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RESEARCH

Characteristics of heavy metals in soil of lead-zinc mining areas and changes of antioxidant enzyme systems in maize leaf under Pb stress

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Abstract

Pb, Cu, Cd, Zn content of soil in mining areas and abandoned land, flats of the Pijiang River and farmlands were investigated. On this basis of soil heavy metal pollution, the changes of antioxidant enzyme system in maize (Qiandan 88) under different Pb concentrations (0, 20, 40, 60, 80, 100, 150, 200, 500, 1000, 2000, 3000 mg/L) stress were studied. The results show that the content of Pb, Cu, Cd, and Zn in soil is the highest in mining areas and abandoned land, followed by flats of the Pijiang River > farmlands, and that the variation range of Pb, Cu, Cd in mining areas and abandoned land are 106.40–2564.72, 14.83–490.88, 22.57–712.77 mg/kg, respectively, which are higher than that of the other land use types. When maize is under stress of 20–500 mg/L Pb concentration, T-SOD activity of maize leaves increase with the increase of Pb concentration and the highest value is 50.21 U/mg prot, but under Pb concentration > 1000 mg/L stress, T-SOD activity of maize leaves decrease gradually. The activity of POD decreases with the increases of Pb concentration, and the lowest POD activity of leaves in maize with the value of 93.24 U/mg prot is appeared in Pb 1000 mg/L concentration treatment group. MDA content in leaves of maize increases with the increase of the Pb concentration and the highest value is 101.98 nmol/mg prot, then the content of MDA decreases gradually when the Pb concentration is more than 500 mg/L, which indicates that the membrane lipid peroxidation of maize leaves under high concentration of Pb stress is serious and leads to the cell damage.

Keywords Heavy metal pollution · Pb stress · Maize · Antioxidant enzyme system

1 Introduction

Soil pollution is a very serious problem in China, specifically for heavy metals. The heavy metal lead (Pb) has been scattered into the soil through smelting, ore weathering, and mining (Wan et al. 2016) leading to adsorption and enrichment of crops. This threats the safety of agricultural products affecting human and animal health (Estival et al. 2012). The assessment of pollution status, spatial distribution, ecological risks and nutritional conditions of heavy metal in mining areas has always been a research hotspot (Song et al. 2011; Pekey and Dodan 2013; Chabukdhara and Nema 2013; Song et al. 2019). In recent years, some studies have focused on the analysis of pollution status and temporal and spatial changes of heavy metal under different land use types, as well as the temporal and spatial distribution characteristics of different forms of heavy metals (Bai et al. 2009), accumulation characteristics (Chen et al. 2005; Guo et al. 2008), and soil heavy metal pollution evaluation (Chen et al. 2005; Chen et al. 2006), while ignoring the impact of different land use changes caused by human activities on heavy metals. In Lanping County of Yunnan Province in Southwest China, the reserves of zinc ore rank first in Asia and second in the world. In Lanping County, Lead zinc tailing wasteland is widely distributed, and the soil is barren, arid and heavily polluted by heavy metals (Lei et al. 2007).

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The most important factor affecting soil heavy metals is human land use activities such as farming or mining.

Heavy metal pollution soil is an extreme ecological environment for plant growth making it difficult for native populations of plants to survive. Plants living on heavy metal contaminated soil have formed a specific tolerance mechanism in their long-term evolution process, which has become a research hotspot for stress ecology. Soil heavy metal pollution is the main abiotic factor that determines the distribution of plants specifically allowing plants containing an antioxidant enzyme mechanism to cope and adapt to abundant environmental stresses in this ecosystem. Under heavy metal stress, plants produce the reactive oxygen species (ROS) as a means to deal with heavy metals. Under the stable conditions, ROS can be removed through various antioxidant defense mechanisms (Jin and Yuan 2007). Plants under the stress of heavy metals, high temperature and salinization accumulate excessive amounts of reactive oxygen species (ROS) leading to harmful cellular effects, such as lipid peroxidation of biofilm damaging plants. Heavy metal tolerant plants have developed an antioxidant mechanism, which can alleviate the damage to a certain extent. The study of the effects of lead stress on the activities of antioxidant enzymes in plants is helpful to reveal the response mode and degree of active oxygen metabolism mechanisms. At present, most of the previous studies focused on aquatic plants, invasive alien plants such as *Chromolaene odorata L.* and tolerant plants in mining wastelands, but little attention has been paid to the formation of tolerant ecotypes of economic crops. The development of the non-ferrous metal mining belt in Lanping County, Yunnan Province has become an important support for the regional economy. However, in the process of zinc mine development, the area of tailings wasteland increased, and it enters into the soil through smelting, ore weathering, mining and other ways, resulting in serious lead pollution (Wan et al. 2016). This pollution threatens croplands used for corn, the most important local cash crop, and becomes the main problem of regional environmental security. In heavy metal polluted farmland, the response mechanism of corn to heavy metal pollution has been the focus of much research. Therefore the survey of heavy metal pollution of different land use types of Lanping County and study on the tolerance of farmland crops to heavy metals is important for sustainable development. In this study, the pollution status of heavy metals in soil of different land use types of Lanping lead-zinc mine was investigated; the antioxidant enzyme system of maize leaves was studied by hydroponics method according to the variation range of lead in different land types.

2 Materials and methods

2.1 Collection of soil samples and analysis of heavy metals in soil

Soil samples were taken in Jinding town, Lanping county of Yunnan Province, southwest of China, which is one of the super-large lead-zinc mining areas in the world. This area has a long history of large-scale mining, with an area of 6.9 km^2. It is a temperate climate with an annual average temperature of 10.4 – 11.8 °C. The altitude is about 2380 m, and the annual average precipitation is 1088.43 mm. Jinding Lead-Zinc mine in Lanping county was discovered and developed in 1980's, with large reserves, long development time and wide area, which caused great damage to the surrounding farmland, forest and river ecosystems, serious lead pollution in soil (Yao et al. 2020; Jing et al. 2016; Liu et al. 2010).

The Lanping Jinding lead-zinc mine is located in the upper reaches of the Pijiang River, and the zinc smelter is located at the foot of the mountain in the mining area. Many maize, rice, and wheat farms reside along the Pijiang River. Samples of soil were taken separated by land use types around Jinding lead-zinc mining area, the mining area, farmland around Pijiang River, mine wasteland and farmland around smelter. In the mining area, sampling sites include the areas to be mined (Fengzi mountain, Jiaya mountain, Bracken Hill), agricultural wasteland and dump (stripping dump of open-pit mining). In this study, the sampling sites were classified according to the distance between the sampling sites and mining areas and smelters. The mining areas and wastelands were classified into one category, which represents the impact of mining activities on heavy metals in the soil; the Pijiang River is a tributary of the Lancang River, which flows through Lanping County. The farmland sampling points are divided into two types: farmland around the Pijiang river and Pijiang river beach. Mining areas and wastelands cannot be used as cultivated land at present, so they are classified as one category; The tidal flats on both sides of the Pijiang River are mostly covered with weeds, and a small part of them is planted with corn and sedum plumbizincicola. and this area has been reclaimed and planted, it also has become cultivated land faster than mining and abandoned land, and is classified into one category. In the existing land use areas where normal agricultural farming has been carried out, they are classified into one category.

According to the distribution of soil types and crop varieties in the mining area, sampling is randomly carried out according to the principles of terrain characteristics, vegetation types. At least 3 mixed agrochemical soil samples were collected for the main agricultural soil types. In this

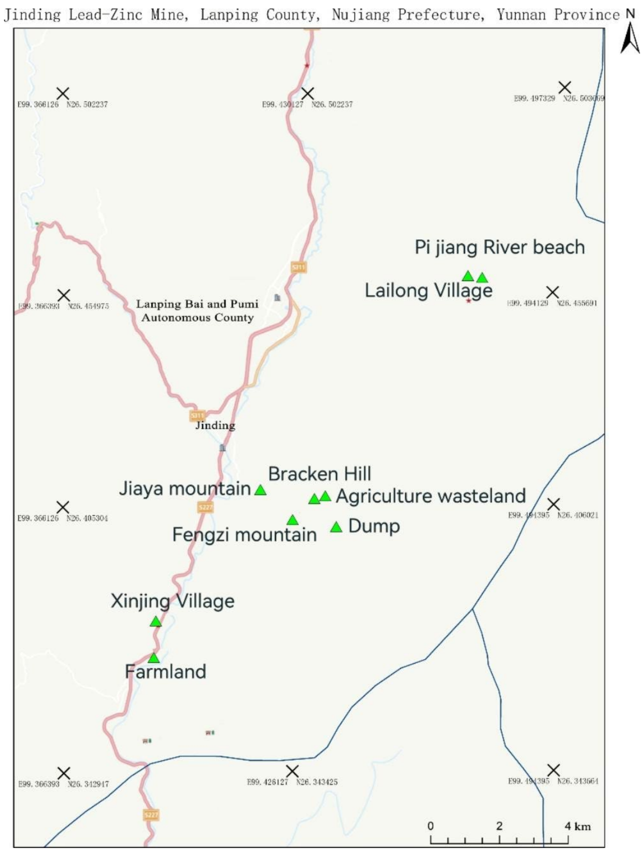

Fig. 1 Soil sampling information

experiment, the chessboard sampling method were used to collect soil samples, and the sampling points should be uniform and random. The sampling depth is 0–20 cm. For sampling, a section of the plough layer should be shoveled out, and the weeds and dead branches should be removed, and then the soil should be shoveled parallel to the section. The mass of the collected soil samples is 1 kg. If the number of soil samples is too large, the soil will be screened by the quadratic method. The sampling points were located using GPS, and the specific 9 soil sampling information is shown in Fig. 1.

The soil samples were naturally air-dried to remove gravel, organic residues, plant roots, etc., and then passed through a sieve of 1 and 0.149 mm to determine copper (Cu), zinc (Zn), cadmium (Cd), and lead (Pb) by flame atomic absorption spectrophotometry and graphite furnace atomic absorption spectrophotometry, respectively. Each soil sample was tested with 3 parallel samples. The determination methods followed the “Soil Monitoring Standards” issued by the Ministry of Environmental Protection of the People’s Republic of China (including: Pb and Cd adopt GB/T 17,141 – 1997; Cu and Zn adopt GB/T 17,138 – 1997). SPSS 22.0 and Origin 2018 software were used to process the data. In order to compare the heavy metal content of Cu, Zn, Cd, and Pb in different land use types, the data of Cu, Zn, Cd and Pb were processed with logarithmic based 10 to complete the data standardization.

2.2 Design of experiment of antioxidant system in maize

According to the Pb content in the farmland soil around the Pijiang River and and the 30-year-old mining area (with vegetation) in the Jinding lead-zinc mining area of Lanping County, Yunnan Province the heavy metal lead (Pb) content was 59.74 and 2564.72 mg/kg, respectively. Therefore, the following Pb concentration gradients were 0, 20, 40, 60, 80, 100, 150, 200, 500, 1000, 2000, 3000 mg/L, and the Pb concentration was 0 mg/L as the control (CK) group. Firstly, 10 maize seeds (Qiandan 88) were sterilized (75% ethyl C_2H_5OH and 30% H_2O_2 solution were prepared in a volume ratio of 4:1, soaked for 30 s and washed with distilled water). These seeds were then transferred to $PbNO_3$-contaminated nutrient solution for hydroponics with three parallel groups per Pb treatment. Each treatment group was set with 3 repeated experiments, and water was supplemented at 8:00 and 20:00 every day. After 20 days, the growth status of maize under different Pb concentrations is shown in Fig. 2, and the maize leaves were collected to determine the relevant indicators. Total superoxide dismutase (T-SOD), malondialdehyde (MDA) and peroxidase (POD) were determined by the kit produced by Nanjing Jiancheng Bioengineering Institute.

3 Results

3.1 Analysis of soil heavy metal pollution of different land use types

The variation law of heavy metal contents in the soil surface under different land use types is shown in Fig. 3. The average value of Pb content in the soil surface layer was in the order of mining area and abandoned land > Pijiang River beach > Farmland around the Pijiang River, and the average values were 2.75, 2.35, 1.83 lg (mg/kg), respectively. The variation range of soil Pb, Cu, Cd in mining areas and abandoned land is higher than that of other land use types, and the variation range of Cd content in the soil surface layer in these land types as mining areas and abandoned land is between 1.35 and 2.85 lg (mg/kg). The average content of Cd in the surface soil of the mining area and wasteland was 2.10 lg (mg/kg), which were higher than the Pijiang River beach and the farmland around the Pijiang River, Cd has the same trend as Pb. It can be seen from Fig. 3d showed that the average Zn content of the surface soil of

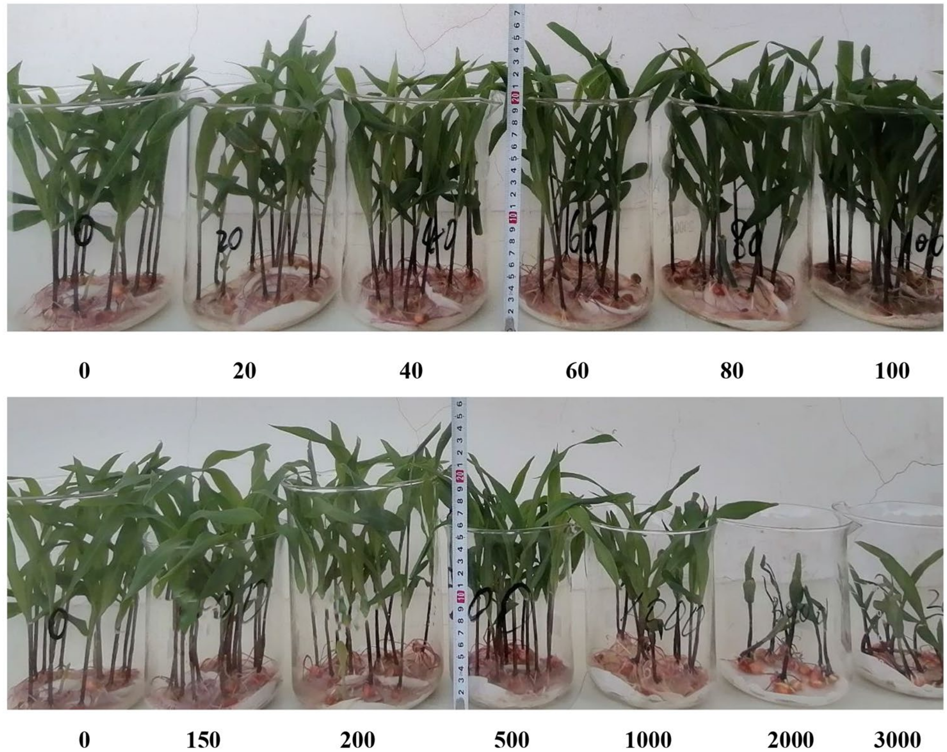

Fig. 2 Growth status of maize under Pb stress

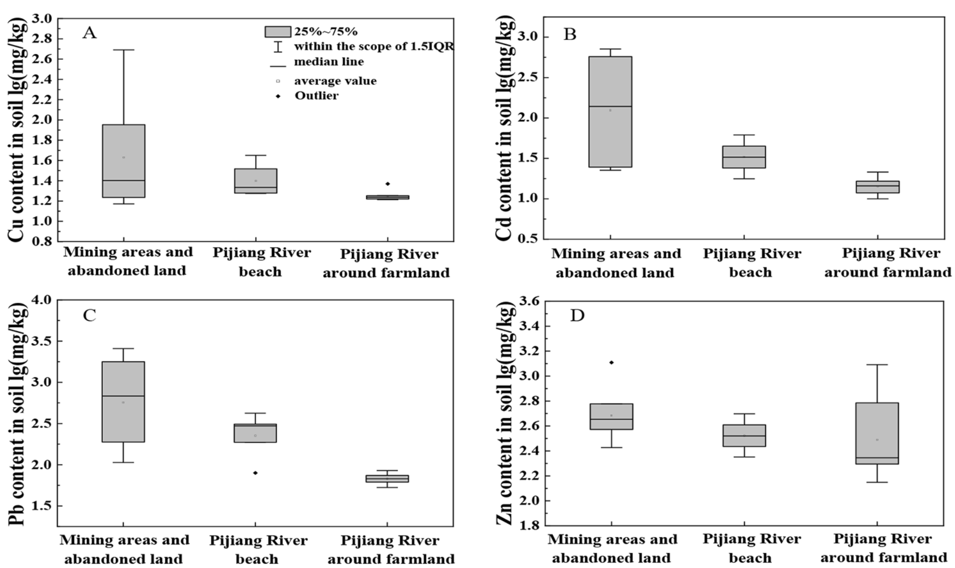

Fig. 3 Comparison of heavy metal content in surface soil of different land types

the mining area and wasteland is the highest with the same value of 2.68 lg (mg/kg), followed by the tidal flats of the Pijiang River, and the average Zn content in the surface soil of farmland around the Pijiang River is the lowest with a value of 2.52 lg (mg/kg). The Cu content is the highest in the surface soil of the mining area and abandoned ground with the value of 1.51 lg (mg/kg), and the variation range is the largest, between 1.17 and 2.69 lg (mg/kg); There is no significant difference in the mean values of Cu content in the Pijiang River beach and the farmland around the Pijiang River, which were 1.28 and 1.23 lg (mg/kg), respectively. The Zn content in the surface soil of the farmland around the Pijiang River had the largest variation, ranging from 2.15 to 3.09 lg (mg/kg), which was higher than that of the other two types of land use.

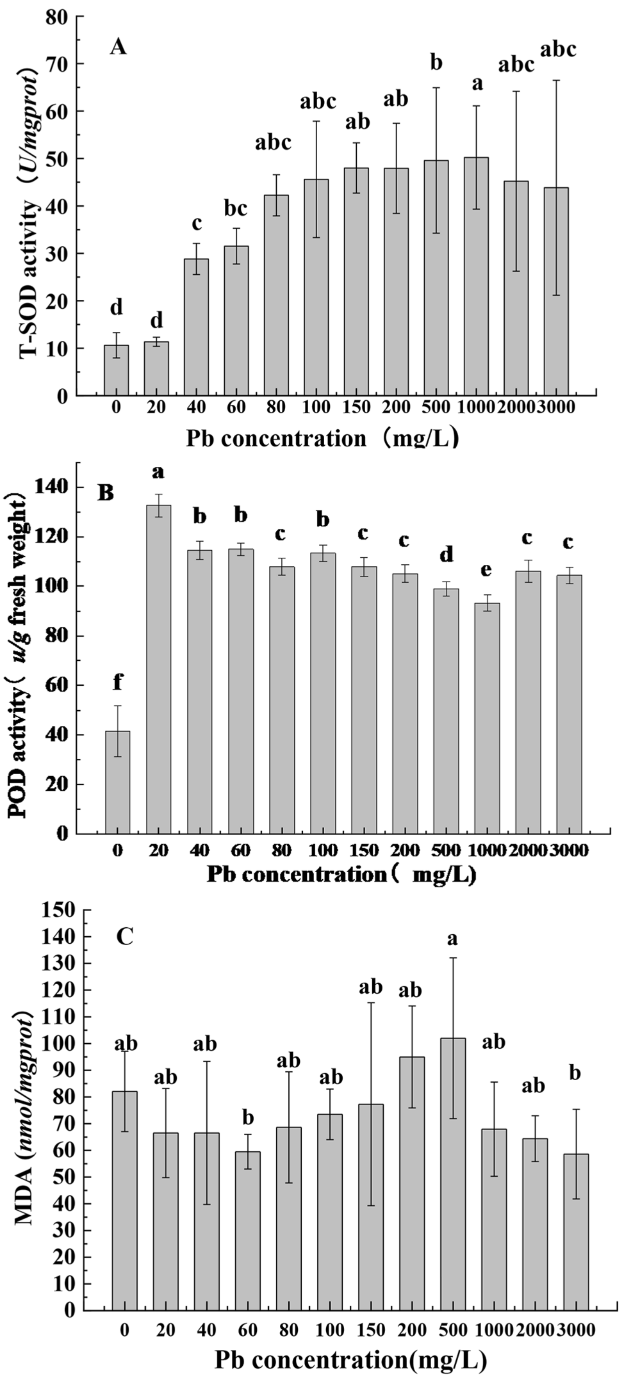

Fig. 4 **a** T-SOD in maize leaves under Pb stress; **b** Changes of POD in maize leaves under Pb stress; **c** Changes of MDA in maize leaves under Pb stress

3.2 Changes of maize leaves antioxidant system under heavy metal lead stress

Under heavy metal Pb stress, the effect of different Pb concentration pollution on the T-SOD activity of maize leaves is shown in Fig. 4a. In the Pb concentration of 0–1000 mg/L treatment group, the T-SOD activity of maize leaves increased with the increase of Pb concentration, and the highest value was 50.21 U/mg prot; In the treatment group with Pb concentration of 2000 and 3000 mg/L, the activity of T-SOD showed a decreasing trend. In the low-concentration treatment group with the Pb concentration of 20, 40 mg/L, the T-SOD of maize leaves with the value of 11.35, 28.81 U/mg prot, respectively, was significantly lower than that of the other treatment groups. these results showed that the T-SOD activity of maize showed that high concentrations of Pb increased the antioxidant enzyme system in leaves except of the 2000 and 3000 mg/L Pb concentration treatment group.

The effect of different Pb concentration pollution on the POD activity of maize leaves is shown in Fig. 4b. In the different Pb concentration treatment groups, the POD activity of maize leaves was higher than that of the high concentration Pb treatment in the low concentration Pb treatment group, and showed a decreasing trend with the increase of Pb concentration. In the Pb concentration of 1000 mg/L treatment group, the POD activity with the value of 93.24 U/mg prot was the lowest, which was significantly different from other treatment groups; In the Pb concentration of 20 mg/L treatment group, the POD activity was the highest, and its value was 132.60 U/mg prot, and there was no significant difference in the POD activity of maize leaves among the 40, 60 and 100 mg/L Pb treatment group, which was lower than that of the 20 mg/L treatment group. In all Pb treatment groups, the POD activity of maize leaves was higher than that of the control group (CK).

In heavy metal stress environment, the metabolism of plants in the growth process may be disordered, breaking the original balance. In the stress environment of different concentrations of Pb, the MDA content of maize leaves is shown in Fig. 4c. In the treatment group with Pb concentration of 20–500 mg/L, the MDA content of maize leaves gradually increased with the increase of Pb concentration. The mazie leaf's MDA content in the 500 mg/L treatment group was the highest with a value of 101.98 nmol/mg prot. In the treatment group with Pb concentration of 1000–3000 mg/L, the MDA content of maize leaves decreased.

4 Discussion

4.1 Soil pollution status of different land use types

In this study, different sampling points were classified according to land use patterns, and 9 sampling points were classified into three types: mining areas and abandoned mines, farmland around the Pijiang River, and farmland in the coastal area of the Pijiang River. The average values and fluctuation ranges of soil Pb, Cd, and Cu in mining areas and mine wastes are higher than the other two types, which indicates that in the process of man-made mining areas, the spatial distribution of Cu, Cd and Pb in the soil in the mining areas and abandoned land is uneven, and the coefficient of variation is larger than that of the farmland with a high artificial replication index. Soil Zn has the lowest average value of farmland around the Pijiang River and the great variation of Zn. This great variation of Zn content in farmland around the Pijiang River may be due to the diversified irrigation and farming methods near the Pijiang River. The chemical properties of soil Zn are unstable, and it can easily spread to the surrounding and deep soil due to factors such as plowing and irrigation, which leads to large changes in the Zn content of the farmland around the Pijiang River under different crops and different water conditions.

The difference in crops planted in the farmland around the Pijiang River will not cause significant differences between the Cu, Cd, and Pb in the 20 cm layer of the soil surface, but the Cd content of the soil surface at all sampling sits in the farmland is higher than that of the "Soil Environmental Quality Standard" secondary standard. The farmland around Pijiang River is mainly planted with maize, sedum with ore, castor and sedum with ore, marigold, rapeseed, Indian mustard. The surrounding farmland presents different changing laws due to different planting and farming methods: there is no significant difference in soil surface Cu, Cd, and Pb content between the Pijiang River and different planting areas around the smelter. The fluctuation range of heavy metal content is 16.35–23.36, 9.98–113.25, 59.74–421.18 mg/kg. The fluctuation range of soil Pb in the farmland is relatively high, which means that the farmland around the Pijiang River and the smelter may cause large fluctuations in Pb due to different farming methods. The smelting plant is located under the mountain in the mining area and is built along the Pijiang River. At the same time, waste water will be discharged during the smelting process. Plants such as maize, rape and wild weeds are mainly grown in the farmland around the smelter. Heavy metals enter the farmland and accumulate in the soil through atmospheric deposition, sewage irrigation, fertilizer and pesticide application, etc. Under traditional tillage conditions, heavy metals can be uniformly distributed in the entire tillage layer through tillage, while under no-tillage conditions, heavy metals are strongly adsorbed after entering the topsoil, making it difficult to migrate to the bottom layer, which may lead to accumulation of heavy metals in the soil surface. Under no-tillage conditions, crop residues were mainly distributed on the soil surface, while under ploughing conditions, the residues were evenly distributed in the tillage layer, which may also have a certain impact on the accumulation of heavy metals. At present, a large number of studies have been carried out on the accumulation of heavy metals under different tillage methods at home and abroad. (Düring et al. 2003) pointed out that under no-tillage conditions, the content of Cd in each layer of soil and the content of Zn in the surface layer were higher than those of conventional tillage.

The Cd content exceeds the "Soil Environmental Quality Standard" secondary standard by 1720%–2336%, and the environmental risk is high. Our study showed that maize growing areas have higher Pb in soil than that of other areas is about 59.74–421.18 mg/kg. This may be due to the discharge of waste gas and liquid waste during the smelting process in the farmland around the smelter, which caused the smelter to settle through the air and be transported by the river. The Cd and Pb contents in the surrounding farmland exceed the "Soil Environmental Quality Standard" secondary standard, which is manifested in the uneven spatial distribution in space.

4.2 Analysis the antioxidant enzyme system of maize leaves under heavy metal Pb stress

The antioxidant enzyme system is an important resistance mechanism for plants to respond to various environmental stresses and plays an important role in the process of plants adapting to adversity (Lin et al. 2007). In Lanping County, Yunnan Province, where the soil is heavily polluted by heavy metals, maize is the main local food crop. Therefore, research on antioxidant systems is very important to explore the tolerance mechanism of plants to heavy metals. Total superoxide dismutase (T-SOD) plays an important role in eliminating superoxide free radicals and reducing lipid peroxidation and membrane damage. The results of this study showed that under the Pb pollution stress, the T-SOD activity of maize increased firstly and then decreased, indicating that T-SOD played a certain role in the process of maize seedlings against the damage caused by heavy metal Pb, but the change characteristics are complex, which may be related to the Enzyme-related metal ions . It is generally believed that the activity of T-SOD of plant will increase under the induction of adapting to adversity (Chen et al. 2017), so that the ability of plant organisms to adapt to the stress environment is improved, and then it makes plant resist adversity and maintain a relatively stable physiological process to some extent. Some studies have also shown that T-SOD enzyme as an inducible enzyme, a certain degree of increase in O_2^- content in plants in a heavy metal Pb stress environment can induce an increase in enzyme activity (Yang 2013), and maintain the normal function of plants to scavenge free radicals. The increase of T-SOD activity is an emergency detoxification measure corresponding to the increase of O_2^- content (Zhang et al. 2006), so as to protect plant cells from the regulatory response of poisoning. However, when high concentrations of Pb stress cause the plant cells to maintain a high O_2^- concentration for a long time, the active substances in the cell including enzymes will also be damaged, resulting in a decrease in T-SOD activity.

Peroxidase (POD) is widely present in various tissues and organs of the plant body, and play a role in removing peroxides, inhibiting its peroxidation of membrane lipids, and avoiding membrane damage and destruction (Wang et al. 2017). The results of this study showed that under the stress of low concentration of Pb (20–500 mg/L), the content of POD in maize was higher than that of the treatment group of high concentration of Pb. Elevated POD is a manifestation of eliminating peroxidative stress, which plays an important role in resistance to heavy metals. In the low-concentration Pb treatment group, the increase in POD activity may be related to the concentration of peroxide substances, that is, when metal ions enter the plant body, the toxic substances produced in the plant body accumulates rapidly, increasing the substrate of the POD enzyme (Yang et al. 2016). Therefore, when the concentration of the substrate is within the normal degradation capacity of the POD enzyme, the POD activity will increase with the increase in the concentration of the enzyme substrate (Xue et al. 2013). Secondly, Under the stress of high concentration of Pb, the active oxygen metabolism in maize is unbalanced, the increase in POD activity may also be related to specific gene expression and cell aging (Sachdev et al. 2021). Usually, plants will produce highly reactive oxygen free radicals, which cause peroxidative damage to biofilms in cells, and cause damage to the functions of chloroplasts, mitochondria and other organelles in plants (Kolupaev et al. 2021), resulting in the decrease of POD enzyme activity in maize.

Plant cell membrane lipid peroxidation produce malondialdehyde (MDA) under heavy metal stress. Therefore, in adversity environments such as salt, drought, nutrient deficiency, etc., plant metabolism may be disordered in the growth process, breaking the original balance, resulting in a large number of harmful substances, such as peroxides MDA, O_2^- etc., which damages the original redox balance. It is toxic to cell membranes and is the most commonly used indicator of membrane lipid peroxidation. The results of this study indicate that under the stress of low concentration of Pb (20–500 mg/L), with the increase of heavy metal pollution concentration, the membrane peroxidation in the maize body will increase and the MDA content will increase. This is similar to the results of recent studies. Many plants showed increased MDA content under different heavy metal stresses (Fatemi et al. 2021). For example, some plants increased the MDA content under high temperature, low temperature, and salt stress (Guo et al. 2021). It is generally believed that there are two reasons for the increase of MDA in plants. Firstly, it is related to free radicals. One of the mechanisms by which O_2^- harms plants is to participate in the activation of membrane lipid peroxidation or membrane lipid degreasing (Kohli et al. 2018). Under the action of the strong oxidant H_2O_2, the more aggressive -OH hydroxyl group is generated through the Habe-weiss biochemical reaction, and membrane lipid peroxidation is initiated (Navabpour et al. 2020), resulting in an increase in MDA. Second, it is related to active oxygen. Reactive oxygen has a damaging effect on many biological functional molecules, such as amino acids, proteins, and carbohydrates (Xie et al. 2016), and eventually cause membrane peroxidation, leading to an increase in the content of membrane lipid peroxidation produced MDA. However, in this study, the MDA content in corn under the stress of high concentration Pb (> 1000 mg/L) was lower than that of low concentration Pb treatment. Plant growth was inhibited or even died under high-concentration lead stress, as shown in Figs. 2 and 4c, which may be because high-concentration lead to damage the function of biomolecules and interferes with the normal life activities of cells, exceeding the limit of plant antioxidant capacity, causing cell metabolism disorder, inhibiting enzyme synthesis, ineffective scavenging of reactive oxygen species, damage to cell membrane system, and inhibition of plant growth (Mao et al. 2018).

5 Conclusions

On the basis of investigation of the soil heavy metal content of different land types around the Jinding lead-zinc mine in Lanping county, Yunnan Province, The variation of Pb is relatively high indicating that large fluctuations in Pb in farmland around the Pijiang River. Based on the current status of heavy metal pollution in this area, a Pb^{2+} stress concentration gradient experiment was set up to determine the antioxidant enzyme system of maize leaves. The T-SOD activity of maize first increased and then decreased, indicating that T-SOD can eliminate heavy metals enrichment in maize seedlings. Under low-concentration Pb (0–500 mg/L) stress, the content of POD in maize leaves was higher than that of the high-concentration Pb stress treatment group. When POD increased, it was a type of elimination of peroxidative stress. But as the concentration of heavy metal pollution increases, the content of MDA that produces serious membrane peroxidation in the corn body also increases, indicating that high Pb concentration will lead to the accumulation of a large amount of MDA in plants.

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