Bioavailability of zinc oxide nano particle with fly ash soil for the remediation of metals by *Parthenium hysterophorus*

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Abstract

To investigate the interaction of zinc oxide nanoparticles (ZnO NPs) with fly ash soil (FAS) for the reduction of metals from FAS by *Parthenium hysterophorus* were studied. The average accumulation of metals by *P. hysterophorus* stem were Fe 79.6%; Zn 88.5%; Cu 67.5%; Pb 93.6%; Ni 43.5% and Hg 39.4% at 5.5 g ZnO NP. The concentration of ZnO NP at 1.5 g did not affect the metals accumulation, however at 5.5 g ZnO NP showed highest metal reduction was 96.7% and at 10.5–15.5 g ZnO NP of 19.8%. The metal reduction rate was R_{max} for Fe 16.4; Zn 21.1; Pb 41.9; Hg 19.1 was higher than Ni 6.4 and Cu 11.3 from the FAS at 5.5 g ZnO NP whereas, the reduction rate of Pb showed highest. With doses of 5.5 g ZnO NP the biomass increased upto 78%; the metal reduced upto 98.7% with the share of 100% ZnO NP from FAS. Further investigation with phytotoxicity the plant reactive oxygen species (ROS) production were affected due was mainly due to the recovery of metals from FAS ($R^2 = 0.99$).

Keywords Fly ash soil · Phytoremediation · ZnO NP · Heavy metals · Mass balance · Reactive oxygen species

Introduction

Fly ash as an industrial waste has been recognized as one of the hazardous contaminants in soil and water. Metal pollution has rapidly intensified since the onset of the industrial revolution posing major environmental and health problems [1]. Metals enter agricultural soils mainly from industrial processes, and then they are transferred to the food chains from the soil [2]. Subsequently, the heavy metals are leached into the surrounding soils including agricultural lands and soils [3]. As metals may cause major environmental and human health problems, effective methods are needed to remediate the pollution from soil [4]. Owing to the serious consequences of toxicity due to metal contamination, increasing efforts have

been made to phytoremediate the toxic metals from the fly ash soil and environment (from both water bodies and soil) [5]. Certain plants such as the *P. hysterophorus* have been reported to have potential to phytosequester heavy metals in large proportion and provide the feasibility of phytoremediation of metals from soil and fly ash soil [6].

The increasing use of zinc oxide nanoparticles (ZnO-NPs) raises concerns about their environmental impacts, but the potential effect of ZnO-NPs on accumulation of metals remains unknown. The increasing use of zinc oxide nanoparticles (ZnO-NPs) by consumers in cosmetics, sunscreen formulation, paints, plastics and industrial products packaging highlights a need to understand their potential applications [7]. Phytoremediation treatment systems use nano technology (NT) that have been studied less extensively than physico-chemical processes; nevertheless, important research has been conducted in this direction [8]. The researcher studied were found that the NPs treatment of the soil with *C. sativus* also reduced total Cu levels up 21% from soil [9]. In fact, the cited reference was found toxicity of nanoparticles CuO, ZnO and TiO₂ to microalga followed the OECD 201 algal growth inhibition test. Plant biomass was significantly increased by NP treatment. ZnO-NPs had a significant effect on biomass, compared with the results of previous studies [10]. The studies conducted by the researchers indicated that the plant with ZnO-NP influences uptake, transport, and inhibit toxicity of

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metals [11]. NPs can adhere to the surfaces of root cells or enter the root cell membrane, depending on the type of metal, the uptake of metal ions, particle aggregation, the presence of hydrophobic NPs, and plant species [12]. The metal accumulation conducted with hydroponic culture of ryegrass was the highest in NP-treated soil (Lin D, Xing 2008).

The ZnO NP influences the metal accumulation and phytoremediation rates by *P. hysterophorus* was studied [13]. The physiological and molecular mechanisms that are responsible for metal hyperaccumulation and tolerance in plants have been extensively studied [14, 15]. The high concentration and accumulation of metals by plants causes retardation of growth, reduction of biomass, generation of reactive oxygen species (ROS), loss of membrane integrity and inhibition of sulfhydryl group enzymes. One of the mechanisms that make a plant species tolerant to heavy metal stress is the presence of a strong antioxidant defense system [16]. Heavy metals induce oxidative stress by generating free radicals and toxic reactive oxygen species (ROS) [16]. Heavy metal toxicity enhances the production of ROS up to 30-fold [17]. Enzymatic and non-enzymatic antioxidant machinery of the plant may alleviate the deleterious effects resulting from the cellular oxidative state [16]. Superoxide dismutases (SODs) are a family of metalloenzymes catalyzing the dismutation of O_2^- to H_2O_2 [18–20].

The main objectives of this study are: (1) to assess the metal uptake rates of *P. hysterophorus* in ZnO NP treated FAS; (2) using mass balance approach to identify the removal pathways of metals from fly ash soil fed with ZnO NP; (3) identifying the role of ZnO NP and importance of plants in removing metals and to compare the plant toxicity and ROS activities; (4) investigating the feasibility of reduction of metals by *P. hysterophorus* from fly ash affected areas with the influence of ZnO NP.

Material and methods

Physico-chemical analysis of fly ash soil

The fly ash soil (FAS) was collected from identified fly ash dumping sites at the Petroleum refiner-PDO, Muscat, Oman. After collection, all samples were brought in polythene bags to the laboratory for analysis. Physico-chemical properties of the FAS and tap water were analyzed according to the method [21]. The FAS was oven dried at 40 °C for 5 days and sieved through a 6 mm mesh. Chemical parameters were soil pH, 7.9; electrical conductivity, 1.13 dsm^{-1} ; total nitrogen, 0.09%; total phosphorus, 0.78% and organic carbon, 4.487%. Levels of sulfate, potassium, carbonate, chloride, magnesium and porosity were estimated according to methods APHA [21]. Water holding capacity was measured by hydrometer.

In this study the Zinc oxide nano particle (ZnO NP) was collected from nanotechnology lab from King Saud University, Saudi Arabia. The stock dispersion of ZnO NPs was produced by adding 0.0; 3.5; 5.5; 10.5 and 15.5 g ZnO-NPs to 1.0 L distilled water (pH 7.0) containing 0.1 mM sodium dodecylbenzene sulfonate (SDBS) (Sigma Aldrich, St. Louis, MO) to enhance the stability of nano-suspension because the particles almost immediately aggregated in surrounding medium [22]. The stock dispersion was sonicated (25 °C, 250 W, 40 kHz) for 1 h to break aggregates before being diluted to concentrations of fly ash soil (FAS).

Experimental design

Parthenium hysterophorus were obtained from the Al-Ansab wetlands, Muscat, Oman. The seeds *P. hysterophorus* were sown and the plants were raised in 12"/12" earthen pots. After sowing the seed, in each pot containing 5 kg of soil, 10 plants were irrigated with ZnO NPs water every day. For convenience, the amendments in per g of FAS are denoted as (1.5 g ZnO NP + FAS); (3.5 g ZnO NP + FAS); (5.5 g ZnO NP + FAS); (10.5 g ZnO NP + FAS) and (15.5 g ZnO NP + FAS soil) series of experiments were performed. Three replicates were taken for each treatment. Various morphological and physiological analyses were carried out in plants one month old (i.e. two months after initiating the irrigation with ZnO NPs water). Plants grown in garden soil served as control. The plants from each treatment were placed under natural conditions. The plants were treated daily and care was taken to avoid leaching of water from the pots. A plastic tub was placed below each pot to collect the leachates. The collected leachates were again returned to the experimental pot. No rainfall was recorded during the period of experiment. The roots from each plant were detached and washed repeatedly using tap water to remove unwanted debris and blotted. Fresh biomass was also recorded.

For metal analysis, FAS samples were analyzed according to Dwivedi et al., [16]. For metal analysis in FAS, dried (1 g) soil from each of the treatments was grounded and digested overnight with 5 ml (FAS, wastewater and *P. hysterophorus*) were digested with $HClO_4/HNO_3$ (1:4 v/v) and diluted with Milli-Q water. The samples were suitably diluted with triple distilled water to measure the concentration of metals. Plant samples were divided into roots and shoots, washed thoroughly with deionised water to remove any adhering soil/wastes particles, blotted and oven dried at 80 °C for 72 h. Metals (Fe, Zn, Cu, Pb, Ni, and Hg) were estimated by metal concentrations in the samples which were determined using on the Inductively-Coupled Plasma Mass Spectrometer, Perkin Elmer Corporation (ICP Optima 3300 RL). The standard reference material of metals (E-Merck, Germany) was used for calibration and quality assurance for each analytical batch. The detection limits of Fe, Zn, Cu, Pb, Ni, and Hg were 4.5,

2.5, 2.0, 0.5, 0.5 and 0.01 $\mu\text{g/l}$. For replicates ($n = 3$) were conducted to assess the precision of the analytical techniques. Triplicate analysis for each metals varied by no more than 5%.

Maximum metals reduction rates

The efficient metal accumulation was calculated by using maximum metals reduction rate (R_{max}) from FAS with the influence of ZnO NP by plants. The metal concentration was accumulated in the aboveground parts of the plant from soil on a dry weight basis. The enrichment coefficient basically depends on the soluble fraction of metals. The efficient accumulation of metals were calculated by using maximum metals plants g biomass of parts rate (R_{max}) and over the remained metal FAS in percentage. To start compartmentalization, 1.5 g ZnO NP initiator was mixed in FAS and the mixture allowed standing for per day basis to promote accumulation of metals increase the soil surface. The scanning electron microscope (SEM) showed soil size and soil granulated was cut into 100 nm shown in Fig. 1.

Mass balance

The compartments were described by mass balance differential methods and plant-specific physiological parameters. For the metals translocation processes within a living plant using mass balance differential methods, a few simplifying assumptions were made: (a) movement of metals between compartments (FAS and plant) occurs by mass flow with the transpiration stream or by diffusion, (b) phloem transport of metals from the leaves back down to the roots is negligible, (c) no significant degradation of metals occurs in the plant system, and. The model is comprised of a set of mass balance methods, one for each plant compartment.

Estimation of various physiological and biochemical parameters

Roots and leaves of the freshly harvested plants were used for the determination of all the physiological and biochemical parameters. Chlorophyll content in the fresh leaves of the

Table 1 Physico-chemical properties of fly-ash soil, Fly-ash affected areas National Thermal Power Corporation (NTPC), Badarpur, New Delhi, India ($n = 3$, mean $\pm$ SD)

Parameters	Concentrations
pH	7.79 ± 0.03
Water holding capacity (%)	72.96 ± 3.95
Total nitrogen (mg/l)	0.045 ± 0.004
Phosphorus (mg/l)	0.67 ± 0.001
Total organic carbon (ppm)	0.76 ± 0.005
Cation exchange capacity	11.25 ± 2.45
Sulfate (%)	12.93 ± 0.68
Potassium (%)	0.95 ± 0.04
Chloride (%)	2.65 ± 0.75
Carbonate (%)	2.09 ± 0.15
Magnesium (%)	1.86 ± 0.08

Values are means of three replicates

plant (100 mg) was estimated following the method [23]. Protein content in the roots and leaves of the treated plants including control was determined using bovine serum albumin (BSA) as a standard protein [24]. The lipid peroxidation in the plant tissue was measured in terms of malondialdehyde (MDA) content, determined by thiobarbituric acid (TBA) reaction [25, 26].

Fresh leaf material was ground in an ice-cooled mortar with ice-cooled homogenization buffer specific for each enzyme. The homogenate, filtered through four layers of muslin cloth, was centrifuged at $12,000 \times g$ for 10 mins at 4°C . The supernatant, i.e., enzyme extract, was used for enzyme determination. For estimation of superoxide dismutase (SOD, EC.1.15.1.1) activity, 0.5 g of fresh plant material was homogenized in 5.0 ml of extraction buffer containing 50 mM phosphate buffer (pH 7.5), 0.1% (w/v), BSA (bovine serum albumin), 0.1% (w/v) ascorbate and 0.05% (w/v) β -mercaptoethanol. SOD activity was assayed by measuring photoreduction of nitroblue tetrazolium (NBT) at 560 nm [24]. One unit of SOD activity is considered as the amount required to inhibit photo reduction of NBT by 50% and is expressed in enzyme units (EU) mg^{-1} protein. All assays were conducted in triplicate and the results were expressed as mean

Fig. 1 TEM analysis of nano- and micro-sized particles. SEM pictures of ZnO nanoparticles at different magnifications A-ZnO nano particle powder size 0.5 nm; B- ZnO nano particle powder mix with FAS after 15 days experiments size 50 nm; C- ZnO nano particle powder mix with FAS after 30 days experiments size 100 nm

Table 2 Metals contents in Fly ash soil (FAS) near Fly-ash affected areas National Thermal Power Corporation (NTPC), Badarpur, New Delhi, India (n = 3, mean $\pm$ SD)

Metal ions	(mg Kg ⁻¹)
Fe	1578.56 $\pm$ 195.12
Zn	63.34 $\pm$ 0.65
Cu	12.14 $\pm$ 0.14
Pb	17.52 $\pm$ 0.75
Ni	20.53 $\pm$ 0.53
Hg	0.24 $\pm$ 0.001

Values are means of three replicates

standard deviation. An analysis of variance (ANOVA) was used to test the significance of results and $p < 0.05$.

Results and discussions

Role of ZnO NP for accumulation of metals and ROS activities

The physico-chemical parameters and metal concentrations in fly ash soil (FAS) are shown in Tables 1 and 2. As shown in Fig. 2, the presence of 1.5–2.5 g of ZnO NP did not affect accumulation of metals, but 5.5 g of ZnO NP show tremendous metals reduction 96.7% and at 10.5–15.5 g of ZnO NP reduced biomass 18.3% and 73.8%. Accumulation of metals by *P. hystrophorus* at 1.5 to 15.5 g ZnO NP dosage is represented in Fig. 2. From this study the accumulation of metals was found 98.6% from FAS at 5.5 g ZnO NP. However, a much lower accumulation of metals was observed at 1.5 g ZnO NP or higher concentrations i.e. 10.5–15.5 g ZnO NP and recovery of metals were also high. At a 5.5 g and lower ZnO NPs dosage, no inhibitory effect was observed. When the dosage of ZnO NPs was 10.5 g, however, the average accumulation of metals decreased to 81.7% of the control, which was further decreased to 24.9% of control as the dosage of ZnO NPs increased to 15.5 g. Apparently, higher concentrations 10.5 and 15.5 of ZnO NPs were capable of inhibiting the

accumulation of metal and toxicity production by release of metals from FAS Fig. 2.

This study was to evaluate the impact of ZnO NPs on metal accumulation by *P. hystrophorus* and ROS production during FAS metals remediation to explore the mechanism Fig. 3. However, the accumulation of metal at ZnO NP 5.5 g/Kg FAS was 176% of the control at a ROS concentration of 56%. When the accumulation of metals was higher, much lower ROS was observed at ZnO NP 5.5 g/Kg FAS Fig. 2 ($p < 0.05$). Figure 3, addressed the toxicity of FAS which came from the released metal ions (Zhang et al. 2015), but others found that the toxicity of ZnO NPs was not caused by the recovery of metals but was caused by ZnO NPs themselves [27]. At higher levels of ZnO NP 10.5–15.5 g/Kg, the ROS production was very high and also had a tremendous effect on accumulation of metals. Thus, the role of ZnO NP for the accumulation of metals, reduces the ROS productions should be taken into account. Moreover, oxidative stress reduces by ZnO NPs with FAS was reported to cause the loss of cell viability, and the increase of intracellular reactive oxygen species (ROS) which was found to be toxic to cytoplasmic lipids, proteins and other intermediates in cells [27].

However, the *P. hystrophorus* showed different accumulation of metals from FAS at dose of ZnO NP 5.5 g/Kg. The accumulation of Fe, Zn, Cu, Pb, Ni and Hg were observed in root (Fe 1584; Zn 1698; Cu 1164; Pb 1592; Ni 775; Hg 205 μ g/g), stem (Fe 2984; Zn 1848; Cu 1253; Pb 1794; Ni 795; and Hg 298 μ g/g) and leaves (Fe 1184; Zn 963; Cu 756; Pb 867; Ni 348; and Hg 80 μ g/g) as shown in Fig. 4. The accumulation of metals Cu, Ni and Hg lowest was observed as compared to Fe, Pb and Zn. However, the biomass of the plants in stem showed an average removal of metals Fe 79.6%; Zn 88.5%; Cu 67.5%; Pb 98.3%; Ni 43.5% and Hg 39.4% at 5.5 g ZnO NP (Fig. 5) from FAS. The accumulation of Fe, Pb and Zn was found to be highest whereas, Cu, Ni and Hg were found minimum in plant biomass ($p < 0.05$). Metal accumulation from the FAS with at 5.5 g ZnO NP in

Fig. 2 Effects of different dosages of ZnO NPs (1.5, 3.5, 5.5, 10.5 and 15.5 g/Kg FAS) and the corresponding released metals and accumulation of metals during FAS treatment. Asterisks indicate statistical differences ($p < 0.05$) from the control. Error bars represent standard deviations of triplicate tests

Fig. 3 Effects of different dosage of ZnO NPs on intracellular ROS production and metals accumulation by *P. hysterophorus* from fly ash soil (FAS). All values are mean of three independent experiments (three replicates each). Asterisks indicate statistical differences ($p < 0.05$) from the control. Error bars represent standard deviations of triplicate tests

plant was found to be in the order Fe 95 > Pb99 > Zn95 > Cu85 > Ni 65 and > Hg 58 (Fig. 5).

Role of ZnO NP on seedling and biomass

The results from this experiment showed that *P. hysterophorus* has the highest seedling lengths (43.5 root, 95.63 stem) and dry weight of root and stem (35 and 65 cm) at 5.5 ZnO NP as compared to 15.5 g ZnO NP where lengths of root and stem was 9.53 and 23.5 cm along with the dry weight of root and stem 12 and 39 g (Fig. 6a). The amount of biomass was increased upto 75 g at 5.5 g ZnO NP ($p < 0.05$), while the

Fig. 4 Accumulation metals by *P. hysterophorus* (Root, Stem and leaf) from fly ash soil (FAS) at 5.5 g ZnO NP. All values are mean of three independent experiments (three replicates each). Asterisks indicate statistical differences ($p < 0.05$) from the control. Error bars represent standard deviations of triplicate tests

Fig. 5 Effects of 5.5 g ZnO NP in FAS on metals accumulation by *P. hysterophorus* from fly ash soil (FAS). All values are mean of three independent experiments (three replicates each). Asterisks indicate statistical differences ($p < 0.05$) from the control. Error bars represent standard deviations of triplicate tests

biomass was decreased upto 45 g at 15.5 g ZnO NP ($p < 0.05$) (Fig. 6b). Biomass growth increased constantly on all the amendments except at 10.5–15.5 g ZnO NP and this reduction were due to metals and ROS production (Fig. 6b). Metals reduced the total chlorophyll contents ($p < 0.05$) as seen by the chlorophyll ‘a’ decreasing with increasing ZnO NP (Fig. 7a). The plant showed highest development of photosynthetic activity at dosage of ZnO NP 5.5 g and adverse effect tended to increase from the lower to the higher concentration of ZnO NP. However, the reduction of the total chlorophyll, chlorophyll ‘b’ and photosynthetic area decreased insignificantly ($p < 0.05$) compared to control (Fig. 7b) when the plant was exposed to 10.5–15.5 g ZnO NP ($p < 0.05$).

Role of ZnO NPs for metals reduction rates R_{max}

As shown in Figs. 1, 2, 3 and 4, when ZnO NP was added to FAS for accumulation of metal, their influence on metals reduction was relevant to the dosage. At a lower ZnO NPs dosage (1.5–3.5 gZnO NP), no good accumulation and no inhibitory effect was observed. When the dosage of ZnO NP was 5.5gZnO NP, however, the highest accumulation of metal increased upto 96.9% with compared to control, which was further decreased to 35.9% of the control as the dosage of ZnO NPs increased to 10.5–15.5 g were capable of inhibition of phytotoxicity activities [27]. The applied of ZnO NP for the accumulation of Fe, Pb and Zn were more in stem compared to the root ($p < 0.05$), but the root accumulated more of Cu, Ni and Hg compared to the stem ($p < 0.05$) (Fig. 4). A

Fig. 6 Effect of different doses of ZnO NP on *P. hysterophorus* root and stem length (cm) [a], biomass (g) [b]. All values are mean of three independent experiments (three replicates each). Asterisks indicate statistical differences ($p < 0.05$) from the control. Error bars represent standard deviations of triplicate tests

considerable amount of Fe, Pb, and Zn metals reduction rates R_{max} from FAS compared with Ni and Hg were less accumulated in *P. hysterophorus* due to the low R_{max} . The metal reduction rate by plant was R_{max} from the experiment we found the reduction rate R_{max} 71.6%, metals remained in FAS 2.17 $\mu\text{g/g}$ and 63.5 un-used ZnO NP at 5.5 g/Kg ZnO NP from the FAS ($R^2 = 0.99$) from the FAS at 5.5 g ZnO NP (Table 3). The metal reduction per gram of FAS metal removed amounted to 0.76 $\mu\text{g/g}$ biomass plant, which was close to the theoretical metal reduction from FAS with 5.5 g ZnO NP, i.e., 47% metals removed [28]. Among various terrestrial plants *P. hysterophorus* was found to be the most significant accumulator with hyperaccumulation factors 4.53 and > 2 for various heavy metals [28, 29]. Similar observations by other researchers have been reported accumulation factor < 2 [30, 31]. Table 4 shows the comparison of maximum accumulation of metal obtained in this study with the literature data from metals reduction and toxicity on plants and soil with other studies [3, 7–9, 11, 12, 14, 22, 27, 28, 32–34]. Clearly, metals and ZnO NP flyash enhance accumulation achieved metals reduction than that from phytoremediation with the soil by plants (Table 4).

Effects of ZnO NPs on ROS

The ROS induced by ZnO NP with FAS was reported to be one of the reasons for their toxicity, which caused the loss of cell viability [32]. ZnO NPs were regarded as an exogenous source of ROS for cells or organisms in some previous reports [33]. As seen in Figs. 3 and 8, an increase of the intracellular ROS production was observed with the increase of ZnO NPs. Usually, ROS, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\text{OH}^\cdot$), are produced in the presence of oxygen [30, 31]. In this experiment, the MDA and ascorbic acid level slowly increases with ZnO NPs 3.5 to 5.5 g were significant and decreased when the plants were subjected to high level of amendments with 10.5–15.5 gZnO NP ($p < 0.05$) (Fig. 8a). ROS production in plants is regulated by the activities of a particular group of oxidant enzymes [16]. The first line of defense against ROS-mediated toxicity is achieved by SOD catalyzes the dismutation of superoxide radicals to H_2O_2 and O_2 ($p < 0.05$). From the Fig. 8, significant increase in SOD activity observed 62% occurred at 5.5 g ZnO NP exposure as compared to control. *P. hysterophorus* at 3.5 to 5.5 g ZnO NP may be a part of this defense mechanism which

Fig. 7 Effect of different doses of ZnO NP on *P. hysterophorus*, photosynthetic area and total chlorophyll (mg g^{-1}) [A], chlorophyll a and chlorophyll b (chlorophyll content in mg g^{-1}) [B]. Asterisks indicate statistical differences ($p < 0.05$) from the control. Error bars represent standard deviations of triplicate tests

Table 3 Mass balance of metals accumulation and metals reduction pathways, ZnO NP utilization in fly ash soil (FAS) of *P. hysterothorus* through experimental periods (n = 3, mean ± SD)

ZnO NP g/Kg	Metals in FAS mg/g	Accumulation in Plant µg/g	Reduction Biomass %	Removal metals %	Metals remained in FAS µg/g	R_{max} % g plant biomass	Recovery metals µg/g	R2
Control	951	115	135	15	27	11	35	0.95
1.5	9.57 ± .843	8360 ± 456	47 ± 2.3	45.8 ± 27.4	539.9 ± 111.4	36.4	215.5 ± 1.5	0.99
2.5	9.65 ± .956	3145 ± 535	39 ± 2.5	63.9 ± 14.5	634.5 ± 110.2	40.1	125.8 ± 3.2	0.95
3.5	4.73 ± .368	1123 ± 345	27 ± 3.2	71.3 ± 12.5	125.3 ± 8.5	51.3	135.3 ± 5.5	0.95
5.5	4.98 ± .427	1894 ± 485	15 ± 1.6	98.7 ± 38.6	2.17 ± 2.9	71.9	63.5 ± 1.2	0.99
10.5	5.58 ± .673	645.5 ± 59	41 ± 7.5	42.5 ± 13.3	41.5 ± 2.3	26.4	345.6 ± 6.9	0.95
15.5	6.19 ± .159	123.90 ± 13	65 ± 15.8	37.5 ± 29.5	23.5 ± 3.5	19.8	553.5 ± 8.5	0.98

was develop [34]. Similar increase in SOD content in response to the ZnO NP had been reported earlier [30, 31]. Recent evidences that have indicated an elevated ZnO NP concentration was correlated with the ability of plants to withstand a metal-induced oxidative stress [13]. The H_2O_2 level in the FAS ZnO NP exposed *P. hysterothorus* was significantly higher than in the 10.5 (Fig. 8b). Similar trends have also been observed in response to other metals [30, 31]. It seems that the H_2O_2 level in the plants of exposed to 5.5 g ZnO NP amendment showed safeguard the cellular activity and did not cause any detrimental oxidative damage to the plant (Fig. 8b). Oxidative damage may occur by either the uncontrolled production or efficient scavenging of ROS by the oxidants at 10.5–15.5 g ZnO NP [21, 30, 31].

In a recent study [7] also reported that the released metals from FAS and ZnO NPs played an important role on causing the adverse effect of ZnO NPs on the performance of phytoremediation process. In the literature the toxicity of ZnO NPs in plants was also observed to come from the released Zn^{2+} , but those studies focused on accumulation of metals instead of toxicity function [7]. The ROS reduced by ZnO NPs was reported to be one reason for their influences on metals accumulation [7, 22], (Fig. 3). ZnO NPs were regarded as an exogenous source of ROS for cells or organisms in some previous reports [7]. As seen in Figs. 3 and 8, an increase of the intracellular oxidative production was observed with the increase of ZnO NPs and release of metals from FAS.

Table 4 Comparison of maximum metals accumulation and reduction from using various plants and nanoparticles

Studies	Plants	Metals Toxicity	Metals Reduction rates	References
Flyash affected area	Algae and plants	Efficient accumulation	Remediate metals FAS	[3]
Silver nanoparticles	Bishop pine	57% reduced biomass	350 mg in soil reduces 57–72%	[7]
silver nanoparticles	live plants.	12.4 wt.% silver	13.6 wt.% silver	[8]
CuO, ZnO and TiO ₂ metal nanoparticles	<i>Pseudokirchneriella subcapitata</i> lettuce seed	72 h EC50 approximately 0.04 mg Zn/l 1000 mg/kg CuO and ZnO NPs	did not differ approximately 0.02 mg Zn/l Effect on growth	[9] [11]
ZnO nanoparticles	<i>Lolium perenne</i>	Reduced root tips shrank, and root	97% Removal	[12]
Cd ²⁺ and Zn ²⁺	<i>Thlaspi caerulescens</i>	High-Zn-grown (500 microm Zn) plants were found to be more Cd-tolerant	(109)Cd root flux, (500) Zn	[14]
Metal oxide nanoparticles	seawater, lagoon, river, and groundwater	nanoparticles (TiO ₂), ZnO and CeO ₂)	Aggregation and rate of sedimentation	[22]
CuO and ZnO nanoparticle	Ps can induce ROS	CuO or ZnO NPs with high reactive activity	NPs role in inducing toxicity	[27]
ZnO engineered nanoparticles	<i>Brassica juncea</i> J.	ZnO ENPs (0, 200, 500, 1000, 1500 mg/l) for 96 h	<i>Brassica juncea</i> accumulate highest 89%	[32]
ZnO nanoparticles	<i>Zea mays</i>	1000 mg/kg CuO and ZnO NPs	Root uptake 8.6% to 43.5%	[33]
ZnO nanoparticle	<i>Zea mays</i>	ZnO NPs at 0–800 mg kg ⁻¹	With ZnO NP root and shoot biomass production	[34]
ZnO NP	<i>Parthenium hysterothorus</i>	Fe 79.6%; Zn 88.5%; Cu 67.5%; Pb 93.6%; Ni 43.5% and Hg 39.4% at 5.5 g ZnO NP	Metal reduced upto 98.7%	Present study

Fig. 8 Effect of ZnO NP on *P. hysterothorus* MDA (mmol g^{-1}) and Ascorbic acid (mg g^{-1} FW) [A], H_2O_2 (nmol g^{-1} FW) and SOD (mg^{-1} protein). Asterisks indicate statistical differences ($p < 0.05$) from the control. Error bars represent standard deviations of triplicate tests

Mass balance

A mass balance was performed on the system in order to removal pathway of metals from FAS and of an average of 92.9% metals were taken up by plants. No lag time existed in tests without ZnO NP and FAS; the reduction rate of metals was $0.76 \mu\text{g/g}$ biomass of *P. hysterothorus* (Table 3). From the experiment we found that the reduction rate R_{max} 71.6%, metals remained in FAS $2.17 \mu\text{g/g}$ and 63.5 un-used ZnO NP at 5.5 g/Kg ZnO NP from the FAS ($R^2 = 0.99$). The amount of metals in FAS was 5960 and transferred in plant $1894 \mu\text{g/g}$ removed from FAS were much higher than that utilized by plant biomass 85%. An average of about 60–83% of Zn removed from soil was utilized by plants depending on the plant type. The results also showed that plant uptake of Zn were dependent on the applied doses of ZnO NP 10.5 to 15.5 g reduced biomass up to 35%. The amount of Ni removed from FAS was also higher than that utilized by the plants from FAS. The amounts of Pb and Hg removed from FAS by the plants ranged from 98% and 34% at 5.5 g ZnO NP showed the highest and lowest reduction rates. The initial metal in FAS, transfer of metals in plants biomass $780 \mu\text{g/g}$ and remained in FAS $17.5 \mu\text{g/g}$ via metals reduction rates was $92 \mu\text{g/g}$ of biomass at 5.5 g ZnO NP. At 5.5 g ZnO NP, the biomass increased upto 78%; the metal reduction 98.7% with the ZnO NP share of 100%. Hence, 10.5–15.5 g ZnO NP mitigated metal reduction and biomass production and reduced ROC efficiency [26, 35]. The mass balances (Table 3) accounted for the ZnO NP needed for metals reduction and plant biomass production [30, 31, 35].

Conclusions

The role of ZnO-NP with FAS containing metals led to the metals significantly accumulated in plant, which can be a potential phytoremediation of 98.2% for Pb, 86% for Ni, 91.4% for Zn, and 39.2% for Hg by *P. hysterothorus*. From the experiment we found the reduction rate R_{max} for Fe 16.4; Zn 21.1; Pb 41.9; Hg 19.1 was higher than Ni 6.4 and Cu 11.3

from the FAS at 5.5 g ZnO-NP whereas, the reduction rate of Pb showed highest. The presence of 1.5 g of ZnO-NP did not affect effective accumulation of metal, but 5.5 g of ZnO-NP show tremendous metals reduction 96.7% and at 10.5 g of ZnO-NP reduced biomass 18.3% and 73.8%. The mass-balance technique can be used routinely to determine accumulation of heavy metals from FAS to the plant influence with the role of ZnO-NP and as an estimate of toxicity to ROS activities. Further investigations with enzyme and tolerance in situ indicated that higher concentration of 10.5–15.5 g ZnO-NP decreased the activity of SOD. With 5.5 g ZnO-NP when biomass increased upto 78%; the metal reduction 98.7% with the ZnO-NP share of 100%.

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Compliance with ethical standards

Disclosure statement For all authors are approved, there is no conflict of interest including experimental, financial and with other issues to carried out this study.

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