agronomy

MDPI

Article

Phytoremediation of Toxic Lead from Contaminated Soil Using *Neyraudia reynaudiana*: Soil of Xuzhou as a Case Study

Fuyao Chen [1,2], Shaoliang Zhang [3,*], Zanxu Chen [2], Yuanyuan Zhang [1] and Bo Cao [1]

[1] Jiangsu Mineral Resources and Geological Design and Research Institute, China National Administration of Coal Geology, Xuzhou 221116, China; chenfuyao@cumt.edu.cn (F.C.); 18622098549@163.com (Y.Z.); 19961921028@163.com (B.C.)
[2] School of Public Policy & Management, China University of Mining and Technology, Xuzhou 221116, China; chenzanxu@cumt.edu.cn
[3] Research Center for Mine Ecological Restoration, Ministry of Education, China University of Mining and Technology, Xuzhou 221116, China
* Correspondence: slzhang@cumt.edu.cn; Tel.: +86-1395-220-1918

Abstract: Lead (Pb), as one of the main pollution elements, has resulted in large-scale soil pollution around the world. Even if phytoremediation can solve this problem, the selection of restoration potential plants has always been a scientific problem. As a multifunctional repair plant, *Neyraudia reynaudiana* can rehabilitate both polluted soils and slopes. *N. reynaudiana* has been widely used in terrain restoration in southern China before. This study was the first to study the growth and Pb absorption and enrichment capacity of *N. reynaudiana* in Xuzhou, north of the Yangtze River. In this study, *N. reynaudiana* was planted in soils with different lead concentrations, and the change of lead content in roots, shoots, and soils, as well as the redox enzyme, was tested and analyzed during each growth stage. The results showed that the roots could absorb Pb and transfer 79.45% to the shoots at most. With the growth of the plant, the ability to accumulate and transfer gradually increased. Moreover, when the soil Pb concentration was above 800 mg kg^{-1}, the ability to accumulate by *N. reynaudiana* was significantly restrained. Furthermore, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) first acted on the redox response in the initial phase, while increasing the pollutant concentration or the growth of *N. reynaudiana* in the later stage, and the glutathione reductase (GR) redox system continued to feed back on the lead stress. This study proved that *N. reynaudiana* is a kind remediation plant for lead pollution soil and could repair soil with a lead pollution concentration lower than 800 mg kg^{-1}. The results provide a theoretical reference for clarifying the action mechanism and threshold value of *N. reynaudiana* in rehabilitating soil lead pollution and provide practical guidance for the planting proportion of *N. reynaudiana*.

Keywords: *Neyraudia reynaudiana*; lead; phytoremediation; soil heavy metal pollution

Citation: Chen, F.; Zhang, S.; Chen, Z.; Zhang, Y.; Cao, B. Phytoremediation of Toxic Lead from Contaminated Soil Using *Neyraudia reynaudiana*: Soil of Xuzhou as a Case Study. *Agronomy* **2024**, *14*, 118. https://doi.org/10.3390/agronomy14010118

Academic Editor: Marzia Vergine

Received: 5 December 2023
Revised: 21 December 2023
Accepted: 29 December 2023
Published: 2 January 2024

1. Introduction

Lead, as a major environmental pollutant worldwide, can pollute agricultural soil and water sources and enter the food chain. Pb can damage the nervous system and liver, resulting in gastrointestinal damage and amentia in children [1–4]. According to the World Health Organization (WHO), lead concentrations in soil should be less than 70 mg kg^{-1} or lower to protect human health. While the U.S. Environmental Protection Agency (EPA) stipulates that lead concentrations in undeveloped land should be less than 400 mg kg^{-1}, the target concentration for lead in developed land is usually 1200 mg kg^{-1}. Pb in soil will reduce soil nutrients, microbial diversity, and soil fertility [5]. Meanwhile, lead interferes with plant nutrient absorption and photosynthesis, slowing plant growth and even death. Pb in soil can lead to a decrease in seed germination rate and plant growth rate and interfere with enzyme activity in plants, causing membrane damage and stomatal closure [6].

Phytoremediation is a technology that uses plants to reduce the amount, toxicity, and mobility of lead in soil, minimizing the disturbances in the soil environment and reducing the spread of lead contamination via water or air, including phytostabilization, phytoextraction, and rhizofiltration [5,7]. *Miscanthus floridulus* has remediation potential for Pb [8]. A co-planting system of *Pteris vittata* L. and the *Ricinus communis* L. in Pb-contaminated soil produced an increased yield of *P. vittata* after Pb was absorbed [9]. Metal hyperaccumulator plant species such as *Eichhornia crassipes*, *Lemna* sp., and *Pistia stratiotes* have been widely used to remediate Pb in soil [10]. In addition to using hyperaccumulator plants to rehabilitate Pb-polluted soil, it can also increase the repair effect by regulating plant growth. Leaf surface control of zinc fertilizer can reduce the Pb concentration in brown rice by reducing the transport ability of Pb in rice roots to straw and then to brown rice [11]. In the upland soils, biochars made from blends of plant and manure materials demonstrated some capacity to reduce soil Pb bioaccessibility [12]. It was first discovered by Blaylockz in 1997 that *Thalaspi arvense* can accumulate Pb in soil in large amounts, and then it was discovered in 2008 that *Sedum alfredii* [13] is also a hyperaccumulator plant for Pb; in 2010 [14], the same was discovered by researchers for *Brassica napus*. In 2012 [15], it was determined that *Chenopodium ambrosioides* could also hyperaccumulate Pb in the soil.

Under heavy metal stress, the redox reaction in plants can demonstrate their response mechanisms [16,17]. *Melia azedarach* and *Ligustrum lucidum* exhibited certain antioxidative defense mechanisms as elevated SOD, POD, and CAT activities, then declined under a high level of Pb–Zn tailing treatment [18]. Higher glutathione (GSH) levels in wheat may allow phytochelatin to bind with excess Pb, resulting in the subcellular sequestration in the root vacuole [19]. Upon Pb exposure, higher levels of flavonols and hydroxycinnamic acids in shoots and triggered GSH in roots and shoots were maintained in *Z. fabago* to overcome Pb toxicity [20]. Tolerance to Pb-induced oxidative stress would result from a synergetic action of both enzymatic and non-enzymatic antioxidant systems, leading to a balanced redox status in rice [21].

N. reynaudiana is a multi-functional restoration plant in southern China, widely used in vegetation restoration projects in soil erosion areas and mining areas [22]. During the growth process, *N. reynaudiana* not only has strong adaptability to damaged terrain but also has barren resistance and adaptability to low-phosphorus and low-potassium soil and can increase nutrient absorption area by increasing the root length and root surface area, to adapt to the environment [23]. At the same time, they have a certain tolerance to acidic soil and can grow normally under pH 3.5 treatment [24]. However, existing studies have not yet studied the time difference of Pb adsorption in the whole life cycle of *N. reynaudiana* and the oxidative metabolism process of heavy metal enrichment. However, revealing the difference in the absorption effect and physiological response mechanism at different periods can provide data support for improving the rehabilitation effect of *N. reynaudiana* and regulating its growth rhythm to further enhance its Pb enrichment ability. In this paper, the gradient concentration stress culture method was used to study the remediation effect on soil Pb pollution and the change characteristics of redox enzyme activity in vivo at different growth stages and to clarify the application range and physiological response mechanism on soil Pb pollution, providing a theoretical basis for the application of heavy metal phytoremediation technology.

2. Materials and Methods

2.1. Plant Material, Culture, and Exposure to Pb

N. reynaudiana seeds were collected from Fuzhou, Fujian province, China. The seeds were surface sterilized with 5% NaClO and germinated with Hoagland nutrient solution in the dark at 26 °C for 7 days. Afterward, the seedlings with 12 cm height were transferred in a pot experiment. The pots were filled with garden soil collected in Xuzhou in the southeast of China, and the seedlings were allowed to grow for 14 days, as shown in Figure 1.

Figure 1. The seedling of *N. reynaudiana*.

In the experiment, Pb $NO_3{}^{-2}$, which contains Pb^{2+}, was chosen as the source of heavy metal. Five concentration gradients were established, with three parallels conducted for each gradient. The pH value was carefully maintained at 6.5. Soil was evenly mixed with Pb $NO_3{}^{-2}$. The control group, designated as Pb0, did not have Pb^{2+} added. The experimental concentrations were determined based on the existing literature, agricultural land in China, and the risk control standard of soil pollution intervention value. The experimental concentrations are given in Table 1. The breeding shed was kept ventilated for a long time during the whole breeding process, and the *N. reynaudiana* and Hoagland nutrient solution were watered regularly.

Table 1. Addition and concentration of heavy metals in soil.

Number	Concentration/mg kg^{-1}	Pb $NO_3{}^{-2}$/g
Pb1	200	1.92
Pb2	400	3.84
Pb3	600	5.75
Pb4	800	7.67
Pb5	1000	9.59

In the Pb1 group, 1.92 g lead nitrate was added to five parallel experimental samples successively, mixed with soil evenly, and used for *N. reynaudiana* cultivating. In the remaining Pb2–Pb5 groups, 3.84 g, 5.75 g, 7.67 g, and 9.59 g lead nitrate were added, respectively, and were also mixed evenly with soil for the planting experiment.

Since the height of the planter was 20 cm, soil mixtures at different depths from 0 to 20 cm were taken as experimental samples during sampling. After natural air drying and grinding, the soil samples were sieved and used for soil Pb content analysis. The shoots of *N. reynaudiana* were collected as samples, along with root samples. They were rinsed with ultra-pure water and then rinsed three times before being dried in a 55 °C oven to a constant weight. After crushing, the plant samples were screened using 100 mesh twice and put into sterile sealed bags for numbering.

To accurately record the heavy metal content and other related indicators of the plants in the whole growing stage, samples were collected five times throughout the growing cycle. They were named as 30 days–young period (YP), 60 days–growth period (GP), 90 days–maturity period (MP), 120 days–old period (OP), and 150 days–apoptosis period (AP). The Figure 2 shows the growth of *N. reynaudiana*.

Figure 2. The picture of *N. reynaudiana*. (Left picture: before adding exogenous Pb in soil. Right picture: after growing for 150 days. From left to right are Pb0 to Pb5).

2.2. Pb Quantification in Soil

The 0.20 g soil sample was weighed (through a 0.2 mm sieve) accurately into the polytetrafluoroethylene crucible and soaked with a little water, 5 mL HCl was added, and it was heated on the electric hot plate of the fume bonnet. When it evaporated to 2–3 mL, 5 mL concentrated HNO_3, 2 mL HF, and 2 mL $HClO_4$ were added successively, and it was heated on the electric hot plate for about 1 h after covering. The crucible was shaken frequently to obtain a good silicon flux effect. The lid was opened and heating was continued until thick white $HClO_4$ smoke rose, the black organic carbide was covered to decompose completely when the black organic matter was on the crucible, and the lid was opened to expel the white smoke and vapor until the liquid was thick. The above decarburization process was repeated according to the decomposition situation. After cooling, the inner wall of the crucible was rinsed with 1% HNO_3 and filtered into a 25 mL colorimetric tube, and the volume was fixed at 25 mL and finally determined with an atomic spectrophotometer. The detail of experimental method were shown in the supplementary.

2.3. Pb Quantification in Different Tissues

A quantity of 5 g of the plant sample was weighed, 5 mL HNO_3 was added, and it was let to stand overnight. Then 2 mL H_2O_2 was added, followed by 4 mL H_2O_2. After shaking well, it was heated at 90 °C for 1 h. Then 5 mL HNO_3 was added, and heating was continued at 150 °C for one hour. After that, the heat was switched to 120 °C until the sample was completely dissolved. It was allowed to cool, and then it was increased to 25 mL with the addition of H_2O_2. It was shaken well for the test. The Pb content was quantified by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500A, Santa Clara, CA, USA). The detail of experimental method were shown in the supplementary.

2.4. Quantification of Antioxidant Activity

(1) The activity of SOD [25] was determined by the nitrogen blue tetrazole method. The crude enzyme extract was prepared from 0.2 g of leaves. After mixing the chromogenic solution with the enzyme solution and a reaction time of 20 min, the enzyme activity was measured with an enzyme marker at 560 nm.

(2) The activity of POD [26,27] was determined by the guaiacol method. To a clean glass tube was added 1.0 mL of enzyme solution, 1.0 mL of 1% guaiacol, and 1.0 mL of 0.18%

H_2O_2; it was shaken well and reacted at 25 °C for 10 min; 0.2 mL of 5% metaphosphate was added to stop the reaction; and the absorbance was determined at 470 nm.

(3) The activity of CAT [26] was determined by the UV absorption method. To each of four tubes was added 0.2 mL crude enzyme solution, 1.5 mL phosphoric acid buffer (pH = 7.8), and 1.0 mL H_2O. After preheating to 25 °C, 0.3 mL of 0.1M H_2O_2 solution was added to each test tube, and the absorbance was measured at 240 nm with violet light. A measurement was taken every 1 min. The total measurement took 4 min.

(4) In the APX method [25] for the determination of activity, after the crude extract of the enzyme was prepared, the reaction system contained 50 mmol/L K_2HPO_4-KH_2PO_4 buffer, pH 7.0, 0.5 mmol/L AsA, 0.1 mmol/L H_2O_2, and 0.5 mmol/L EDTA. The total volume of the reaction was 1 mL. The reaction was initiated with an enzyme solution containing 40 μg protein. The decrease in absorbance value due to AsA oxidation at 290 nm was measured at room temperature 25 °C. The enzyme amount of 1 h oxidizing 1 μmol AsA was used as 1 enzyme activity unit.

(5) The GR activity [25] assay reaction system contained 100 mmol/L K_2HPO_4-KH_2PO_4 buffer, pH = 7.5; 2.5 mmol/L EDTA; 0.75 mmol /L DTNB; 0.1 mmol/L NADPH; 1 mmol/L GSSG; and an enzyme solution containing 40 μg protein. The total volume of the reaction was 1 mL, and the reaction was started with 0.1 mmol/L NADPH at 25 °C. The product had an absorption peak at 412 nm, and the amount of enzyme consuming 1 μmol/L NADPH in 1 h was equivalent to 1 unit of enzyme activity.

The list of abbreviation were shown in the supplementary.

2.5. Statistical Analysis

To quantify the adsorption capacity and differences of Pb at different growth stages, the following evaluation indices were developed. Experimental data were input and calculated using SPSS 25.0, and Origin 2018 was used for plotting. *AF* (absorption factor) is the proportion of soil heavy metal elements in the roots (Formula (1)). *TF* (transport factor) [28] is the proportion of heavy metals migrating from the roots to the shoots (Formula (2)). *BCF* (bio concentration factor) [29] is the commonly used indicator to evaluate the ability of plants to accumulate heavy metals in aboveground parts (Formula (3)). *PRI* (pollution rehabilitation index) is the index of soil rehabilitative effect of *N. reynaudiana* (Formula (4)).

$$AF = RP/SP \times 100\% \quad (1)$$

$$TF = PP/RP \times 100\% \quad (2)$$

$$BCF = PP/SP \quad (3)$$

$$PRI = (Cs - Ce)/Cs \quad (4)$$

where *AF* is the absorption factor and *RP* is the heavy metal content (mg kg^{-1}) in the root. *SP* is the heavy metal content in the soil (mg kg^{-1}). *TF* is the transport factor, and *PP* is the heavy metal content in the shoots (mg kg^{-1}). *RP* is the content of heavy metals in the roots (mg kg^{-1}). *BCF* is the bio concentration factor, and *SP* is the heavy metal content of the corresponding soil sample (mg kg^{-1}). *PRI* is the pollution rehabilitation index, and *Cs* is the initial concentration of Pb in the soil before planting. *Ce* is the concentration of Pb in the soil after harvesting.

3. Results and Discussion

3.1. Change of Pb Content in Soil

The amount of Pb accumulated in the soil decreased significantly with the growth of *N. reynaudiana*. In the Pb1, Pb2, and Pb3 groups, the Pb decreased to the lowest values at the AP stage, which were 17.86 mg kg^{-1}, 27.96 mg kg^{-1}, and 28.50 mg kg^{-1}, respectively. In the Pb4 and Pb5 groups, the soil lead content decreased to the lowest values of 344 mg kg^{-1} and 412 mg kg^{-1} at OP stage, respectively, and then the soil Pb content increased during the AP stage. Therefore, when the soil lead content exceeded 800 mg kg^{-1}, the migration

of lead in the soil was significantly affected. The change of Pb content in soil were shown in Figure 3.

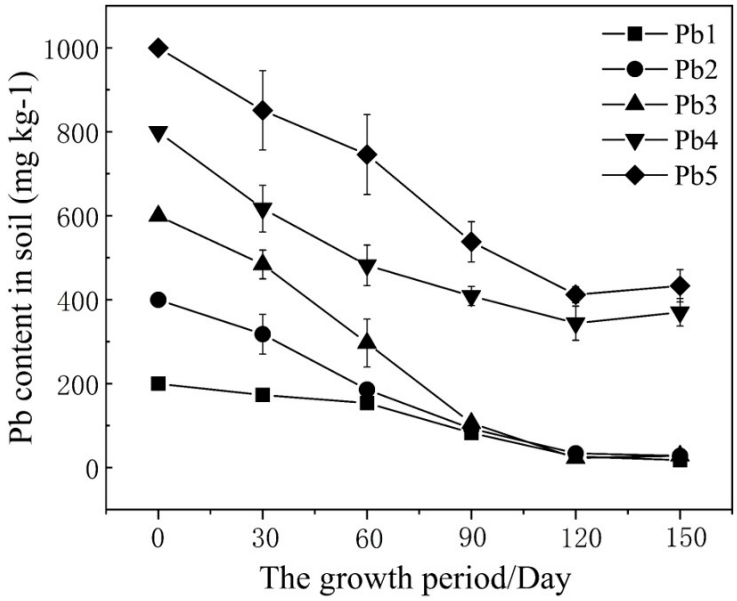

Figure 3. Changes of Pb content in soil during each growth period. Results are expressed as means ± SD (standard deviation). N = 5.

The ultimate goal of phytoremediation is to address Pb contamination in the soil. Therefore, we analyzed the SRI under different concentrations of pollution in different time periods. The results are presented in Table 2.

Table 2. The SRI of Pb in each growth period of *N. reynaudiana*.

Number	Growth Period				
	YP (30 Days)	GP (60 Days)	MP (90 Days)	OP (120 Days)	AP (150 Days)
Pb1	0.14	0.23	0.59	0.87	0.91
Pb2	0.21	0.54	0.77	0.92	0.93
Pb3	0.19	0.51	0.82	0.96	0.95
Pb4	0.23	0.4	0.49	0.57	0.54
Pb5	0.15	0.25	0.46	0.59	0.57

The SRI directly reflected the degree of soil remediation, and the larger the value, the better the remediation effect of soil Pb pollution. With the addition of different Pb content in the soil, the remediation effect on soil Pb pollution was also different. In the Pb1 and Pb2 groups, the soil rehabilitation index also increased with plant growth and peaked at the AP growth stage. However, in the Pb3, Pb4, and Pb5 groups, the SRI of *N. reynaudiana* peaked at the OP stage and then gradually decreased.

3.2. Change of Pb Content in Roots

The Pb content in the roots was measured, and the results showed that the Pb content in the roots gradually increased with the growth of *N. reynaudiana*. The maximum value growth for 30 days was 350.75 mg kg^{-1}, and the maximum value for 150 days was 689.31 mg kg^{-1}, which was more than double the YG stage. The change of Pb content in roots were shown in Figure 4.

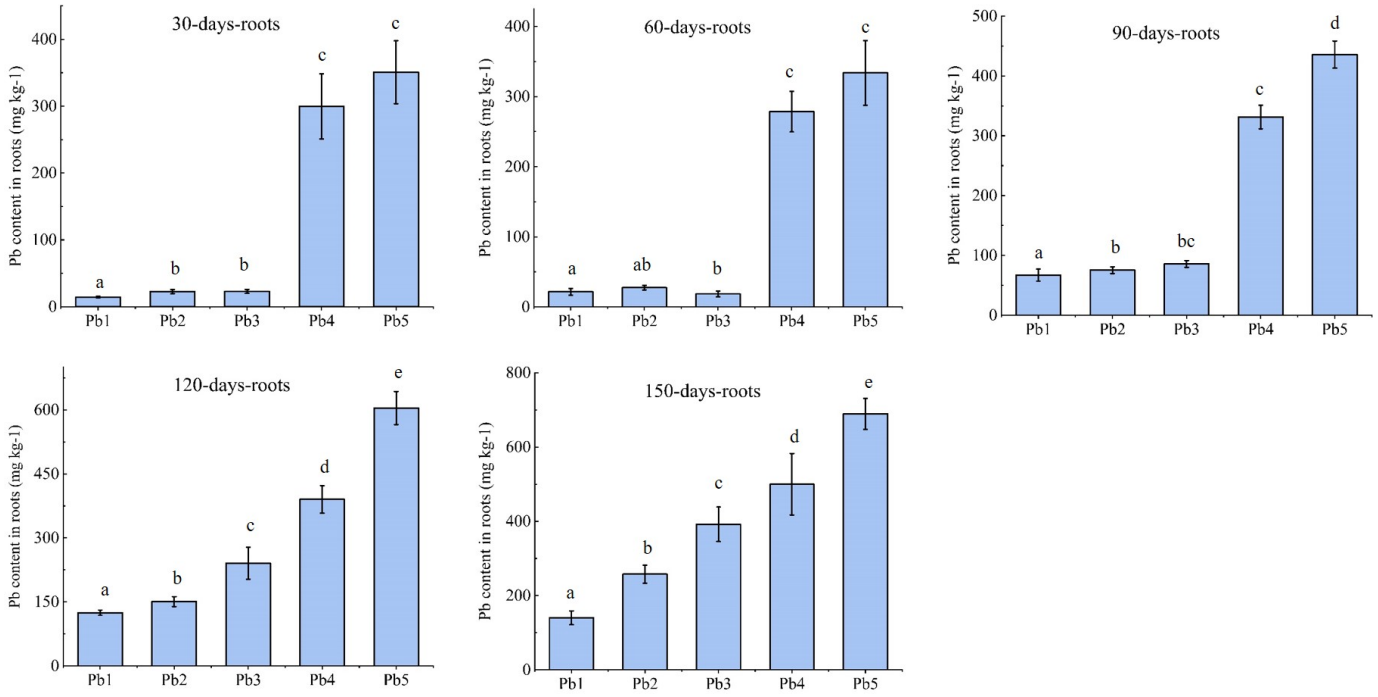

Figure 4. Change of Pb content in root (mg kg^{-1}). Results are expressed as means ± SD (N = 5). Different lowercase letters indicate significant differences between Pb concentrates at $p < 0.05$.

The Pb content in the root varied at each growth stage. During the YP and GP periods, the Pb content in the roots of the Pb4 and Pb5 groups was significantly different from that of the Pb1, Pb, and Pb3 groups. However, with the growth of *N. reynaudiana*, the difference between each concentration was more significant. When the growth of *N. reynaudiana* reached 150 days, there were significant differences in the root concentration at each concentration.

The AR reflects the extent of the transfer of Pb in the soil to the roots. As the first step of the link between soil heavy metals and plants, the roots directly affect the damage of plants in root cells, and roots are important organs for the uptake of water and mineral elements by plants. Therefore, the AR of *N. reynaudiana* was analyzed (Table 3). The AR of Pb increased with the growth of plants and reached 1375.58% in the Pb3 group at the AP stage. However, when the soil Pb concentration reached 800 mg kg^{-1}, the maximum absorption rate of the Pb decreased significantly.

Table 3. The AR of Pb in each growth period of *N. reynaudiana*.

Number	Growth Period				
	YP (30 Days)	GP (60 Days)	MP (90 Days)	OP (120 Days)	AP (150 Days)
Pb1	8.36%	14.07%	81.10%	466.32%	784.60%
Pb2	7.12%	14.81%	231.48%	443.12%	921.24%
Pb3	4.77%	6.27%	567.99%	1045.96%	1375.58%
Pb4	48.57%	57.81%	87.52%	113.49%	135.07%
Pb5	41.21%	44.73%	56.320%	146.67%	159.19%

3.3. Change of Pb Content in Shoots

As *N. reynaudiana* grow, the Pb content in the shoot gradually increases. In the Pb1 and Pb2 groups, the Pb content in the shoots gradually increased and reached the peak values of 107.43 mg kg^{-1} and 128.94 mg kg^{-1} at the AP stage, respectively. However, in the Pb3, Pb4, and Pb5 groups, the pattern of Pb accumulation in the shoots changed. The

maximum appeared at the OP stage, and the Pb contents in the shoots were 127.03 mg kg^{-1}, 125.99 mg kg^{-1}, and 124.7 mg kg^{-1}. At the AP stage, the Pb content in the shoots decreased. The change of Pb content in shoots were shown in Figure 5.

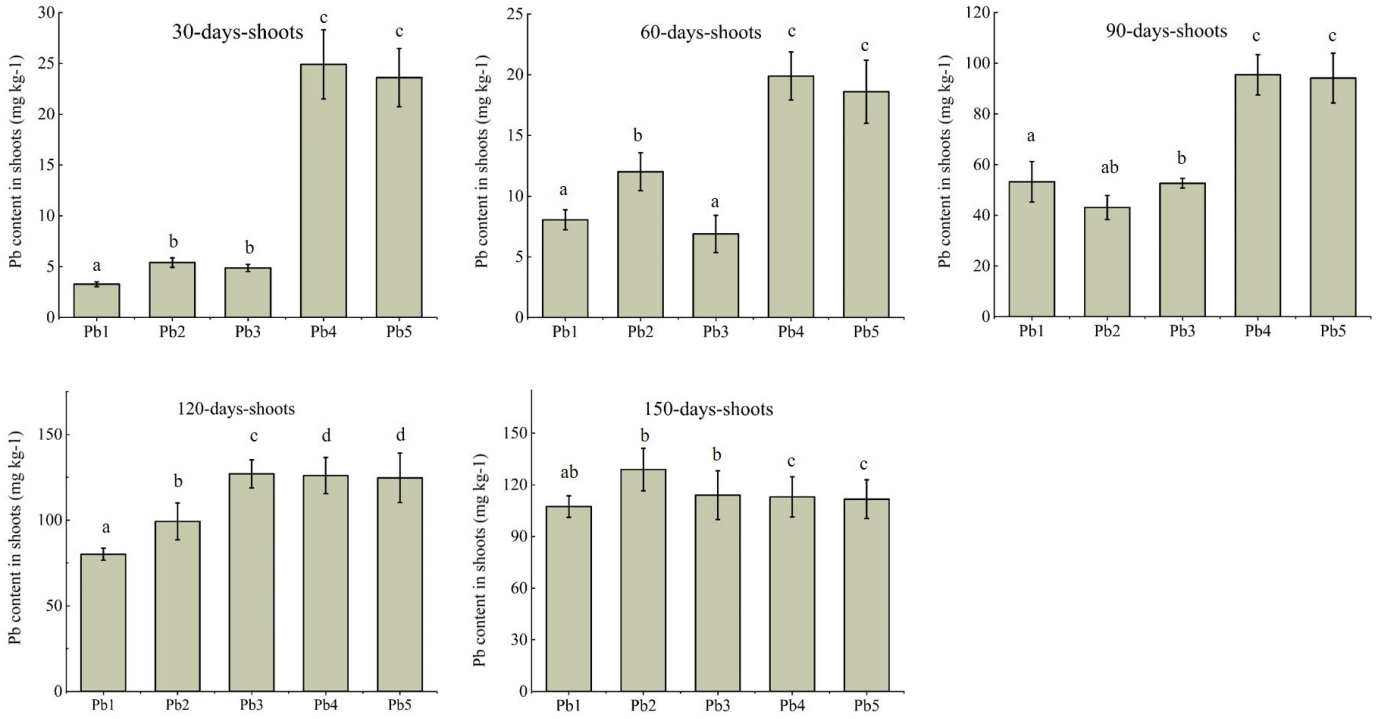

Figure 5. Change of Pb content in shoots (mg kg^{-1}). Results are expressed as means ± SD (N = 5). Different lowercase letters indicate significant differences between Pb concentrates at $p < 0.05$.

Depending on the results of the statistical analysis, there were significant differences in the effect of soil Pb concentration on Pb content in leaves at each growth stage of *N. reynaudiana*. Lead levels in the Pb4 and Pb5 shoots were consistently significantly different from those in the three groups with lower concentrations.

The TR reflects the transfer process of Pb in *N. reynaudiana*. The higher the transfer rate, the more Pb was accumulated in the shoots, while less Pb was accumulated in the root. Therefore, we analyze and discuss the TR, and the results are shown in Table 4. The transfer rate showed a trend of increasing first and then decreasing, reaching its maximum value at the MP stage.

Table 4. The TR of Pb in each growth period of *N. reynaudiana*.

Number	Growth Period				
	YP (30 Days)	GP (60 Days)	MP (90 Days)	OP (120 Days)	AP (150 Days)
Pb1	22.60%	37.15%	79.45%	64.25%	76.66%
Pb2	23.84%	43.61%	57.21%	65.88%	50.06%
Pb3	21.13%	36.98%	61.34%	52.80%	29.09%
Pb4	8.31%	7.14%	28.81%	32.27%	22.61%
Pb5	6.73%	5.57%	21.60%	20.64%	16.21%

To analyze the remediation ability of *N. reynaudiana* against soil Pb contamination, this study also investigated the Pb enrichment rate of *N. reynaudiana*. The Pb accumulation rate at different growth stages was calculated and analyzed (Table 5).

Table 5. The BR of Pb in each growth period of *N. reynaudiana*.

Number	Growth Period				
	YP (30 Days)	GP (60 Days)	MP (90 Days)	OP (120 Days)	AP (150 Days)
Pb1	1.89%	5.23%	64.35%	299.59%	601.51%
Pb2	1.70%	6.46%	46.34%	291.94%	461.16%
Pb3	1.01%	2.32%	49.69%	552.30%	400.14%
Pb4	4.04%	4.13%	23.33%	36.63%	30.54%
Pb5	2.77%	2.49%	17.50%	30.27%	25.80%

In the Pb1 and Pb2 groups, BR increased and peaked at the AP stage. However, in the Pb3, Pb4, and Pb5 groups, the Pb accumulation rate gradually decreased after peaking during the OP period. Therefore, the soil Pb concentration significantly affected the BR of *N. reynaudiana*.

3.4. Changes of Redox Enzyme Activity

Under Pb stress, the activities of SOD, CAT, APX, GR, and POD were carried out at each growth stage, as shown in Figure 6. Heavy metal enrichment is mainly achieved through the enzyme activity in *N. reynaudiana*, which determines the absorption capacity of the plants, and the activity levels of different enzymes are different at different growth stages and under different concentration stress.

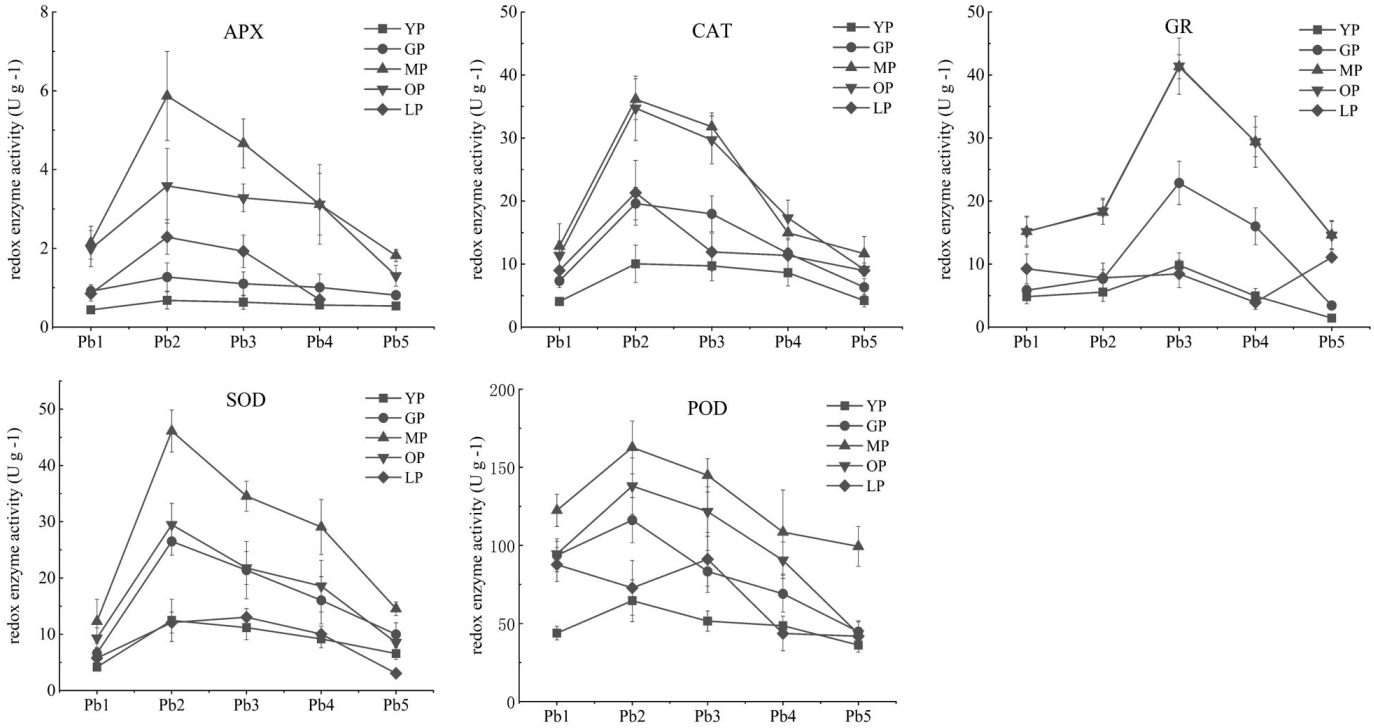

Figure 6. Changes in redox enzyme activity (U g^{-1}). Results are expressed as means ± SD (standard deviation), N = 5.

With the addition of exogenous Pb, the activity of SOD, POD, CAT and APX increased and then decreased with plant growth. The peak values measured in the Pb2 group at MP (90 days) stage were 46.11 U g^{-1}, 162.75 U g^{-1}, 12.85 U g^{-1}, and 5.87 U g^{-1}. Even though the GR activity also increased first and then decreased, the peak value appeared in the OP (120 days) stage of the Pb3 group, which was 41.41 U g^{-1}. As the protective enzymes in the redox enzyme system, these five enzymes can reduce the damage caused

by harmful components produced in the process of free radical oxidation by converting the O_2 produced in *N. reynaudiana* under external stress pressure into H_2O_2.

SPSS 22.0 was used to analyze the activity changes of the different redox enzymes, and the results are shown in Table 6. It was found that the activity of the redox enzyme gradually decreased with the increase in the Pb concentration in the soil. In the one-way ANOVA, it was found that there were significant differences in enzyme activity between the group with high Pb concentrations and the group with low Pb concentrations.

Table 6. The comparative analysis of the redox activity under the Pb stress in *N. reynaudiana*.

	Pb0	**Pb1**	**Pb2**		**Pb3**	**Pb4**	**Pb5**
Average	28.83	116.18	174.77		166.13	119.92	79.71
SD	13.84	39.05	73.13		70.43	51.70	36.45
F-value				5.57			
Pb0		−145.94 *	−137.31 *		−91.09 *	−50.88	50.88
Pb1			−49.95		−3.74	36.48	−36.48
Pb2					54.84	95.06 *	−95.06 *
Pb3						86.43 *	−86.42 *
Pb4							−40.22

* Significant differences at the 0.05 level ($p < 0.05$).

With the increase in exogenous Pb content, the SRI increased gradually, peaking in the 800 mg kg^{-1} group and then decreasing significantly. The AR results indicated that the roots of *N. reynaudiana* were irreversibly damaged when the soil concentration exceeded 800 mg kg^{-1}, which affected the absorption of Pb. The maximum value of the transfer rate tested in the MP stage indicated that the damage of *N. reynaudiana* existed from the beginning. When the soil concentration exceeded 800 mg kg^{-1}, the bioconcentration rate was significantly damaged. Not only could *N. reynaudiana* accumulate Pb in the environment, *Eichhornia crassipes* could also be defined as a feasible hyperaccumulation plant, which grows well at 1000 mg L^{-1} Pb concentration. The MDA content slightly decreased in leaf and root tissues when the Pb treatment was over 400 mg L^{-1} [30].

3.5. Discussion

With the addition of different amounts of exogenous Pb, the activity increased and then decreased with the plant growth. By comparing the activities of other enzymes, it was found that the peak activity of GR was one test period (30 days) later than that of other redox enzymes. Therefore, the GR redox enzyme system can continue redox metabolism in the later stages of plant growth with high lead content. Maintaining high GSH levels in both roots and shoots and high phenolics in shoots, mainly flavanols and HCAs, helped the *Z. fabago* seedlings counteract Pb toxicity in the Pb–Zn mining area. Thus, higher innate levels of antioxidant compounds and the differential organ-specific response of redox metabolites contributed to the exhibited tolerance to Pb [20]. Pb induced increased formation of ROS (O_2, OH, and H_2O_2) and enhanced the activities of the antioxidant enzymes APX, SOD, CAT, and POD. The compounds glutathione (GSH) and ascorbic acid are important non-enzymatic antioxidants present in the cell. The oxidized forms of these compounds are dehydroascorbic acid (DAH) and reduced glutathione (GSSH). GR is involved in this process. Therefore, the peak activity of GR is measured one period later than that of others [6,17].

Because of the extreme carcinogenicity, genotoxicity, and neurotoxicity that heavy metals pose, remediation of the soils is urgently required [31]. Phytoremediation is an economically and socially acceptable, environment friendly, and esthetically pleasant approach to remediate Pb-polluted soils [10]. Even defining the accumulation concentration and elements of plants, the scope of the application of phytoremediation can be defined [32], but there are still many limitations in the practical application of superaccumulator plants such as *N. reynaudiana*. Chemical additives are the main methods to enhance phytoreme-

diation [33]. Melatonin could mitigate these negative effects of *Cynodon dactylon* about decreases in biomass and chlorophyll production, degradation of thylakoid membranes, reduced photosynthesis and PSII (reaction center of photosystem II) efficiency, and elevated oxidative stress after growth in high-exposure Pb soil [34]. Biosolids can be used for immobilizing heavy metals and mitigating their risk in soils [35,36]. Future studies might determine the exact expression of genes in plants under heavy metal stress, the processes involved, and how they can be efficiently utilized to improve plant tolerance to toxic metals and facilitate the remediation process [37]. The Figure 7 show the effect of Pb on redox enzyme activity.

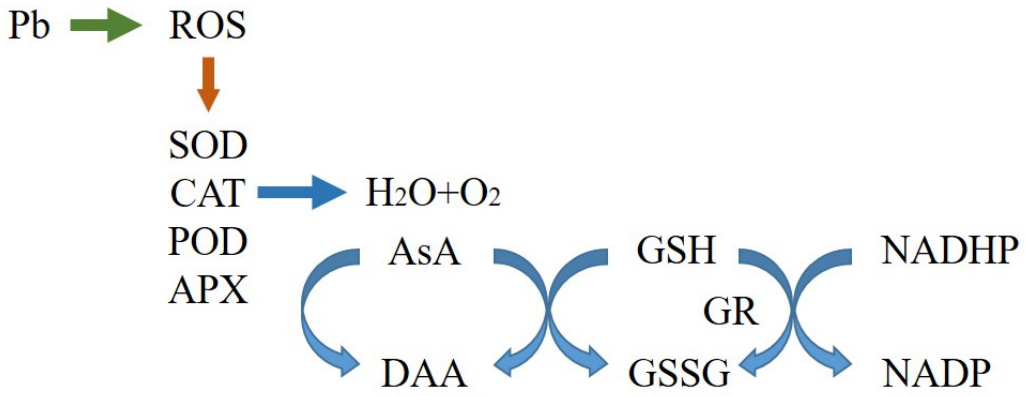

Figure 7. Effect of Pb on redox enzyme activity.

The enrichment of Pb in soil is affected by many other factors. The soil pH can affect the solubility and absorbability of Pb in the soil. Low pH (acidic soil) can increase the solubility of Pb and make it more easily absorbed by plants. The lower soil pH value increases the absorption and accumulation of Pb by tea trees, thus increasing the Pb content in tea [38]. Soil with high organic matter content can form strong combinations with Pb, thus reducing the bioavailability of Pb and reducing the absorption of Pb by plants [39]. The biological availability of heavy metals in soil is low, and fewer of them can be absorbed by plants, which seriously affects the effect of phytoremediation. The use of chelating agents can effectively reduce the migration of Pb in soil and reduce the loss of Pb to plants and damage to the environment. Zn in soil will affect the absorption of Pb because Zn will occupy the absorption site of Pb on plant roots, so the content of Zn in soil is high, and the absorption effect of the plant is poor [40,41]. Therefore, reasonable use of these influencing factors can improve the remediation effect of lead in the environment.

4. Conclusions

As a kind of terrain restoration plant widely used in China, *N. reynaudiana* is a very excellent environmental control plant with a wide range of cultivation and rapid growth. In this study, it was found that *N. reynaudiana* had the ability to repair soil lead pollution. The rehabilitative ability of *N. reynaudiana* to Pb in soil was different in each growth stage. As the plant grows, it increases first and declines after peaking at 120 days. At the same time, there is a threshold value for the restoration of Pb. When the soil Pb content exceeds 800 mg kg^{-1}, the protein structure will be destroyed, resulting in internal tissue damage and decreased adsorption capacity. This finding can provide a basis for the selection of site restoration of *N. reynaudiana*, and can avoid the blindness planting. The activity of oxidoreductase determines the absorptive capacity of *N. reynaudiana*, and the activity levels of different enzymes are different. SOD, CAT, APX, and POD first act on the initial stage of redox reaction, and when the pollution is aggravated or the growth of *N. reynaudiana* enters the middle and late stage, the GR redox system will continue to work in the later stage of growth polluted by high Pb concentration. The enzyme activity measured in this

study is only a part of the redox reaction. In the future, it is possible to further increase the repair ability and repair range of *N. reynaudiana* by adjusting the metabolic process.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14010118/s1, Text S1: experimental method; Text S2: the list of abbreviation.

Author Contributions: All authors made significant contributions to the preparation of this manuscript. Conceptualization, F.C. and S.Z.; data curation, F.C. and Z.C.; formal analysis, Y.Z.; investigation, Z.C.; project administration, S.Z.; supervision, S.Z.; validation, F.C.; writing—original draft, F.C.; writing—review and editing, F.C. and B.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Open fund of Key Laboratory of Coal Measures Mineral Resources of China National Administration of Coal Geology (KFKT-2020-5), the Science and Technology Innovation Foundation of Xuzhou city (KC23381),the Science and Technology Innovation Foundation of Jiangsu province (BE2023855) and the National Natural Science Foundation of China (51874307).

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: We would like to thank Huping Hou for her help in the processes regarding plant resources such as seed acquisition, seedling cultivation, and planting management. We are also indebted to Hui Xu and Lei Cao for their support in obtaining the funding.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kan, X.; Dong, Y.; Feng, L.; Zhou, M.; Hou, H. Contamination and health risk assessment of heavy metals in China's lead–zinc mine tailings: A meta–analysis. *Chemosphere* **2021**, *267*, 128–133. [CrossRef] [PubMed]
2. Silver, M.K.; Li, X.; Liu, Y.; Li, M.; Mai, X.; Kaciroti, N.; Kileny, P.; Tardif, T.; Meeker, J.D.; Lozoff, B. Low-level prenatal lead exposure and infant sensory function. *Environ. Health* **2016**, *15*, 65–79. [CrossRef] [PubMed]
3. Boskabady, M.; Marefati, N.; Farkhondeh, T.; Shakeri, F.; Farshbaf, A.; Boskabady, M.H. The effect of environmental lead exposure on human health and the contribution of inflammatory mechanisms, a review. *Environ. Int.* **2018**, *120*, 404–420. [CrossRef] [PubMed]
4. Ebrahimi, M.; Khalili, N.; Razi, S.; Keshavarz-Fathi, M.; Khalili, N.; Rezaei, N. Effects of lead and cadmium on the immune system and cancer progression. *J. Environ. Health Sci. Eng.* **2020**, *18*, 335–343. [CrossRef] [PubMed]
5. Alengebawy, A.; Abdelkhalek, S.T.; Qureshi, S.R.; Wang, M.Q. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics* **2021**, *9*, 42. [CrossRef] [PubMed]
6. Zulfiqar, U.; Farooq, M.; Hussain, S.; Maqsood, M.; Hussain, M.; Ishfaq, M.; Ahmad, M.; Anjum, M.Z. Lead toxicity in plants: Impacts and remediation. *J. Environ. Manag.* **2019**, *250*, 83–98. [CrossRef] [PubMed]
7. Kushwaha, A.; Hans, N.; Kumar, S.; Rani, R. A critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies. *Ecotoxicol. Environ. Safe.* **2018**, *147*, 1035–1045. [CrossRef] [PubMed]
8. Cheng, S.F.; Huang, C.Y.; Chen, K.L.; Lin, S.C.; Lin, Y.C. Phytoattenuation of lead-contaminated agricultural land using Miscanthus floridulus-an in *situ* case study. *Desalination Water Treat.* **2016**, *57*, 7773–7779. [CrossRef]
9. Yang, J.X.; Yang, J.; Huang, J. Role of co-planting and chitosan in phytoextraction of As and heavy metals by Pteris vittata and castor bean—A field case. *Ecol. Eng.* **2017**, *109*, 35–40. [CrossRef]
10. Kumar, A.; Kumar, A.; Cabral-Pinto, M.M.S.; Chaturvedi, A.K.; Shabnam, A.A.; Subrahmanyam, G.; Mondal, R.; Gupta, D.K.; Malyan, S.K.; Kumar, S.S.; et al. Lead Toxicity: Health Hazards, Influence on Food Chain, and Sustainable Remediation Approaches. *Int. J. Environ. Res. Public Health* **2020**, *17*, 2179. [CrossRef]
11. Hu, J.Y.; Tao, R.H.; Cao, C.; Xie, J.H.; Gao, Y.X.; Hu, H.X.; Ma, Z.W.; Ma, Y.H. Effect of Leaf Surface Regulation of Zinc Fertilizer on Absorption of Cadmium, Plumbum and Zinc in Rice (*Oryza sativa* L.). *Sustainability* **2023**, *15*, 1877. [CrossRef]
12. Plunkett, S.A.; Eckley, C.S.; Luxton, T.P.; Johnson, M.G. The effects of biochar and redox conditions on soil Pb bioaccessibility to people and waterfowl. *Chemosphere* **2022**, *294*, 361–374. [CrossRef] [PubMed]
13. Xiong, J.; He, Z.; Liu, D.; Mahmood, Q.; Yang, X. The role of bacteria in the heavy metals removal and growth of Sedum alfredii Hance in an aqueous medium. *Chemosphere* **2008**, *70*, 489–494. [CrossRef] [PubMed]
14. He, L.Y.; Zhang, Y.F.; Ma, H.Y.; Su, L.N.; Chen, Z.J.; Wang, Q.Y.; Qian, M.; Sheng, X.F. Characterization of copper-resistant bacteria and assessment of bacterial communities in rhizosphere soils of copper-tolerant plants. *Appl. Soil Ecol.* **2010**, *44*, 49–55. [CrossRef]

15. Zhang, W.-H.; Huang, Z.; He, L.-Y.; Sheng, X.-F. Assessment of bacterial communities and characterization of lead-resistant bacteria in the rhizosphere soils of metal-tolerant Chenopodium ambrosioides grown on lead–zinc mine tailings. *Chemosphere* **2012**, *87*, 1171–1178. [CrossRef] [PubMed]
16. Bela, K.; Horváth, E.; Gallé, A.; Szabados, L.; Tari, I.; Csiszár, J. Plant glutathione peroxidases: Emerging role of the antioxidant enzymes in plant development and stress responses. *J. Plant Physiol.* **2015**, *176*, 192–201. [CrossRef]
17. Cejudo, F.J.; González, M.-C.; Pérez-Ruiz, J.M. Redox regulation of chloroplast metabolism. *Plant Physiol.* **2020**, *186*, 9–21. [CrossRef]
18. Huang, X.H.; Zhu, F.; He, Z.X.; Chen, X.Y.; Wang, G.J.; Liu, M.S.; Xu, H.Y. Photosynthesis Performance and Antioxidative Enzymes Response of Melia azedarach and *Ligustrum lucidum* Plants Under Pb-Zn Mine Tailing Conditions. *Front. Plant Sci.* **2020**, *11*, 12–25. [CrossRef]
19. Rahman, M.M.; Swaraz, A.M.; El-Shehawi, A.M.; Elseehy, M.M.; Alam, M.F.; Kabir, A.H. The Mechanistic Basis of Sulfur-mediated Alleviation of Pb Toxicity in Wheat. *Gesunde Pflanz.* **2022**, *74*, 571–581. [CrossRef]
20. López-Orenes, A.; Santos, C.; Dias, M.C.; Oliveira, H.; Ferrer, M.Á.; Calderón, A.A.; Silva, S. Genotoxicity and Cytotoxicity Induced in *Zygophyllum fabago* by Low Pb Doses Depends on the Population's Redox Plasticity. *Horticulturae* **2021**, *7*, 455. [CrossRef]
21. Khan, M.; Al Azzawi, T.N.I.; Imran, M.; Hussain, A.; Mun, B.G.; Pande, A.; Yun, B.W. Effects of lead (Pb)-induced oxidative stress on morphological and physio-biochemical properties of rice. *Biocell* **2021**, *45*, 1413–1423. [CrossRef]
22. Youfa, L.; Yonggui, W.; Tianling, F.; Jiajia, Z.; Qingjun, M.; Cong, Y. Ecological restoration effect and potential of Neyraudia reynaudiana vegetation on AMD polluted soil. *J. Soil Water Conserv.* **2016**, *30*, 8–20.
23. Liping, C.; Pengfei, W.; Xiaolong, H.; Xiangqing, M.; Shuo, J.; Jingjing, R. Effects of phosphorus stress on photosynthetic characteristics of pioneer plant Neyraudia reynaudiana on soil and water conservation. *J. Soil Water Conserv.* **2012**, *26*, 1–5.
24. Jialong, W.; Jun, D.; Hong, F.; Ting, D.; Huan, Q. Effects of acidity and aluminum stress on the growth of Neyraudia reynaudiana. *J. Agric. Environ. Sci.* **2018**, *37*, 8–16.
25. Ahammed, G.J.; Xu, W.; Liu, A.; Chen, S. Endogenous melatonin deficiency aggravates high temperature-induced oxidative stress in *Solanum lycopersicum* L. *Environ. Exp. Bot.* **2019**, *161*, 303–311. [CrossRef]
26. Xu, Z.; Regenstein, J.M.; Xie, D.; Lu, W.; Ren, X.; Yuan, J.; Mao, L. The oxidative stress and antioxidant responses of *Litopenaeus vannamei* to low temperature and air exposure. *Fish Shellfish Immunol.* **2018**, *72*, 564–571. [CrossRef] [PubMed]
27. Machado, B.R.; Silva, P.G.P.; Garda-Buffon, J.; Santos, L.O. Magnetic fields as inducer of glutathione and peroxidase production by *Saccharomyces cerevisiae*. *Braz. J. Microbiol.* **2022**, *53*, 1881–1891. [CrossRef] [PubMed]
28. Wu, Q.H.; Wang, S.Z.; Thangavel, P.; Li, Q.F.; Zheng, H.; Bai, J.; Qiu, R.L. Phytostabilization Potential of *Jatropha curcas* L. in Polymetallic Acid Mine Tailings. *Int. J. Phytoremediat.* **2011**, *13*, 788–804. [CrossRef]
29. Baker, A.J.M. Accumulators and excluders—Strategies in the response of plants to heavy metals. *J. Plant Nutr.* **1981**, *3*, 643–654. [CrossRef]
30. Malar, S.; Shivendra Vikram, S.; Jc Favas, P.; Perumal, V. Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. *Bot. Stud.* **2014**, *55*, 54–63. [CrossRef]
31. Luo, X.; Wu, C.; Lin, Y.; Li, W.; Deng, M.; Tan, J.; Xue, S. Soil heavy metal pollution from Pb/Zn smelting regions in China and the remediation potential of biomineralization. *J. Environ. Sci.* **2023**, *125*, 662–677. [CrossRef] [PubMed]
32. Stofejová, L.; Fazekas, J.; Fazekasová, D. Transfer of Potentially Toxic Elements in the Soil-Plant System in Magnesite Mining and Processing Areas. *Processes* **2022**, *10*, 720. [CrossRef]
33. Shen, X.; Dai, M.; Yang, J.; Sun, L.; Tan, X.; Peng, C.; Ali, I.; Naz, I. A critical review on the phytoremediation of heavy metals from environment: Performance and challenges. *Chemosphere* **2022**, *291*, 132–143. [CrossRef] [PubMed]
34. Xie, C.C.; Pu, S.Y.; Xiong, X.; Chen, S.Y.; Peng, L.L.; Fu, J.Y.; Sun, L.X.; Guo, B.M.; Jiang, M.Y.; Li, X. Melatonin-assisted phytoremediation of Pb-contaminated soil using bermudagrass. *Environ. Sci. Pollut. Res.* **2021**, *28*, 44374–44388. [CrossRef] [PubMed]
35. Yang, X.; Pan, H.; Shaheen, S.M.; Wang, H.L.; Rinklebe, J. Immobilization of cadmium and lead using phosphorus-rich animal-derived and iron-modified plant-derived biochars under dynamic redox conditions in a paddy soil. *Environ. Int.* **2021**, *156*, 1421–1433. [CrossRef] [PubMed]
36. Sharma, B.; Sarkar, A.; Singh, P.; Singh, R.P. Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. *Waste Manag.* **2017**, *64*, 117–132. [CrossRef]
37. Wang, J.; Duan, X.C.; Wang, Y.Z.; Sheng, J.J. Transcriptomic and physiological analyses of *Miscanthus lutarioriparius* in response to plumbum stress. *Ind. Crops Prod.* **2022**, *176*, 6–19. [CrossRef]
38. Ye, J.; Zhang, Q.; Liu, G.; Lin, L.; Wang, H.; Lin, S.; Wang, Y.; Wang, Y.; Zhang, Q.; Jia, X.; et al. Relationship of soil pH value and soil Pb bio-availability and Pb enrichment in tea leaves. *J. Sci. Food Agric.* **2021**, *7*, 10–29. [CrossRef]
39. Wang, H. Effects of EDTA on growth and lead-zinc accumulation in maize seedlings grown in amendment substrates containing lead-zinc tailings and soil. *Acta Ecologica Sinica.* **2011**, *31*, 7125–7132.

40. Díaz, G.; Azcón-Aguilar, C.; Honrubia, M. Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of Lygeum spartum and Anthyllis cytisoides. *Plant Soil* **1996**, *180*, 241–249. [CrossRef]
41. Zheng, R.; Li, C.; Sun, G.; Xie, Z.; Chen, J.; Wu, J.; Wang, Q. The influence of particle size and feedstock of biochar on the accumulation of Cd, Zn, Pb, and As by *Brassica chinensis* L. *Environ. Sci. Pollut. Res.* **2017**, *24*, 22340–22352. [CrossRef] [PubMed]