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The effect of phosphate application in Pb-contaminated soil on the oxidative stress of leaves, Pb accumulation in biomass of maize and Pb speciation in rhizosphere soil

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Abstract: Reducing the bioavailability of Pb in soil is the key to alleviate the toxicity of Pb to plant. Maize were exposed to Pb 100 mg/kg of soil with three fertilizer levels of control (T1), nitrogen, phosphorus and potassium of 204 mg/kg (T2) and nitrogen, potassium of 204 mg/kg (T3). The phosphate supplement lead to the reduction by 24.92%, 29.73% and 25.31% respectively in activity of total superoxide dismutase (T-SOD), peroxidase (POD) and concentration of lipid peroxidation (MDA) in maize leaves, and reduced Pb accumulation in above- and below-ground biomass of maize by 39.20% and 37.58%. In T2 treatment group, the water soluble Pb, ionic fraction and carbonate fraction Pb in rhizosphere soil decreased by 37.57%, 36.36% and 43.24%, and organic fraction Pb and residual fraction Pb was the highest with the value of 11.67 and 18.57 mg/kg; the soil aluminum bound (Al-P) and iron bound phosphate (Fe-P) were the highest with 93.53 mg/kg and 230.32 mg/kg, indicating that the phosphate supplement increases the soil ionic P and transforms the chemically mobilized P (such as O-P, Ca-P) into the bioavailable P. Moreover, the soil organic fraction and residual fraction immobilized Pb was positively correlated with the bioavailable Al-P and Fe-P, indicating that the ionic fraction P (Al-P and Fe-P) react with Pb and produce residual P-Pb compounds. Therefore, phosphate supplement to Pb contaminated soil could transfer unstable fraction Pb into stable fraction Pb by P-induced Pb immobilization, reduce the bioavailability of Pb and alleviate the toxicity of heavy metal to plant.

Keywords： antioxidant enzyme, heavy metal Pb, phosphate speciation, Pb speciation, accumulation of Pb in below- and above-ground biomass

Introduction

High rates of industrial and agricultural development have led to land contamination by global pollutants such as heavy metal Lead (Pb), especially in Yunnan province, southwest of China. Pb is a highly toxic environmental pollutant that has high mobility in the soil-plant system and negatively impacts plants and animals. Its concentration in some land types such as mining area and farmland around the mining area has increased due to industry, mining and smelting (He et al. 2015; Kubier and Pichler 2019；Deng et al. 2022; Chen et al. 2022; Zhang et al. 2021; Sun et al. 2020; Yan et al. 2019). Pb can be retained in the soil through a series of reactions such as adsorption, ionic exchange and precipitation, and formed various Pb-compounds which have different effects on oxidative stress of plant and enrichment in plant organs (Selim 2015; Wan et al. 2015). In general, the Pb-compounds with high mobility are more bioavailability and more environmental risk for plant. Heavy metal Pb has been divided into four speciation: soluble/exchangeable fraction (F1), reducible fraction (F2), oxidizable fraction (F3) and residual fraction (F4), and the mobility is decreased in the order of: F1>F2>F3>F4 (Ure et al. 1993). The residual fraction of Pb has been recognized as the most stable fraction which causes less effect on the oxidative stress of plant than that of other fraction of Pb (Zeng et al. 2017). Based on this theory, some researches and remediation strategies designed to promote the transformation of Pb from unstable fraction (F1, F2 and F3) to mineral structure (F4) was focused, aiming to protect against Pb-induce oxidative stress and enrichment for Pb in plant's organs ((Awan et al. 2020; de Anicésio and Monteiro 2021；He et al. 2020; Hua et al. 2020; da Silva et al. 2018; Moreira et al. 2018).

In recent years, phytoremediation for heavy metal contaminated some mining areas, such as the hyperaccumulator plants of *Pteris vittata L.*, tanzania guinea grass and *Arabis alpina L.var.parviflora Franch* has been studied under conditions of phytotoxicity and the different fraction of heavy metals ((de Souza Cardoso and Monteiro 2021; Wang et al. 2022; Guo et al. 2017; Zhang et al. 2020). These hyperaccumulator plants has shown promise for phytoextraction of metals due to its high amount of heavy metals absorption and antioxidant mechanism which have the function of eliminating ROS, transforming them into non-toxic compounds (Nematian and Kazemeini 2013; Xu et al. 2020; Kathal et al. 2016). However, the toxic effects of Pb depend on speciation of Pb. The physiological changes of plant rhizosphere in Pb-contaminated soil can affect the metal transformation through acidification effect and mycorrhizae effect (Bolanet al. 2014; Zhang and Van Gestel 2019; Baruah et al. 2020; Bilias et al. 2021). For farmland crops, such as maize, the supplement of fertilizer is inevitable. Whether the application of fertilizer changes the speciation of Pb is also an important aspect of its tolerance mechanism. The application of water-soluble P-compounds of fertilizer could dissolve in soil with the formation of dicalcium phosphate and phosphoric acid (H_3PO_4), and then H_3PO_4 dissociates into dihydrogen phosphate and hydrogen ions, reducing the soil pH to a low level (Zeng et al. 2013; Basta and McGowen 2004). Theoretically, the decrease of soil pH will promote the transformation of residual fraction Pb into soluble/exchangeable fraction Pb and increase the bioavailable Pb for plant (Cao et al. 2008). In addition, phosphorus, nitrogen and potassium supplement can reduce the deleterious effects of Cd toxicity by increasing photosynthesis in tomato (*Solanum lycopersicum L.*) plants (Naciri et al. 2021) and antioxidant enzymes activities and proline and polyamines synthesis in tanzania guinea grass (de Anicésio and Monteiro 2021). However, few studies have investigated the physiological processes and the changes of speciation of Pb involved in the role of phosphorous and nitrogen in alleviating Pb toxicity in plants, especially in farmland crops, such as maize.

The phosphate supplement inhibits the absorption of Pb and improves the P/Pb ratio in plants by the competitive absorption of phosphorus and heavy metal Pb in plants, so as to alleviate the toxicity of Pb to plants, which shows that the plants has high tolerance to heavy metal Pb (Lessl and Ma 2013; Han et al. 2017). However, phosphate fertilizer supplement in Pb-contaminated soil will produce a large amount of bioavailable phosphorus and Pb in the rhizosphere, and increases the chance of chemical reaction of residual P-Pb compounds. Therefore, it is difficult to judge whether the absorption of Pb by plants is blocked due to the formation of residual P-Pb compounds in the rhizosphere, or the competitive absorption between phosphorus and lead improves plant tolerance. Therefore, it needs to be further studied on the relationship between phosphate application, and Pb speciation and physiological changes of plants, especially in farmland crop. This study was carried out to evaluate the effect of phosphate on rhizosphere soil Pb speciation, enrichment of Pb by plant and changes in the antioxidant system of maize under Pb toxicity. The hypothesis is that improving phosphorus nutrient can transform Pb speciation in rhizosphere soil and then affects the antioxidant system, increasing the tolerance of maize to this heavy metal.

Materials and methods

Experiment design

Jinding lead zinc mining area in Lanping County of Yunnan province, southwest of China has a long mining history, causing varying degrees of heavy metal pollution to the surrounding land (Zhan et al. 2019). The mining area and wasteland, farmland around the mining area, river beach in the mining area, farmland around the smelter in the mining area and other land types have serious soil heavy metal pollution, especially lead pollution (Fu et al. 2017). Among these land types, the highest content of heavy metal Pb in soil is 2562.74 mg/kg, and the variation range of Pb content in farmland around the mining area is 59.74-310.69 mg/kg (He et al. 2021; Zhang et al. 2002; Zhang et al. 2021). According to the Pb content of the farmland soil around the mining area, the concentration of heavy metal Pb ($PbNO_3$) was set as 100 mg/kg in pot experiments. The soil used in pot experiments was red soil collected from farmland in Lanping County. Red soil and humus are mixed by 1:1 weight, and then the heavy metal Pb ($PbNO_3$) is added to make the Pb content in potted soil 100 mg/kg. After that, the soil is kept moist and placed for 30 days, so that the heavy metal Pb can react with soil ions to achieve even distribution of the pollutants.

Black plastic bags were used to cover the interior of the pots to prevent the loss of soil solution including water soluble pollutants and nutrients. The maize variety Qiandan 88 planted in large area was used as the research object. Firstly, 10 maize seeds (Qiandan 88) are sterilized (75% C_2H_5OH and 30% H_2O_2 solution is prepared in a volume ratio of 4:1, soaked for 30s and then washed with distilled water). Then, these seeds were transferred to the soil with $PbNO_3$ pollution 100 mg/kg soil pot and each Pb treatment group was performed in quintuplicate. After 20 days of maize growth, phosphate (P), nitrogenous (N) and potassium (K) fertilizer were supplied to the soil. Therefore, the experimental treatments were as following: (i) 100 mg/kg Pb contaminated soil (T1 treatment group); (ii) 100 mg/kg Pb contaminated soil supplied with N, P and K of 204 mg/kg (T2 treatment group); (iii) 100 mg/kg Pb contaminated soil supplied with N, K of 204 mg/kg (T3 treatment group). Soil moisture in the pots was maintained at 50% of the full moisture capacity by daily watering, the need for which was determined by weighing the pots. The plants were grown under greenhouse conditions at 24–28/20–22 °C and under natural illumination for 60 days.

Plant analyses

At the growth of 2 months，the plants were removed from the pots, the above-ground biomass (including stems and leaves) and below-ground biomass (roots) were separated from the maize, and the roots were shaken and washed free of soil with tap water. Plant biomass of maize was weighed and sampled for biochemical and chemical analyses, and dried to constant weight in an oven at 75 °C. The dried plant biomass was digested with HNO_3-H_2O_2, and the content of heavy metal Pb in above- and below-ground biomass was determined by ICP-MS spectrometry. According to the methods of Tara and Ewa (Tara and Urszula 2021; Ewa and Beata 2002), the enrichment coefficient of heavy metal Pb and the content of Pb in above- and below-ground biomass of maize were calculated.

Samples of leaves (1 g) were ground in a mortar with quartz sand and Na/K-phosphate buffer (pH 7.0-7.4) with leaves samples/solution ratio of 1:4 and 1:9 respectively for total superoxide dismutase (T-SOD) and peroxidase (POD). 1 g leaves of maize were ground in a mortar with quartz and 10 ml trichloroacetic acid (5% $C_2HCl_3O_2$) for malondialdehyde (MDA). The homogenate was centrifuged for 10 min, the supernatant liquid was decanted, the pellet was suspended in 2 mL of the same buffer, and the suspension was centrifuged again. Then T-SOD and POD activities and MDA concentration were determined by commercial kits produced by Nanjing Jiancheng Bioengineering Instituted with mechanisms described in Li et al. (2013).

Measure of chemical properties in maize rhizosphere soil

The samples of rhizosphere soil were removed from maize rhizosphere for soil enzyme activity, and air-dried and grounded in a ball mill. After thorough mixing, 200 mg of the sample, required for analysis, was collected in triplicate. The soil urease was measured by sodium phenolate colorimetry, which was calculated from the mass of NH_3-N (mg) produced by 1.0 g soil cultured at 37 °C for 24 h; the acid phosphatase was measured by disodium diphenyl phosphate colorimetry, which was calculated by the mass of phenol released by 1.0 g soil cultured for 24 h; the sucrase activity was measured by 3,5-dinitrosalicylic acid colorimetry, which was calculated by the mass of glucose (mg) produced by 1.0 g soil cultured at 37 °C for 24 h (Geng et al. 2017; Ma et al. 2018). Each sample was performed in triplicate. The dried and grounded soil was used to determine water-soluble phosphorus, aluminum bound phosphorus (Al-P), iron bound phosphorus (Fe-P), occluded phosphorus (O-P) and calcium bound phosphorus (Ca-P) by Sequential Extraction method (Zhang 1982； Lu 2000； Wang and Lu 2020). Then the different soil extraction solution was determined by molybdenum-antimony colorimetric method. Seven speciation Pb of soil, including water soluble Pb, carbonate fraction Pb, ionic fraction Pb, humic acid fraction Pb, Fe-Mn oxide fraction Pb, organic fraction Pb and residual fraction Pb, are extracted by Seven-step extraction method according to the standard of China Geological Survey (Dong 2017). Then these soil extraction solutions were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). 10 g soil was sampled and dissolved by 25 mL of deionized water at 20-25 °C, then soil pH was measured by pH meter.

Statistics

Data were processed by calculating the means of at least three replicates. Standard deviations and confidence intervals were used at $p \leq 0.05$. SPSS 22.0 software was used to analyze the effects of different fertilization treatments with the same Pb concentration toxicity on rhizosphere soil enzyme activity, phosphorus and Pb speciation in maize rhizosphere soil, antioxidant enzyme activity in maize leaves, and accumulation and enrichment coefficient of heavy metal Pb by maize. And Duncan's multiple range tests were applied in analysis of variance (ANOVA) (p <0.05). Pearson parameter was applied in analysis of the correlation among the changes of pH, different P speciation and Pb speciation in rhizosphere soil **(**$^{**}p < 0.01$; $^{*}p < 0.05$**)**. Originpro 2018 software is used to perform linear fitting or nonlinear curve fitting (exponential) on the relationship between phosphorus speciation and chemically stable Pb (organic fraction Pb+ residual fraction Pb) of maize rhizosphere soil.

Results

Changes of antioxidant enzyme system in maize leaves under Pb stress

The total superoxide dismutase (T-SOD), peroxidase (POD) and lipid peroxidation (MDA) in maize leaves under the stress of 100 mg/kg Pb concentration with the different N，P and K supplement are shown in Fig.1. In T1, T2 and T3 treatments, the activities of T-SOD, POD and MDA in maize leaves were T1 treatment group >T3 treatment group>T2 treatment group. The activities of T-SOD and POD in maize leaves in T2 treatment group were significantly different from those in the other two groups (p <0 .05), and there was no significant difference between T1 and T3 treatment groups (p < 0.05). The maximum value of T-SOD and POD appeared in T1 treatment with the value of 60.48 U/mgprot, 72.11 U/mgprot, respectively. Compared to T1 treatment group, T-SOD and POD in maize leaves of T2 and T3 treatment decreased by 24.92% and 2.15%, 29.73% and 23.20%, respectively. There was significant difference in concentration of MDA in maize leaves among T1, T2 and T3 treatments ($p < 0.05$)，and the highest value of it appeared in T1 treatment group with the value of 103.30 nmol/mgprot. The concentration of MDA of T2, T3 decreased by 25.31% and 13.14%, respectively, comparing to T1 treatment group.

Fig.1 Maize superoxide dismutase (SOD), peroxidase (POD) and lipid peroxidation (MDA) in leaves

Accumulation characteristics of Pb in maize

The accumulation of Pb in above- and below-ground biomass of maize is shown in Fig.2. The accumulation of Pb in below-ground biomass of maize was higher than that of the above-ground biomass among these treatment groups. In both aboveground and below-ground biomass of maize, the accumulation of Pb in T1 treatment group was significantly higher than that in the other two groups, and there was no significant difference between T2 and T3 treatment groups ($p < 0.05$). In T2 and T3 treatment groups, the accumulation of heavy metal Pb by above- and below-ground biomass of maize decreased by 39.20% and 37.58%, 30.82% and 29.18%, respectively, compared with T1 treatment group. The enrichment coefficient for Pb in maize was similar to the accumulation of Pb in above- and below-ground biomass of maize. The highest enrichment coefficient for Pb in maize appeared in T1 treatment group with the value of 0.80, and this parameter of maize in T2 and T3 treatment groups has no significant difference and were significantly lower than that of T1 treatment group ($p < 0.05$). The enrichment coefficient for Pb in maize in T2 and T3 treatment groups decreased by 12.03% and 6.22%, respectively, compared with T1 treatment group.

Fig.2 Enrichment coefficient of heavy metal Pb in above- and below-ground biomass of maize

Pb and phosphorus speciation and enzyme activities in soil

The speciation of Pb in maize rhizosphere soil is shown in Fig.3. The soil water soluble Pb, ionic fraction Pb and carbonate fraction Pb content have significant difference ($p < 0.05$) and showed T1 treatment group>T3 treatment group>T2 treatment group. However, the contents of organic fraction and residual fraction Pb in T2 and T3 treatment groups were higher than those in T1 treatment group and both speciation of Pb showed T2 treatment group>T3 treatment group>T1 treatment group. The highest content of water soluble Pb in the rhizosphere soil of maize appeared in the T1 treatment group with the value of 0.05 mg/kg. Compared with T1 treatment, the water soluble Pb in maize rhizosphere soil in T2 and T3 treatments decreased by 37.57% and 28.65% respectively, and ionic fraction and carbonate fraction Pb in both treatment groups decreased by 36.36% and 28.16%, 43.24% and 36.37%, respectively, which were significantly lower than those in T1 treatment group. In T2 and T3 treatments, there was no significant difference in ionic Pb in maize rhizosphere soil, but there was significant difference in carbonate fraction Pb in rhizosphere soil ($p < 0.05$). The content of humic acid fraction Pb in maize rhizosphere soil in T2 treatment group was lower than that of the other two groups. The content of Fe-Mn oxides Pb in maize rhizosphere soil of T2 treatment group was significantly lower than that in T1 treatment group, and the contents of organic fraction Pb and residual fraction Pb in rhizosphere showed the maximum value with 11.67 and 18.57 mg/kg, respectively, which were significantly higher than those in other groups.

Fig.3 heavy metal Pb speciation in maize rhizosphere soil

The speciation of phosphorus in maize rhizosphere soil are shown in Fig.4.The contents of aluminum bound phosphate (Al-P) and iron bound phosphate (Fe-P) in maize rhizosphere soil showed T2 treatment group>T3 treatment group>T1 treatment group. The content of Al-P and Fe-P in maize rhizosphere soil showed T2 treatment group>T3 treatment group>T1 treatment group. The highest value of maize rhizosphere soil Al-P appeared in T2 treatment group with the value of 93.53 mg/kg, and increased by 109.62% compared with T1 treatment group. In T2 treatment group, the content of Fe-P in maize rhizosphere soil showed the maximum value with 230.32 mg/kg. The contents of occluded phosphate (O-P) and calcium bound phosphate (Ca-P) in maize rhizosphere soil were T1 treatment group>T3 treatment group>T2 treatment group, and the rhizosphere soil O-P contents in T1, T2 and T3 treatment groups were 39.38, 29.89 and 38.17 mg/kg, respectively. And the content of Ca-P in maize rhizosphere soil in T1 treatment group was the highest with the value of 922.38 mg/kg, but in T2 and T3 treatment groups, the rhizosphere soil Ca-P content decreased by 57.05% and 20.51% respectively compared with T1 treatment.

Fig.4 Soil phosphorus speciation

The maize rhizosphere soil pH of T1, T2 and T3 treatment groups is shown in Fig.5A. The soil pH was T1 treatment group>T2 treatment group>T3 treatment group of maize rhizosphere soil, indicating that the supplement of N, P, K to the soil reduces the pH of the soil. Compared with T1 treatment group, the rhizosphere soil pH in T2 and T3 treatment groups decreased by 0.46 and 0.50, respectively. The activities of urease, acid phosphatase and sucrase in maize rhizosphere soil are shown in Fig.5B, 5C and 5D. The range of soil urease activity was 8.88-10.26 mg/g.24h^{-1}, and there was no significant difference among these groups ($p < 0.05$). The activity of acid phosphatase in maize rhizosphere soil was the highest in T3 treatment group, followed by T2 treatment group and the lowest in T1 treatment group. The soil acid phosphatase activity in the T1 treatment group was the lowest with the value of 1443.18 mg/g.24h^{-1}, and the activity of soil acid phosphatase in T2 and T3 treatment groups increased by 18.11% and 117.43%, respectively, compared with T1 treatment group. The maize rhizosphere soil sucrase activity was T3 treatment group>T1 treatment group>T2 treatment group, and there was significant difference among these groups ($p < 0.05$), and the highest soil invertase activity in T3 treatment group was 41.66 mg/g.24h^{-1}.

Fig.5 The maize rhizosphere soil pH and enzyme activity

Correlation analysis of soil Pb and phosphorus speciation

The correlation analysis between soil pH, different phosphorus speciation and different Pb speciation is shown in Table 1. The soil Al-P, residual fraction Pb and organic fraction Pb were significantly negatively correlated ($p < 0.01$) or significantly negatively correlated ($p < 0.05$) with the maize rhizosphere soil pH, but water soluble Pb, ionic fraction Pb and carbonate fraction Pb were positively correlated with soil pH ($p < 0.01$). The soil Al-P was negatively correlated with Ca-P, water soluble Pb, ionic fraction Pb, and carbonate fraction Pb ($p < 0.01$), positively correlated with organic fraction Pb and residual fraction Pb ($p < 0.01$), and negatively correlated with Fe-Mn oxide fraction Pb ($p < 0.05$). There was a significant positive correlation between O-P and Ca-P ($p < 0.05$). The Ca-P was positively correlated with water soluble Pb and ionic fraction Pb ($p < 0.01$), and negatively correlated with organic fraction Pb and residual fraction Pb ($p < 0.01$). The water soluble Pb has a significantly positive correlation with ionic fraction Pb and carbonate fraction Pb ($p < 0.01$), indicating that these three speciation of Pb is extremely easy to transform each other. Water soluble Pb, ionic fraction Pb, carbonate fraction Pb and Fe-Mn oxide fraction Pb all have a extremely significantly negative correlation with organic fraction Pb and residual fraction Pb ($p < 0.01$), because the chemical properties of organic fraction Pb and residual fraction Pb are stable and it is difficult to convert other speciation of Pb.

Table 1 Correlation analysis of Pb and P

P compound in soil reacts directly or indirectly with heavy metal Pb. In this study, regression analysis is carried out on the data of P and Pb speciation to explore the relationship between P speciation and Pb speciation (Fig.6). The contents of immobilized Pb (organic fraction+residual fraction) in soil were positively correlated with Al-P and Fe-P, and the correlation coefficient R^2 were 0.91 and 0.92 respectively and negatively correlated with Ca-P. The change of P-Pb relationship model is consistent with the correlation analysis (Table 1), which indicates that mobilized phosphorus (such as Al-P, Fe-P) may react with easily absorbed Pb (such as water soluble Pb, ionic fraction Pb and carbonate fraction Pb), and Pb will transform to a stable state Pb (organic fraction Pb, residual fraction Pb, etc.) to reduce the mobility and bioavailability of Pb.

Fig.6 Regression analysis of residual fraction and organic fraction Pb and P speciation （Al-P、Fe-P、O-P、Ca-P）

Correlation analysis of antioxidant enzyme system, accumulation characteristics and Pb speciation in maize leaves

The correlation analysis between enrichment coefficient for Pb in maize and Pb speciation in soil is shown in Table 2. The enrichment coefficient for Pb in maize was significantly positively correlated ($p < 0.05$ or $p < 0.01$) with water soluble Pb, ionic fraction Pb, carbonate fraction Pb and Fe-Mn oxide fraction Pb, and extremely significantly negatively correlated with organic fraction Pb and residual fraction Pb ($p < 0.01$). These results indicated that the enrichment of heavy metal Pb by plants depends on the content of bioavailable Pb in soil to a certain extent, which can promote the high enrichment of Pb by plants.

Table 2 Correlation between enrichment coefficient and Pb speciation

The correlation analysis between enzyme activities of T-SOD, POD, concentration of MDA in maize leaves and soil different Pb speciation is shown in Table 3. The activity of T-SOD was significantly positively correlated ($p < 0.01$or $p < 0.05$) with MDA content in maize leaves, soil water soluble Pb, carbonate fraction Pb and Fe-Mn oxide fraction Pb. The activity of POD was extremely significantly positively correlated with MDA content in maize leaves, soil water soluble Pb ($p < 0.01$), ionic fraction Pb and carbonate fraction Pb ($p < 0.05$). The activity of T-SOD, POD and the content of MDA in maize leaves were extremely significantly negatively correlated with organic fraction Pb and residual fraction Pb ($p < 0.01$). This result indicated that if the bioavailable Pb for plant in soil (such as water soluble Pb, ionic fraction Pb and carbonate fraction Pb) is transformed into stable Pb (such as organic fraction Pb and residual fraction Pb), the toxicity of heavy metal Pb on plants will be alleviated.

Table 3 Correlation between SOD、POD、MDA and soil Pb speciation

Discussion

Most studies have shown that aluminum bound phosphate (Al-P) and iron bound phosphate (Fe-P) in soil are bioavailable phosphorus for plant, and Fe-P is converted into phosphate by chemical reduction of Fe^{3+} in the reduction reaction process (Qin et al., 2021). In the reduction process in soil, the calcium bound phosphate (Ca-P) in the soil is easy to transform into A1-P and Fe-P to increase the proportion of bioavailable phosphorus sources (Al-P, Fe-P) in the soil and then improves the potential phosphorus supply capacity of the soil for plant (Fan et al. 2021). The occluded phosphate (O-P) in soil is amorphous aluminum iron phosphate wrapped by Fe_2O_3 to form stable compounds. The main soil type in Yunnan province is red soil, which is rich in iron, aluminum compounds, which provides more adsorption sites for P adsorption and increases the Fe-P content. Moreover, PO_4^{3-} will replace -OH groups and adsorb on the surface of aluminum oxide, and Al^{3+} will combine with organic matter to form "Al-organic matter-P" complex, resulting in the increase of Al-P content (Baquero et al. 2012; Song et al. 2020; Asenso et al. 2018). With the decrease of soil pH, amorphous and crystalline ferric phosphate were hydrolyzed to release phosphorus, and a large amount of H^+ reduced the variable negative charge on the soil surface and promoted the desorption of adsorbed phosphorus (Temporetti et al. 2019; Rehman et al. 2000; Xu et al. 2015; Ansari and Thakur 2015). In this study, maize rhizosphere soil pH decreased in the T2 and T3 treatment groups (see Fig. 5) because the chemical fertilizer increase H^+ in the soil and the absorption of salt-based cations by plants releases the same amount of H^+ into the soil, resulting in soil acidification (Zhang et al. 2017; Zhang et al. 2019; And and Sridhar 2004; Dong et al. 2019). The O-P and Ca-P content of the maize rhizosphere soil in T2 and T3 treatment groups were lower than that of T1 treatment group. Moreover and the Al-P of maize rhizosphere soil was significantly negatively correlated with soil pH ($p < 0.05$) while the Ca-P in soil has a significant positive correlation with soil pH ($p < 0.01$, in Table 1). This result indicates that the application of chemical fertilizers in soil not only increases the content of ionic P in maize rhizosphere soil, but also transforms the chemically mobilized P (such as O-P, Ca-P) into the ionic state due to the decrease of soil pH. In this process, although mobilized P is conducive to plant absorption (Schillaci et al. 2021; Istenič and Božič 2021; Pedro et al. 2021; Suresh et al. 2021), and can also increase the risk of soil phosphorus loss with surface runoff (Boero 1977; Li et al. 2013; Temporetti et al. 2019). Some studies have shown that the organic acids exuded by plant roots reduce the pH of the rhizosphere soil and promotes the release of ionic P. And with the decrease of H^+ and organic acid in the soil, the insoluble phosphorus compounds (such as calcium phosphate compounds, hydroxy aluminum phosphate (iron) compounds) in the soil are hydrolyzed and dissociated into $H_2PO_4^-$, HPO_4^{2-} and other ionic states ((Hayes et al. 2000; Boafo et al. 2018). It is generally believed that the increase of ionic P will promote the absorption of by plants, and high phosphorus in rhizosphere soil will inhibit the activity of acid phosphatase and reduce the transformation of soil organic phosphorus into inorganic phosphorus (Su et al. 2019; Wang and Lu 2020; Peng et al. 2015). But when the phosphorus is deficient in soil, plants can promote the hydrolysis of organic phosphorus compounds by increasing the activity of acid phosphatase in rhizosphere soil, generate inorganic phosphorus that can be absorbed by plants and improving the ability of soil to supply phosphorus (Hayes et al. 2000; Wang et al. 2021; Cheng et al. 2021; Zhan et al. 201). However, in this study, the soil phosphatase activity in the T2 and T3 treatment groups was higher than that in T1 treatment group, indicating that the ionic P in the soil was still deficient relative to the plants. This may be due to some chemical reaction between the ionic fraction P and the heavy metal Pb in rhizosphere the soil, which reduced the bioavailability of P.

The water soluble, ionic fraction and carbonate fraction of heavy metal Pb are easy to be absorbed by plants, Fe-Mn oxide fraction and organic fraction will be released under oxidation and reduction, and the residue Pb is insoluble, which can be transformed into soluble state by chemical reaction (Eyenubo 2021; Wang et al. 2021; Ida and Eva 2021; Wang et al. 2021). The soil pH will affect the change of heavy metal Pb form to a great extent. Soil acidification will catalyze the complex biochemical reaction in the soil and increase the ionic fraction of heavy metal Pb, so as to transform them into water soluble, ionic fraction and carbonate fraction, and correspondingly enhance the toxicity to plants. Based on this, many scholars applied alkaline compounds, such as lime, to the soil to increase the soil pH (Cheng et al. 2021; Han et al. 2021), and the Fe^{2+} and Mn^{2+} are hydrolyzed and combined into $Fe(OH)_2$ and $Mn(OH)_2$, so that the Fe-Mn oxide fraction Pb content increases. In this process, Pb combines with organic fraction to form insoluble complex (organic combined state) to reduce the availability of heavy metals Pb (Wan et al. 2022; Ming et al. 2022; Meng et al. 2021; Xiao et al. 2021; Zhang et al. 2021). In this study, the soil pH in T2 and T3 was lower than that in T1 treatment, but the soil water soluble Pb, ionic fraction Pb and carbonate fraction Pb in the two treatment groups were lower than that in T1 treatment group, and the contents of organic fraction and residual fraction Pb increased, indicating that of the supplement of ionic fraction phosphate fertilizer in maize rhizosphere soil (see above discussion) produces more chemically stable P-Pb compounds (organic and residual fraction Pb). In table1, soil pH was significantly negatively correlated with chemically stable Pb (organic and residual fraction Pb) ($p < 0.05$). The pH decreased in soil, but the residual fraction Pb increased. The reason may be that the supplement of chemical fertilizer acidifies the soil, and then a large amount of ionic Pb is produced in the soil, which increases the opportunity of chemical reaction with ionic fraction P and generates residual P-Pb compound precipitation. In addition, a large amount of ionic fraction P (Al-P and Fe-P) in the soil provides an opportunity for its reaction with Pb. Correlation analysis of P and Pb form in soil showed that the mobilized Pb (water soluble, ionic fraction, carbonate fraction) and chemically stable Pb (organic and residual fraction) show significantly negative correlation and significantly positive correlation with the soil Al-P respectively ($p < 0.01$, in table 1). And the soil chemically stable Pb (organic and residual fraction) were extremely significant negative correlation with the Ca-P content ($p < 0.01$, in table 1). Through regression analysis of the speciation of chemically stable Pb (organic bound Pb+residual Pb) and P, it is found that the amount of organic fraction and residue fraction Pb increases linearly with Al-P and Fe-P, and decreases with the increase of O-P and Ca-P. The application of phosphate fertilizer in Pb-contaminated farmland can alleviate the toxicity of heavy metals on maize plants by changing the speciation of Pb. The result of this study is consistent with these findings that the supplement of hydroxyapatite, dipotassium hydrogen phosphate (K_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) to Pb-contaminated soil can form a large amount of residual fraction lead phosphate precipitation in the rhizosphere soil of plants, reduce the mobility of Pb and their bioavailability (Wang and Song 2019; Narwade and Khairnar 2018; Aliabadi et al. 2014).

Heavy metal Pb stress can make plants produce reactive oxygen species (ROS), destroy membrane lipids and hinder the formation of proteins, pigments and nucleic acids (Kasperczyk et al. 2015). Moreover, a large amount of malondialdehyde (MDA) accumulated in the plant, and the increase of its content indicates that the plant is under high-level antioxidant stress (Rehman and Sheikh 2000). In order to eliminate the harm of oxidative stress on cells, plants catalyze generation of hydroxyl radical (OH^-) and the transformation of superoxide (O_2^-) to H_2O_2 through Haber Weiss and Fenton reaction or other mechanisms, so as to eliminate the harm of reactive oxygen. Therefore, the change of antioxidant enzyme system of plant is the emergency response mechanism of plants under heavy metal stress (Rahbari et al. 201). The activity of plant antioxidant enzyme depends on the degree of plant tolerance, heavy metal ion concentration, exposure time and toxicity, and reflects the redox state of cells to a certain extent (Bahrami et al. 2016; Muradoglu et al. 2015). The activities of SOD and POD of plants, such as *Dendrobium officinale* (Guan et al. 2016), *Medicago sativa* L. (Zhu et al. 2018), *Ricinus communis L.* (Yi et al. 2016) increased linearly with the increase of heavy metal content. When the concentration of heavy metals exceeded the regulation range of plant antioxidant enzyme system, the activities of SOD and POD decreased, indicating that the mechanism of removing pollutants by upregulating antioxidant enzymes system is limited under the stress environment of high concentration of heavy metals. In this study, under the same heavy metal Pb stress (100 mg/kg), the activities of SOD, POD and MDA in maize leaves of T2 and T3 treatment groups were lower than those of T1 treatment group, especially the three parameter values of T2 treatment group were the lowest. And the accumulation amount and enrichment coefficient for Pb in the above- and below-ground biomass of maize in T2 and T3 were lower than those in T1 treatment group, especially the maize of T2 treatment group has the lowest of accumulation amount and enrichment coefficient for Pb. This may be due to the supplement of N, P and K in T2 and T3 treatment groups reduced the content of bioavailable Pb in soil, thus reducing the toxicity on maize plant, which is consistent with the results that the application of phosphate fertilizer reduced the absorption and accumulation of Pb in roots, stems, leaves and grains of *Oryza sativa L.* plants (Gao et al. 2015). However, some studies believe that the inorganic salt ions such as phosphorus compete with heavy metal Pb for adsorption (Hua et al. 2020), which reduces the absorption of Pb by plants. In this experiment, it was found that a large amount of residual fraction Pb was produced in maize rhizosphere soil (see above discussion), which reduced its toxicity on plants. The application of N、K elements can improve the antioxidant enzyme defense system of plants under heavy metal Pb stress, enhance the ability of eliminating reactive oxygen species (ROS) and reduce the accumulation of malondialdehyde (MDA) in plant (Lin 2011). And the content of soil urease in T3 treatment group was higher than that of T1 treatment group, which may be because the increase of N and K elements promoted the increase of plant biomass, resulting in the increase of urease content in soil, which was conducive to the circulation of soil nitrogen. Potassium application increased the contents of glutamate acid, proline and arginine in *guinea* grass roots under Cd pollution stress; thereby improve the activities of SOD, POD and other enzymes (de Anicésio and Monteir 2021). However, in this study, SOD and POD activity of maize leaves decreased in T3 (supplement of N, K) treatment group (Fig. 1), and its mechanism needs to be further discussed.

Conclusion

The supply of phosphorus fertilizer can reduce the Pb-induced oxidative stress in maize, which is evidenced by the decrease in MDA concentration in the leaves of maize. In addition, the activities of antioxidant enzymes (SOD and POD) in maize leaves decreased, and the accumulation amount and enrichment coefficient of Pb in above- and below-ground biomass of maize decreased after the application of phosphorus fertilizer. Mitigation of Pb toxicity and accumulation amount of Pb in biomass of maize is because soil ionic phosphorus reacts with Pb to form residual P-Pb compounds, which is evidenced by the increase in maize rhizosphere soil organic fraction Pb and residual fraction Pb with supply of phosphorus fertilizer, which reduces the mobility and bioavailability of heavy metal Pb. Therefore, the form of Pb in soil can alleviate the stress of Pb on maize, change its physiological indexes, and finally reduce the enrichment of Pb in plants.

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Reference

Aliabadi M, Irani M, Ismaeili J, Najafzadeh S (2014) Design and evaluation of chitosan/hydroxyapatite composite nanofiber membrane for the removal of heavy metal ions from aqueous solution. Journal of the Taiwan Institute of Chemical Engineers 45: 518-526

And A, Sridhar MKC (2004) Soil quality near the chemical fertilizer industry in Port Harcourt, Nigeria. African Journal of Environmental Assessment and Management 8: 19-26

Ansari MI, Thakur DG (2015) Effect of pH bath on phosphorus content in electroless nickel-phosphorus deposit. International Journal for Technological Research In Engineering 2: 1120-1124

Asenso E, Li JH, Lian H, Issake F, Tian K, Zhang L, Zhang LY, Chen H B (2018) Effects on soil biochemical properties and maize grown in latosolic red soil of southern China. Applied and Environmental Soil Science 42: 344-353

Awan SA, Ilyas N, Khan I, Raze MA, Rehman AU, Rizwan M, Rastogi A, Tariq R, Brestic M (2020) Bacillus siamensis reduces cadmium accumulation and improves growth and antioxidant defense system in two wheat (*Triticum aestivum L.*) varieties. Plants 9: 878-891

Bahrami M, Heidari M, Ghorbani H (2016) Variation in antioxidant enzyme activities, growth and some physiological parameters of bitter melon (Momordica charantia) under salinity and chromium stress. Journal of Environmental Biology 37: 529-535

Baquero JE, Ralisch R, Medina CC, Filho JT, Guimarase MF (2018) Soil physical properties and sugarcane root growth iIn a red oxisol. Brazilian Journal of Soil Science, 36: 63-70

Baruah N, Gogoi N, Farooq M (2020) Influence of biochar and organic soil amendments on bioavailability and immobilization of copper and lead to common cocklebur in acidic sandy loam soil. Journal of Environmental Chemical Engineering 8: 104480

Basta NT, Mcgowen SL (2004) Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. Environmental Pollution 127: 73–82

Bilias F, Nikoli T, Kalderis D, Gasparatos D (2021) Towards a soil remediation strategy using biochar: effects on Soil chemical properties and bioavailability of potentially toxic elements. Toxics 9: 184-218

Boafo DK, Kraisornpornson B, Panphon S, Owus BE (2018) Effect of organic and reduced mineral fertilisation on pH, nutrient content and microbial properties of acid soil. Annual Research & Review in Biology 24: 1-11

Boero GE (1977) Phosphatase activity, pH, and phosphorus in the rhizosphere of corn. Kansas State University.

Bolan N, Kunhikrishnan A, Thangarajan R, Kumpiene J, Park J, Makino T, Kirkham MB, Scheckel K (2014) Remediation of heavy metal (loid)s contaminated soils to mobilize or to immobilize? Journal of hazardous materials 266: 141–166

Cao XD, M LQ, Singh SP, Zhou QX (2008) Phosphate-induced lead immobilization from different lead minerals in soils under varying pH conditions. Environmental Pollution 152: 184–192

Chen Y, Yan K, He Y M, Zhen FD, Zu YQ, Li Y, Chen JJ, Li B (2022) Effect of shading on the accumulation characteristics of Cd, Pb and Zn of Sedum plumbizincicola. Chinese Journal of Eco-Agriculture https://kns.cnki.net/kcms/detail/13.1432.S.20211220.1756.001.html

Cheng J, Liu MH, Xiao NW, Yang L, Du HY, Du LZ, Zhang K Q (2021) Effects of pig farm slurry application on heavy metals in the cabbage and soil. Journal of Agro-Environment Science 40: 2559-2567

Chen X, Fu DG, He GX, Zhang YJ, Yu MH, Wang Y, Liu CE, Duan CQ (2021) Differences of *pinus yunnanensis* community and soil phosphorus between phosphorus-rich and phosphorus-poor areas of central Yunnan. Journal of soil Water Conservation 35: 285-292

da Silva EB, de Oliveira LM, Wilkie AC, Li YG, Ma LQ (2018) Arsenic removal from As-hyperaccumulator *P. vittata* biomass: Coupling extraction with precipitation. Chemosphere 193: 288-294

de Anicésio ÉCA, Monteiro FA (2021) Potassium reduces oxidative stress in tanzania guinea grass under cadmium toxicity. Environmental Science and Pollution Research 29: 1184–1198

de Souza Cardoso AA, Monteiro FA (2021) Sulfur supply reduces barium toxicity in Tanzania guinea grass (Panicum maximum) by inducing antioxidant enzymes and proline metabolism. Ecotoxicology and Environmental Safety 208: 111643

Deng JD, Zhang C, Li Y, Li B, Zu YQ, Li Y, Chen JJ, Zhang Z (2022) Effect of ecological buffer patterns on abating soil erosion and heavy metal diffusion in riverbanks near mining waste catchments. Journal of Agro-Environment Science https:// kns.cnki. net/kcms/detail/ 12. 1347. S. 20220124. 1920.002. html

Dong HC (2017) Study of extraction method for different mercury fractionations in soil. Beijing University of Chemical Technology.

Ewa P, Beata K (2002) Trace metal (Cd, Cu, Pb, Zn) and sulphur content in soils and selected plant species of Iceland. A pilot study Icelandic Agricultural Sciences 15: 3-9

Eyenubo OB (2021) Variation in heavy metal distribution and speciation analysis of soil contaminated with E-Waste in Abraka, Delta State, Nigeria. Journal of environmental pollution and management 3: 102-115

Fan HL, Wang XG, Chen ZH, Guo DY, Sun lR, Jiao NY, Shi ZY (2021) Effects of flooding on transformation of inorganic phosphorus fraction in calcareous soils. Journal of Plant Nutrition and Fertilizers 27: 1311-1320

Fu XL., Song TT, Chen Y, Wei YM, Wang QJ (2017) Inversion analysis of heavy metal pollution in soil in mining disturbed areas based on remote sensing data: A case study of Lanping Zn-Pb mining area. Journal of residuals science and technology

14: 85-93

Gao C, Li J, Han Y, Huang YC, Meng B (2015) Effects of different types of phosphate fertilizers on lead accumulation in rice. Acta Scientiae Circumstantiae 35: 288-293

Geng Y, Wang D, Yang W (2017) Effects of different inundation periods on soil enzyme activity in riparian zones in Lijiang. Catena 149: 19-27

Guan CL, Wang QL, Hu X F (2016) Effects of different fertilizers on growth, antioxidant enzyme activity and polysaccharide accumulation of dendrobium officinale. Journal of Zhejiang University (Natural Sciences Edition) 35: 933-938

Guo H, Chen JR, Zhong B, Liu C, Wu JS, He LZ, Ye ZQ, Liu D(2017) Heavy metal concentration, enzyme activity, and physical and chemical properties of rhizosphere and non-rhizosphere soils containing Moso bamboo. Acta Ecologica Sinica 37: 6149-6156

Han F Y, Tang YK, Lai ZY, Qi JJ, Li Z (2021) A study of lead remediation in soil by phosphate-solubilizing fungus and bone char. Journal of Nanjing Agricultural University 44: 887-894

Han YH, Liu X, Rathinasabapathi B, Li HB, Chen YS, Ma LQ (2017) Mechanisms of efficient As solubilization in soils and As accumulation by As-hyperaccumulator *Pteris vittata*. Environmental Pollution 227: 569-577

Hayes JE, Richardson AE, Simpson RJ (2000) Components of organic phosphorus in soil extracts that are hydrolysed by phytase and acid phosphatase. Biology and fertility of soils 32: 279-286

He SX, Wang X, Wu X, Yin YL, Ma LQ (2020) Using rice as a remediating plant to deplete bioavailable arsenic from paddy soils. Environment International,141: 105799

He SY, He ZL, Yang XE, Stoffella PJ, Baligar VC (2015) Soil biogeochemistry, plant physiology, and phytoremediation of cadmium contaminated soils. Advances in Agronomy 134: 135–225

HeYJ, Jiang RR, Wang HX, Wang F, Wang ZL, Zhang M, Pi XF, Tang XH, Hou XL (2021) The character of soil nutrients and heavy metals under different land. Journal of Anhui Agricultural Sciences 49: 63-69

Hua CY, Chen J, Cao, Y, Li HB, Chen YS, Ma LQ (2020) *Pteris vittata* coupled with phosphate rock effectively reduced As and Cd uptake by water spinach from contaminated soil. Chemosphere 247: 125916

Huang N, Zhou Y, Wu XN, Fu DG, Zhao LQ (2019) Effects of dominant species on soil enzyme activity in the phosphorus-enriched degraded mountain area in the Lake Dianchi watershed of southwestern China. Research of Soil and Water Conservation 26: 171-176

Ida S, Eva T (2021) Removal of heavy metals during primary treatment of municipal wastewater and possibilities of enhanced removal: a review. Water 13: 1121-1146

Tara G, Urszula N, (2021) Effects of garden amendments on soil available lead and plant uptake in a contaminated calcareous soil. Applied Sciences 11: 5777-5789

Istenič D, Božič G (2021) Short-rotation willows as a wastewater treatment plant: biomass production and the fate of macronutrients and metals. Forests 12: 554-559

Kasperczyk A, Dobrakowski M, Czuba ZP, Horak S, Kasperceyk S (2015) Environmental exposure to lead induces oxidative stress and modulates the function of the antioxidant defense system and the immune system in the semen of males with normal semen profile. Toxicology and applied pharmacology 284: 339-344

Kathal R, Malhotra P, Kumar L (2016) Phytoextraction of Pb and Ni from the polluted soil by *Brassica juncea L.* Journal of Environmental Analytical Toxicology 6: 2161-0525

Kubie A, Pichler T (2019) Cadmiumin groundwater—a synopsis based on a large hydrogeochemical data set Science of the Total Environment 689(1) 831–842

Lessl JT, Ma LQ (2013) Sparingly-soluble phosphate rock induced significant plant growth and arsenic uptake by *Pteris vittata* from three contaminated soils. Environmental Science Technology 47: 5311–5318

Li HX, Xiao Y, Cao LL, Yan X, Li C, Shi HY, Wang JW, Ye YH (2013) Cerebroside C increases tolerance to chilling injury and alters lipid composition in wheat roots. Plos one 8: e73380

Li W, Zhang HY, Sun HZ, Zeng F, Gao YN, Zhu L (2018) Influence of pH on short-cut denitrifying phosphorus removal. Water Science and Engineering 11: 17-22

Lu RK (2000) Soil sgrochemical analysis methods Bei Jing: China Agricultural Science and Technology Press

Ma XM, Liu Y, Zarebanadkouki M, Razavi BS, Blagodatskaya E, Kuzyakov Y (2018) Spatiotemporal patterns of enzyme activities in the rhizosphere: effects of plant growth and root morphology. Biology and Fertility of Soils 54: 819-828

Meng Y, Tao G, Huang DQ, Yao XJ (2022) Diversity of phosphate-solubilizing fungi and their applications in agriculture and ecology. Journal of Agricultural Science and Technology 2: 1-10

Ming JJ, Xiang JQ, Kang Y, Huang L, Chen YB, Qu Y, Hu BS, Yin HQ (2022) Effect of different passivators on cadmium contaminated soil in enshi Chinese. Agricultural Science Bulletin 38: 93-99

Moreira LJD, da Silva EB, Fontes MPF, Liu X, Ma L Q(2018) Speciation bioaccessibility and potential risk of chromium in Amazon forest soils. Environmental Pollution 239: 384-391

Muradoglu F, Gundogdu M, Ercisli S, Encu T, Balta F, Jaafar HZ, Zia-Ul-Haq M (2015) Cadmium toxicity affects chlorophyll a and b content antioxidant enzyme activities and mineral nutrient accumulation in strawberry Biological Research 48(11) 1-7

Naciri R, Lahrir M, Benadis C, Chtouki M, Oukarroum A (2021) Interactive effect of potassium and cadmium on growth root morphology andchlorophylla fluorescence in tomato plant. Scientific Reports 11: 1-10

Narwade VN, Khairnar RS, Kokol V (2018) In situ synthesized hydroxyapatite-cellulose nanofibrils as biosorbents for heavy metal ions removal. Journal of Polymers and the Environment 26: 2130-2141

Nematian MA, Kazemeini F (2013) Accumulation of Pb Zn Cu and Fe in plants and hyperaccumulator choice in Galali iron mine area Iran. International Journal of Agriculture and Crop Sciences 5: 426-431

Pedro M, de Souza Campos PM, Borie F, Cornejo P, Meier S, López-Ráez JA, López-Garcia A, Seguel A (2021) Wheat root trait plasticity nutrient acquisition and growth responses are dependent on specific arbuscular mycorrhizal fungus and plant genotype interactions. Journal of Plant Physiology 256: 153297

Peng ZG, Xu JX, Wang JZ (2015) Relationship between soil enzyme activity and inorganic phosphorus forms in high phosphorus region of Xiangxi River watershed. Environmental Engineering 33: 116-120

Qin XM, Nong YQ, Luo YF, Lu JM, Qin HY, Chen YQ, Wei JJ, Wei CZ (2022) Effects of phosphorus application on inorganic phosphorus speciation and phosphorus Upuptake in maize-soybean intercropping rhizosphere red soil. Soil and Fertilizer Sciences in China 2: 1-12

Rahbari A, Fatemi H, Esmaiel PB, Rizwan M, Soltani AA (2021) Lead (Pb)-resistant bacteria inhibit Pb accumulation in dill (Anethum graveolens L) by improving biochemical physiological and antioxidant enzyme response of plants. Environmental Science and Pollution Research 28: 5704–5713

Rehman OU, Sheikh AA, Gill KH (2000) Available phosphorus and pH status of attock soils. Pakistan Journal of Agricultural Sciences 37: 74-76

Schillaci M, Kehelpannala C, Martinez-Seidel F, Smith P, Arsova B, Watt M, Roessner U (2021) The metabolic response of brachypodium roots to the interaction with beneficial bacteria is affected by the plant nutritional status Metabolites 11(6) 358-381

Song FF, Xu MG, Duan YH, Cai ZJ, Wen SL, Chen XN, Shi WQ, Colinet G (2020) Spatial variability of soil properties in red soil and its implications for site-specific fertilizer management. Journal of Integrative Agriculture 19: 2313-2325

Su JQ, Li XR, Bao JT (2014) Effects of nitrogen addition on soil physico-chemical properties and enzyme activities in desertified steppe Chinese. Journal of Applied Ecology 25: 664-670

Sun JB, Yao D, Zhao HY, Mei Q, Cai QL, Chen FD, Li TG, LiY (2020) Effect of planting density on marigold (*Tagetes erecta L*) biomass and cadmium and lead accumulation. Journal of Yunnan Agricultural University (Natural Science) 35: 673-681

Suresh G, Guru G, Ravichandran V (2021) Effect of nutrient levels and plant growth regulators on test weight protein content and post-harvest soil nutrient status of pearl millet. International Journal of Current Microbiology and Applied Sciences 6: 271-277

Temporetti P, Beamud G, Nichela D, Baffico G, Pedrozo F (2019) The effect of pH on phosphorus sorbed from sediments in a river with a natural pH gradient. Chemosphere 228: 287-299

Ure A, Quevauviller P, Muntau H, Griepink B (1993) Speciation of heavy metals in soils and sediments An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of the European Communities. International journal of environmental analytical chemistry 51: 135–151

Wan J, Zeng G, Huang D, Wan J, Zeng G, Huang D, Huang C, Lai C, Li N, Wei Z, Xu P, He XX, He Y (2015) The oxidative stress of phanerochaete chrysosporium against lead toxicity. Applied biochemistry and biotechnology 175: 1981–1991

Wan QL, Li Q, Peng Y, Zhao C, Huang T, Zhao R, Peng DP (2022) Feasibility of soilization on applying fulvic acid in phosphogypsum dealkalized red mud. Environmental Engineering 2: 1-8

Wang AD, Zou DS, Zeng XY, Chen B, Zheng XC, Li LC, Zhang LQ, Xiao ZH, Wang H (2021) Speciation and environmental risk of heavy metals in biochars produced by pyrolysis of chicken manure and water-washed swine manure. Scientific Reports 11: 994-1007

Wang DG, Zhang QY, Cao WC, Zhou GF, Wang JC (2021) Effects of different Land use types on soil phosphorous vailability and phosphate-solubilizing bacterial community structure. Chinese Journal of Tropical Crops 42: 3646-3654

Wang FL, Song NN (2019) Salinity-induced alterations in plant growth antioxidant enzyme activities and lead transportation and accumulation in Suaeda salsa: implications for phytoremediation. Ecotoxicology 28: 520-527

Wang QL, Lu SC (2020) Effects of two conditioners on the absorption and transformation of phosphorus in facility phosphorus-rich soil. Northern Horticulture 35: 87-92

Wang XF, Liu TT, Gong XJ, Yuan XZ, Liu H, Kong WW (2020) Phosphorus form in rhizosphere soil of four typical plants in the Littoral Zone of the Three Gorges Reservoirs. Acta Ecologica Sinica 40: 1342-1356

Wang Y, Feng FY, Ge J, Li Y, Yu XY (2022) Effects and mechanisms of plant root exudates on soil remediation. Acta Ecologica Sinica 37: 1-14

Xiao YC, Yu HB, Song YH (2022) Spatial differentiation characteristics and response relationship of DOM nutrients and heavy metals in river sediments Environmental Science 2(6) 1-18

Xu WM, Xiang P, Liu X, Ma LQ (2020) Closely-related species of hyperaccumulating plants and their ability in accumulation of As Cd Cu Mn Ni Pb and Zn. Chemosphere 251: 126334

Xu YF, Hu H, Liu JY, Luo JH, Qian GG, Wang AJ (2015) pH dependent phosphorus release from waste activated sludge: contributions of phosphorus speciation Chemical Engineering Journal 267: 260-265

Yan XL, Ma LZ, Li Y, Chen JJ (2019) Screening zea mays cultivars based upon accumulation of Cd and Pb in the farmland around the Lead-zinc mine area. Journal of Yunnan Agricultural University (Natural Science) 34: 1076-1083

Yi XY, Jiang LJ, Chen JZ, Liu Q, Yi SM (2016) Effects of lead/zinc tailings on photo synthetic characteristics and antioxidant enzymesys-tem of *Ricinus communis L.* Chinese Journal of Ecology 35: 880-887

Zeng G, Chen M, Zeng Z (2013) Shale gas: surface water also at risk. Nature 499: 154-154

Zeng GM, Wan J, Huang DL, Hu L, Huang C, Cheng M, Xue WJ, Gong XM, Wang RZ, Jiang DN (2017) Precipitation adsorption and rhizosphere effect: the mechanisms for phosphate-induced Pb immobilization in soils—a review. Journal of Hazardous Materials 339: 354–367

Zhan FD, Zeng WZ, Yuan XC, Li TG, Zu YQ, Jiang M, Li Y (2019) Field experiment on the effects of sepiolite and biochar on the remediation of Cd- and Pb-polluted farmlands around a Pb–Zn mine in Yunnan province China. Environmental Science and Pollution Research 26: 7743-7751

Zhan XY, Zhang L, Zhang XB, Zhang SX (2013) Phosphorus uptake by wheat with different phosphorus efficiencies and differences in rhizosphere soil phosphorus components under high phosphorus conditions. Journal of Nuclear Agricultural Sciences 27: 1762-1770

Zhang JN, Zhang XX, Sun HF, Wang C, Zhou S (2021) Effects of different slow release fertilizers on nitrogen loss and cadmium migration in vegetable. Journal of Zhejiang University (Agriculture and Life Sciences) 47: 619-627

Zhang JW, Huang JQ, Wang ZH, Wang X (2019) Effects of nitrogen fertilizer on soil chemical property in citrus orchard. IOP Conference Series: Earth and Environmental Science 310: 42-66

Zhang L, Zhang YX, Song B, Wu Y, Zhou ZY (2020) Potential of accumulation and application of dominant plants in Lanping Lead-zinc mine Yunnan province. Acta Ecologica Sinica 41: 4210-4217

Zhang LL, Van Gestel CAM (2019) Effect of ageing and chemical form on the bioavailability and toxicity of Pb to the survival and reproduction of the soil invertebrate *Enchytraeus crypticus*. Science of the Total Environment 664: 975-983

Zhang Q, Liu JJ, Shao SX, Liu ZH (2002) An estimate of the lead isotopic compositions of upper mantle and upper crust and implications for the source of lead in the Jinding Pb-Zn deposit in western Yunnan China. Geochemical Journal 36: 271-287

Zhang SJ (1982) Availability and determination of phosphorus in paddy soil. Soils 4:152-154

Zhang XH, Ma AJ, Lu JL, Liu JH, Cao QG (2017) Effects of microbial fertilizer on forage growth and soil chemical properties. Chemical Engineering Transactions 59: 751-756

Zhang Z, Zhang D, Zu YQ, Li Y, Chen FD, Li B, Chen JJ (2021) Growth and heavy metal accumulation characteristics of five plants in mining area. Environmental Science Technology 35: 673-681

Zheng XR, Wu KH, Sun PJ (2021) Effects of substrate types on the transformation of heavy metal speciation and bioavailability in an anaerobic digestion system. Journal of Environmental Sciences 101: 361-372

Zhu SM, Song HL, Zhang L, Yan J, Shi XJ, Yan H, Liu L, Li HL (2018) Effects of lead stress on growth and physiological and biochemical indexes of tobacco. Plant Physiology Journal 54: 465-472

Zu YQ, Li Y, Chen JJ (2005) Hyperaccumulation of Pb Zn and Cd in herbaceous grown on lead–zinc mining area in Yunnan China. Environment international 31: 755-762

Figures

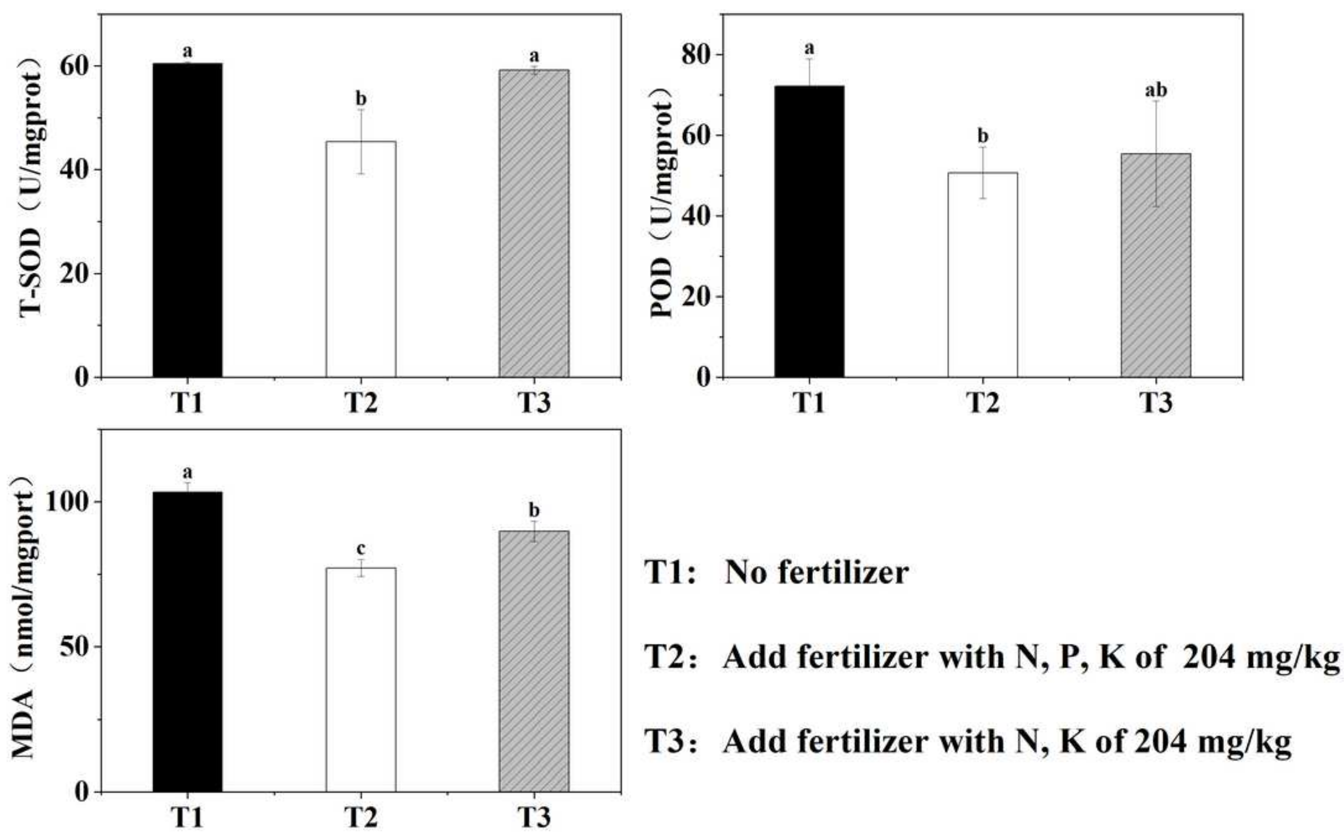

Figure 1

Maize superoxide dismutase (SOD), peroxidase (POD) and lipid peroxidation (MDA) in leaves

Figure 2

Enrichment coefficient of heavy metal Pb in above- and below-ground biomass of maize

Figure 3

heavy metal Pb speciation in maize rhizosphere soil

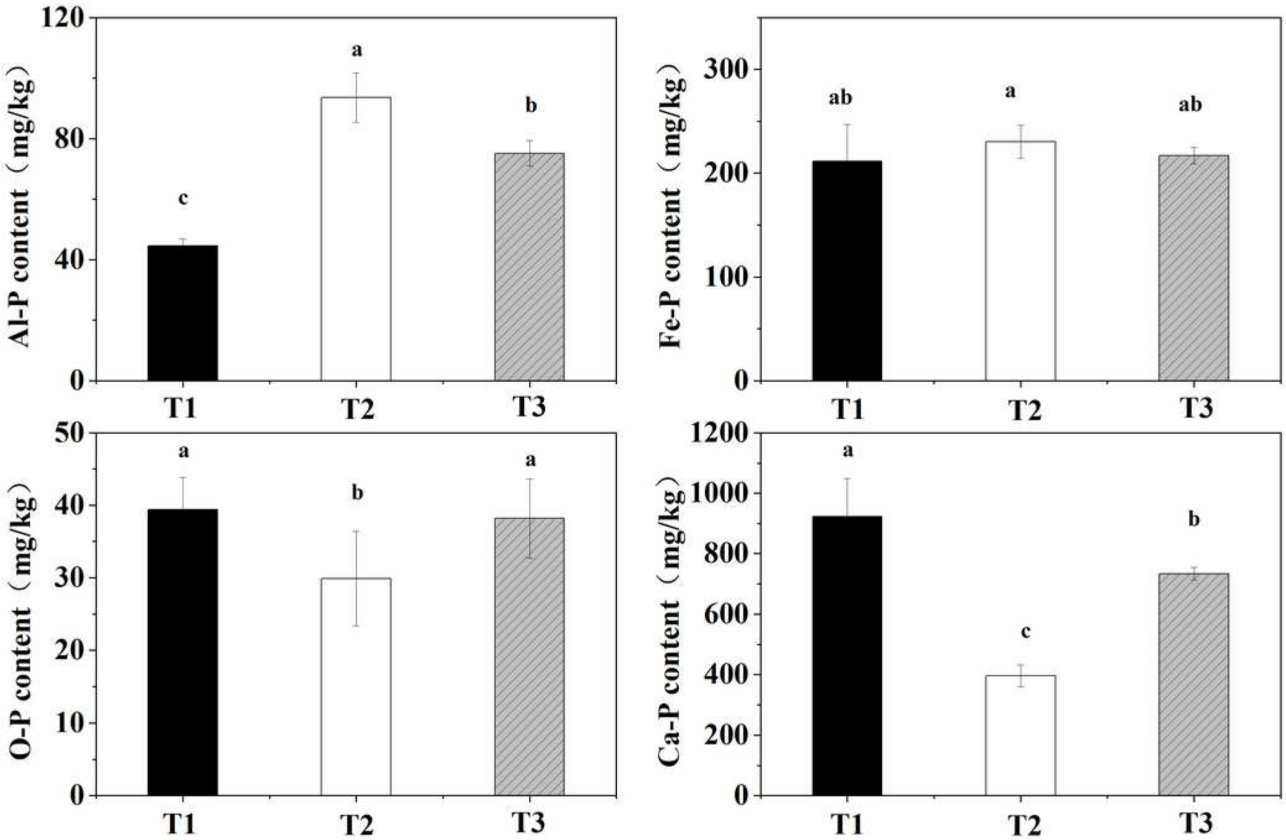

Figure 4

Soil phosphorus speciation

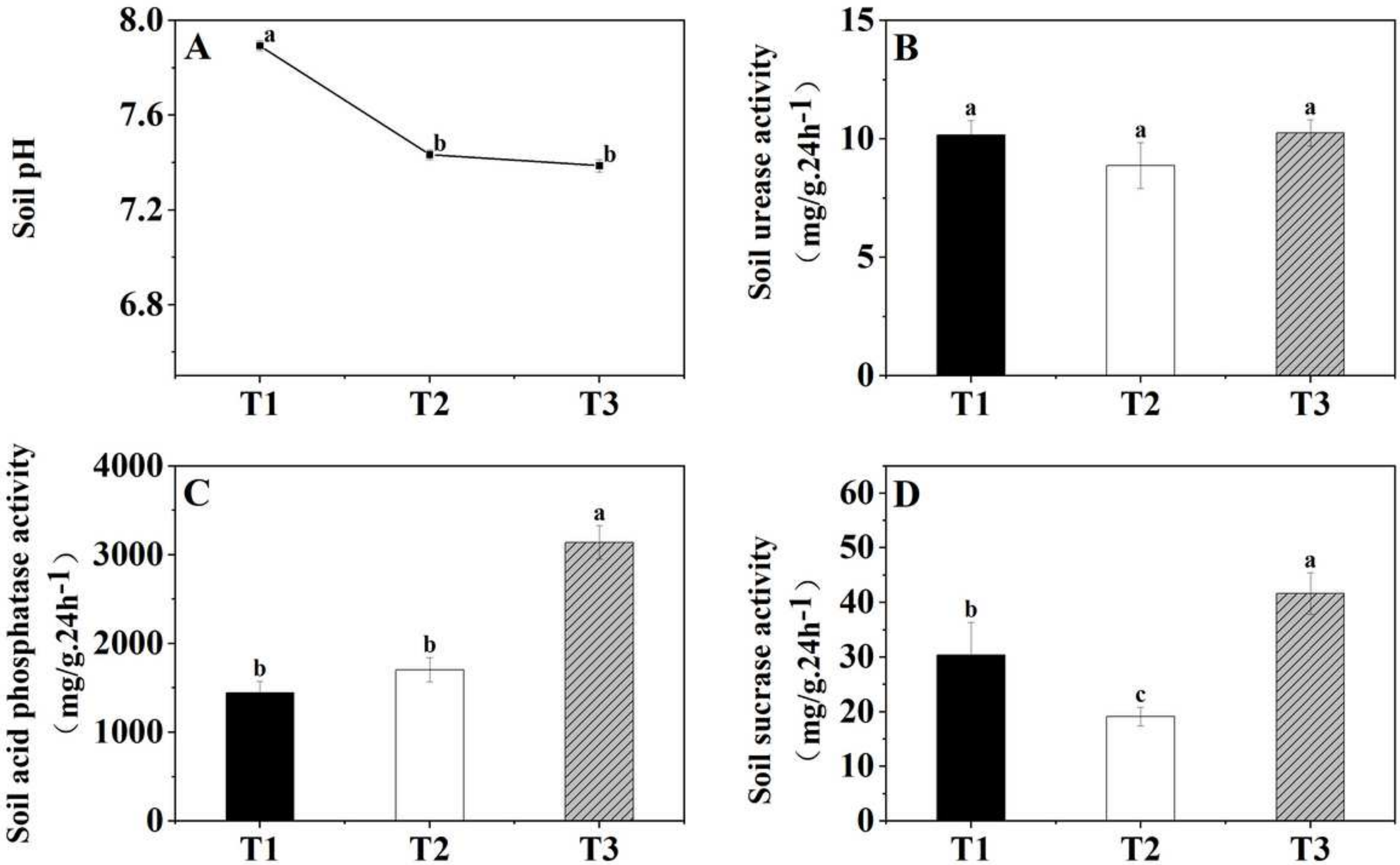

Figure 5

The maize rhizosphere soil pH and enzyme activity

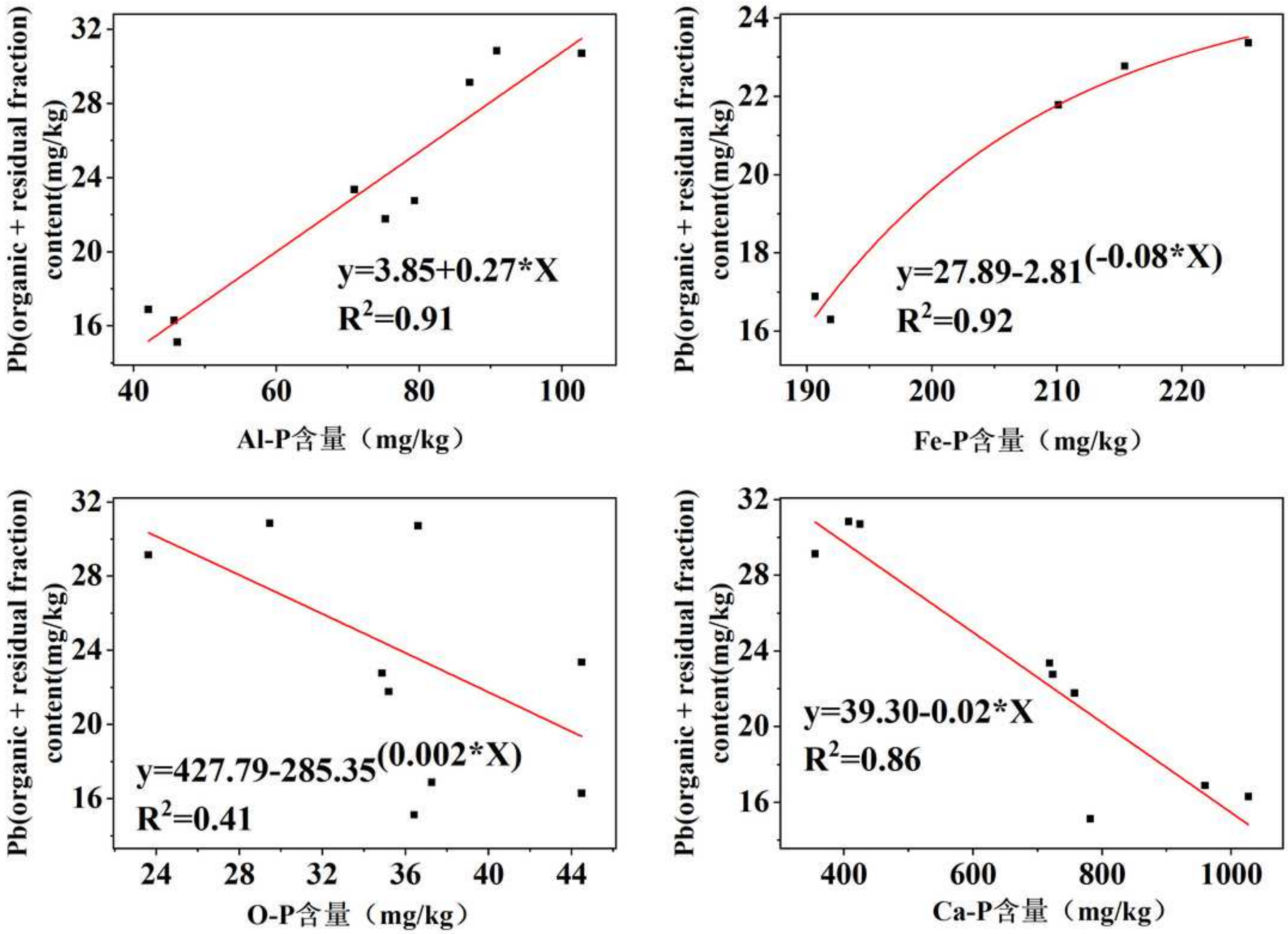

Figure 6

Regression analysis of residual fraction and organic fraction Pb and P speciation （Al-P、Fe-P、O-P、Ca-P）