, on APX Activity in *Helianthus annuus*

Accumulation of Heavy Metals (Pb & Cd)

The results of the present study revealed that ZnO NPs enhanced the heavy metal accumulation in shoots as compared to roots. In the present study, the middle dose of Pb (3.7mg/l) and Cd (0.2 mg/l) is treated as control. The maximum accumulation of Pb (320.03, 471.92 & 688.41 mg kg^{-1} dry wt) and Cd (306.72, 473.72 & 675.94mg kg^{-1} dr. wt) was noticed with the highest dose of ZnO NPs (75mg/l, respectively) in 20, 40 & 60-day-old shoots of *Helianthus annuus* (Fig.5).

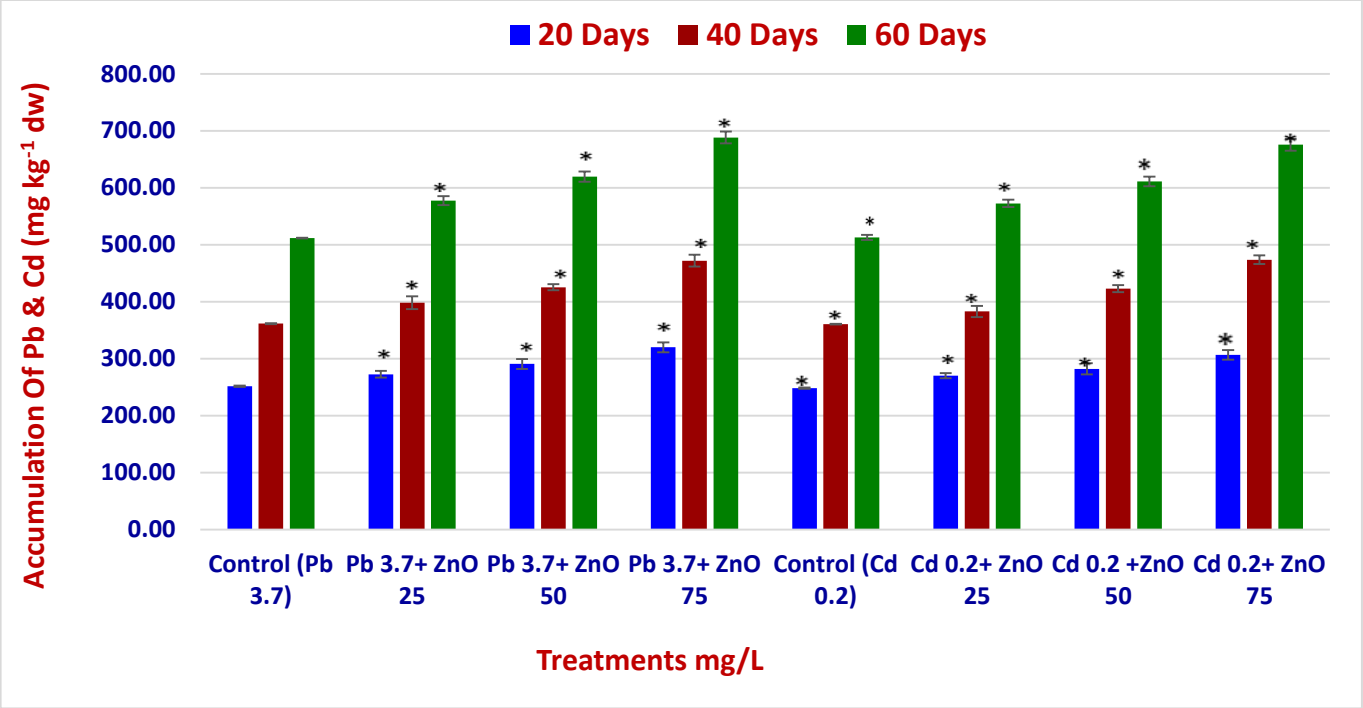


Fig. 5 ZnO NPs assisted phytoremediation of Pb and Cd through *Helianthus annuus* in their Shoots.

Similarly to shoots, the middle dose of Pb (3.7mg/l) and Cd (0.2 mg/l) was also treated as a control. Combined treatments of heavy metals (Pb & Cd) showed a concentration-dependent increase in Pb & Cd accumulation, which was noticed in 20, 40, and 60-day-old plants. The maximum accumulation of Pb (79.64, 94.19, & 109.09 and Cd (76.01, 93.97 & 108.79 mg kg^{-1} dry wt., respectively) was noticed with the highest dose of ZnO NPs (75mg/L) as compared to their respective controls in 20, 40 & 60-day-old roots of *Helianthus annuus* (Fig.6).

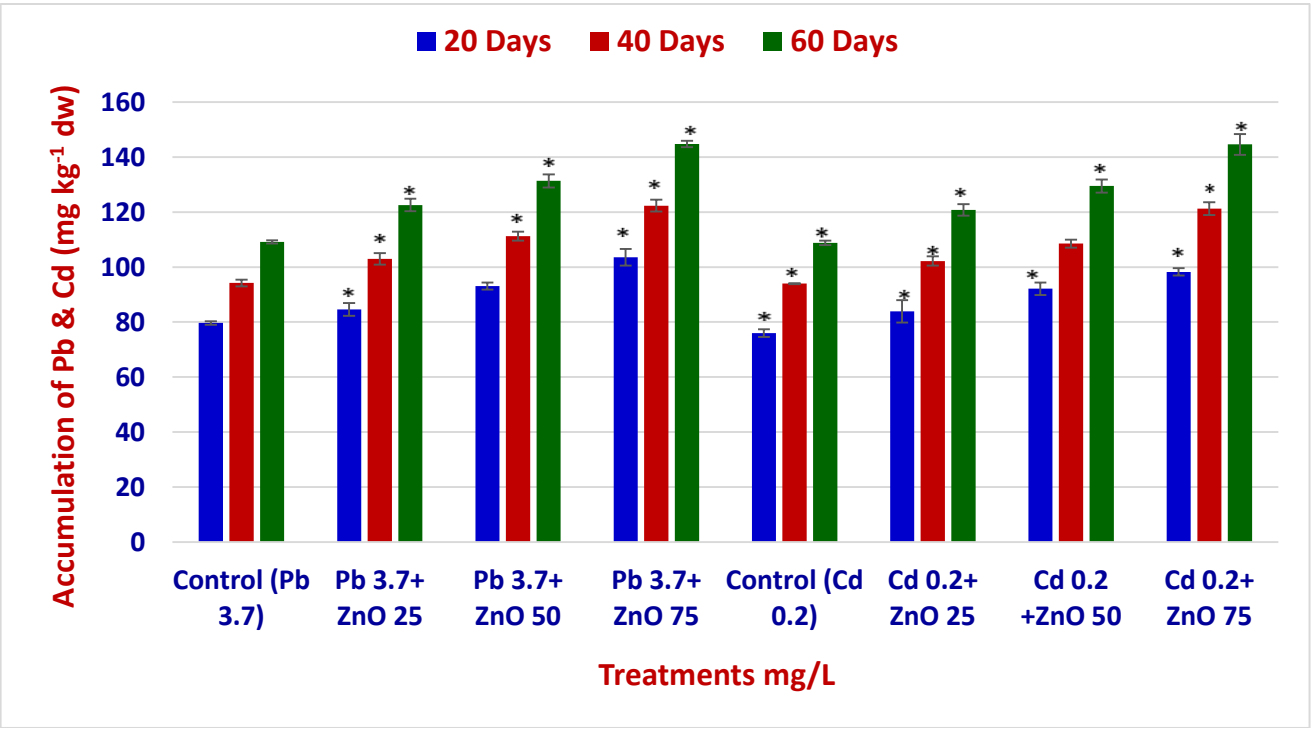


Fig.6 ZnO NPs assisted phytoremediation of Pb and Cd through *Helianthus annuus* in their roots

DISCUSSION

Seed Germination:
The results of the present investigation clearly indicate that heavy metals (Pb and Cd) exert significant impacts on seed germination of *Helianthus annuus.* Further, seed germination was enhanced with different doses of ZnO NPs (25, 50 & 75 mg/L) and in combined treatments of heavy metals (Pb & Cd) and ZnO NPs. Thus, it is clear that ZnO nanoparticles can mitigate heavy metal toxicity in *Helianthus annuus*. (Al-Sudani et al., 2024) evaluated the impact of ZnO NPs on the seed germination of Sunflower and noticed that seed germination was enhanced using ZnO NPs. They observed that seed germination significantly increased with ZnO NP treatments and concluded that ZnO NPs influenced seed germination, with impacts dependent on plant species, nanoparticle type, and doses. These findings align with those reported by (Santás-Miguel et al., 2023).

Seedling Height:
The present study clearly indicates that seedling height was slightly impacted by the different doses of Pb (2.7, 3.7, and 4.7 mg/L) and Cd (0.1, 0.2, and 0.3 mg/L). The ZnO nanoparticles were found to be effective in enhancing the seedling height in combined treatments of Pb and Cd. The different doses of ZnO NPs (25, 50, and 75 mg/L) enhanced seedling height more effectively than the combined treatments. Thus, ZnO NPs in combination with Pb and Cd effectively mitigate the metal toxicity in *Helianthus annuus.* (Prakash and Chandran, 2023) concluded that nanoparticles ranging between 1 and 100 nm have the potential to enhance the absorption of heavy metals through phytoremediator plants and also enhance the plant height, root size, and number of leaves. (Rajiv, Vanathi and Thangamani, 2018) Also suggested that ZnO NPs improve sunflower growth and development. (Mathin et al., 2022) Synthesized the ZnO nanoparticles from *Anthocephalus cadamba* Lin. and observed that such ZnO nanoparticles successfully remediate the toxic metals and positively enhance the plant growth of green gram seeds. Prajapati (2024) evaluated the impacts of Zinc oxide nanoparticles on root, shoot, seedling length, and seed germination of Maize plants and observed that lower concentrations of ZnO nanoparticles (80 ppm) increased the above-mentioned growth parameters, but at higher concentrations (640 - 5120 ppm), these parameters were decreased. (Rattan et al., 2009) and (Shukla et al., 2016) concluded that Zinc plays an important role in plant metabolism and functions as an essential element for plant growth and development. Further, Zn also acts as a cofactor in many vital enzymes involved in regulating the activity of numerous metabolic enzymes. In recent times, the application of nanotechnology in agricultural practices has spurred researchers to explore and develop new techniques for increased productivity and disease resistance. Prajapati et al. (2024) observed that ZnO nanoparticles have drawn attention for their unique physicochemical properties, including antimicrobial activity and potential as nano-fertilizers at the optimum concentrations for various crop plants. Further, they concluded that the application of ZnO NPs in agriculture also raises concerns regarding their potential toxicity and environmental impacts at elevated concentrations.

Fresh and Dry Weight:
The findings of the present study clearly reveal that different doses of Pb (2.7, 3.7, and 4.7 mg/L) and Cd (0.1, 0.2 & 0.3 mg/L) slightly reduced the fresh & dry weight of plants as compared to their respective controls in 10, 20 & 30-day-old plants. The maximum reduction occurred with the higher doses of Pb & Cd (4.7 mg/L and 0.3 mg/L as compared to lower doses of Pb (2.7 & 3.7 mg/L) and Cd (0.1 & 0.2 mg/L) and control. ZnO NPs treatment showed marked improvement in the fresh and dry weight of the plant. The maximum increase in fresh and dry weight of plants was noticed with the higher doses of ZnO nanoparticles (75 mg/L). In combined treatments of heavy metals (Pb & Cd) and ZnO nanoparticles, ZnO nanoparticles mitigated the metal toxicity.

The maximum mitigation of metal toxicity was noticed with the lowest doses of Pb & Cd (2.7 & 0.1 mg/L, respectively) and the highest dose of ZnO NPs (75 mg/L). (Faizan et al., 2021) reported that Zinc oxide nanoparticles mitigate the adverse impacts of Cd toxicity in *Oryza sativa* by the alteration of the photosynthetic and antioxidant defense system. They explored that rice plants supplemented with ZnO nanoparticles under Cd toxicity significantly enhanced the shoot length 34%, root fresh weight 30%, shoot dry weight 23.07%, and root dry weight 12.24%. (Rastogi et al., 2017) revealed that nanoparticles play a crucial role in the growth and development of plants under stress conditions. They are being used to deliver the proteins, specific chemicals, and nucleotides to the specific sites. Further, (Hassan et al., 2024) studied the impacts of ZnO nanoparticles to mitigate cadmium toxicity and noticed that plants readily absorb heavy metal (Cd) and subsequently disrupt the various physiological and biochemical processes and enhance the formation of ROS (reactive oxygen species), which induces the oxidation of cellular components and structure, resulting in significant growth losses. Thus, the growth of plants and biomass productivity is reduced. They further explored that ZnO nanoparticles mitigate Cd toxicity and improve chlorophyll synthesis and photosynthetic efficiency, which results in enhanced biomass (fresh and dry weight of plants).

Antioxidant Defense System

Heavy metals (Pb, Cd, Cr, Ni, Cu, As, etc) induce hazardous oxidative stress in the sunflower (*Helianthus annuus*) plant. *Helianthus annuus* triggers an enhanced antioxidant defense system, including enzymes like SOD, APX, CAT & POD to scavenge the reactive oxygen species. The sunflower plants are a hyperaccumulator plant that stands these toxins through inducing a robust and upregulated enzymatic response that protects the tissues during the accumulation of heavy metals, but high levels of these metals can still reduce the growth & development of plants.

SOD (Superoxide Dismutase) Activity:

Superoxide dismutase (SOD) is a critical antioxidant enzyme that acts as the first line of defense against oxidative stress. SOD neutralizes the harmful superoxide radicals into H_2O_2 and O_2. SOD also protects the mitochondria and maintains the redox homeostasis in cells. In the present investigation, the effects of different doses of Pb and Cd were examined, revealing that these heavy metals induce toxic effects on the growth and development of plants in terms of reduced growth and biochemical parameters. SOD activity slightly increased due to Pb & Cd stress as a result of the defense system of sunflower plants. The different concentrations of ZnO NPs increased the SOD Enzyme activity significantly in 10-, 20-, and 30-day-old plants as compared to their respective controls and heavy metals (Pb & Cd) treatments. In combined treatments of heavy metals and ZnO NPs, the increase in SOD activities was also noticed. The result of the present investigation clearly indicates that higher heavy metal concentration induces oxidative stress, requiring more SOD for detoxification. At 30 days, ZnO NPs (75mg/L) show a mean of 22.90, which is 142.23% as compared to the control. This indicates that ZnO NPs at higher concentrations stimulate the enzyme production. It is suggested that ZnO NPs in combination with heavy metals (Pb & Cd) decrease the SOD activity back towards the control. Thus, ZnO NPs help the sunflower plant to mitigate the oxidative stress caused by heavy metals, allowing the enzyme levels to be stabilized. (Akhtar et al., 2022) noticed that heavy metals (Cd, Cr, Cu & Pb) increase the oxidative stress, which results in increased SOD, CAT & APX activity. ZnO NPs mitigate the heavy metal stress by reducing H_2O_2 content. Ramjan et al. (2022) studied the synergistic effect of Zinc oxide nanoparticles to alleviate Cd toxicity and noticed that superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) & peroxidase (POD) were increased when ZnO NPs were applied to Cd-stressed seedlings of linseed (*Linum usitatissium* L.). Furthermore, the introduction of ZnO NPs reduced the level of malondialdehyde (MDA) and H_2O_2 in the linseed plant grown in Cd-toxic conditions.

CAT (Catalase) Activity

Catalase is a strong antioxidant enzyme that protects cells from oxidative damage. It is typically a homo-tetrameric protein having four isomorphic polypeptide subunits. Each subunit binds a heme (ferriprotoporphyrin) group, which is crucial for the reaction. The gene CAT encodes the catalase enzyme, which is synthesized in the cytosol. After synthesis, the apoprotein part of catalase folds and acquires its home group before it is transported to its primary location, the peroxisome. Catalase is predominantly located in peroxisomes but is also present in the cytoplasm and mitochondria in some cell types. (Baker et al. 2023). They further stated that catalase is a core antioxidant enzyme in most organisms that decomposes the H_2O_2 (Hydrogen peroxide) into water and oxygen. In the present study, different doses of Pb (2.7,3.7 & 4.7 mg/L) and Cd (0.1,0.2 & 0.3 mg/L) slightly increased the catalase activity as compared to their respective controls in 10,20 & 30 days old sunflower plants. The application of ZnO NPs at various levels (25,50& 75 mg/L) significantly enhanced the catalase activity in 10,20 and 30-day-old plants compared to their respective controls.

(Sinhal, 2016) stated that the activities of antioxidant enzymes like APX (Ascorbate peroxidase), SOD (superoxide dismutase), and CAT (Catalase) were increased with the Pb treatments (2.7,3.7 &4.7 mg/L). Hussain et al. (2024) studied that ZnO NPs alleviated the phytotoxicity induced by Cd, in terms of reduced chlorophyll content and carotenoid contents upto 52.87 % and 23.31%, respectively. They evaluated that Cd stress enhanced the proline, catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) when plants were treated with cadmium as compared to control plants. Due to cadmium toxicity, the stress indicators such as MDA (Malondialdehyde), H_2O_2 (Hydrogen peroxidase), and non-enzymatic antioxidants in plant leaves. Venkatachalam et.al. (2017) described that ZnO NPs reversed the oxidative stress induced by heavy metals (Pb&Cd) in *Leucana leucocephala* plants. They concluded that ZnO NPs efficiently mitigated the heavy metal-induced toxicity. The antioxidative enzymes like SOD, CAT & POD were elevated in heavy metal-exposed leaves supplied with ZnO NPs, and oxidative stress-induced toxicity was also confirmed by the reduced MOA contents and the elevated levels of antioxidative enzyme activities in leaves.

APX (Ascorbate Peroxidase) Activity

Ascorbate peroxidase (APX) is a major antioxidant enzyme found in plants and other organisms. It is a very strong shield of antioxidant defense system converting harmful H_2O_2 (Hydrogen peroxide) into H_2O (water). Here, APX enzyme uses ascorbate as an electron donor and thus prevents oxidative damage. APX is synthesized on free ribosomes in the cytoplasm. It exists in various isoforms which specifically function in the cytosol, mitochondria, chloroplasts, peroxisomes, or apoplast to safely break down toxic H_2O_2 (Hydrogen peroxidase) into water. Further, it is a key component of the ascorbate –

glutathione cycle, which crucially scavenges the harmful ROS (reactive oxygen species) in plants and microbial cells (Shengchun, 2023). Further, APX effectively participates in many plants metabolic processes and thus participates in many growth and development processes like nodule development, leaf senescence, seed germination, lateral root formation, and programmed cell death. In the present investigation, under the influence of different doses of Pb & Cd, APX activity slightly increased as compared to their respective controls in 10,20 &30 days old plants. This may be due to enhanced oxidative stress induced by heavy metals. ZnO NPs significantly enhanced the APX activity, but the maximum increase in activity was noticed with the highest doses of ZnO NPs, which mitigated the heavy metal-induced toxicity.

Hussain et al. (2024) studied that ZnO NPs ameliorated the phytotoxic impacts of Cd toxicity in maize plants by regulating primary metabolites and antioxidant activity. They noticed that Cd toxicity induced oxidative stress in terms of reduced protein and total free amino acids. The application of ZnO NPs mitigated such stress and increased the activity of APX, SOD, POD & CAT. (Adrees et al., 2020) investigated that when ZnO NPs were applied at different levels (0,25,50 &100 mg/L), the growth and antioxidant enzyme activities were enhanced significantly.

Accumulation of Heavy Metals

In the present investigation, the accumulation of heavy metals (Pb & Cd) under the influence of different concentrations of ZnO NPs (25,50 &75 mg/L) was significantly enhanced. The middle dose of Pb (3.7 mg/L) & Cd (0.2 mg/L) has been treated as a control in both the shoots and roots. ZnO NPs assisted the accumulation of heavy metals more in shoots than roots. The maximum accumulation of Pb and Cd was noticed with the highest dose of ZnO NPs (75mg/L) as compared to controls (Pb,3.7 & Cd,0.2 mg/L) in 20-day-old, 0-day-old, and 0-day-old sunflower plants. Similar results were also noticed by (Pérez-Hernández et al., 2023). They evaluated the ZnO NPs assisted phytoremediation of soil contaminated with Pb & As through sunflower plants. They noticed that the ZnO NPs assisted the phytoremediation potential of sunflower was increased significantly. ZnO NPs also enhanced the bioconcentration and translocation of metals in sunflowers. (Prakash and Chandran, 2023) noticed that nanoparticles mitigate the toxic effect of heavy metals due to their small (1 to 100 nm), high surface volume ratio, and electrical charge, which results in improvement in plant height, root size, and number of leaves. Thus, ZnO NPs are able to enhance the plant biomass. They further stated that, due to the physicochemical properties of ZnO NPs, they can absorb or eliminate heavy metals through redox (Oxidation-reduction) reactions. They concluded that ZnO NPs increased the size of the plant and bioconcentration and translocation of As & Pb into the plants. Furthermore, the nanotechnology combined with phytoremediation with *Helianthus annuus* and ZnO NPs may be a novel technique for the removal of heavy metals from polluted soil. (Usman et al., 2022) stated that ZnO NPs significantly enhanced the metal accumulation potential of sunflowers through the phytoextraction technique.

CONCLUSION

The present investigation clearly indicates that heavy metals (Pb and Cd) exert phytotoxic effects on the growth of *Helianthus annuus*. Furthermore, ZnO nanoparticles (ZnO NPs) had a positive impact by enhancing the growth of *Helianthus annuus*. The results of antioxidant enzyme activities revealed that ZnO NPs effectively alleviated the oxidative stress induced by heavy metals in sunflower plants. The combined treatment of heavy metals and zinc oxide nanoparticles clearly demonstrated the mitigation of metal toxicity in *Helianthus annuus*. Moreover, ZnO NPs enhanced the accumulation potential of *Helianthus annuus* for Pb and Cd. Thus, the present investigation suggests that *Helianthus annuus* can be successfully cultivated in soils contaminated with heavy metals. The phytoremediation efficiency of *Helianthus annuus* for the accumulation of Pb and Cd can be further enhanced through the application of zinc oxide nanoparticles.

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