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Extenuating lead toxicity in Chinese cabbage using organic acids: Comparative efficacy of acetic, citric, and tartaric acids

Muhammad Rizwan [a,b], Umair Riaz [c,*], Humera Aziz [d], Wajiha Anum [e], Muhammad Rizwan [d], Ghulam Murtaza [f], Jean Wan Hong Yong [g,*], Hong Chen [a,*]

[a] Key Laboratory of Water-Sediment Sciences and Water Disaster Prevention of Hunan Province, School of Hydraulic and Environmental Engineering, Changsha University of Science & Technology, Changsha 410114, China
[b] Department of Environmental Engineering, Faculty of Civil Engineering, Yildiz Technical University, Esenler, Istanbul 34220, Türkiye
[c] Department of Soil & Environmental Sciences, MNS-University of Agriculture, Multan 60000, Pakistan
[d] Department of Environmental Sciences, Government College University, Faisalabad 38040, Pakistan
[e] Department of Agronomy, Regional Agriculture Research Institute, Bahawalpur, Agriculture Department. Government of Punjab, Pakistan
[f] School of Agriculture, Yunnan University, Kunming, Yunnan 650504, China
[g] Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Alnarp, 23456, Sweden

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ABSTRACT

Lead (Pb) toxicity in the environment and plants has emerged as a significant global concern, primarily due to its entry into the human body through the food chain. Several physicochemical and biological techniques are being explored for Pb removal/immobilization. This study explored the efficacy of organic acids—citric acid (CA), acetic acid (AA), and tartaric acid (TA) in mitigating Pb toxicity in plants parts. The treatments included a control (CK, no acid treatment), citric acid (CA) at 0.25 mM (CA0.25) and 0.5 mM (CA0.5), tartaric acid (TA) at 100 mM (TA100) and 200 mM (TA200), and acetic acid (AA) at 100 mM (AA100) and 200 mM (AA200), each replicated in triplicate. Chinese cabbage (*Brassica rapa*) was used as the test crop. Our results revealed significant reductions in Pb concentrations in both plant parts (roots and leaves) as well as in soil following the addition of organic acid. Among the three acid treatments at different concentrations, tartaric acid (TA200) at 200 mM delivered the best results in reducing Pb accumulation in soil-plant system. It notably increased catalase activity (144 μmol g^{-1}), ascorbic acid (12,344 mg L^{-1}), superoxide dismutase (178 U mg^{-1} protein), and glutathione (98.0 μg g^{-1} FW), compared to citric and acetic acids treatments. Further, Pearson's correlation showed a strong negative relationship between Pb content and antioxidant activities, such as catalase, glutathione, and peroxidase ($r > 0.90$). This study revealed that the application of tartaric acid (TA), particularly at 200 mM through both exogenous and soil treatments, could be an effective strategy to minimize Pb accumulation in polluted areas.

1. Introduction

Excessive emissions of trace metals, predominantly in particulate form, lead to the contamination of surface environments. Once airborne, these particulates contribute to atmospheric pollution and pose significant risks of bioaccumulation and biomagnification within the food chain (Clemens and Ma, 2016; Rizwan et al., 2024a; Rizwan et al., 2024b). Lead (Pb) is extensively used in modern industries, including automotive batteries, construction materials, sealants (e.g., chimney tops), electrical and electronic components (e.g., solder, cathode ray tube glass), weights, ammunition, fishing gear, and as stabilizers in polyvinyl chloride (PVC) products. Over time, the degradation and disposal of these Pb-based products contribute to environmental contamination, with soil acting as the ultimate sink for Pb accumulation. Isotopic analyses have confirmed that while direct ingestion of lead-based paint chips is a primary route of human exposure, the continuous build-up of Pb in soils poses long-term risks through indirect pathways, including plant uptake and entry into the food chain (Shan et al., 2025). Naturally, Pb is present in soil as a result of weathering and other pedogenic processes acting on the soil parent material. Vegetables are a potential supply of essential nutrients and also serve as a significant functional dietary components by providing protein, vitamins, iron, and

* Corresponding authors.
E-mail addresses: mrizwan17@hotmail.com (M. Rizwan), umair.riaz@mnsuam.edu.pk (U. Riaz), humeraaziz@gcuf.edu.pk (H. Aziz), wajiha_anum@live.com (W. Anum), mrizwan@gcuf.edu.pk (M. Rizwan), murtazabotanist@gmail.com (G. Murtaza), jean.yong@slu.se (J.W.H. Yong), chenh@csust.edu.cn (H. Chen).

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calcium, all of which have significant health benefits (Musah, 2025). Environmental lead (Pb) exposure is a serious issue for human health. Children are susceptible to Pb poisoning, which damages the central nervous system and can be fatal in severe situations (Kumar et al., 2024). There is a severe problem for the coming generations regarding Pb pollution is increasing rapidly in all segments of life. Several processes, including phytoextraction, phyto stabilization, phytovolatilization, and rhizo-filtration, are used in bioremediation when heavy metals are absorbed or accumulated by plants (Li et al., 2024; Rahman and Hasegawa, 2011). There are two techniques to phytoremediation: (1) use of hyper accumulators or high biomass generating plants (2) use of phyto chelators like organic acids including citric acid, malic acid, oxalic acid, and TA and synthetic chelators including ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) (Liu et al., 2024). Organic acids are significant plant root exudates and microbial metabolites because of their capacity to accelerate the dissolution of metals from extremely insoluble mineral phases in soil, thus boosting metal mobility in the vicinity of roots and raising their availability to plants. Organic acids release protons (H^+) into the soil solution, lowering the pH of the rhizosphere. It also contains functional groups like carboxyl (-COOH) and hydroxyl (-OH), which act as ligands to form stable complexes with metal(oid)s ions. Organic acids compete with inorganic anions like hydroxides (OH^-), carbonates (CO_3^-), or phosphates (PO_3^-) that bind metals in insoluble forms. This exchange releases metals from these complexes and enhances their mobility. Organic acids can dissolve minerals by breaking down their crystal structure through complexation or acid hydrolysis. Some organic acids facilitate redox reactions that change the valence state of metals, making them more soluble. Organic acids, therefore, act as natural agents that manipulate soil chemistry to release metals from insoluble forms, enhance their mobility, and improve their availability for plant uptake, making them key players in phytoremediation and soil management strategies (Cao et al., 2025). Compared to other metals, the interactions between organic acids and lead (Pb) are less well understood. However, chelating agents play a key role in enhancing metal mobility. Organic acids such as citric, gallic, malic, and tartaric acids—naturally produced by plants and microorganisms in the rhizosphere—act as effective chelators. They improve the solubility of mineral nutrients and facilitate the formation of organo-mineral complexes. (Balint and Boaja, 2024). Acetic acid (CH_3COOH), citric acid ($C_6H_8O_7$), and tartaric acid ($C_4H_6O_6$) are organic acids capable of forming organo-mineral complexes in polluted soils. They enhance the solubility and mobility of heavy metals, making them potential agents for soil remediation. Among these, citric and tartaric acids show strong chelating abilities, while acetic acid acts as a simple mobilizer. However, their comparative efficacy as soil treatments remains largely unexplored and warrants further investigation at different levels. To address this gap, the present study examines the use of exogenous applications of these organic acids at varying levels to reduce Pb accumulation in soil and edible parts of Chinese cabbage. This research offers a novel approach toward developing sustainable, plant-based strategies for managing Pb pollution and safeguarding the nutritional quality of crops grown in contaminated areas.

2. Materials and methods

2.1. Experiment design & setup

The naturally contaminated soil was collected from the Changsha, Hunan Province, China. The soil type was loam, developed from granite and Haplic Alisols. Soil analyses were carried out following the protocols described in the ICARDA manual (Ryan et al., 2001). Electrical conductivity (EC_w) was measured using a 1:1 soil-to-water extract with a conductivity meter. Soil texture was estimated by the saturation percentage method. Soil reaction (pH_w) was determined from a 1:10 soil-to-water suspension using a calibrated pH meter. Organic matter (OM) content was analyzed by the Walkley method through wet oxidation with potassium dichromate. Total nitrogen was quantified using the Kjeldahl method. Extractable phosphorus was determined using the sodium bicarbonate (Olsen) method, while extractable potassium was assessed through the sodium acetate extraction method. Extractable lead (Pb) was measured by the DTPA extraction method followed by atomic absorption spectrophotometry (AAS). Total lead content was determined through di-acid digestion ($HNO_3 + HClO_4$) and subsequent analysis by AAS in soil and plant samples. The detailed results showed in Table 1. The test crop was Chinese cabbage, treated with three different organic acids in the following concentration combinations; CK (Control), CA0.25 (Citric acid @ 0.25 mM), CA0.5 (Citric acid @ 0.5 mM), TA100 (Tartaric acid @ 100 mM), TA200 (Tartaric acid @ 200 mM), AA100 (Acetic acid @ 100 mM) and AA200 (Acetic acid@ 200 mM). A pot experiment was conducted in a greenhouse using a total of seven treatments, each replicated three times. Each pot was filled with 10 kg of soil, and fertilizers and pesticides were applied during crop growth according to standard recommendations. After maturity, plants were harvested

2.2. Plant physiological and enzymatic characteristics

Plant physiological parameters were determined by following standard test methods. The soluble protein contents were determined by the colorimetric method using Thomas Brilliant Blue (Simonian and Smith, 2006). The malondialdehyde (MDA) content was determined by the thiobarbituric acid (TBA) colorimetric method (Simonian and Smith, 2006). The superoxide anion content was determined by the hydroxylamine method (Kono, 1978). The hydrogen peroxide (H_2O_2) content was determined by spectrophotometry (Zhou et al., 2006). Hydroxyl radical scavenging capacity was determined by the fluorometric method. Superoxide dismutase (SOD) activity was determined by the nitro blue tetrazolium (NBT) photochemical reduction method (Flohe, 1984). Catalase (CAT) activity was determined by the potassium permanganate titration method (Goldblith and Proctor, 1950). Peroxidase (POD) activity was determined by the guaiacol colorimetric method (Senthilkumar et al., 2021). Dehydroascorbic acid (DHA) content was determined by the fluorometric method (Kampfenkel et al., 1995). Ascorbic acid (AsA) content was determined by the colorimetric method (Kampfenkel et al., 1995). The content of glutathione (GSH) was determined by the fluorometric method (Creissen, 1999). The content of proline was determined by the colorimetric method using ninhydrin (Wang, 2023).

2.3. Translocation and bioaccumulation factor

The translocation factor (TF) and biological concentration factors (BCF) were estimated as the ratio between plants roots to soil (Cui et al., 2007).

$$TF = \frac{Cin\ edible\ parts}{Cin\ roots} \tag{1}$$

Table 1
The pre-experiment soil characteristics used in experiment Values are means ± SD.

Characteristics	Units	Value
Electrical conductivity (EC_w)	dS m^{-1}	1.23 ± 0.09
Texture	–	Loam
Soil reaction (pH_w)		8.21 ± 0.06
Organic Matter (OM)	%	0.62 ± 0.12
Total Nitrogen	%	0.021 ± 0.08
Extractable Phosphorus	mg kg^{-1}	12 ± 1.89
Extractable Potassium	mg kg^{-1}	239 ± 12.32
Extractable Pb	mg kg^{-1}	15.12 ± 1.33
Total Pb	mg kg^{-1}	132 ± 10.32

$$BCF = \frac{Cin\ root}{Cin\ soil} \quad (2)$$

2.4. Quality assurance

The chemicals used in this study were chromatographic pure. All laboratory consumables, including centrifuge tubes, were soaked overnight in a 20 % HNO_3 solution prepared with ultrapure water (TDS<2 ppm), followed by thorough rinsing with ultrapure water. Measurements on the ICP were conducted in triplicate, and results are presented as the mean ± standard deviation of three replicates. The instrument was calibrated using certified standard solutions, and calibration curves with correlation coefficients (R^2) greater than 0.999 were employed. Quality control measures included the analysis of procedural blanks, certified reference materials (CRMs), and spike recovery tests, which confirmed the accuracy and precision of the method. The detection limit for Pb was 0.01 ppm. Internal standards were used to correct for instrument drift, and all analyses were performed at room temperature.

2.5. Statistical analysis

Sampling was performed across each treatment group and replication to ensure representative data collection. The data were analyzed using OriginPro software (OriginLab Corporation). Prior to conducting statistical tests, assumptions of normality and homogeneity of variance were checked. Normality of data distribution was assessed using the Shapiro-Wilk test, and homogeneity of variances was verified using Levene's test. Where necessary, data transformations were applied to meet these assumptions. One-way analysis of variance (ANOVA) was performed to determine treatment effects, and mean comparisons among treatments were carried out using the Least Significant Difference (LSD) test at a 0.05 probability level (Fisher, 1954). Pearson's correlation analysis was used to examine relationships among plant physiological parameters, enzymatic activities, and Pb distribution in plants under different organic acid applications. Additionally, Principal Component Analysis (PCA) was performed to further confirm significant relationships and patterns among the measured variables (Sedgwick, 2012).

3. Results and discussion

3.1. The effects of organic acids on Pb accumulation in Chinese cabbage

The organic acids applications to Chinese cabbage plants significantly ($P < 0.05$) affected the Pb translocation from soil to leaf parts. Our results discovered that the maximum Pb concentrations i.e., 3.65 ± 0.18, 1.65 ± 0.08 and 11.32 ± 0.57 mg kg^{-1} Pb in roots, leaf and soil, respectively from control (soil with no organic acids). Among organic acids, the TA was effectual in reducing Pb contents in Chinese cabbage roots up to 73.15 % (0.98 ± 0.05 mg kg^{-1}) and the subsequent soil also contained least Pb contents (0.76 mg kg^{-1}) which was lower than 42.23 % as compared to control. Interestingly, the trend in leaf parts were different, where acetic acid @ 200 mM showed least Pb concentration in leaf as 0.67 mg kg^{-1} followed by TA 200 mM as 0.76 mg kg^{-1}. From the results we affirm that acetic acid reduces translocation of Pb in leaf of chinses cabbage while TA and citric acid were less effective in reducing Pb concentration leaf (Table 2). Results showed that TF varied from 0.41 to 0.78 whereas BCF varied from 0.08 to 0.15. In general, the TF in all treatments were lower than 1 and described in the following order: TA200 >CA0.25 >TA100 >CA0.5 >AA100 >CK>AA200. Our results confirmed by Bareen (2012), he affirms that organic chelating agents have less leaching hazard and higher biodegradability. Therefore, it can use as chelating agents with optimized levels of applications. Our results showed that citric acid applications least immobilize the Pb-translocation in plants, same thing was discovered by Wu et al. (2004) who stated that citrate at lower concentrations of 3 mmol kg^{-1} did not significantly immobilize of heavy metals like Zn, Cd, Cu and Pb in *Brassica juncea.* whereas Evangelou et al. (2007) elaborated that citrate at higher concentrations of 10 and 20 mmol kg^{-1} increased Cd uptake by 1.5–3 folds. Similarly, in tobacco plants (*Nicotiana tabacum*) at lower concentration (5 mmol kg^{-1}) of citrate, oxalate or tartrate did not show significant reduction in Pb uptake however at higher concentration (60 mmol kg^{-1}) citrate doubled Pb content in shoots (Evangelou et al., 2007). Our results showed that there is different Pb-translocation from soil to leaves with respect to organic acid application (Table 2). This is because of formation of Pb-complex between the organic acid and metal (Pb). Similar findings explain by Yang et al., (2022) and Khan et al., (2022) about formation of complexes between organic acids and Pb (via complexation process) as the basic pathway for reducing ROS. After entering into a plant system, heavy metals bind with proteins and enzymes complexes which leads to increased ROS production. Plant responds to heavy metals toxicity by producing phytochelatins and organic acids are also categorized are phytochelatins produced in cytosol. The subsequent phytochelatin metals and metalloid complexes are stored in vacuolar compartments which are less sensitive to the harmful impacts of heavy metals (Dago et al., 2014; Chen et al., 2025). Polak et al. (2014) suggested that binding and compartmentalization of metal ions occur in plant in the presence of photoheating (in our case organic acids). Our results also confirmed by Osmolovskaya et al. (2018) who depicted that that citrate, tartaric and malate acids form strong bonds with ions of heavy metals under chelating process and also affect their carboxyl groups which are responsible to transport donor functions in metal ligands. Plants root naturally exudates TA (2, 3-dihydroxysuccinic acid) is identified to impact bioavailability of heavy metals (Chen et al., 2020). Tartaric acid is a 4-carbon carboxylic acid formed by plants via vitamin C metabolism (Melino et al., 2009). Tao et al. (2020) confirmed that Cd hyperaccumulating plants releases more TA in comparison to non-hyper accumulating plants. Contrary to our results Chen et al. (2020) stated a significant effect of TA on Pb mobility since it helps in metal desorption. Khan et al. (2016) tested six different chelates and found TA as the most effective in Pb uptake and translocation in spinach. The root Pb contents reduced from 3.65 in control to 0.98 mg kg^{-1} under influence of TA (Table 2). Our results are in accordance with the findings of Khan et al., (2016) who also find a decrease in root Pb concentration and attributed such response to chelate based solubility of Pb. Acetic acid (CH_3COOH, acetate) is a well-recognized organic acid that have shown strength to plants for withstanding against abiotic stresses. Hossain et al. (2020) and Wang et al. (2023) has highlight the positive influence of acetic acid in heavy

Table 2
Lead (Pb) concentration distribution in soil and the different organs of Chinese cabbage

Treatment	Lead contents (mg kg^{-1})			Pb translocation	
	Roots	Leaves	Soil	TF	BCF
CK	3.65 ± 0.18 a	1.65 ± 0.08 a	11.32 ± 0.57 a	0.45 ± 0.02d	0.15 ± 0.008 a
CA0.25	2.12 ± 0.11b	1.44 ± 0.07 b	9.43 ± 0.47 b	0.68 ± 0.03 b	0.15 ± 0.008 a
CA0.5	1.55 ± 0.08 d	0.95 ± 0.05c	8.98 ± 0.45c	0.61 ± 0.03 b	0.11 ± 0.006 d
TA100	1.65 ± 0.08 d	1.02 ± 0.05c	7.65 ± 0.38 e	0.62 ± 0.03 b	0.13 ± 0.007b
TA200	0.98 ± 0.05 e	0.76 ± 0.04d	6.54 ± 0.33 f	0.78 ± 0.04a	0.12 ± 0.006c
AA100	1.98 ± 0.10c	1.00 ± 0.05c	8.76 ± 0.44c	0.51 ± 0.03c	0.11 ± 0.006d
AA200	1.65 ± 0.08 d	0.67 ± 0.03d	8.44 ± 0.42 d	0.41 ± 0.02 d	0.08 ± 0.004 e

TF: translocation factor, BCF: Bioconcentration factor. The values are means ± SE and alphabetical letters represent the statistically significant groups according to LSD@ 0.01.

metals toxics effects mitigation. However hormonal biosynthesis and signaling effect of acetic acid is species specific. Zhu et al. (2021) confirmed involvement of acetic acid in lower accumulation of Cd in tomato plants and specify root endophytic bacteria for this output. Acetic acid stimulates jasmone acid pathway which in turn reduce root nitric acid and alleviates root Fe shortage caused due to heavy metal stress. The Cd translocation was reduced from roots to shoots, a more strengthen antioxidant defense system was activated and oxidative stress indicators were reduced in lentil seedlings (Hossain et al., 2022). In plants, Pb may be transported into plant parts via apo plastic pathway or non-selective cation channels (Pourrut et al., 2013). Our findings are in agreement with Ghnaya et al. (2013) who revealed that Pb is probably bound by citrate and further elaborated a positive correlation between Pb and citrate concentration. Higher translocation of Pb was found in sportulacastrum in comparison to *Brassica juncea* due to higher concentration of citrate in xylem. Similar results were found by Pourrut et al. (2013) who reported limited transport of Pb in phloem due to Pb-comlex formation.

3.2. The effects of organic acids on plant physiological and enzymatic activity

The CAT activity was significantly increased under exogenous application of organic acids in comparison to control (Fig. 1A) the least activity was observed in control (46.52 umol g^{-1}). At lowest concentration of organic acids, acetic acids showed maximum value (144 umol g^{-1}) however by increasing the concentration to double, the values decreased in all treatments, for example in acetic acids it reduces from 144 umol g^{-1} to 122 umol g^{-1}. Similarly, TA decreased CAT from 143.33 umol g^{-1} to 133.00 umol g^{-1}. However, the citric acid increased CAT from 88.18 umol g^{-1} (0.25 mM) to 95.18 umol g^{-1} (0.5 mM). The least SOD activity was observed in control (144.03 U mg $^{-1}$ protein) while in all other treatments, the SOD activity was increased (Fig. 1A). Acetic acid (100 mM) and citric acid (0.25 mM) showed highest SOD as178.50 U mg^{-1} protein and 178.27 U mg^{-1} protein (Fig. 1A). POD activity significantly increased under TA (344.17 μmol g^{-1} FW), citric acid (321.17 μmol g-1 FW) and acetic acid (299.27 μmol g-1 FW). The detailed results of POD activity are shown in Fig. 1A. The MDA content was decreased in all organic acid treatments in comparison to control (Fig. 1B). Citric acid (0.50 Mm) followed by TA (200 Mm) was most effective in decreasing MDA content·H_2O_2 contents were also highest in control (Fig. 1B). At lower concentrations of applied organic acids, citric acid (0.25 mM) and TA (100 Mm) showed similar H_2O_2 contents as 25.7 μmol • g^{-1} DW (Fig. 1B). The GSH was highest in control and acetic acid (100 mM) as 98.0 μmol L^{-1} while all other treatments showed lesser values as shown in Fig. 1C. Citric acid (0.50 mM) and TA (200 mM) were most effective in reducing GSH activity as 56.0 μmol L^{-1} and 65.0 μmol L^{-1} respectively as shown in Fig. 1C. We observed that at lower concentration, acetic acid (91.2 μmol mg^{-1}) was most efficient in scavenging hydroxyl radical. However, at higher concentrations, all organic acids increased their hydroxyl radical scavenging ability and maximum was obtained in citric acids (0.50 mM) as 92.1 (Fig. 1C). At lower

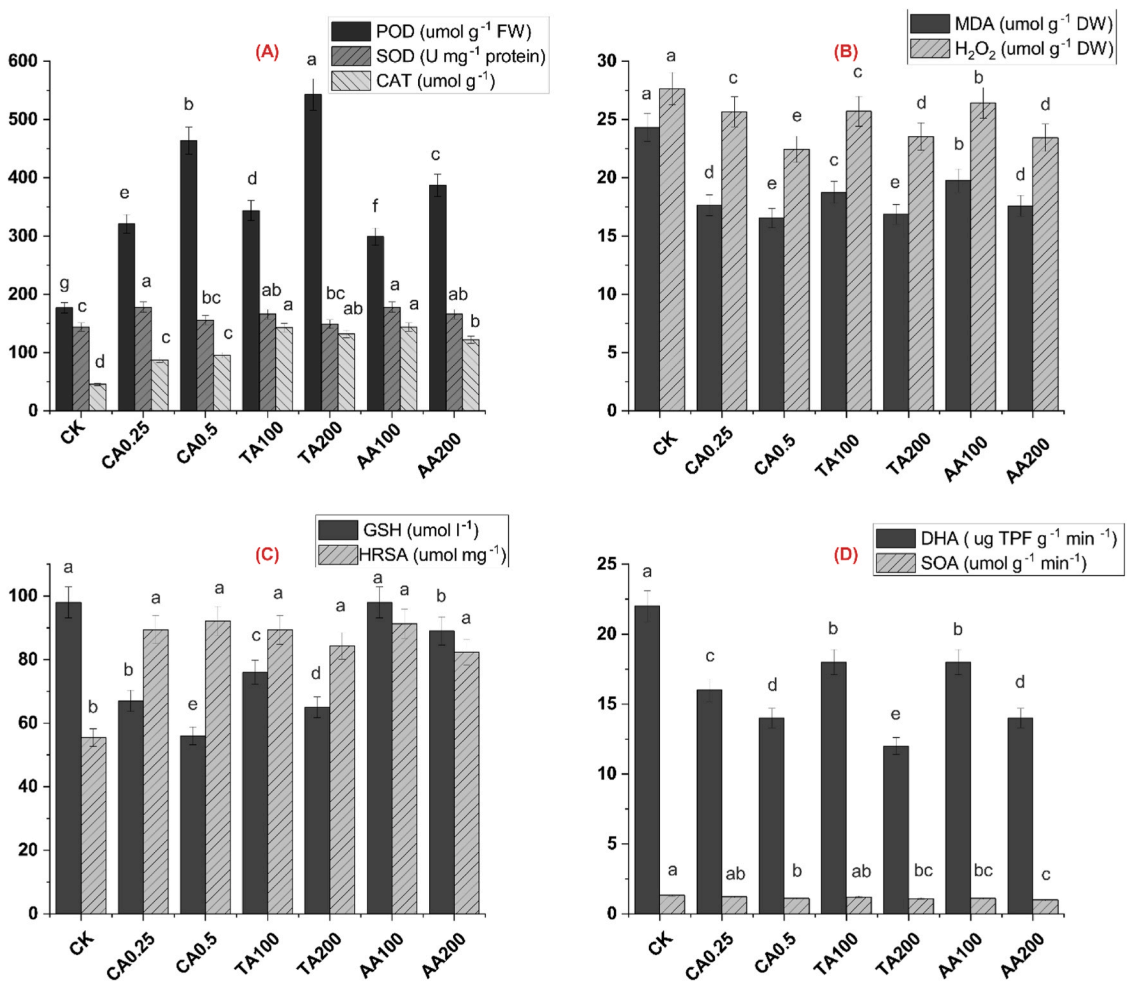

Fig. 1. The effects of organic acids on (A) POD, SOD and CAT activity (B) MDA and H_2O_2 content (C) GSH and HRSA and (D) DHA and SOA in Chinese cabbage. The y axis represents the respective activities/content and x axis represents the treatments. The histogram shows mean values ± SE ($n = 3$). The alphabetical letters on bars shows statistically significant groups according to LSD @ 0.01.

application rates the DHA content was also reduced as compared to control however more reduction was observed at higher application rate (Fig. 1D). We observed highest SOA in control. While among organic acid treatments the highest concentration of SOA was in citric acid 0.25 mM and TA 200 mM (Fig. 1D). Our study confirmed that Severe oxidative damage occurs when plants are exposed to Pb stress due to excessive ROS production (Yang et al., 2022; Pal and Sukul 2022). Non conducive environment can lead to stress in plants and give rise to free radicals posing toxic impacts on plants cell membranes (Kurtyka et al., 2018). The role of organic acids in anti-oxidant defense mechanism adopted by plants is multifaceted, and mostly determined through the type of stress and how it stimulates a plant. Many studies exist in literature underlining the capacity of organic acids as protectors against ROS (Chen et al., 2024; Zia-ur-Rehman et al., 2023). Antioxidant enzyme system helps to remove active oxygen free radicals for protecting plants. Substantially, plants strive against heavy metals through intracellular chelation, beginning through organic and amino acids synthesis, neutralizing ROS (Kocaman, 2023). Howbeit, exogenous exertion of diverse organic acids has potential to aid plants against abiotic stresses. We observe increased SOD (178.0 U mg^{-1} protein) and CAT (144 umol g^{-1}) activity in acetic acid treatment whereas POD (543.0 μmol g^{-1} FW) was highest in TA (Fig. 1A). Yet either of them was superior in strengthening enzymatic activities in Chinese cabbage. Increase in ROS scavenging is a key mechanism to cope with HM and is regulated by different antioxidant enzymatic activities in the plants (Smeets et al., 2002). Our results are in concurrence with Santos et al. (2018) who observed escalation APX, CAT and SOD in pepper leaves. In a further study Wang et al. (2010) tested seeds of *Vicia faba* in Pb polluted soils and exhibited an increase in O^- and lipid peroxidation in roots, soil Pb activated SOD, POD and APX enzymes and displayed a biphasic curve. Upregulation of POD and APX might be the chief scavengers for increased H_2O_2 when CAT activities were reduced after plant suscept to soil Pb. SOD is an important antioxidant enzyme that provides the first line of defense against superoxide anions by catalyzing the conversion of O_2^- to H_2O_2, and O_2. CAT has high conversion rate and can catalyze the decomposition of H_2O_2 into H_2O and O_2. POD also promotes decomposition of H_2O_2 (El-Beltagi and Mohamed, 2013; Rizwan et al., 2024b). Guo et al. (2017) specify increased gene expression of antioxidant enzymes like POD1, Cu/Zn-SOD and GPX1 induced by organic acids. A study on citric acid application in cabbage plants under Cd stress resulted in enhanced antioxidant activities (Catalase, superoxide dismutase and peroxidase) (Faraz et al., 2020). This may be related to the chelation of Pb by organic acids, which alleviates HM induced oxidative stress and ROS, and H_2O_2 accumulation would consequently greatly decrease. An alleviated concentration of MDA, H_2O_2 in control (soil without any organic acid) (Fig. 1b) indicated oxidative stress. Highest MDA (24.3 nmol g^{-1} DW) and H_2O_2 (27.7 umol g^{-1} DW) in control compared with other organic acids corroborates their effectiveness in reducing MDA and H_2O_2 content. Debnath et al. (2021) elaborated that MDA and H_2O_2 are basically byproducts of metabolic processes particularly happening during abiotic stresses. Our study is also in agreement with Shi et al. (2023) who also found increased MDA, H_2O_2 and O_2^- ions in *R. chinensis*. Overall, Pb stress (500–1500 mg kg^{-1} Pb $(NO_3)_2$ increased leaflet MDA, H_2O_2, and O_2^- accumulation by 1.27–2.14 folds, 1.12–1.76 fold, and 1.13–1.88 fold, respectively. The increasing GSH and APX activities indicated GSH-ascorbate cycle was also involved in eliminating H_2O_2 (Wang et al., 2010). We observed reductions in AsA content in Pb stressed plants whereas an increasing trend was observed in plants treated with organic acids (the highest in acetic acid) as shown in Fig. 1C. Our results are supported by Ighodaro and Akinloye (2018) who reported that CAT degrades H_2O_2 into H_2O and O_2 whereas MDHAR participates in AsA-GSH cycle, amid regenerating AsA from monodehydroascorbate. AsA and GSH scavenge ROS by electron donation to the free radicals also called sacrificial nucleophiles. Hussain et al. (2024) also observed increased AsA contents in acetate pretreated lentil plants exposed to Cu stress. Tan et al., (2022) reported inhibited AsA-GSH system in Pakchoi under Pb stress.

3.3. Principal component analysis and Pearson's correlation matrix

The PCA loading plot Figure 5 (a) showed the relationship of the observed parameters with each other. It was observed that H_2O_2, DHA and MDA were present in the same quadrant and had very small angles between the vectors thus sharing a close and positive correlation between them. The PCA loading plot (Fig. 2a) showed the relationships among the observed variables, where Pb uptake and enzymatic activities loaded strongly on PC1, while physiological parameters were mainly associated with PC2, indicating their distinct contribution to variance. The score plot (Fig. 2b) indicated that treatments AA200 and AA100 clustered closely, suggesting a similar impact on the measured variables. In contrast, the control (CK) appeared far from other treatments, reflecting its distinct performance compared to the organic acid applications. Our data confirmed that the applied treatments significantly recued the translocation of Pb from soil to leaves as compared to control. Heat map of Pearson's correlation matrix (Fig. 3) showed varied interactions/relationships between the observed variables. A strong and positive correlation existed between CAT and AsA (r = 0.96), CAT and HRSA (r = 0.93), DHA and SOA (r = 0.91), DHA and MDA (r = 0.99), DHA and H_2O_2 (r = 0.99). SOA also showed strong and positive relationship with MDA, H_2O_2, TF, BF, Root, shoot and soil Pb contents (Fig. 3). However, HRSA showed negative but strong correlation with

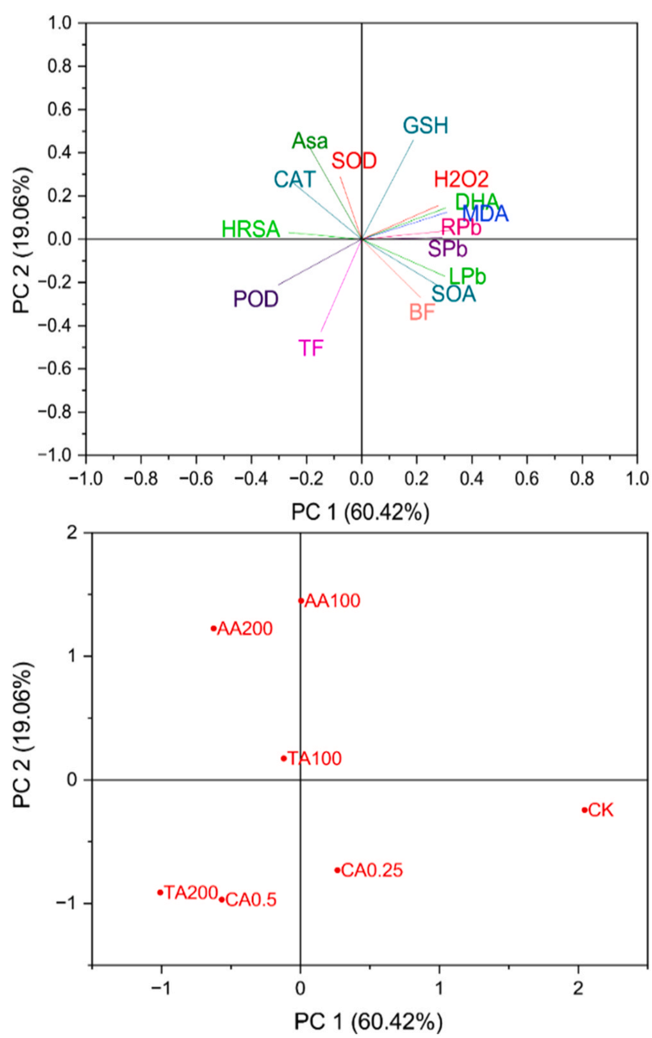

Fig. 2. (a): The PCA loading plot showing relationship **(b):** PCA scores plot between the observed variables in Chinese cabbage..

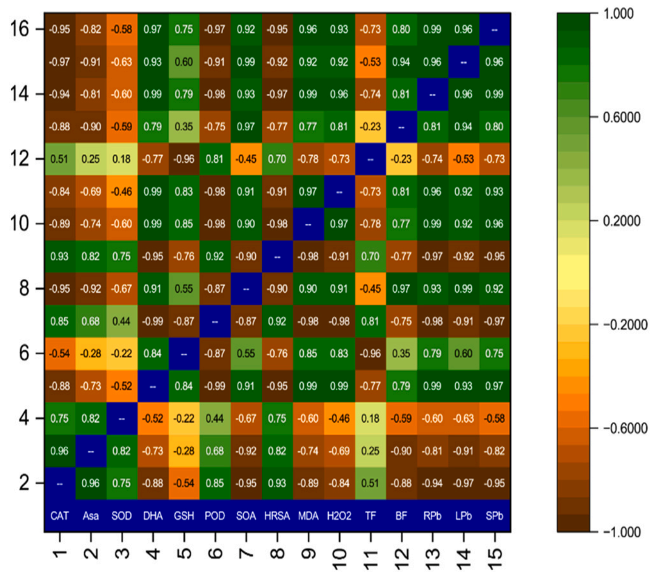

Fig. 3. The Heat map of Pearson's correlation matrix among various observed parameters in Chinese cabbage..

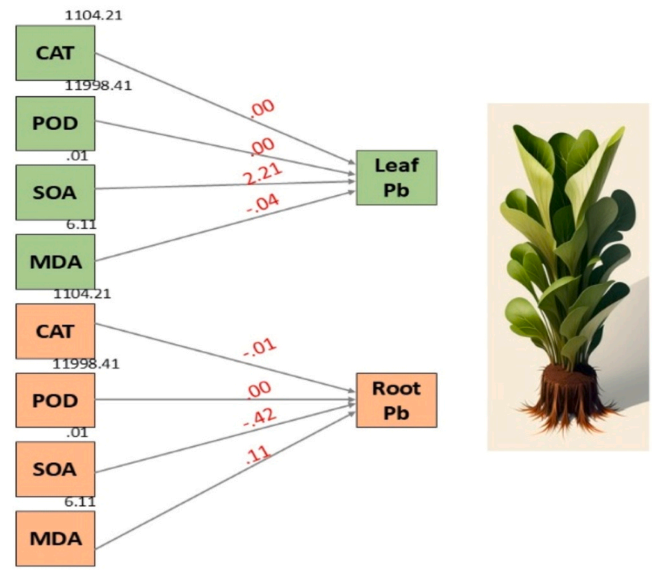

Fig. 4. The path analysis of Pb contents in roots and shoots of Chinese Cabbage.

MDA, H_2O_2, Root, shoot and soil Pb contents. From the summary of path analysis, we observed that leaf Pb was negatively co-related to CAT, POD and MDA whereas it shared a positive relationship with SOA. While Root Pb was showed negative relationship with CAT, POD and SOA while MDA had positive influence on the Root Pb. Hydrogen peroxide (H_2O_2) is a reactive oxygen species that accumulates during oxidative stress. DHA, being an unsaturated fatty acid, is prone to oxidation and can react with H_2O_2. Thus, as H_2O_2 levels increase under stress, DHA could undergo oxidation, resulting in a positive correlation. Additionally, both DHA and H_2O_2 can be involved in signaling pathways related to stress responses, further linking them. High concentrations of SOA in the soil may enhance the mobility of heavy metals like lead (Pb), making them more available for uptake by plants. Therefore, higher SOA could be associated with higher soil Pb contents. The unstandardized estimates obtained for both leaf and root Pb are indicated in red in the Fig. 4. The Pb contents accumulation in plants reduced the CAT, POD and MDA activity as confirmed by Tan et al., (2022). These negative correlations affirm our findings with previous reported results (Bareen, 2012, Faraz et al., 2020).

4. Conclusion

With the growing threat of pollution and heavy metal toxicity, both humans and ecosystems face significant health and toxicity risks (Clemens and Ma, 2016; Shi et al., 2023). Plants, in particular, are vulnerable to heavy metal stress, which impairs seed germination, growth, and natural functioning. Lead (Pb) toxicity induces oxidative stress, causing damage to key cellular components such as lipids, proteins, and DNA. This study demonstrates that the application of organic acids—AA, TA, and CA—offers an environmentally sustainable approach to reducing Pb uptake and translocation in Chinese cabbage. These acids effectively mitigate Pb-induced stress by activating the plant's antioxidant defense system, minimizing oxidative damage. The findings revealed that organic acids, especially tartaric acid, can enhance Pb tolerance in plants, supporting their potential as effective tools for managing abiotic stress. This research emphasizes the promising role of organic acids in promoting environmental adaptation and improving plant resilience against heavy metal contamination.

CRediT authorship contribution statement

Hong Chen: Supervision, Conceptualization. **Jean Wan Hong Yong:** Funding acquisition, Data curation, Conceptualization. **Ghulam Murtaza:** Writing – review & editing, Validation, Investigation. **Muhammad Rizwan:** Methodology, Investigation. **Muhammad Rizwan:** Formal analysis, Data curation, Conceptualization. **Wajiha Anum:** Visualization, Validation. **Humera Aziz:** Data curation. **Umair Riaz:** Writing – original draft, Supervision.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper. No financial support or other benefits from commercial sources have been received for the research. The authors have no personal or professional relationships with organizations or individuals that could influence the content or outcomes of this study.

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Data availability

Data will be made available on request.

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