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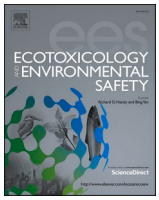

Microwave seed priming and ascorbic acid assisted phytoextraction of heavy metals from surgical industry effluents through spinach

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ABSTRACT

The prevalence of inorganic pollutants in the environment, including heavy metals (HMs), necessitates a sustainable and cost-effective solution to mitigate their impacts on the environment and living organisms. The present research aimed to assess the phytoextraction capability of spinach (*Spinach oleracea* L.), under the combined effects of ascorbic acid (AA) and microwave (MW) irradiation amendments, cultivated using surgical processing wastewater. In a preliminary study, spinach seeds were exposed to MW radiations at 2.45 GHz for different durations (15, 30, 45, 60, and 90 seconds). Maximum germination was observed after the 30 seconds of radiation exposure. Healthy spinach seeds treated with MW radiations for 30 s were cultivated in the sand for two weeks, after which juvenile plants were transferred to a hydroponic system. Surgical industry wastewater in different concentrations (25 %, 50 %, 75 %, 100 %) and AA (10 mM) were provided to both MW-treated and untreated plants. The results revealed that MW-treatment significantly enhanced the plant growth, biomass, antioxidant enzyme activities and photosynthetic pigments, while untreated plants exhibited increased reactive oxygen species (ROS) and electrolyte leakage (EL) compared with their controls. The addition of AA to both MW-treated and untreated plants improved their antioxidative defense capacity under HMs-induced stress. MW-treated spinach plants, under AA application, demonstrated relatively higher concentrations and accumulation of HMs including lead (Pb), cadmium (Cd) and nickel (Ni). Specifically, MW-treated plants with AA amendment showed a significant increase in Pb concentration by 188 % in leaves, Cd by 98 %, and Ni by 102 % in roots. Additionally, the accumulation of Ni increased by 174 % in leaves, Cd by 168 % in roots, and Pb by 185 % in the stem of spinach plant tissues compared to MW-untreated plants. These findings suggested that combining AA with MW irradiation of seeds could be a beneficial strategy for increasing the phytoextraction of HMs from wastewater and improving overall plant health undergoing HMs stress.

1. Introduction

Excessive concentrations of heavy metals (HMs) and metalloids are highly toxic, posing significant environmental impacts and ongoing threats to food security due to their persistent accumulation and non-biodegradable nature (Yong et al., 2010b; Khalid et al., 2017; Saleem et al., 2020; Tow et al., 2018; Qureshi et al., 2024; Shaghaleh et al., 2024). These undesirable metals enter the food chain and the environment through various pathways and can adversely affect the all living organisms. The pollution of water resources by HMs and metalloids have intensified due to extensive industrialization, urbanization, agricultural practices, and climate change (Abeed et al., 2022; Yong et al., 2010b;

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Hossain et al., 2020). Water resources contaminated with HMs have been increasing due to extensive industrialization, urbanization, agricultural practices, and climate changes (Azam et al., 2015, Khanna et al., 2019; Shaghaleh et al., 2024). Also, the global demand for medical technologies has driven substantial growth in the surgical equipment manufacturing sector, which has experienced an annual expansion rate of 7.8 % in recent years (Tariq et al., 2013). Like the world's boost of the surgical industry, Pakistan alone have established network of 2500 active surgical units all over the country (Junaid et al., 2016). Stainless steel is extensively used, comprising along titanium (Ti), aluminium (Al), tungsten (W), beryllium (Be), copper (Cu), molybdenum (Mo), and cobalt (Co), and chromium (Cr) (Spry, 2005). Cadmium (Cd), nickel (Ni), manganese (Mn) and lead (Pb) are also crucial metals used in surgical equipment manufacturing. These industries used a huge quantity of water in the process of manufacturing equipment and directly discharge their effluents into water bodies and on soil due to lack of proper water treatment systems. Discharge of excessive effluents can lead to environmental contamination, due to the presence of toxic metals like Pb, Cd, and Ni entering into the environment from various industrial processes (Masood et al., 2023; Tariq et al., 2013b). These HMs can later accumulate in the soils due to their high persistence; becoming readily available under certain scenarios for uptake by plants and subsequently entering the humans via the food chain (Liu et al., 2012; Song et al., 2019; Sigamani et al., 2024).

Lead (Pb), is one of the heaviest divalent metals, which can significantly affect the plant seed germination, growth, and physiological processes like photosynthesis and respiration (Gupta et al., 2024). Studies have revealed the accumulation of high levels of Pb in the roots of various plants, such as *Lactuca sativa* (L.) (Ikkonen and Kaznina, 2022) and *Brassica juncea* (L.) (Shehzad et al., 2022). Similarly, higher concentrations of Ni in the effluents used as growth medium can hinder the growth, seed germination, and may cause oxidative damage in the plants (Hassan et al., 2019). Research has revealed that high Ni concentrations reduced growth in wheat (*Triticum aestivum* L.) plants by causing excessive oxidative stress (Uruç Parlak, 2016). Cd accumulation in edible crops is a major concern for human exposure (Liu et al., 2012; Wang et al., 2023), with studies showing disruptions in chloroplast structures, lower chlorophyll contents and reduced growth in *Zea mays* (L.); attributed to high Cd levels (Rigby and Smith, 2020).

Uncontrolled industrial aqueous effluents polluted with HMs is a global issue. To combat HMs pollution of industrial effluents and soils accumulated with HMs, various physiochemical and biochemical remediation methods can be employed; whereas the phytoremediation is considered cost-effective, environmentally friendly, efficient, and easy to implement compared with all other treatments (Razzak et al., 2022; Song et al., 2019). Phytoremediation, using plants to mitigate pollutants via adsorption, mobilization, and extraction, is a particularly promising approach (Yong et al., 2010b; Kamran et al., 2020, Zhang et al., 2020). In phytoremediation, several plant species, such as *Brassica juncea*, *Juncus maritimus*, *Schima superba*, and *Alyssum murale* can be used for delivering effective and sustainable results (Yan et al., 2020). Importantly, plant species selected for phytoremediation should have the potential to accumulate a high level of the target pollutant, while the pollutant should not be the growth limiting factor. Secondly, phytoremediation should have a high threshold level for the target pollutant, and it should be easily cultivable, preferably native species, and adapted to the local environment. Above all, these plants should be resistant to pathogens and pests, and with the preferred traits of a highly effective hyperaccumulator (van der Ent et al., 2013; Ali et al., 2013).

Spinach, *Spinacia oleracea* (L.), belongs to the family Amaranthaceae, and is a commonly cultivated as an edible plant around the globe especially in Asia due to its comparatively fast growth and higher biomass production. Spinach can uptake the HMs with faster rate due to its wider lamina and excessive uptake of water content from soil (Agarwal et al., 2018). Spinach having characteristics of faster growth and response towards various HMs stress been remained in consideration in research studies previously (Khaliq et al., 2022). Spinach can be subjected to higher HMs stress owing to its powerful antioxidant defence mechanisms (Shahid et al., 2020) which makes it a potential candidate for the treatment of contaminated sites (Khaliq et al., 2022). Moreover, the phytoremediation process of the plants can be improved by the addition of chelating agents those have specific mechanisms for metal chelation (Paz-Ferreiro et al., 2014).

Several chelating agents are used to extract HMs by increasing the bioavailability for plants to absorb through roots and translocation to shoots, that enhance the phytoextration process (Alwutayd et al., 2024; Diarra et al., 2021). The frequent use of chelating agents in home, farming, and industrial contexts can produce HMs-complexes that are responsible for the bioavailability of HMs in water (Qiang et al., 2018). Due to their unique ability in mobilizing metals, the chelator induced phytoextractions do raise possible environmental concerns (Ben Massoud et al., 2019). Ascorbic acid (AA) is more biodegradable as compared to other chelating agents with larger molecular mass and complex chemical formula to bind HMs for their extraction (Thinh et al., 2021). The addition of AA improved the photosynthesis process and can prevent the oxidative damage in plants undergoing HMs stress (Gallie, 2013), and the growth-promoting role of AA for the fenugreek plants exposed to Cr stress has been reported by Azzouzi et al. (2024).

Electromagnetic radiation of various wavelengths and frequencies have many important characteristics and their application in plant growth systems is not much reported in the literature. Although previous research has highlighted the positive effects of radiations on growth, chlorophyll content, and antioxidant enzymes in plants, the effects of microwave (MW) radiations on plant growth undergoing HMs stress remained understudied (Gupta et al., 2013; Abbey et al., 2017). MW-treated okra plants, for instance, exhibited a rapid increase in protein contents and photosynthetic pigments (Iwuala et al., 2021). However, the combined application of MW radiation and chelating agents for phytoremediation remained unexplored. The present study was therefore planned with the prime objective to assess the impacts of MW radiation on spinach seed germination, and evaluation of surgical wastewater effects on spinach's morpho-physiological and biochemical attributes. More importantly, to assess the phytoextraction potential of spinach plants with AA amendment, and to examine the combined effects of MW radiation and AA chelator on the phytoextraction potential of spinach plants cultivated using wastewater derived from surgical processes.

2. Material and methods

2.1. Seeds collection, preparation and cultivation

Seeds of the spinach were collected from Ayub Agriculture Research Institute (ARRI) Faisalabad and disinfected by treating them with 3.0 % H_2O_2 for 5 minutes, followed by washing and rinsing with deionized water. Subsequently, the seeds were air dried and exposed to 2.45 GHz microwave radiation for varying durations: 0, 15, 30, 45, 60, and 90 seconds by using the microwave oven. Afterward, the MW-treated seeds were placed in methanol solution and incubated at 25 °C in an IB-05 G incubator made by JEIO Tech, Korea, for a period of two weeks to assess germination rates. The germination rates observed were 7.76, 8.33, 9.33, 6.33, 4.33 and 2.67 out of 10 at 0, 15, 30, 45, 60, and 90 seconds of MW, respectively, as the mean of triplicate. Maximum seed germination occurred at 30 seconds of exposure to a frequency of 2.45 GH.

Seeds of spinach irradiated for 30 seconds were selected for cultivation in sand pots for two weeks. After the germination, the juvenile plants were transferred to a hydroponic system made up of glass square bowls with capacity up to 5.0 L filled with water and Hoagland's nutrient solution. The composition of the Hoagland's nutrient solution was as 0.22 mg/L $(NH_4)_6Mo_7O_{24}$, 0.02 mg/L $ZnSO_4{\cdot}7\ H_2O$, 2.86 mg/L H_3BO_3, 0.08 mg/L $CuSO_4{\cdot}7H_2O$, 20 mg/L $Na_2Fe{\cdot}EDTA$ and 2.13 mg/L

$MnSO_4 \cdot 4\ H_2O$. The experimental setup was aerated using constant airflow pumps coupled with the assembly. Surgical industry wastewater (WW) alone and in combination with AA was applied to the hydroponic system having control and MW treated spinach plants. Each treatment was applied in triplicate, and the experimental setup followed a complete randomised design (C.R.D.).

2.2. Wastewater sampling and the Application of Treatments

Wastewater samples of the surgical instrument manufacturing industry were taken from the Sialkot city. Collected wastewater's physicochemical properties were analysed by standard methods of analysis of wastewater (Clescerl et al., 1998). The pH was evaluated by using a desktop pH meter. The chemical oxygen demand (COD) was determined by using chemical oxygen demand COD plus colorimeter (model: La-motte, code-1922/1922-EX2). Biological oxygen demand (BOD) was determined titrimetrically, and total suspended solids (TSS) were measured by gravimetrically (Supplementary Table 1).

After spending two weeks in hydroponic system, the MW-treated and untreated juvenile plants were treated with various concentrations of surgical effluents and ascorbic acid (AA) with three replications as; T_1: control, T_2: MW30, T_3: A.A 10, T_4: MW 30 + A.A 10, T_5: W.W 25 %, T_6: W.W 25 % + MW 30, T_7: W.W 25 %+ A.A 10, T_8: W.W 25 % + MW 30 + A.A 10, T_9: W.W 50 %, T_{10}: W.W 50 % + MW 30, T_{11}: W.W 50 % + A.A 10, T_{12}: W.W 50 % +MW 30 + A.A 10, T_{13}: W.W 75 %, T_{14}: W.W 75 % + MW 30, T_{15}: W.W 75 % + A.A 10, T_{16}: W.W 75 % +MW 30 + A.A 10, T_{17}: W.W 100 %, T_{18}: W.W 100 % + MW 30, T_{19}: W.W 100 % + A.A 10, T_{20}: W.W 100 % +MW 30 + A.A 10. For the next four weeks, all the treatments were exogenously provided to the hydroponic system at one-week intervals.

2.3. Evaluation of agronomic traits

After four weeks of continuous treatments, the plants were harvested and assessed for morpho-physiological and biochemical attributes. The fresh biomass of plant parts like root, stem, and leaves were measured with an analytical weighing balance of Sartorius company (Denmark). Similarly, dry biomass was also measured after drying the plant material in an oven at 70 ^{0}C until complete dryness and constant weight. A ruler was used to measure the length of plant shoot, root and leaf area.

To estimate the soluble protein content in the roots and leaves, the analysis was performed by following the methodology of Bradford (1976) where a standard (albumin) and a dye (Coomassie brilliant blue G-250) was used. The second-topmost and fully expanded leaves was assessed non-destrucively for foliar chlorophyll content (SPAD-502 m, Zhejiang Top Instruments Co. Ltd., China)(Yong et al., 2010a). To determine the carotenoids, chlorophyll a, chlorophyll b, and total chlorophyll contents in leaves with a spectrophotometer (UV-Visible, Halo DB20 / DB-20S, Dynamica Labs, London, UK), the protocols given by Arnon (1949) and Metzner et al. (1965) were followed; and with some minor modifications (Song et al., 2020).

2.4. Estimation of Antioxidant Enzymes catalase (CAT) and ascorbate oxidase

The activities of antioxidant enzymes such as catalase (CAT) and ascorbate oxidase (APX) in the spinach leaf and root were measured using the methods described by Nakano and Asada (1981) and Aebi (1984) respectively. The Zhang (1992) methodology was followed to assess the concentrations of peroxidases (POD) and superoxide dismutase (SOD).

2.5. Determination of Malondialdehyde (MDA), Hydrogen Peroxide (H_2O_2) and Electrolyte Leakage (EL)

The colorimetric approach suggested by Jana and Choudhuri (1981) was utilized to assess the H_2O_2 level in spinach roots and leaves. The content of MDA was assessed by using Heath and Packer's (1968) TBA reaction approach which has been further refined by Dhindsa et al. (1981) and Zhang and Kirkham (1994).

The EL in spinach roots and leaves were measured using the technique outlined by Dionisio-Sese and Tobita (1998).

Further calculations were carried out by the following equation,

$$EL = \left(\frac{EC1}{EC2}\right) \times 100$$

The EC1 is the initial conductivity, and EC2 is the final conductivity, which was measured by a conductivity meter 720 (INCOLAB Company, Kuwait).

2.6. Analysis of heavy metals in plant biomass

The oven dried-plant samples were milled into fine powder by using the coffee grinder. A portion of 0.5 g of each sample was taken and concentrated sulphuric acid (H_2SO_4) and hydrochloric acid (HCL) in the ratio of 3:1 were added into it and placed on a hot plate at 250–300 C^0 for complete digestion of the sample. Hydrogen peroxide (H_2O_2) 30 % was then added, drop by drop, until the digested sample was completely oxidized and decoloured. The samples were further analysed using atomic absorption spectrophotometer NOVA A400, made by Analytik Jena, Germany to determine the HMs concentration according to the technique described by Ehsan et al. (2013). All the treatments were analysed in triplicates and the results were calculated by plotting the standard calibration curve of standard solutions prepared for desired concentration.

Plant metal content (mg kg^{-1}) was calculated by multiplying the dilution factor by the digested sample's metal concentration (mg L^{-1}).

Where;

$$\text{Dilution factor} = \frac{\text{Total sample volume(mL)}}{\text{Plant material weight(g)}}$$

The metal accumulation in the entire plant body was estimated by using the equation given below (Zayed et al., 1998).

Metal Accumulation = plant organ's dry weight (g) × concentration of metal in plant organ (μg g^{-1})

2.7. Statistical Analysis

All the treatments were performed in triplicate and for all the treatments means of three replicates and standard deviation were calculated and presented in results. The Statistix 10.0 software was used for finding the significance ($p<0.05$) of results through analysis of variance (ANOVA) followed by Tukey's post hoc test.

3. Results

3.1. Preliminary study to determine the optimal microwave radiation exposure time

Seeds of spinach were irradiated with MW and the response for different time durations (15, 30, 45, 60 and 90 s) found is depicted as in Section 2.1. Observations confirmed that the longer irradiation of seeds resulted in suppression of seeds germination. Germination at 30 seconds, observed was maximum whereas a significant reduction in seed germination was observed at time interval of 60 s and 90 s when exposed to same frequency of 2.45 GHz. It may be concluded that observation of increase and decrease in seed germination may depend upon on both intensity and duration of exposure time of irradiation. Seeds with an optimized time of 30 s for germination were further selected and used in the hydroponic study under AA and WW.

3.2. Agronomic traits of the spinach plants

Previously optimized plants were subjected to the treatment of surgical industry effluents and AA as chelating agent. Spinach plants used in experiments were observed and agronomic traits were evaluated. Plant growth characteristics of spinach exhibited substantial reduction under wastewater stress (Supplementary Table 2). The growth attributes of spinach significantly decreased with increasing the concentration of wastewater in comparison to the control treatment. The addition of chelating agent, ascorbic acid (AA) 10 mM alone and in combination MW significantly improved the growth characteristics of spinach in seeds of 30 s irradiation. A linear trend of reduction in plant germination and growth is found in MW treated maximum effect was observed at 100 % WW as compared to the respective controls where plant height was decreased by 65 & 57 %, leaf area by 49 & 51 %, root length by 57 & 53 %, and number of leaves per plant by 53 & 48 % respectively. The combined effects of 30 s MW irradiated, AA (10 mM) under WW, the plant height increased in the range of 8–49 %, leaf area increased by 8–42 %, root length increased by 5–34 %, and number of leaves per plant by 7–50 % respectively compared to the corresponding treatments without AA and MW 30 s.

The spinach plant biomass decreased under WW treatment (Supplementary Table 2) and found it in a dose-dependent manner. Spinach plants treated with 100 % WW and MW showed maximum reduction in fresh weight of roots by 75 & 64 %, stem by 61 & 54 %, and leaves by 65 & 59 %, respectively compared with control treatment. Similarly, the dry weight of leaves was decreased by 73 & 61 %, stem by 58 & 53 % and root by 62 & 59 % compared with respective controls. The combined treatment of AA (10 mM) and MW 30 s under WW (25, 50, 75 & 100 %) significantly improved the fresh weight of roots by 10−95 %, stem by 8−50 %, leaves by 8−52 %, and the dry biomass of root by 9−48 %, stem by 6−43 %, leaves by 11−98 %, compared with the corresponding treatments without AA and MW 30 s.

3.3. Photosynthetic pigments, soluble proteins and carotenoids contents

Spinach plants under investigation in various treatments of WW, MW and AA and in control were analysed for carotenoids and chlorophyll contents to evaluate the oxidative damage caused by HMs to spinach (Fig. 1). Results showed more oxidative stress due to high doses of WW and also significantly reduces chlorophyll and carotenoid contents. When MW-treated and untreated plants were compared to controls, the maximum reduction was observed under WW 100 %, with reductions of 68 & 66 % for chlorophyll *a*, 70 & 64 % for chlorophyll b, 70 & 67 % for total chlorophylls, and 80 & 73 % for carotenoids, respectively. However, adding AA (10 mM) to both MW-treated and untreated plants under WW enhanced chlorophyll and carotenoids content compared to controls. The combined treatment of AA (10 mM) and MW 30 s under WW (25, 50, 75 & 100 %) significantly improved the chlorophyll *a* content in the range of 6−46 %, *chlorophyll b* 8−90 %, total chlorophylls 8−53 % and carotenoids 10−93 % as compared to the respective treatments without AA and MW 30 s.

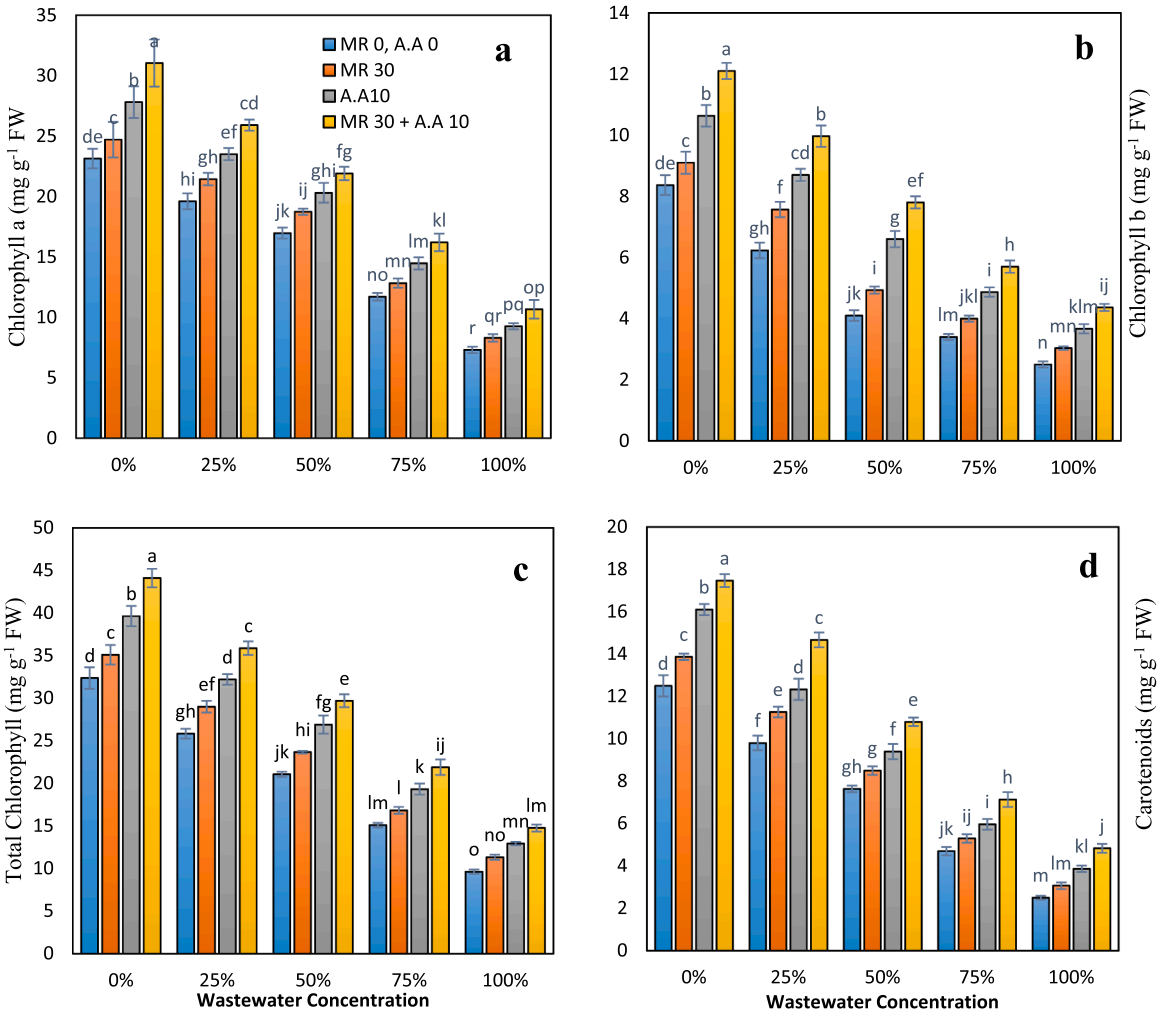

Fig. 1. The combined effects of MR and AA under surgical industry effluents on physiological traits of spinach, such as Chl a (a), Chl b (b), total Chl (c), and carotenoids (d), is presented as the mean of three replicates ± standard deviation. The significance of values at $P < 0.05$ is indicated by different small alphabets.

Spinach showed a significant decrease in SPAD value and SP content of both roots and leaves compared with control (Fig. 2). MW treated and untreated plant of Spinach showed maximum reduction at WW 100 % in SP of roots by 58 % & 56 %, SP in leaves by 72 & 68 %, and SPAD value by 80 & 79 % respectively compared with control plants. On other hand, both MW 30 s treated and untreated spinach plants showed significant increase in SPAD and SP content under AA (10 mM) compared with controls. The combined treatment of AA (10 mM) and MW 30 s under WW (25, 50, 75 & 100 %) significantly enhanced the SP content of leaves in the range of 17–67 %, SP in the root by 14–71 %, and in SPAD value by 19–70 % as compared with respective treatments without AA and MW 30 s.

3.4. MDA, H_2O_2 and EL concentration

Increased lipid peroxidation and EL were observed in the roots and leaves of both MW-treated and untreated Spinach plants (Fig. 3). Correspondingly, the maximum increase in spinach (MW 30 s treated and untreated) was recorded at WW 100 % for EL in roots (184 & 299 %), EL in leaves (159 & 260 %), H_2O_2 in roots (138 & 184 %), H_2O_2 in leaves (235 & 365 %). MDA in root (185 & 267 %) and MDA in leaves (166 & 230 %) compared with control plants. The application of AA 10 mM and MW 30 s under different concentrations of WW (0, 25, 50, 75, and 100 %) significantly decreased the EL and lipid peroxidation in both MW-treated and untreated Spinach. The combined treatment of AA (10 mM) and MW 30 s under WW (25, 50, 75 & 100 %) significantly decreased the EL content in root by 5–42 %, EL, in leaves by 8–42 %, H_2O_2 in root by 6–32 %, H_2O_2 in leaves by 8–46 %, and MDA in root by 9–52 % and MDA in leaves by 5–42 % as compared with respective treatments without AA and MW 30 s.

3.5. Antioxidant defence system of plants

As the concentration of applied WW increased, the antioxidative responses of spinach plants treated with and without MW also increased (Fig. 4). In both MW-treated and untreated plants, the enzymatic activities begin to rise at low concentrations (25 %) of HMs contaminated WW, peak at 50 % WW and then begin to decline towards 100 % of the WW. Under WW, the maximum increase in antioxidant enzymes activities of plants treated with and without MW was recorded where APX in leaves was increased by 13 & 8 %, APX in root by 84 & 37 %, CAT in leaves by 2 & 2 %, CAT in roots by 70 & 46 %, POD in leaves by 58 & 53 %, POD in root by 6 & 5 %, SOD in leaves by 72 & 34 %, SOD in root by17 & 21 % respectively compared with control. Further, the combined application of AA (10 mM) and MW (30 s) under WW (25, 50, 75 & 100 %) significantly enhanced the content of CAT in leaves by 5–52 %, CAT in the roots by 6–50 %. APX in leaves by 7–86 %, APX in root by 7–62 %, POD in leaves by 8–42 %, POD in root by 6–30 %, SOD in leaves by 6–96 %, and SOD in the root by 4–34 % respectively as compared with respective treatments without AA and MW 30 s.

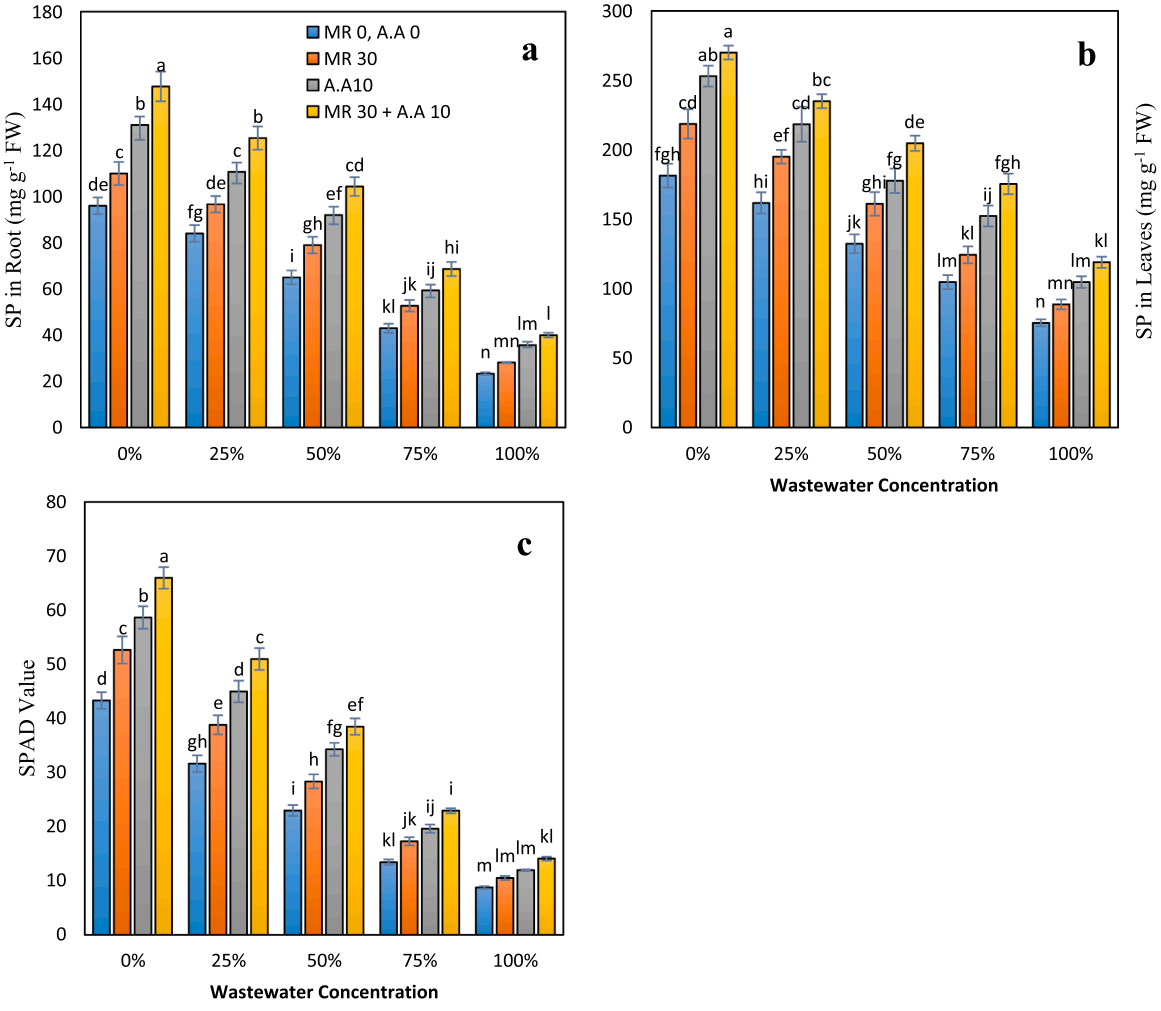

Fig. 2. The combined effects of MR and AA under surgical industry effluents on physiological traits of spinach, such as SP in root (a), SP in leaves (b), and SPAD value (c), is presented as the mean of three replicates ± standard deviation. The significance of values at $P < 0.05$ is indicated by different small alphabets.

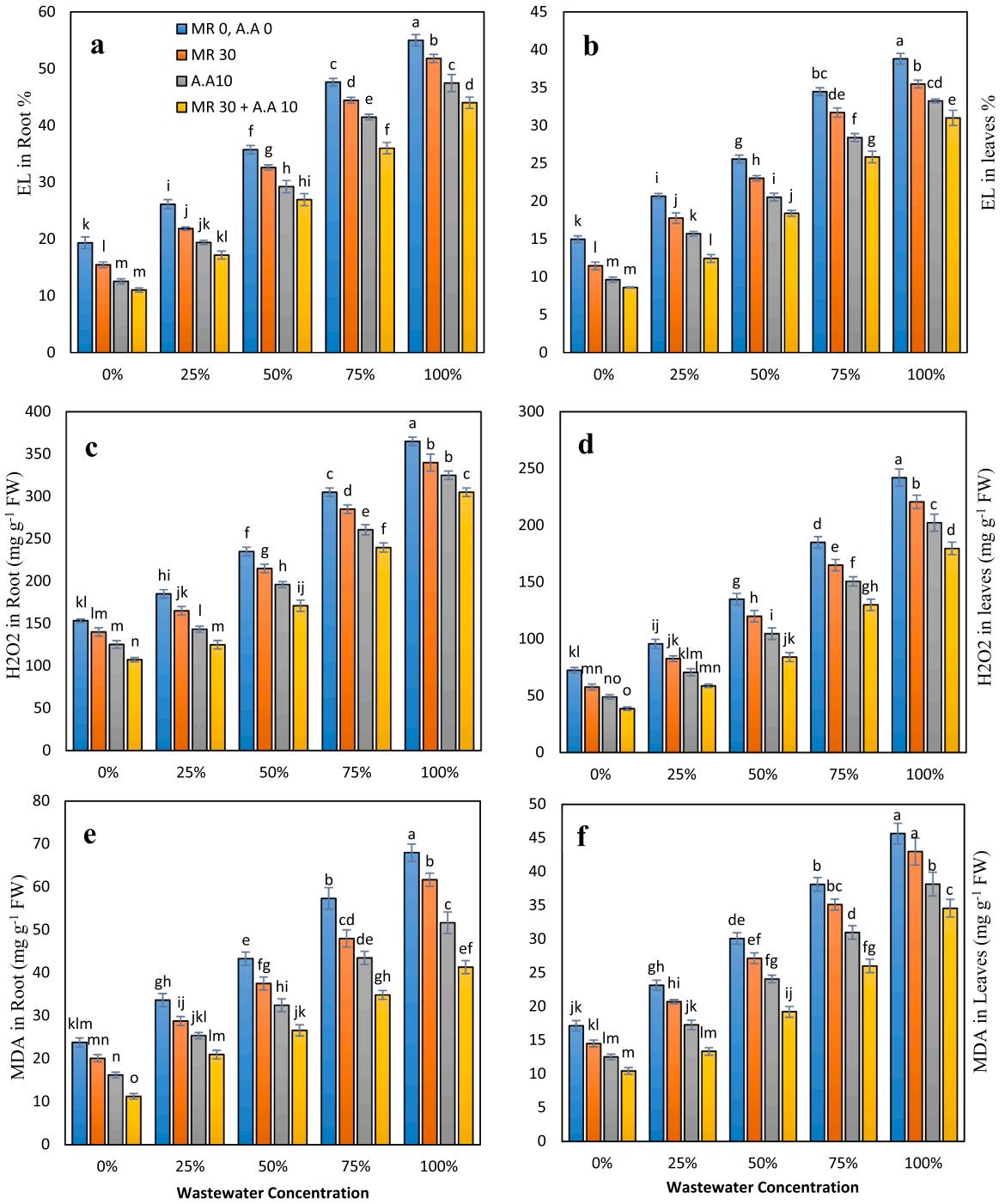

Fig. 3. The combined effects of MR and AA under surgical industry effluents on molecular attributes of spinach, such as EL in root (a), EL in leaves (b), H2O2 in root (c), H2O2 in leaves (d), MDA in root (e), and MDA in leaves (f), is presented as the mean of three replicates ± standard deviation. The significance of values at $P < 0.05$ is indicated by different small alphabets.

3.6. The levels of HMs in plants

The levels of HMs in the spinach plants increased significantly in a dose dependant manner with increasing concentration of applied WW (Tables 1-3). The results showed that in both MW-treated and untreated plants, the concentration and accumulation of Ni, Cd, and Pb were enhanced with and without AA 10 mM application. The maximum increase in concentration of HMs of both MW-treated and untreated plant was recorded at WW 100 % where Ni concentration in root was increased by 644 & 393 %, in stem by 435 & 274 %, in leaves by 557 & 299 %, Cd concentration in root increased by 572 & 316 %, in stem by 521 & 317 %, in leaves by 462 & 271 %, Pb concentration in root increased by 469 & 234 %, in stem by 691 & 334 %, in leaves by 1104 & 372 % respectively compared with control plants. Similarly, parallel to this, the accumulation of Ni in root increased by 222 & 134 %, in stem by 160 & 101 %, in Leaves by 121 & 90 %, Cd accumulation in root increased by 192 & 99 %, in stem by 201 & 123 %, in leaves by 89 & 77 %. accumulation of Pb in root increased by147 & 59 %, in stem by 283 & 132 %, in leaves by 306 & 125 % respectively compared with control.

Furthermore, the combined treatment of AA (10 mM) and MW 30 s under WW (25, 50, 75 & 100 %) significantly increased the

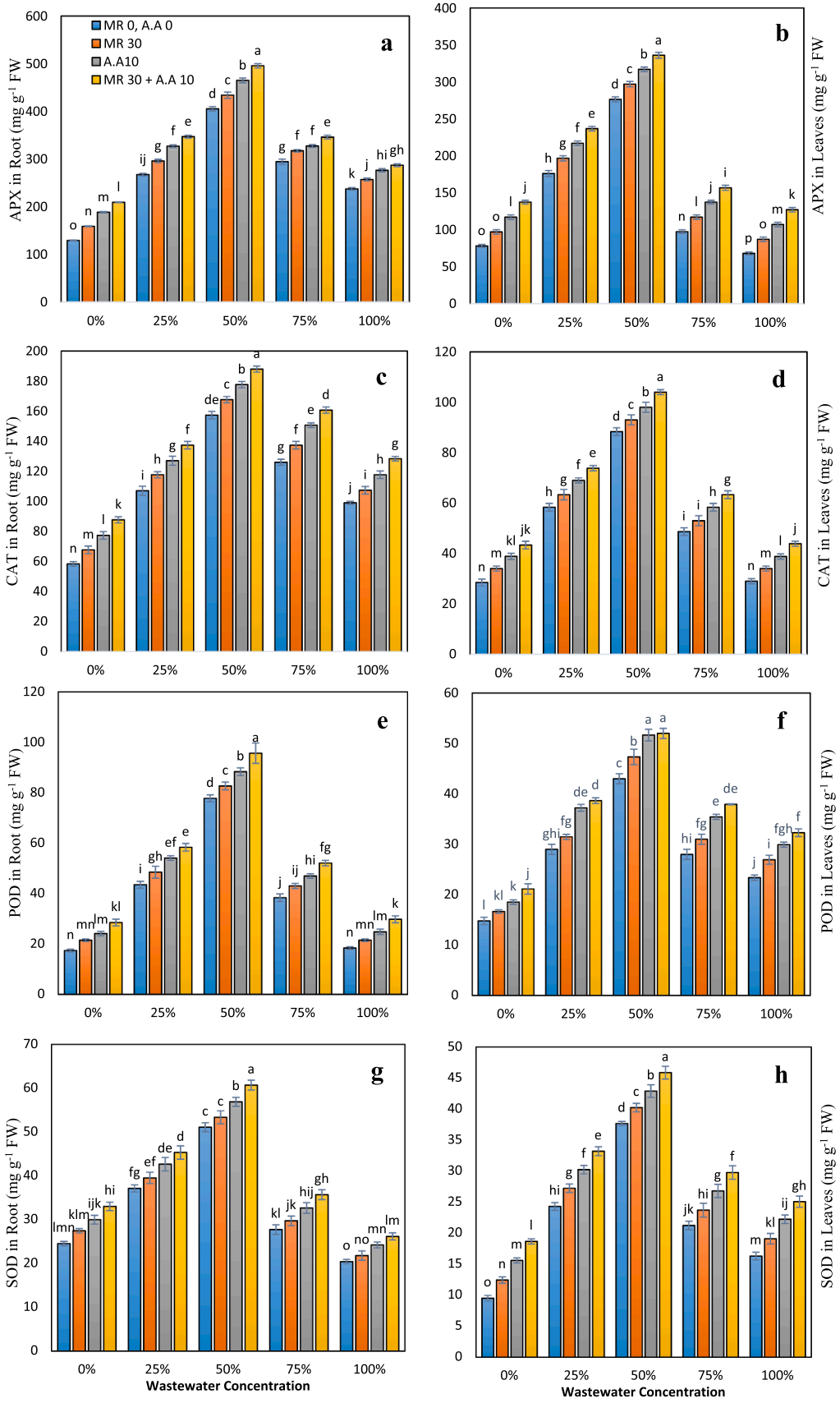

Fig. 4. The combined effects of MR and AA under surgical industry effluents on the antioxidative defense capacity of spinach, such as APX in root (a), APX in leaves (b), CAT in root (c), CAT in leaves (d), POD in root (e), POD in leaves (f), SOD in root (g), and SOD in leaves (h), is presented as the mean of three replicates ± standard deviation. The significance of values at $P < 0.05$ is indicated by different small alphabets.

Table 1
The combined effects of microwave radiations (MR) and ascorbic acid (AA) under surgical industry effluents on the Ni concentration and accumulation by spinach is presented as the mean of three replicates ± standard deviation. The significance of values at $P < 0.05$ is indicated by different small alphabets.

Treatments	Nickel Concentration (mg kg^{-1})					Nickel Accumulation (μg plant^{-1})				
	0 %	**25 %**	**50 %**	**75 %**	**100 %**	**0 %**	**25 %**	**50 %**	**75 %**	**100 %**
	Leaf					**Leaf**				
MR0, A.A 0	0.00 ± 0.00 n	136.81 ± 15.04 m	340.58 ± 10.45j	628.33 ± 27.53 g	898.33 ± 12.58c	0.03 ± 0.02i	1256.29 ± 170.59 h	2487.97 ± 163.38fg	3183.66 ± 159.06ef	2785.66 ± 128.55ef
MR 30	0.00 ± 0.00 n	171.66 ± 12.58Lm	385 ± 15j	701.66 ± 17.55 f	953.33 ± 15.27b	0.07 ± 0.04i	1806.66 ± 218.30gh	3145.16 ± 165.83ef	4422.83 ± 250.99c	3973.66 ± 205.98 cd
A.A10	0.05 ± 0.03 n	216.66 ± 15.27kl	456.66 ± 25.16i	760 ± 20e	1001.66 ± 17.55b	0.78 ± 0.56i	2566.66 ± 238.30 f	4142 ± 273.00 cd	5526 ± 335.54b	5209.83 ± 191.43b
MR 30 + A.A 10	0.07 ± 0.02 n	266.67 ± 12.58k	531.66 ± 32.53 h	821.66 ± 22.54d	1058.33 ± 12.58a	1.10 ± 0.32i	3442.83 ± 254.25de	5593.33 ± 607.99b	6685.16 ± 309.01a	6527.66 ± 238.60a
	Stem					**Stem**				
MR 0, A.A 0	0.00 ± 0.00o	249.43 ± 11.02 n	548.33 ± 17.56 L	933.33 ± 32.53 h	1335 ± 32.78d	0.07 ± 0.017 L	3102.2 ± 196.07k	5357.33 ± 279.76ij	7498.83 ± 308.68fg	8054.66 ± 220.69efg
MR 30	0.19 ± 0.32o	317.66 ± 7.50 n	648.33 ± 33.29k	1039.66 ± 37.28 g	1459 ± 25.94c	3.11 ± 5.16 L	4291 ± 260.00jk	6944.33 ± 575.18ghi	9226.13 ± 655.27de	9829.2 ± 617.16 cd
A.A10	0.8 ± 0.1o	395.1 ± 4.85 m	731.66 ± 30.13j	1131.66 ± 30.13 f	1573.33 ± 30.55b	13.60 ± 2.05 L	5833.81 ± 79.84hij	9105.16 ± 667.81def	117,77.33 ± 764.34b	12,012.67 ± 465.01b
MR 30 + A.A 10	1.08 ± 0.12o	466.66 ± 15.27 m	830 ± 30i	1227.66 ± 41.54e	1746.66 ± 15.27a	19.59 ± 2.11 L	7361 ± 401.28gh	11,215 ± 820.04bc	14,214 ± 1101.22a	14,734.33 ± 832.95a
	Root					**Root**				
MR 0, A.A 0	0.1 ± 0.05 n	260.1 ± 20.0 m	860 ± 20 J	1408.33 ± 33.29 g	1936.77 ± 50.17d	0.82 ± 0.38k	1902.73 ± 224.01j	5304.66 ± 229.99gh	5919.33 ± 420.38gh	6132.67 ± 256.42fgh
MR 30	0.38 ± 0.23 n	340 ± 20Lm	956.66 ± 27.53 J	1505 ± 22.91fg	2156.91 ± 66.99c	3.56 ± 2.09k	2790.66 ± 232.01ij	6445.16 ± 378.12efg	7227 ± 410.16def	7550.54 ± 359.29cde
A.A10	0.72 ± 0.06 n	423.33 ± 25.16 L	1086.66 ± 50.33i	1593.33 ± 35.11 f	2426.71 ± 25.08b	7.49 ± 0.96k	3927 ± 337.98i	7904.66 ± 635.25 cd	8713.66 ± 430.85c	9952.19 ± 514.06b
MR 30 + A.A 10	0.95 ± 0.04 n	526.66 ± 27.54k	1275 ± 27.83 h	1710 ± 30e	2596.80 ± 55.08a	10.96 ± 0.85k	5199.16 ± 350.00 h	10,120.17 ± 613.19b	10,262 ± 351.00b	12,212.32 ± 777.54a

Table 2
The combined effects of microwave radiations (MR) and ascorbic acid (AA) under surgical industry effluents on the Cd concentration and accumulation by spinach is presented as the mean of three replicates ± standard deviation. The significance of values at $P < 0.05$ is indicated by different small alphabets.

Treatments	Cadmium Concentration (mg kg^{-1})					Cadmium Accumulation (μg plant^{-1})				
	0 %	25 %	50 %	75 %	100 %	0 %	25 %	50 %	75 %	100 %
	Leaf					Leaf				
MR0, A.A 0	0.00 ± 0.00q	119.31 ± 8.11p	275 ± 13.22 L	508.33 ± 7.63 h	670 ± 10d	0.03 ± 0.02k	1095.03 ± 106.50j	2009.83 ± 167.63hi	2575.67 ± 76.00fg	2077.67 ± 98.00hi
MR 30	0.00 ± 0.00q	159.15 ± 7.62o	336.66 ± 17.55k	546.66 ± 8.32 g	720.33 ± 11.67c	0.05 ± 0.01k	1673.68 ± 159.25i	2750.5 ± 177.41 f	3445.07 ± 160.74de	3002.5 ± 156.36ef
A.A10	0.04 ± 0.01q	187.87 ± 6.51 n	406.66 ± 15.27j	576 ± 6.55 f	760.66 ± 4.04b	0.68 ± 0.24k	2224.32 ± 126.92gh	3688 ± 177.00 cd	4186.67 ± 191.48b	3966.73 ± 96.95bc
MR 30 + A.A 10	0.06 ± 0.01q	218.37 ± 6.31 m	458.33 ± 7.63i	610 ± 10e	809.13 ± 8.73a	0.95 ± 0.16k	2816.92 ± 111.09 f	4815 ± 307.77a	4962.33 ± 173.98a	4990.54 ± 176.92a
	Stem					**Stem**				
MR 0, A.A 0	0.00 ± 0.00p	145.94 ± 6.68o	345 ± 15 L	646.66 ± 15.27 h	907 ± 17.53d	0.03 ± 0.03 m	1816.38 ± 140.68 L	3371 ± 208.07ij	5195.33 ± 153.05fg	5472.2 ± 115.08efg
MR 30	0.00 ± 0.00p	168.57 ± 7.02no	424.53 ± 24.03k	704.33 ± 22.72 g	960 ± 10c	0.09 ± 0.02 m	2278.07 ± 178.72kl	4548.2 ± 408.90gh	6249.83 ± 422.22de	6466 ± 359.03d
A.A10	0.06 ± 0.02p	199.04 ± 8.07 n	483.33 ± 12.58j	780 ± 20 f	1003.33 ± 15.27b	1.02 ± 0.47 m	2940.54 ± 168.68jk	6012.83 ± 350.64def	8117.33 ± 520.02bc	7660.33 ± 270.59c
MR 30 + A.A 10	0.07 ± 0.01p	255.95 ± 19.04 m	570 ± 15i	850 ± 10e	1068.33 ± 7.63a	1.32 ± 0.26 m	4040.27 ± 398.23hi	7700 ± 487.51c	9835 ± 548.33a	9011.67 ± 495.28ab
	Root					**Root**				
MR 0, A.A 0	0.00 ± 0.00p	184.51 ± 7.17o	493.33 ± 15.27 L	875 ± 22.91 h	1240 ± 20d	0.03 ± 0.03i	1346.66 ± 68.02 h	3043 ± 146.05fg	3678 ± 269.45ef	3926.67 ± 156.03de
MR 30	0.00 ± 0.00p	232.66 ± 8.02no	576.66 ± 15.28k	961.66 ± 17.55 g	1335 ± 25c	0.06 ± 0.02i	1907.27 ± 54.91 h	3885 ± 221.47e	4618.33 ± 276.64 cd	4671.67 ± 117.95c
A.A10	0.07 ± 0.02p	281.77 ± 7.12 n	661.66 ± 22.54j	1067.6 ± 18.71 f	1430 ± 30b	0.72 ± 0.29i	2611.36 ± 107.94 g	4811.83 ± 328.79c	5838.11 ± 264.46b	5866 ± 357.20b
MR 30 + A.A 10	0.08 ± 0.01p	366.66 ± 15.27 m	750 ± 20i	1145 ± 15e	1528.33 ± 30.13a	0.92 ± 0.08i	3619.33 ± 206.00ef	5954 ± 395.39b	6871 ± 204.50a	7187.17 ± 447.32a

concentration of Ni in root by 6−102 %, in stem by 9−87 %, in leaves by 6−94 %, concentration of Cd in root by 7−98 %, in stem by 5−75 %, in leaves by 7−83 % and concentration of Pb in root by 5−104 %, in stem by 6−125 %, in leaves by 4–188 % respectively. Similarly, the accumulation of Ni in the root increased by 21–173 %, in the stem by 22−137 %, in leaves by 26−174 %, accumulation of Cd in root by18−168 %, in stem by 18−122 %, in leaves by 33−103 %, accumulation of Pb in root by 16−176 %, in stem by 18−185 %, in leaves by 38−304 % respectively as compared with respective treatments without AA and MW 30 s.

Table 3
The combined effects of microwave radiations (MR) and ascorbic acid (AA) under surgical industry effluents on the Pb concentration and accumulation by spinach is presented as the mean of three replicates ± standard deviation. The significance of values at $P < 0.05$ is indicated by different small alphabets.

Treatments	Lead Concentration (mg kg^{-1})					Lead Accumulation (μg plant^{-1})				
	0 %	25 %	50 %	75 %	100 %	0 %	25 %	50 %	75 %	100 %
	Leaf					Leaf				
MR0, A.A 0	0.00 ± 0.00o	51.22 ± 7.73 n	198.8 ± 11.55k	409.26 ± 8.92 g	616.9 ± 7.90c	0.04 ± 0.02 g	470.80 ± 84.75 f	1453.15 ± 134.17e	2073.67 ± 67.32d	1912.9 ± 85.77d
MR 30	0.00 ± 0.00o	81.36 ± 6.90 m	252.8 ± 11.36j	454.73 ± 15.93 f	646.23 ± 6.43b	0.04 ± 0.02 g	855.48 ± 97.08 f	2065.38 ± 122.93d	2866.94 ± 191.05c	2693.25 ± 124.13c
A.A10	0.05 ± 0.02o	119.4 ± 7.64 L	296.41 ± 9.99i	514 ± 18.33e	674.8 ± 3.86ab	0.82 ± 0.30 g	1414.08 ± 119.41e	2687.64 ± 101.54c	3738.13 ± 261.20b	3509.21 ± 87.05b
MR 30 + A.A 10	0.04 ± 0.02o	147.58 ± 8.93 L	346.06 ± 15.76 h	577 ± 11.53d	697.85 ± 2.30a	0.68 ± 0.40 g	1905.7 ± 164.67d	3638.93 ± 338.87b	4694.07 ± 180.51a	4303.43 ± 109.21a
	Stem					**Stem**				
MR 0, A.A 0	0.00 ± 0.00p	75.41 ± 8.52o	235.8 ± 5.40 L	430 ± 10 h	596.76 ± 7.22d	0.04 ± 0.03k	939.19 ± 127.17j	23,204.65 ± 187.73 h	3454.67 ± 102.78ef	3600.53 ± 59.29def
MR 30	0.00 ± 0.00p	112.30 ± 7.98 n	287.6 ± 6.48k	474.66 ± 10.01 g	635.33 ± 5.50c	0.05 ± 0.02k	1518.63 ± 160.21ij	3078.87 ± 171.70fg	4210.67 ± 235.42 cd	4279 ± 229.99c
A.A10	0.05 ± 0.02p	140.36 ± 5.00 n	321.76 ± 14.45j	507.93 ± 7.12 f	696.56 ± 15.43b	0.96 ± 0.35k	2073.26 ± 98.84hi	4004.20 ± 304.22cde	5284.35 ± 275.04b	5318.58 ± 2220.12b
MR 30 + A.A 10	0.04 ± 0.02p	170.12 ± 4.61 m	380.9 ± 20.60i	556.66 ± 10.40e	739.26 ± 5.05a	0.78 ± 0.45k	2682.47 ± 106.18gh	5148.98 ± 467.12b	6442.33 ± 403.82a	6234.97 ± 315.32a
	Root					**Root**				
MR 0, A.A 0	0.00 ± 0.00q	108.3 ± 6.29p	258.33 ± 7.63 L	454 ± 8.54 h	616. ± 66 ± 7.63d	0.03 ± 0.03 m	790.91 ± 63.46 L	1593.67 ± 81.15j	1907.93 ± 126.42hij	1951.67 ± 70.48ghi
MR 30	0.00 ± 0.00q	145.52 ± 9.26o	299.63 ± 9.45k	485 ± 5 g	652 ± 8.18c	0.05 ± 0.02 m	1192.08 ± 47.30k	2018.8 ± 124.56fghi	2328.67 ± 121.00ef	2281.63 ± 50.79efg
A.A10	0.05 ± 0.02q	180.58 ± 7.81 n	346 ± 11.53j	530.33 ± 11.67 f	685 ± 5b	0.58 ± 0.21 m	1674.65 ± 117.13ij	2516.17 ± 170.01de	2900.27 ± 142.43c	2809 ± 136.37 cd
MR 30 + A.A 10	0.06 ± 0.02q	221.7 ± 10.61 m	391.66 ± 7.64i	570 ± 10e	740 ± 10a	0.69 ± 0.31 m	2188.52 ± 138.35efgh	3108.83 ± 186.93bc	3420.67 ± 117.00ab	3479.33 ± 195.00a

4. Discussion

The present study demonstrated that exposures to higher volumes of contaminated WW with a high concentration of HMs would influence the physio-biochemical processes, morphological characteristics, and membrane permeability of plants (Alwutayd et al., 2024; Kiran et al., 2022) and similarly in the present study the application of HMs contaminated surgical effluent affected the growth parameters such as fresh weight, dry weight, plant height and root length and inhibited the overall growth and biomass of spinach plants. The silver lining of the research study designed with the addition of chelating agent AA along with MW treated plants resulted in improved plant growth and biomass under HMs stress (Wu et al., 2024). Metals-induced toxicity in plants affects the photosynthesis process through a significant reduction in plant pigments; there effects were observed in the present study. One of the reasons supporting the phenomenon of pigment reduction is the capacity of some HMs (Pb) to prevent plants from absorbing essential minerals with functionality delivered by Mg and Fe and ultimately inhibiting chlorophyll synthesis (Giannakoula et al., 2021). As expected, an increase in ROS and enzyme activities under HMs stress was observed in the experimental plants. Specifically, the application of the chelating agent AA enhanced the protein content many folds, higher chlorophyll, and SPAD values; with concomitant lowering of ROS production and EL in plants, thereby improving the overall growth of MW-treated and untreated spinach plants.

Interestingly, Farid et al. (2017) reported similar results and highlighted that microwave irradiated seeds germinated better, grew faster and were able to accumulate higher amounts of HMs during their. Farid et al. (2017) also observed higher germination rates of *B. napus* under MW treatment for 30 s which was similar to results of present study. In another similar study carried out by Lin (2004), with exposures to high MW frequencies (5.70 and 9.30 GHz) for a longer time, resulted in having less seed germination due to the ability of MW to damage the cell membrane; which was contrary to the present research study in which MW frequency applied was 2.5 GHz. Interestingly, it was discovered that an optimized level of radiation could be useful for regulating the seed germination processes; rendering the level of applied radiation as an essential factor to be considered for experimentation. Gupta et al. (2013) presented that exposure of seed to low MW frequency (2.45 GHz, for 30 s at 650 W) resulted into higher seed germination in mustard plants, and results are similarly found with spinach plants. Furthermore, research studies of Gul et al. (2014) and Vashisth et al., (2013) highlighted the better germination rate in shisham (*Dalbergia sissoo* L.) and tomato (*Solanum lycopersicum* L.) under the influence of magnetic field and similar effect of seed germination was observed in *B. napus*. Effects obtained from present study and in literature, might be the reason of improved cell membrane structure and disinfection of bacterial colonization on the seed surface.

Experimentation provided with surgical WW containing HMs produced a significant impact with reduction in plant development and biomass production as compared to controlled experiments where no such experience observed (Chaturvedi et al., 2019, Namdjoyan et al., 2020). With the increasing volume of WW and enhanced concentration of HMs, the agronomic traits such as plant height and biomass along with chlorophyll contents significantly affected by HMs toxicity. Effect on different physiological and biochemical processes such as nutrient uptake and mineral transportation were also observed by Jibril et al. (2017). Previous studies reports that exposure of spinach plants to higher concentrations of metals such as Ni, Cd and Pb reduced and hindered the morpho-physiological attributes (Agarwal et al., 2018, Üçüncü et al., 2013). In contrast to this, Hannan et al. (2021) reported that plants treated with chelating agents such as organic acid had higher chlorophyll content and Mattar et al. (2022) reported that organic acids are the important addition to increase the plant tolerance when plants have HMs stress. This may happen by improving the antioxidant defence mechanism and net photosynthetic rates. A study reported by Ma et al. (2016) presented that Cr toxicity significantly reduced the production of Chl *a*, Chl *b*, total Chls, and plant carotenoids in rice seedlings when exposed to HMs stress. However, the application of metal chelators such as AA significantly increased the carotenoid content and photosynthetic pigments of plants (Azizi et al., 2021).

Another very important aspect observed in present study is the effect

of higher concentrations of HMs in surgical WW which ultimately reduced the soluble protein and SPAD value in spinach plant and this might be due to the generation of ROS as reported by Kohli et al. (2018). Another evidence of the higher ROS production responsible for suppressed gas exchange properties of the plants under HMs stress is reported by Jing et al. (2022). Similar results were found in wheat (Liu et al., 2023), *B. juncea* (Kohli et al., 2018), and malabar spinach (*Basella alba*) (Zewail et al., 2020). The level of MDA and H_2O_2 increased with the increase in the generation of ROS and same trend of results was observed in the present study. Moreover, higher lipid peroxidation, H_2O_2 and EL production have been observed in tomato plants under the HMs stress (Bali et al., 2019). The higher levels of EL suggested that HMs-induced toxicity in plants affect the nutrient translocation and electron transport chain (Li et al., 2023). Research has revealed that due to the interference of discharged K^+ in the electron transport chain, an increase in H_2O_2, EL, OH^-, O^{2-} and MDA production occurred in plants when subjected to HMs stress (Mohiuddin et al., 2022; Dotaniya et al., 2022). Similar to HMs stress, salinity, drought, and pesticides are among the additional types of stresses which can increase the production of ROS in plants (Tripathy and Oelmüller, 2012). Controlled experimental plants when compared with the MW-treated plants the comparison hence demonstrated a less oxidative damage and higher tolerance to HM stress. Similarly, ascorbic acid (AA) in contrast to other organic acids such as citric acid, acetic acid promoted the antioxidant system of the plants (Kosar et al., 2015; Gill et al., 2015). In the present study, due to the higher production of ROS, the antioxidant enzyme activities were increased to protect the plant's protein and similar kind of results were described by Abbey et al. (2017) for *B. rapa* (Chinese cabbage) when exposed to MW at 400 W compared with 200 and 800 W. On the other hand, *B. napus* (L.) showed tolerance under less Ni stress by improving enzymatic activities. However, a sudden reduction was observed at a higher level of Ni stress which might be in response to severe oxidative damage (Hannan et al., 2021). The addition of AA as a chelating agent minimized the oxidative stress stimulated by HMs by enhancing the antioxidant enzyme activities of plant (Azzouzi et al., 2024). The previous studies revealed that the absorption of HMs by plants caused the oxidative stress which may be resulted into reduced enzymatic activity and hence reduce the plant growth (Singh et al., 2013). In comparison to plants treated with surgical WW, the plants supplemented with AA enhanced the enzymatic potential both in MW-treated and untreated plants. Present results are supported by Njus et al., (2020) which proved that AA, as a chelator, plays a significant role in the plant's normal functioning through improved antioxidants of plants under HMs stress. In the present study the analysis depicts that the high concentrations of Ni, Cd and Pb in all parts of plants treated with MW- and untreated plants provided with WW and further addition of AA accumulated higher content of these HMs (Tables 1,2, & 3). In a research study conducted by Ullah et al., (2015), concluded that the accumulation of higher content of HMs posed severe negative effects on plant physiology and biochemistry. The permissible concentration of Ni in plant tissues ranged from 0.5 to 5 mg kg^{-1}, however, its concentration in soil may vary from 5 to 150 mg kg^{-1} (Kacálková et al., 2014). Under organic acids amendment, an increased level of Ni accumulation was recorded in *B. napus* (L.) (Rodríguez-Vila et al., 2015). Similarly, the increased concentration of Cd and Pb was also observed in all parts of *Lycopersicon esculentum*, tomato, and *B. juncea* (L.) grown under HMs stress (Khanna et al., 2019, Kohli et al., 2018). The chelation effect of AA additionally enhanced the accumulation of Pb, Cd, and Ni in the plant roots and shoots in both kind of experiments with MW and controlled (Njus et al., 2020). The results suggested that the combination of AA as chelating agent and application of microwaves on seeds could be useful arrangement to remediate the HMs from surgical industry wastewater when it is applied to some useful application in soil.

5. Conclusions

A laboratory scale hydroponics study was carried out to assess the phytoextraction potential of MW-irradiated spinach plants for the removal of HMs from surgical processing industry wastewater under AA application. This study concluded that the seed germination of spinach was increased when exposed to low-frequency of MW for a brief period (30 s). The surgical industry effluents adversely affected the physiology and biochemistry of spinach plants; specifically, the higher levels of WW lowered growth and increased accumulation of HMs. In plants subjected to MW treatments, the uptake and accumulation of Ni, Cd, and Pb was higher as compared to untreated control plants. When AA was introduced to MW-treated plants, it enhanced the phytoextraction process and also promoted spinach growth. The most significant positive effects were observed when AA was applied to MW-treated plants exposed to WW and leading to higher growth and metal accumulation. This study suggested that the MW-treated plants could be a potential protocol for the treatment of industrial effluents under organic acid amendments. Moving forward, further studies at the field scale are required to understand the performance of spinach plants under realistic conditions.

Ethics approval and consent to participate

Not applicable

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CRediT authorship contribution statement

Suliman M.S Alghanem: Writing – review & editing, Resources, Methodology. **Muhammad Abubakar:** Writing – original draft, Investigation, Formal analysis. **Haifa A.S Alhaithloul:** Writing – review & editing, Resources, Methodology. **Jean W.H Yong:** Writing – review & editing. **Muhammad Rizwan:** Writing – original draft, Supervision, Methodology. **Amany H.A Abeed:** Writing – review & editing, Funding acquisition. **Mujahid Farid:** Writing – original draft, Supervision, Conceptualization. **Ibtisam M Alsudays:** Writing – original draft, Software, Resources, Data curation. **Sheharyaar Farid:** Software, Formal analysis. **Muhammad Zubair:** Validation, Resources, Formal analysis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Authors' contributions

All authors contributed to the study's conception and design. [Mujahid Farid]: Conceptualization, Project administration, Supervision, Writing- original draft. [Muhammad Abubakar]: Conceptualization, Writing – review & editing. [Suliman Mohammed Suliman Alghanem]: Writing – original draft, Writing – review & editing. [Muhammad Zubair]: Methodology, Writing – review & editing. [Muhammad Rizwan]: Conceptualization, Project administration, Writing – original draft, [Haifa Abdulaziz Sakit Alhaithloul]: Data curation, Formal analysis, Methodology, Writing – original draft. [Sheharyaar Farid]: Writing – original draft, Writing – review & editing. [Ibtisam Mohammed Alsudays], Writing – review & editing. [Amany H. A. Abeed], [Jean Wan Hong Yong]: Conceptualization, Project, Funding Acquisition, review & editing. All authors gave their feedback and input during the write-up, experimentation, analysis, data validation and proofreading of the present research work.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116731.

References

Abbey, L., Udenigwe, C., Mohan, A., Anom, E., 2017. Microwave irradiation effects on vermicasts potency and plant growth and antioxidant activity in seedlings of Chinese cabbage (*Brassica rapa subsp. pekinensis*). J. Radiat. Res. Appl. Sci. 13–26. https://doi.org/10.1016/j.jrras.2017.01.002.

Abeed, A.H.A., Ali, M., Eissa, M.A., et al., 2022. Impact of sewage water irrigation on Datura innoxia grown in sandy loam soil. BMC Plant Biol 22, 559. https://doi.org/10.1186/s12870-022-03935-9.

Aebi, H., 1984. Catalase in vitro methods. Enzymol., 105, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Agarwal, A., Gupta, S.D., Barman, M., Mitra, A., 2018. Photosynthetic apparatus plays a central role in photosensitive physiological acclimations affecting spinach (*Spinacia oleracea* L.) growth in response to blue and red photon flux ratios. Environ. Exp. Bot. 156, 170–182. https://doi.org/10.1016/j.envexpbot.2018.09.009.

Ali, H., Khan, E., Sajad, M.A., 2013. Phytoremediation of heavy metals—concepts and applications. Chemosphere 91 (7), 869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075.

Alwutayd, K.M., Alghanem, S.M.S., Alwutayd, R., Alghamdi, S.A., Alabdallah, N.M., Al-Qthanin, R.N., Abeed, A.H., 2024. Mitigating chromium toxicity in rice (Oryza sativa L.) via ABA and 6-BAP: Unveiling synergistic benefits on morphophysiological traits and ASA-GSH cycle. Science of The Total Environment 908, 168208.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24 (1), 1–15. https://doi.org/10.1104/pp.24.1.1.

Azam, I., Afsheen, S., Zia, A., 2015. Evaluating insects as bio indicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan. Biomed. Res. Int. 168, 289–300. https://doi.org/10.1155/2015/942751.

Azizi, F., Farsaraei, S., Moghaddam, M., 2021. Application of Exogenous Ascorbic Acid Modifies Growth and Pigment Content of Calendula officinalis L. Flower Heads of Plants Exposed to NaCl Stress. J. Soil Sci. Plant Nutr. 21 (4), 2803–2814. https://doi.org/10.1007/s42729-021-00567-0.

Azzouzi, S., Khamar, M., Nounah, A., Cherkaoui, E., 2024. Effect of ascorbic acid on chromium remediation in fenugreek (Trigonella foenum-gracium L.) germination. Biorem. J. 1–9. https://doi.org/10.1080/10889868.2024.2318558.

Bali, S., Jamwal, V.L., Kaur, P., Kohli, S.K., Ohri, P., Gandhi, S.G., Bhardwaj, R., Al-Huqail, A.A., Siddiqui, M.H., Ahmad, P., 2019. Role of P-type ATPase metal transporters and plant immunity induced by jasmonic acid against lead (Pb) toxicity in tomato. Ecotoxicol. Environ. Saf. 174, 283–294. https://doi.org/10.1016/j.ecoenv.2019.02.084.

Ben Massoud, Sakouhi, L., Chaoui, A., 2019. Effect of plant growth regulators, calcium and citric acid on copper toxicity in pea seedlings. J. Plant. Nutr. 42, 1230–1242. https://doi.org/10.1080/01904167.2019.1609506.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Chaturvedi, R., Favas, P.J., Pratas, J., Varun, M., Paul, M.S., 2019. Metal(loid) induced toxicity and defense mechanisms in *Spinacia oleracea* L. Ecological hazard and Prospects for phytoremediation. Ecotoxicol. Environ. Saf. 183, 109–570. https://doi.org/10.1016/j.ecoenv.2019.109570.

Clescerl, L.S., Greenberg, A.E., Eaton, A.D., 1998 Standard methods for the examination of water and wastewater‖, 20th edn(American Public Health Association, American water works association, water environ. Feder.

Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A., 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Exp. Bot. *32* (1), 93–101. https://doi.org/10.1093/jxb/32.1.93.

Diarra, I., Kotra, K.K., Prasad, S., 2021. Assessment of biodegradable chelating agents in the phytoextraction of heavy metals from multi–metal contaminated soil. Chemosphere *273*, 128483. https://doi.org/10.1016/j.chemosphere.2020.128483.

Dionisio-Sese, M.L., Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. *135* (1), 1–9. https://doi.org/10.1016/S0168-9452(98)00025-9.

Dotaniya, M.L., Pipalde, J.S., Jain, R.C., Selladurai, R., Gupta, S.C., Das Vyas, M., Kumar, A., 2022. Nickel-mediated lead dynamics and their interactive effect on lead partitioning and phytoremediation indices in spinach. Environ. Monit. Asses. 194 (5), 1–10. https://doi.org/10.1007/s10661-022-09935-4.

Ehsan, S., Ali, S., Noureen, S., Farid, M., 2013. Comparative assessment of different heavy metals in urban soil and vegetables irrigated with sewage/industrial waste water. Ecoterra 35, 37–53.

Farid, M., Ali, S., Rizwan, M., Saeed, R., Tauqeer, H.M., Sallah-Ud-Din, R., Raza, N., 2017. Microwave irradiation and citric acid assisted seed germination and phytoextraction of nickel (Ni) by *Brassica napus* L.: morpho-physiological and biochemical alterations under Ni stress. Environ. Sci. Pollut. Res. 24 (26), 21050–21064. https://doi.org/10.1007/s11356-017-9751-5.

Gallie, D.R., 2013. Ascorbic Acid: A Multifunctional Molecule Supporting Plant Growth and Development. Scientifica 2013, 795964. https://doi.org/10.1155/2013/795964.

Giannakoula, A., Therios, I., Chatzissavvidis, C., 2021. Effect of lead and copper on photosynthetic apparatus in citrus (*Citrus aurantium* L.) plants. The role of antioxidants in oxidative damage as a response to heavy metal stress. Plants 10 (1). https://doi.org/10.3390/plants10010155.

Gill, R.A., Zang, L., Ali, B., Farooq, M.A., Cui, P., Yang, S., Zhou, W., 2015. Chromium-induced physio-chemical and ultrastructural changes in four cultivars of *Brassica napus* L. Chemosphere 120, 154–164. https://doi.org/10.1016/j.chemosphere.2014.06.029.

Gul, S., Tanvir, M.A., Nawaz, M.F., Rizwan, M., Haq, Z., 2014. Magnetic field application as pre-sowing seed treatment enhances the seed germination potential of Shisham (*Dalbergia sissoo*). World Appl. Sci. J. 32 (5), 792–796. https://doi.org/10.5829/idosi.wasj.2014.32.05.14533.

Gupta, M., Dwivedi, V., Kumar, S., Patel, A., Niazi, P., Yadav, V.K., 2024. Lead toxicity in plants: mechanistic insights into toxicity, physiological responses of plants and mitigation strategies. Plant Signal. Behav. 19 (1), 2365576 https://doi.org/10.1080/15592324.2024.2365576.

Gupta, A., Singh, R., Lehana, P., 2013. Effect of microwaves treated soil on growth of mustard plants. Int J. Eng. Adv. Technol. 2, 808–812.

Hannan, F., Huang, Q., Farooq, M.A., Ayyaz, A., Ma, J., Zhang, N., Ali, B., Deyett, E., Zhou, W., Islam, F., 2021. Organic and inorganic amendments for the remediation of nickel contaminated soil and its improvement on *Brassica napus* growth and oxidative defense. J. Hazard. Mater. 416, 125921 https://doi.org/10.1016/j.jhazmat.2021.125921.

Hassan, M.U., Chattha, M.U., Khan, I., Chattha, M.B., Aamer, M., Nawaz, M., Ali, A., Khan, M.A.U., Khan, T.A., 2019. Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities—a review. Environ. Sci. Pollut. Res. 26 (13), 12673–12688. https://doi.org/10.1007/s11356-019-04892-x.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125 (1), 189–198. https://doi.org/10.1016/0003-9861(68)90654-1.

Hossain, M.S., Abdelrahman, M., Tran, C.D., Nguyen, K.H., Chu, H.D., Watanabe, Y., Hasanuzzaman, M., Mohsin, S.M., Fujita, M., Tran, L.S.P., 2020. Insights into acetate-mediated copper homeostasis and antioxidant defense in lentil under excessive copper stress. Environ. Pollut. 258, 113544 https://doi.org/10.1016/j.envpol.2019.113544.

Ikkonen, E., Kaznina, N., 2022. Physiological responses of lettuce (*Lactuca sativa* L.) to soil contamination with Pb. Horticul 8 (10). https://doi.org/10.3390/horticulturae8100951.

Iwuala, E., Ajewole, T., Osundinakin, M., Popoola, M., Abiodun, I., Agbolade, J., 2021. Impact of controlled microwave radiation in enhancing the productivity of *Abelmoschus esculentus* seedlings (L.) Moench. J. Plant Inter. 16 (1), 179–186. https://doi.org/10.1080/17429145.2021.1912422.

Jana, S., Choudhuri, M.A., 1981. Glycolate metabolism of three submersed aquatic angiosperms: Effect of heavy metals. Aquatic Botany 11, 67–77. https://doi.org/10.1016/0304-3770(81)90047-4.

Jibril, S.A., Hassan, S.A., Ishak, C.F., Megat Wahab, P.E., 2017. Cadmium toxicity affects phytochemicals and nutrient elements composition of lettuce (*Lactuca sativa* L.). Adv. Agric., 1236830 https://doi.org/10.1155/2017/1236830.

Jing, M., Zhang, H., Wei, M., Tang, Y., Xia, Y., Chen, Y., Shen, Z., Chen, C., 2022. Reactive oxygen species partly mediate DNA methylation in responses to different heavy metals in pokeweed. Front. Plant Sci. 13 https://doi.org/10.3389/fpls.2022.845108.

Junaid, M., Hashmi, M.Z., Malik, R.N., 2016. Evaluating levels and health risk of heavy metals in exposed workers from surgical instrument manufacturing industries of Sialkot, Pakistan. Environ. Sci. Pollut. Res. 23 (18), 18010–18026. https://doi.org/10.1007/s11356-016-6849-0.

Kacálková, L., Tlustoš, P., Száková, J., 2014. Chromium, nickel, cadmium, and lead accumulation in maize, sunflower, willow, and poplar. Pol. J. Environ. Stud. 23 (3).

Kamran, M., Danish, M., Saleem, M.H., Malik, Z., Parveen, A., Abbasi, G.H., Jamil, M., Ali, S., Afzal, S., Riaz, M., 2020. Application of abscisic acid and 6-benzylamino-purine modulated morpho-physiological and antioxidative defense responses of tomato (*Solanum lycopersicum* L.) by minimizing cobalt uptake. Chemosphere 263, 128169. https://doi.org/10.1016/j.chemosphere.2020.128169.

Khalid, S., Shahid, M., Dumat, C., Niazi, N.K., Bibi, I., Gul Bakhat, Abbas, G., Murtaza, B., Javeed, H.M., 2017. Influence of groundwater and wastewater irrigation on lead accumulation in soil and vegetables: implications for health risk assessment and phytoremediation. Int. J. Phytorem. 19 (11), 1037–1046. https://doi.org/10.1080/15226514.2017.1319330.

Khaliq, M.A., Javed, M.T., Hussain, S., Imran, M., Mubeen, M., Nasim, W., Fahad, S., Karuppannan, S., Al-Taisan, W.A.A., Almohamad, H., Al Dughairi, A.A., 2022. Assessment of heavy metal accumulation and health risks in okra (*Abelmoschus esculentus* L.) and spinach (*Spinacia oleracea* L.) fertigated with wastwater. Int. J. Food Contam. 9 (1), 11. https://doi.org/10.1186/s40550-022-00097-2.

Khanna, K., Kohli, S.K., Ohri, P., Bhardwaj, R., Al-Huqail, A.A., Siddiqui, M.H., Alosaimi, G.S., Ahmad, P., 2019. Microbial fortification improved photosynthetic efficiency and secondary metabolism in *Lycopersicon esculentum* plants under Cd stress. Biomolecules 9 (10), 581. https://doi.org/10.3390/biom9100581.

Kiran, Bharti, R., Sharma, R., 2022. Effect of heavy metals: an overview. Mater. Today.: Proc. 51, 880–885. https://doi.org/10.1016/j.matpr.2021.06.278.

Kohli, S.K., Handa, N., Sharma, A., Gautam, V., Arora, S., Bhardwaj, R., Wijaya, L., Alyemeni, M.N., Ahmad, P., 2018. Interaction of 24-epibrassinolide and salicylic acid regulates pigment contents, antioxidative defense responses, and gene expression in *Brassica juncea* L. seedlings under Pb stress. Environ. Sci. Pollut. Res. 25, 15159–15173. https://doi.org/10.1007/s11356-018-1742-7.

Kosar, F., Akram, N.A., Ashraf, M., 2015. Exogenously-applied 5-aminolevulinic acid modulates some key physiological characteristics and antioxidative defense system in spring wheat (*Triticum aestivum* L.) seedlings under water stress. South Afr. J. Bot. 96, 71–77. https://doi.org/10.1016/j.sajb.2014.10.015.

Li, C., Liu, J., Wei, Z., Cheng, Y., Shen, Z., Xin, Z., Huang, Y., Wang, H., Li, Y., Mu, Z., Zhang, Q., 2023. Exogenous melatonin enhances the tolerance of tiger nut (*Cyperus esculentus* L.) via DNA damage repair pathway under heavy metal stress (Cd^{2+}) at the sprout stage. Ecotoxicol. Environ. Saf. 265, 115519 https://doi.org/10.1016/j.ecoenv.2023.115519.

Lin, J.C., 2004. Evaluating scientific literature on biological effects of microwave radiation. IEEE Microw. Mag. 5, 34–37. https://doi.org/10.1109/MMW.2004.1284940.

Liu, P., Li, L., Ippolito, J.A., Xing, W., Wang, Y., Wang, Y., Cheng, Y., Qiu, K., 2023. Heavy metal distribution in wheat plant components following foliar Cd application. Chemosphere *322*, 138177. https://doi.org/10.1016/j.chemosphere.2023.138177.

Liu, L., Zhang, Q., Hu, L., Tang, J., Xu, L., Yang, X., Yong, J.W.H., Chen, X., 2012. Legumes can increase cadmium contamination in neighboring crops. Plos One, 7, e42944. https://doi.org/10.1371/journal.pone.0042944.

Ma, J., Lv, C., Xu, M., Chen, G., Lv, C., Gao, Z., 2016. Photosynthesis performance, antioxidant enzymes, and ultrastructural analyses of rice seedlings under chromium stress. Environ. Sci. Pol. Res. 23 (2), 1768–1778. https://doi.org/10.1007/s11356-015-5439-x.

Masood, N., Irshad, M.A., Nawaz, R., Abbas, T., Abdel-Maksoud, M.A., AlQahtani, W.H., Abeed, A.H., 2023. Green synthesis, characterization and adsorption of chromium and cadmium from wastewater using cerium oxide nanoparticles; reaction kinetics study. Journal of Molecular Structure 1294, 136563.

Mattar, G., Haddarah, A., Haddad, J., Pujola, M., Sepulcre, F., 2022. New approaches, bioavailability and the use of chelates as a promising method for food fortification. Food Chem. *373*, 131394 https://doi.org/10.1016/j.foodchem.2021.131394.

Metzner, H., RauH, Senger H., 1965. Untersuchungen zur synchronisierbarkeit einzelner pigmentmangel-mutanten von Chlorella Plant 65: 186–194. https://doi.org/10.1007/BF00384998.

Mohiuddin, K.M., Saha, P., Hossain, M., Nahar, K., Ahmed, I., Hoque, A., Rahman, M., 2022. Assessment of health risk due to consumption of spinach (*Spinacia oleracea*) cultivated with heavy metal polluted water of bhabadah water-logged area of Bangladesh. Earth Syst. Environ. 1–14. https://doi.org/10.1007/s41748-022-00302-4.

Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. *22* (5), 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232.

Namdjoyan, S., Soorki, A.A., Elyasi, N., Kazemi, N., Simaei, M., 2020. Melatonin alleviates lead-induced oxidative damage in safflower (*Carthamus tinctorius* L.) seedlings. Ecotoxicol 29 (1), 108–118. https://doi.org/10.1007/s10646-019-02136-9.

Njus, D., Kelley, P.M., Tu, Y.J., Schlegel, H.B., 2020. Ascorbic acid: The chemistry underlying its antioxidant properties. Free Radic. Biol. Med. *159*, 37–43. https://doi.org/10.1016/j.freeradbiomed.2020.07.013.

Paz-Ferreiro, J., Lu, H., Fu, S., Méndez, A., Gascó, G., 2014. Use of phytoremediation and biochar to remediate heavy metal polluted soils: a review. Solid Earth 5 (1), 65–75. https://doi.org/10.5194/se-5-65-2014.

Qiang, T., Fan, G., Yufeng, G., Toru, I., Takeshi, K., 2018. Desorption characteristics of Cr (III), Mn(II), and Ni(II) in contaminated soil using citric acid and citric acid-containing wastewater. Soil Found. 58, 50–64. https://doi.org/10.1016/j.sandf.2017.12.001.

Qureshi, F.F., Ashraf, M.A., Rasheed, R., Hussain, I., Rizwan, M., Iqbal, M., Yong, J.W.H., 2024. Microbial-assisted alleviation of chromium toxicity in plants: A critical review. Plant Stress 11, 100394. https://doi.org/10.1016/j.stress.2024.100394.

Razzak, S.A., Faruque, M.O., Alsheikh, Z., Alsheikhmohamad, L., Alkuroud, D., Alfayez, A., Hossain, S.M.Z., Hossain, M.M., 2022. A comprehensive review on conventional and biological-driven heavy metals removal from industrial wastewater. Environmental Advances 7, 100168. https://doi.org/10.1016/j.envadv.2022.100168.

Rigby, H., Smith, S.R., 2020. The significance of cadmium entering the human food chain via livestock ingestion from the agricultural use of biosolids, with special reference to the UK. Environ. Int 143, 105–844. https://doi.org/10.1016/j.envint.2020.105844.

Rodríguez-Vila, A., Covelo, E.F., Forján, R., Asensio, V., 2015. Recovering a copper mine soil using organic amendments and phytomanagement with *Brassica juncea* L. J. Environ. Manag. 147, 73–80. https://doi.org/10.1016/j.jenvman.2014.09.011.

Saleem, M.H., Ali, S., Rehman, M., 2020. Jute: a potential candidate for phytoremediation of metals-a review. Plant 9 (2), 258. https://doi.org/10.3390/plants9020258.

Shaghaleh, H., Azhar, M., Zia-ur-Rehman, M., Hamoud, Y.A., Hamad, A.A.A., Usman, M., Rizwan, M., Yong, J.W.H., Alharby, H.F., Al-Ghamdi, A.J., Alharbi, B.M., 2024. Effects of agro based organic amendments on growth and cadmium uptake in wheat and rice crops irrigated with raw city effluents: Three years field study. Environ. Pollut. 344, 123365. https://doi.org/10.1016/j.envpol.2024.123365.

Shehzad, J., Mustafa, G., Arshad, H., Ali, A., Naveed, N.H., Riaz, Z., Khan, I., 2022. Morpho-physiological and biochemical responses of *Brassica* species toward lead (Pb) stress. Acta Physiol. Plant. 45 (1), 8. https://doi.org/10.1007/s11738-022-03493-5.

Sigamani, S., Dhrisha, J.A., YT, D.M., Subiksha, S., Balaji, U., Kolandhasamy, P., Syed, A., Elgorban, A.M., 2024. Bioaccumulation and health risk of metal contamination from different tiers of food chain in Ennore estuary, Southeast coast of India. Mar. Pollut. Bull. 200, 116154 https://doi.org/10.1016/j.marpolbul.2024.116154.

Singh, H.P., Mahajan, P., Kaur, S., Batish, D.R., Kohli, R.K., 2013. Chromium toxicity and tolerance in plants. Environ. Chem. Lett. 11 (3), 229–254. https://doi.org/10.1007/s10311-013-0407-5.

Song, J., Finnegan, P.M., Liu, W., Xiang, L., Yong, J.W.H., Xu, J., Zhang, Q., Wen, Y., Qin, K., Guo, J., Li, T., Zhao, C., Zhang, Y., 2019. Mechanisms underlying enhanced Cd translocation and tolerance in roots of *Populus euramericana* in response to nitrogen fertilization. Plant Science 287, 110206. https://doi.org/10.1016/j.plantsci.2019.110206.

Song, Q., Liu, Y., Pang, J., Yong, J.W.H., Chen, Y, Bai, C., Gille, C., Shi, Q., Wu, D., Han, X., Li, T., Siddique, K.H.M., Lambers, H., 2020. Supplementary calcium restores peanut (*Arachis hypogaea*) growth and photosynthetic capacity under low nocturnal temperature. Front. Plant Sci. 10, e1637. https://doi.org/10.3389/fpls.2019.01637.

Spry, C., 2005. Essentials of perioperative nursing. Jones Bartlett Publ. Ed. 3rd 215–217.

Tariq, S., Iqbal, F., Ijaz, A., 2013. Assessment and multivariate analysis of metals in surgical instrument industry affected top soils and groundwater for future reclamation. Int. J. Environ. Pollut. Solut. https://doi.org/10.7726/ijeps.2013.1006.

Thinh, N.Van, Osanai, Y., Adachi, T., Vuong, B.T.S., Kitano, I., Chung, N.T., Thai, P.K., 2021. Removal of lead and other toxic metals in heavily contaminated soil using biodegradable chelators: GLDA, citric acid and ascorbic acid. Chemosphere 263, 127912. https://doi.org/10.1016/j.chemosphere.2020.127912.

Tow, T.S.W., Eng, Z.X., Wong, S.P., Ge, L., Tan, S.N., Yong, J.W.H., 2018. Axonopus compressus (Sw.) Beauv.: A potential biomonitor for molybdenum in soil pollution. Int. J. Phytoremediat. 20, 1363–1368. https://doi.org/10.1080/15226514.2016.1207599.

Tripathy, B.C., Oelmüller, R., 2012. Reactive oxygen species generation and signaling in plants. Plant Signal. Behav. 7 (12), 1621–1633. https://doi.org/10.4161/psb.22455.

Üçüncü, E., Tunca, E., Fikirdeşici, Ş., Özkan, A.D., Altındağ, A., 2013. Phytoremediation of Cu, Cr and Pb mixtures by *Spinacia oleracea* L. Bull. Environ. Cont. Toxicol. 91 (5), 600–604.

Ullah, A., Heng, S., Munis, M.F.H., Fahad, S., Yang, X., 2015. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: a review. Environ. Exp. Bot. 117, 28–40. https://doi.org/10.1016/j.envexpbot.2015.05.001.

Uruç Parlak, K., 2016. Effect of nickel on growth and biochemical characteristics of wheat (*Triticum aestivum* L.) seedlings. NJAS - Wagening. J. Life Sci. 76, 1–5. https://doi.org/10.1016/j.njas.2012.07.001.

van der Ent, A., Baker, A.J., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362, 319–334. https://doi.org/10.1007/s11104-012-1287-3.

Vashisth, A., Singh, R., Joshi, D.K., 2013. Effect of static magnetic field on germination and seedling attributes in tomato (*Solanum lycopersicum* L). J. Argic Physic 13, 182–185.

Wang, R., Sang, P., Guo, Y., Jin, P., Cheng, Y., Yu, H., Xie, Y., Yao, W., Qian, H., 2023. Cadmium in food: Source, distribution and removal. Food Chem. 405, 134666 https://doi.org/10.1016/j.foodchem.2022.134666.

Wu, P., Li, B., Liu, Y., Bian, Z., Xiong, J., Wang, Y., Zhu, B., 2024. Multiple physiological and biochemical functions of ascorbic acid in plant growth, development, and abiotic stress response. Int. J. Mol. Sci. 25 (3) https://doi.org/10.3390/ijms25031832.

Yan, A., Wang, Y., Tan, S.N., Mohd Yusof, M.L., Ghosh, S., Chen, Z., 2020. Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. Front. Plant Sci. 11 https://doi.org/10.3389/fpls.2020.00359.

Yong, J.W.H., Ng, Y.F., Tan, S.N., Chew, A.Y.L., 2010a. Growth observations and the effects of fertilizer applications on physiology of *Jatropha curcas* L. Photosynthetica 48, 208–218. https://doi.org/10.1007/s11099-010-0026-3.

Yong, J.W.H., Tan, S.N., Ng, Y.F., Low, K.K.K., Peh, S.F., Chua, J.C., Lim, A.A.B., 2010b. Arsenic hyperaccumulation by *Pteris vittata* and *Pityrogramma calomelanos*: A comparative study of uptake efficiency in arsenic treated soils and waters. Water Sci. Technol. 61, 3041–3049. https://doi.org/10.2166/wst.2010.223.

Zayed, A., Lytle, C.M., Qian, J.H., Terry, N., 1998. Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta 206, 293–299. https://doi.org/10.1007/s004250050403.

Zewail, R.M.Y., El-Desoukey, H.S., Islam, K.R., 2020. Chromium stress alleviation by salicylic acid in Malabar spinach (*Basella alba*). J. Plant Nutr. 43 (9), 1268–1285. https://doi.org/10.1080/01904167.2020.1727504.

Zhang, X.Z., 1992. The measurement and mechanism of lipid peroxidation and SOD POD and CAT activities in biological system. In: Zhang, X.Z. (Ed.), Research Methodology of Crop Physiology. Beijing Agric Press, pp. 208–211.

Zhang, J., Kirkham, M.B., 1994. Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. Plant Cell Physiol. 35 (5), 785–791. https://doi.org/10.1093/oxfordjournals.pcp.a078658.

Zhang, H., Zhang, L.L., Li, J., 2020. Comparative study on the bioaccumulation of lead, cadmium and nickel and their toxic effects on the growth and enzyme defence strategies of a heavy metal accumulator, *Hydrilla verticillat* L. Royle. Environ. Sci. Pollut. Res 19: 069-680. https://doi.org/10.1007/s11356-019-06968-0.