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Research article

Alleviating effects of silicate, selenium, and microorganism fertilization on lead toxicity in ginger (*Zingiber officinale* Roscoe)

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ABSTRACT

The aim of this work was exploring the effects of silicon, selenium, and a microorganism fertilizer on alleviating the effects of lead (Pb) toxicity in ginger. Ginger plants were grown in soil containing 500 mg/kg $Pb(NO_3)_2$ without (CK) or with Si, Se, or microorganism fertilizer (T1, T2, T3) as soil conditioners. Morphology indexes, Pb accumulation and distribution rates, and antioxidant enzyme activities were investigated. The Pb transfer and Pb absorption coefficients were calculated, and Pb accumulation in plant organs at various developmental stages were determined. All three soil conditioners alleviated Pb stress in ginger plants. The rhizome fresh weight in T1, T2, and T3 was increased by 96.06, 85.81, and 41.58%, respectively, compared with CK. The accumulation of Pb in organs was lower in all treatments than in CK. The chlorophyll and carotenoid contents in leaves, and root activity, root length, and the tolerance index, were higher in the treatments than in CK. The reactive oxygen species content in ginger leaves and roots was significantly lower in all treatments than in CK. Soil conditioners alleviated the negative effects of Pb stress on ginger plants: Si was the most effective, followed by Se, and then the microorganism fertilizer.

1. Introduction

Heavy metals contamination of soils is a serious problem because it affects food safety (Emamverdian et al., 2018; Tiwari and Lata, 2018). The release of heavy metals into agricultural soils has increased dramatically worldwide during the last few centuries (Nocito et al., 2006; Peng et al., 2010). These metals are toxic to plants, animals, and human beings and their removal is required to prevent toxicity. Reducing the heavy metals content in crops will decrease potential health risks. According to the Environmental Protection Agency, lead (Pb) is one of the most widespread heavy metals contaminants (Carpenter et al., 2003). Excessive Pb is released in various ways. Rapid industrialization and the indiscriminate use of chemical fertilizers and pest control agents are the primary sources of heavy metals contamination in agricultural soils. Plants accumulate heavy metals from the soil (metal mining, chemical fertilizers and pesticides), water (heavy metals residues), or air (coal mining) (Sengar et al., 2008). Lead is readily absorbed, accumulated, and transferred in plant tissues. The root is the primary point of Pb uptake and accumulation (Kumar et al., 2011).

Heavy metals pollution can be alleviated by various substances including silicon (Si), selenium (Se), and sodium chloride. Previous studies have shown that Si can promote the growth of many plant species such as rice, sugarcane, and some cyperaceous plants (Epstein, 1994; Liang et al., 2005; Ma et al., 2002). It also alleviates stresses caused by various biotic (plant diseases and insect pests) and abiotic (nutrient deficiency, metal toxicity, drought and salt stress) factors in several plant species (Liang et al., 2007; Ma and Yamaji, 2006). Silicon has been shown to dramatically reduce the toxicity of various metals in plants, for example, cadmium (Cd) toxicity in pakchoi (Song et al., 2009), manganese toxicity in cucumber and cowpea (Fuhrs et al., 2009; Pereira et al., 2018; Shi et al., 2005), Al toxicity in wheat, rice, maize and soybean (Ma et al., 2004). Silicon is deposited in the cell walls of leaves, stems, and roots, and alleviates the toxic effects of heavy metals (Ma and Yamaji, 2006). Previous studies have reported that silica deposition in wheat leaves inhibited transpiration and salt accumulation (Campbell et al., 2000; Peleg et al., 2010). In tomato, silicate crystals

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deposited in the epidermal cells formed a barrier that decreased water loss, which contributed to salt dilution and reduced salt toxicity (Romero-Aranda et al., 2006). In barley and cucumber, Si was shown to improve salt tolerance and alter antioxidant enzyme activity(Liang et al., 2003; Wei et al., 2004). In rice, the alleviation of zinc (Zn) toxicity by Si was correlated with Zn transport and antioxidant reactions (Song et al., 2014).

Selenium is an essential element for human beings, but not for plants. However, Se has been shown to alleviate the negative effects of some abiotic stresses such as drought, cold, water logging, salinity, and heavy metals (Feng et al., 2013). Excess Se may damage plants by causing oxidative stress (Jiang et al., 2015; Tang et al., 2015). Selenium plays roles in reactions catalyzed by both glutathione peroxidase and thioredoxin reductase. Several studies have shown that Se can protect against toxicity of toxic elements such as Cd, Pb, antimony, arsenic, copper, and mercury (Feng et al., 2013; He et al., 2004; Kaur et al., 2017; Qingqing et al., 2019). In rice, Se application significantly reduced Cd and Pb concentrations, and Se application at 0.5 mg per kg of soil dramatically reduced metals mobility in soil (Hu et al., 2014).

For thousands of years, farmers in China have utilized organic wastes in agriculture to maintain plant production and soil fertility (Westerman and Bicudo, 2005). Long-term application of manure has increased the organic C content and stabilized the soil profile (Mandal et al., 2007). However, the use of manure to fertilize agricultural fields has drastically decreased since the 1980s because of the increased use of inorganic fertilizers (Ju et al., 2005). Consequently, since 1981, China has become the largest fertilizer consumer, with a 90% increase in fertilizer use (Liu et al., 2004). Large-scale application of inorganic fertilizers has greatly increased crop yield, but has also caused many problems, including increased greenhouse gas emissions, nitrate leaching, and eutrophication (Baghdadi et al., 2018).

Ginger (*Zingiber officinale*) has been grown for many years in tropical and subtropical regions such as India, Malaysia, China, and Korea. Ginger is famous for its aromatic odor and warm and pungent taste. Ginger extracts are used extensively in the food and beverage industries, in chutney, marmalade, pickles, biscuits, ginger beer, and other bakery products. Ginger is also used as herbal medicine for treating pains, vomiting, indigestion, fever and infectious diseases. The detection of toxic heavy metals in ginger rhizomes is crucial for protecting consumers against heavy metals toxicity (Wagesho and Chandravanshi, 2015). Despite the extensive use of ginger worldwide, only few studies have analyzed its heavy metals profile (Mishra et al., 2007). Gupta et al. (2010) analyzed the concentrations of volatile and non-volatile toxic elements (arsenic, mercury, Pb and Cd) in the rhizome of ginger using atomic absorption spectrophotometry (AAS).

Lead is a non-essential element and is potentially toxic for plants, animals, and humans. Therefore, Pb pollution has become a major problem with the development of modern industry and agriculture. There is a need to develop strategies to alleviate the damaging effects of heavy metals toxicity in polluted ecosystems. Ginger is particularly vulnerable to heavy metals contamination, because the edible part is the rhizome that grows in direct contact with soil. Therefore, the aims of this study were: (1) to determine the effects of different soil conditioners on the growth of ginger under Pb stress; (2) to quantify the effects of soil conditioners on Pb accumulation and distribution in various organs of ginger under Pb stress; and (3) to determine the effect of soil conditioners on root activity, root length, and the tolerance index of ginger.

2. Materials and methods

2.1. Plant material and experimental design

The ginger variety 'LaiWuDaJiang' was grown at the experimental station situated in ShanDong Agricultural University, Tai'an (36° 09′ N, 117° 09′ E), ShanDong Province, China. The pH of the soil was 7.3 and the contents of alkali-hydrolyzed N, available phosphorus (P), and available potassium (K) were 100.5 mg kg^{-1}, 63.4 mg kg^{-1}, and 127.8 mg kg^{-1}, respectively. The experimental design is summarized in Table 1. In each treatment, $Pb(NO_3)_2$ (PuTian company, Taian, ShanDong Province) was added to the soil at the rate of 500 mg kg^{-1}. Then, different soil conditioners were added to the different treatments (see Table 1). Si and Se were provided by Huarun Biotechnology Company, Laiwu, ShanDong Province and microorganism fertilizer was provided by Maikezhen Biotechnology Company,ShanDong Province. Each treatment had 45 pots. Three biological replicates for each treatment were used for every analysis. Ginger rhizomes were harvested on July 15th, August 15th, September 15th, and October 15th.

Table 1
Experimental design.

Treatment	Pb (mg·kg^{-1})	Soil conditioner
CK	500	/
T1	500	Si [1.5 g kg^{-1}(soil)]
T2	500	Se [1 ml kg^{-1}(soil)]
T3	500	Microorganism fertilizer [0.5 ml kg^{-1}(soil)]

2.2. Measurement of growth parameter and yield

Samples were taken at the seedling stage, tillering stage, and rhizome expansion stage. Five plants in each treatment were selected to measure plant height, stem diameter, branch number, leaf number, root length, fresh weight, and dry weight. At harvest time, the rhizomes were weighed to determine the yield per plant.

2.3. Quality evaluation

The soluble proteins content was determined using the Coomassie Brilliant Blue method (Jiang and Huang, 2002). Free amino acid content was determined by the ninhydrin method (Wu et al., 2005). The soluble sugars content was measured by anthrone colorimetry (Jiang and Huang, 2002).

To determine the gingerols content, 1 g of ginger powder was added to a 100-ml volumetric flask, then 70 ml acetone was added and the mixture was shaken at 50 °C for 1 h. The mixture was cooled and then the volume was completed to 100 ml with acetone. The filter liquor was used for the determination of gingerols.

To determine the vitamin C content, 10 g of fresh tissue was ground with 2 ml 2% oxalic acid solution, and the vitamin C content was measured against a 2,6-dichloro indophenol standard (Karpinski et al., 1997).

2.4. Soil nutrient analyses

Natural air-dried soil samples were sieved through a 1-mm mesh size sieve. Soil pH, alkali-hydrolyzed N, available P, and available K were determined. The pH of the soil suspension was measured using a pH meter, the content of soil alkali-hydrolyzed N was determined by the alkali-hydrolysis diffusion method, the available P content was determined by molybdenum-antimony anti-colorimetry, and the available K content was determined by flame photometry.

2.5. Antioxidant enzyme activities

Oxidative damage was evaluated by determining the malondialdehyde (MDA) content by the thiobarbituric acid method (Wada et al., 2011). The activity of the antioxidant system was evaluated by determining the activities of superoxide dismutase (SOD) using the nitroblue tetrazolium method (Chapman et al., 2019) and the activities of peroxidase (POD) and catalase (CAT) as described by (Roldan et al., 2008).

2.6. $O_2^{-\cdot}$ Generation rate and H_2O_2 content

The superoxide ($O_2^{-\cdot}$) generation rate was measured by the hydroxylamine oxidation method (Rauckman et al., 1979). The H_2O_2 content was determined as described by (Gay and Gebicki, 2000).

2.7. Measurement of element contents

To determine the contents of various elements, exactly 1 g of dried sample (root, stem, leaf, rhizome) was added to a 25-mL conical flask containing 10 ml HNO_3: $HClO_4$ (4:1 vol/vol). After leaching for 24 h, the sample was digested (200 °C) using an electric furnace. The mixed acid was added when the solution turned dark brown. White smoke was produced and the digestion solution became transparent and yellowish. After cooling, the digestion solution was washed into a 50-ml volumetric flask. The flask was washed with ultrapure water, which was added to the mixture. The contents of Pb, calcium (Ca), magnesium (Mg), iron (Fe), Mn, copper (Cu), and Zn were determined by flame atomic absorption spectrophotometry.

2.8. Root length, root activity, index of tolerance

Roots were scanned using an Epson Expression 4990 scanner (Epson, Nagano, Japan). Root length data were analyzed using WinRHIZO (Shiya Technology, Shijiazhuang, China). Root activity was measured using the triphenyl tetrazolium chloride (TTC) method (Qiman et al., 2011).

The tolerance index was calculated as follows (Gupta et al., 2013):

Index of tolerance = average root length in treatment/ average root length in CK.

2.9. Determination of proline content

Sample was grounded and diluted with deionized water. Then centrifuge at 3000g for 10 min.750 μL of the diluted supernatant was added to the same volume of formic acid and vortex-mixed for 2 min. A 750 μL of ninhydrin 3% in dimethyl sulfoxide was then added. The mixture was heated at 100 °C in a heating block for 15 min. The number was measured at 520 nm on the Biochrom (UK) Libra S12 spectrophotometer.

2.10. Pb morphometry

The contents of ethanol-extracted Pb, hydrochloric acid-extracted Pb, and residual Pb in ginger root were measured by the two-step continuous leaching method.

2.11. Primary transfer coefficient and absorption coefficient of Pb

The primary transfer coefficient (PTI) and secondary transfer coefficient (STI) were calculated as follows:

PTI = Pb content of rhizome/ Pb content of root

STI = Pb content of aboveground part/ Pb content of rhizome

The various absorption coefficients were calculated using the following formulae:

RAI (root absorption coefficient) = Pb content of root/ Pb content of the soil

UAI (underground absorption coefficient) = Pb content of underground (root and rhizome)/Pb content of soil

AAI (aboveground absorption coefficient) = Pb content of aboveground/ Pb content of soil

Finally, the BCF (biological concentration coefficient) was calculated as follows:

BCF = Pb content of the plant/ Pb content of the soil

2.12. Statistical analyses

The physiological and morphological parameters of ginger were compared among different treatments using analysis of variance. Differences among treatments were determined using Fisher's least significance difference test at P = 0.05. All data were analyzed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Effect of soil conditioners on growth of ginger under Pb stress

Plants have developed a number of ways to adapt to and tolerate heavy metals stress. Different soil conditioners can alleviate Pb stress at various stages of plant growth. At the seedling stage, there was no significant difference among the treatments. However, when plant height and shoot number increased at the tillering stage, there were marked differences among the treatments. The plant height in the T1, T2, and T3 treatments was increased by 28.6, 24.53, and 14.79%, respectively, compared with CK. The shoot number in T1, T2, and T3 was increased by 178.38, 124.32, and 89.19%, respectively, compared with CK. The leaf fresh weight was increased by 103.10, 99.38, and 44.27% in T1, T2, and T3, respectively, compared with CK.

At the rhizome expansion stage, the rhizome fresh weight differed markedly among the treatments. It was increased by 96.06, 85.81, and 41.58% in T1, T2, and T3, respectively, compared with CK. The T1 treatment had the strongest effect to alleviate Pb toxicity. The final rhizome fresh weight was increased by 21.98, 32.12, and 51.36% in T1, T2, and T3, respectively, compared with CK (Table 2). All three soil conditioner treatments alleviated Pb toxicity, and were ranked from most to least effective as follows: T1 > T2 > T3 (Fig. 1).

3.2. Effect of soil conditioners on yield and quality of ginger under Pb stress

As shown in Table 3, the ginger yield under Pb stress differed markedly among the different soil conditioner treatments. Compared with the yield of CK, the yields of T1, T2, and T3 were increased by 32.21, 31.36, and 18.47%, respectively. There were no significant differences among the treatments in dry matter, crude cellulose content, and gingerols content. The treatments were ranked, from highest to lowest contents of soluble sugars, soluble proteins, and free amino acids as follows: T3 > T2 > T1 > CK. In T3, the contents of soluble sugars, soluble proteins, and free amino acids were increased by 50.00, 66.67, and 59.38%, respectively, compared with CK. However, the gingerols content was highest in T2, at 79.31% higher than that in CK (Table 3).

3.3. Effect of soil conditioners on Pb contents in organs of ginger plants at different growth stages

As shown in Fig. 2, the Pb contents in various organs differed among treatments. The Pb contents in organs of ginger under Pb stress were reduced by all treatments, with the largest decrease in T1, followed by T2, and a smaller decrease in T3. The Pb content in the roots of T1, T2, and T3 was decreased by 61.07, 49.22, and 31.79%, respectively, and that in the rhizome was decreased by 58.85, 45.29, 27.63%, respectively, compared with CK. The Pb content in the stem of T1, T2, and T3 was reduced by 72.52, 65.19, and 40.36%, respectively, compared with CK. The Pb content in the leaf of T1, T2, and T3 was reduced by 81.97, 73.13, and 61.37%, respectively, compared with CK (Fig. 2).

The Pb content differed among rhizomes of different ages (Fig. 3).

Table 2
Effect of different soil conditioner on the growth of ginger under Pb stress.

Growth stage		Plant height (cm)	Stem diameter (mm)	Shoot number	Root FW (g/plant)	Stem FW (g/plant)	Leaf FW (g/plant)	Rhizome FW (g/plant)
Seedling stage (07–15)	CK	45.5 ± 1.0b	7.2 ± 0.1b	1.3 ± 0.6a	13.6 ± 0.5c	51.5 ± 1.2b	16.4 ± 0.1b	40.5 ± 3.3c
	T1(Si)	56.9 ± 1.5a	8.8 ± 0.5a	2.7 ± 0.6a	16.7 ± 1.5a	64.2 ± 0.6a	26.9 ± 0.9a	49.4 ± 4.3 ab
	T2(Se)	55.9 ± 2.6a	8.6 ± 0.2a	2.3 ± 0.6a	16.1 ± 0.6 ab	63.8 ± 0.4a	26.4 ± 0.8a	50.9 ± 2.1a
	T3(Mi)	53.2 ± 2.0a	8.5 ± 0.1a	1.7 ± 0.6a	15.5 ± 0.6b	62.5 ± 0.4a	24.2 ± 0.2a	44.9 ± 2.8bc
Tillering stage (08–15)	CK	53.4 ± 2.3c	8.0 ± 0.4c	3.7 ± 1.2c	20.8 ± 1.4c	59.5 ± 0.4c	32.3 ± 0.7d	85.0 ± 2.3c
	T1(Si)	68.7 ± 3.3a	10.9 ± 1.1a	10.3 ± 1.5a	27.6 ± 1.6a	97.3 ± 0.8a	65.6 ± 0.6a	112.3 ± 11.0a
	T2(Se)	66.5 ± 3.7 ab	10.3 ± 1.0 ab	8.3 ± 1.5 ab	26.1 ± 1.6 ab	96.6 ± 1.2a	64.4 ± 0.7b	101.3 ± 9.6 ab
	T3(Mi)	61.3 ± 2.9b	8.9 ± 0.6bc	7.0 ± 1.7b	24.2 ± 0.3b	80.8 ± 0.4b	46.6 ± 0.4c	97.4 ± 1.9bc
Rhizome enxpanding stage (09–15)	CK	64.6 ± 4.0b	9.5 ± 0.1c	5.3 ± 0.6c	29.7 ± 1.7b	73.6 ± 1.6d	42.4 ± 2.9c	109.2 ± 8.5c
	T1(Si)	78.6 ± 2.5a	13.2 ± 0.7a	12.0 ± 1.7a	41.3 ± 2.6a	178.8 ± 4.1a	91.0 ± 0.4a	214.1 ± 4.9a
	T2(Se)	76.8 ± 3.0a	12.6 ± 0.5a	11.3 ± 1.5a	40.8 ± 1.5a	170.6 ± 4.6b	89.2 ± 0.2a	202.9 ± 6.4a
	T3(Mi)	71.5 ± 2.9a	10.9 ± 0.2b	9.3 ± 0.6b	37.3 ± 0.6a	122.9 ± 5.1c	65.4 ± 1.3b	154.6 ± 7.2b
Harvest Stage (10–15)	CK	65.9 ± 2.5c	10.8 ± 0.3c	6.3 ± 0.6c	35.3 ± 1.2c	91.8 ± 3.0c	68.6 ± 3.3c	212.8 ± 8.0d
	T1(Si)	80.9 ± 3.6a	14.5 ± 0.6a	13.7 ± 1.2a	45.3 ± 1.4a	245.8 ± 9.0a	119.4 ± 3.3a	322.1 ± 11.2a
	T2(Se)	77.9 ± 4.2 ab	15 ± 0.3a	12.3 ± 1.5a	44.7 ± 1.7a	238.3 ± 7.6a	116.0 ± 0.2a	289.3 ± 10.7b
	T3(Mi)	73.1 ± 3.0b	12.9 ± 0.2b	9.7 ± 1.2b	39.5 ± 1.2b	152.5 ± 3.4b	93.7 ± 3.7b	254.5 ± 3.3c

Note: Under the same column, values followed with the same letter was not significant at $P = 0.05$.

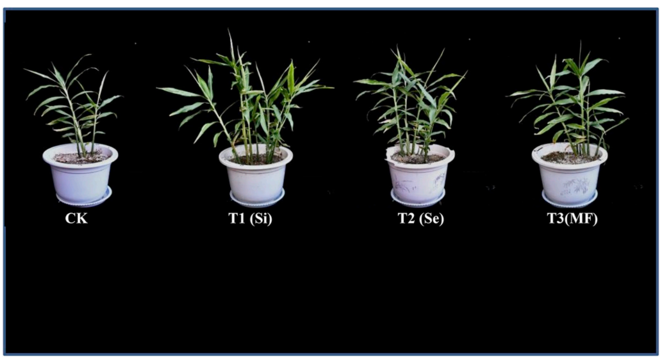

Fig. 1. The morphology of ginger under different soil conditioner treatment at tillering stage. T1:Si treatment. T2:Se treatment. T3:microorganism fertilizer.

The Pb content was highest in the original planted rhizomes, followed by the first-produced rhizomes, and then the last-produced rhizomes. In the T1 treatment, for example, the Pb contents in the three ages of rhizomes were 6.96, 1.71, and 0.85 mg kg^{-1}, respectively, which were 0.41, 0.34, and 0.58 that in CK (Fig. 3).

3.4. Effect of soil conditioners on Pb accumulation and distribution rate in organs of ginger under Pb stress

All the soil conditioner treatments reduced Pb accumulation in different organs (Table 4). In the rhizome, Pb accumulation was lowest in T1, followed by T2, and then T3. The Pb distribution rate into the rhizome was also decreased in the soil conditioner treatments. The Pb distribution rate in T1, T2, T3 was 26.65, 28.64, and 30.13%, respectively (Table 4). Careful observations of Pb accumulation and distribution rates among the different levels of ginger showed that, compared with CK, T1 showed the lowest Pb accumulation, with decreases of 46.03, 57.48, and 35.94% in mother-ginger, son-ginger, and grandson-ginger, respectively (Table 5).

3.5. Effect of soil conditioner on Pb transfer coefficient and Pb absorption coefficient of ginger

As shown in Table 6, under the same degree of Pb stress, the Pb transfer coefficient and absorption coefficient in various organs of ginger differed significantly between the treatments and the control. The CK and treatments were ranked, from the highest values of these indexes to the lowest, as follows: CK > T3 > T2 > T1. The root is the first point of contact with Pb in soil. All the soil conditioner treatments reduced the root absorption coefficient and primary transfer coefficient. The coefficient values were less than 0.5, indicating that Pb absorption and transfer by the plant root system were inhibited. This was beneficial for reducing Pb accumulation and the biological concentration coefficient, ultimately resulting in the alleviation of Pb toxicity. Biological concentration coefficient of all treatments were lower than 1, indicating that ginger is not Pb-accumulator plant. The soil conditioner treatments were ranked, from most to least effective alleviation of Pb toxicity, as follows: Si > Se > microorganism fertilizer (Table 6).

3.6. Effect of soil conditioner on chlorophyll and carotenoid contents of ginger leaves under Pb stress

As shown in Fig. 4, the chlorophyll and carotenoid contents were significantly higher in T1, T2, and T3 than in CK at different growth stages. During the experiment, the chlorophyll and carotenoid contents in ginger leaves first increased and then decreased. The highest

Table 3
Effect of different soil conditioner on ginger yield and quality under Pb stress.

	Yield (g/plant)	Dry matter (%)	Soluble sugars (%)	Crude cellulose (mg/g)	Soluble proteins (mg/g)	Free amino acids (mg/g)	Gingerol (%)	Vc (mg/100g FW)
CK	216.71 ± 9.81d	8.86 ± 0.11a	0.38 ± 0.06c	0.55 ± 0.07a	0.21 ± 0.06c	0.32 ± 0.02c	0.96 ± 0.02c	0.29 ± 0.04b
T1(Si)	319.68 ± 9.61a	8.05 ± 0.28b	0.57 ± 0.02a	0.39 ± 0.04b	0.35 ± 0.02a	0.51 ± 0.01a	1.32 ± 0.10a	0.48 ± 0.01a
T2(Se)	284.67 ± 11.21b	8.38 ± 0.14b	0.54 ± 0.06 ab	0.37 ± 0.04b	0.31 ± 0.02a	0.47 ± 0.01 ab	1.17 ± 0.13 ab	0.52 ± 0.13a
T3(Mi)	256.60 ± 5.49c	8.42 ± 0.13b	0.46 ± 0.04bc	0.43 ± 0.05b	0.27 ± 0.01b	0.44 ± 0.03b	1.23 ± 0.09b	0.35 ± 0.03a

Note: Under the same column, values followed with the same letter was not significant at $P = 0.05$.

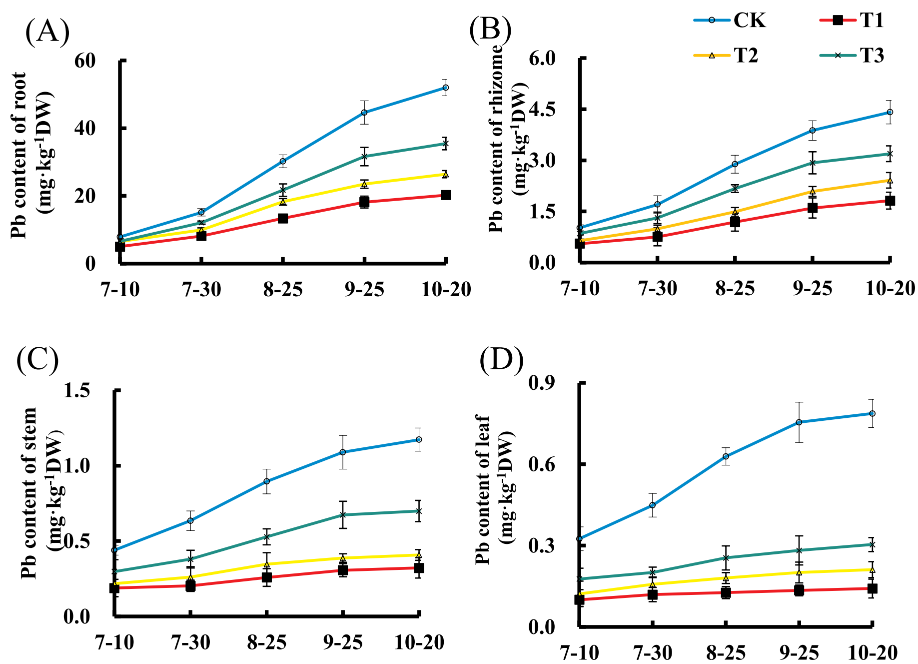

Fig. 2. Effect of soil conditioner on Pb content of different organ at different growth stage. (A) Pb content of root in CK,T1,T2,T3 on7-10,7–30,8-25,9–25,10-20. (B) Pb content of rhizome in CK,T1,T2,T3 on 7–10,7-30,8–25,9-25,10–20. (C) Pb content of stem in CK,T1,T2,T3 on 7–10,7-30,8–25,9-25,10–20. (D) Pb content of leaf in CK,T1,T2,T3 on 7–10,7-30,8–25,9-25,10–20. Error bars stand for the standard errors.

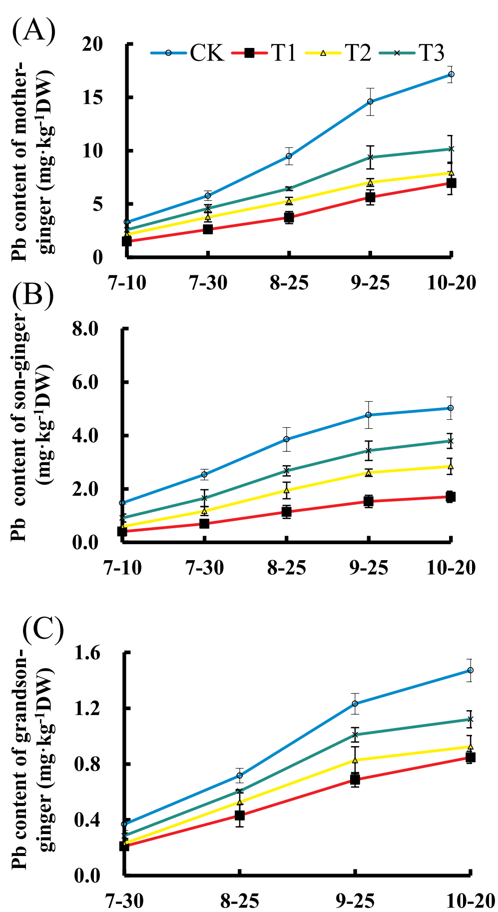

Fig. 3. Effect of soil conditioner on Pb content of ginger rhizome at different growth stage. (A)Pb content of mother-ginger in CK,T1,T2,T3 on 7–10,7-30,8–25,9-25,10–20. (B) Pb content of son-ginger in CK,T1,T2,T3 on 7–10,7-30,8–25,9-25,10–20. (C) Pb content of grandson-ginger in CK,T1,T2,T3 on 7–10,7-30,8–25,9-25,10–20. Error bars stand for the standard errors.

chlorophyll content was on September 20th, when it was 25.97, 23.22, and 15.44% higher in T1, T2, and T3, respectively, than in CK. Thus, all three soil conditioner treatments increased the pigment contents in ginger leaves. As antenna pigments, carotenoid transfer their absorbed light energy to chlorophyll for further photochemical reactions. The trends in carotenoid contents were the same as the trends in chlorophyll contents. The carotenoid contents were highest on September 20, when it was 21.29, 17.85, and 11.27% higher in T1, T2, and T3, respectively, than in CK (Fig. 4).

3.7. Effect of soil conditioner on reactive oxygen and antioxidant enzyme activities in ginger leaves and roots

As shown in Fig. 5, Pb stress led to a continuous increase in ROS levels for CK. The ROS content in ginger leaves and roots was significantly lower in all the treatments than in CK. The generation rate of O^{2-} in CK leaves and roots peaked on October 14th. The generation rate of O^{2-} in T1, T2, and T3 was decreased by 66.25, 39.71, and 32.76%, respectively, in leaves and by 49.16, 30.89, and 41.40%, respectively, in roots, compared with CK. The H_2O_2 content in the leaves and roots of ginger was consistent with the O^{2-} generation rate. The H_2O_2 content in the leaves and roots of CK peaked on October 14th. The H_2O_2 content in the leaves of T1, T2, and T3 was reduced by 22.14, 16.32, and 11.39%, respectively, and that in the roots was reduced by 41.06, 38.51, and 33.46%, respectively, compared with CK (Fig. 5).

In plants, SOD is an important protective enzyme that scavenges ROS. It can effectively remove excess O^{2-} and weaken its peroxidation effect on membrane lipids. Both POD and CAT are oxido-reductase enzymes; POD has high activity and catalyzes the oxidation and decomposition of toxic substances. As shown in Fig. 6, SOD activity in leaves and roots of ginger first increased and then decreased during the experiment, with peak activity on September 6th. At that time, SOD activity in the leaves of T1, T2, and T3 was 10.17, 7.83, and 4.19% higher, respectively, and that in roots was 23.70, 14.48, and 5.18% higher, respectively, than in CK. The SOD activity in leaves and roots decreased over time, but was always higher in the treatments than in CK. The trends in POD and CAT activity were similar to that of SOD activity. The activities of SOD, POD, and CAT in leaves and roots were

Table 4
Effect of soil conditioner on Pb accumulation and distribution rate in various organ of ginger under Pb stress.

	Accumulation (μg/plant)				Distribution rate(%)			
	Root	Rhizome	Stem	Leaf	Root	Rhizome	Stem	Leaf
CK	176.29 ± 4.99a	100.33 ± 0.13a	9.11 ± 0.09a	4.96 ± 0.05a	58.17	32.33	2.94	1.60
T1(Si)	85.23 ± 2.41c	51.74 ± 0.05d	5.34 ± 0.05c	1.34 ± 0.01c	62.10	26.65	2.75	0.69
T2(Se)	107.99 ± 3.05b	64.83 ± 0.07c	6.59 ± 0.07b	1.950.01c	60.09	28.64	2.91	0.86
T3(Mi)	128.75 ± 3.64b	79.28 ± 0.09b	8.26 ± 0.08b	2.26 ± 0.02b	58.68	30.13	3.14	0.86

Note: Under the same column, values followed with the same letter was not significant at $P = 0.05$.

Table 5
Effect of soil conditioner on Pb accumulation and distribution rate in rhizome at different growth stage.

Treatment		Rhizome		
		Mother-ginger	Son-ginger	Grandson-ginger
Pb Accumulation (μg/plant)	CK	46.75 ± 1.33a	39.16 ± 1.12a	13.16 ± 0.38a
	T1(Si)	25.23 ± 0.72d	16.65 ± 0.48d	8.43 ± 0.24c
	T2(Se)	28.67 ± 0.82c	25.73 ± 0.73c	9.02 ± 0.26b
	T3(Mi)	36.54 ± 1.04b	31.46 ± 0.90b	9.88 ± 0.28b
Pb distribution rate (%)	CK	46.60	39.03	13.12
	T1(Si)	48.76	32.18	16.29
	T2(Se)	44.22	39.69	13.91
	T3(Mi)	46.09	39.68	12.46

Note: Under the same column, values followed with the same letter was not significant at $P = 0.05$.

Table 6
Effect of soil conditioner on Pb transfer coefficient and Pb absorption coefficient of ginger.

	PTI	STI	RAI	UAI	AAI	BCF
CK	0.5121 ± 0.005a	0.1402 ± 0.001a	0.3918 ± 0.004a	0.5925 ± 0.006a	0.0281 ± 0.0003a	0.6206 ± 0.006a
T1(Si)	0.3811 ± 0.004c	0.1292 ± 0.001c	0.2715 ± 0.003c	0.375 ± 0.004d	0.0134 ± 0.0001c	0.3884 ± 0.004d
T2(Se)	0.4238b ± 0.004c	0.1317 ± 0.001b	0.3060 ± 0.003bc	0.4356 ± 0.004c	0.0171 ± 0.0002b	0.4527 ± 0.005c
T3(Mi)	0.4574 ± 0.005b	0.1327 ± 0.001b	0.3467 ± 0.003b	0.5052 ± 0.005b	0.0210 ± 0.0002b	0.5263 ± 0.005b

Note: PTI (primary transfer coefficient); STI(secondary transfer coefficient);RAI(root absorption coefficient);UAI(underground absorption coefficient); AAI(above-ground absorption coefficient);BCF(Biological concentration coefficient). Values followed with the same letter was not significant at $P = 0.05$.

higher in T1, T2, and T3 than in CK, indicating enhanced scavenging of excess O^{2-} and antioxidant protection in the treatments (Fig. 6).

On September 6th, the MDA content in leaves was decreased by 38.23, 32.36, and 23.48% in T1, T2, and T3, respectively, compared with CK. Similarly, the MDA content in roots was decreased by 43.00, 36.12, and 26.61% in T1, T2, and T3, respectively, compared with CK. All the soil conditioner treatments resulted in decreased MDA contents, indicating that they inhibited membrane lipid peroxidation to reduce damage caused by excess Pb. The Si treatment had the strongest protective effect (Fig. 6).

3.8. Effect of soil conditioners on mineral element contents in ginger under Pb stress

Excessive Pb stress disrupts the balance of nutrients in plant cells and causes ion toxicity, which ultimately leads to cell leakage and/or the inhibition of absorption of other elements. Table 7 summarizes the contents of various mineral elements in the root, rhizome, stem, and leaf of ginger in the different treatments. The absorption and transfer rates differed among organs. The contents of Ca, Mg, Fe, Mn, Cu, and Zn in organs were lowest in CK, and higher in all the treatments (Table 7).

3.9. Effect of Pb stress on root activity, root length, index of tolerance, and extracted-state of Pb

As shown in Table 8, the root activity of ginger first increased and then decreased during the experiment. The root activity of ginger was higher in T1, T2, and T3 than in CK. At the seedling stage, the root activity of T1, T2, and T3 was 22.90, 17.48, and 9.63% higher, respectively, than that in CK. The root activity peaked at the tillering stage, and was 44.66, 35.57, and 19.38% higher in T1, T2, and T3, respectively, than in CK. The root activity decreased over time. On October 14th, the root activity of CK was only 29.56, and those of T1, T2, and T3 were 127.79, 102.08, and 82.64% higher, respectively (Table 8).

As shown in Fig. 7, the root length of ginger was significantly greater in the treatments than in CK. The tolerance index in the T1, T2, and T3 treatments was always greater than 1, whereas that in CK was less than 1. Under Pb stress, plants in T1, T2, and T3 showed strong Pb resistance, indicating that all the soil conditioners improved root growth to improve Pb tolerance.

The proportions of the different forms of Pb in ginger differed between the treatments and CK. In the three treatments, the main extracted state was hydrochloric acid-extracted Pb, accounting for

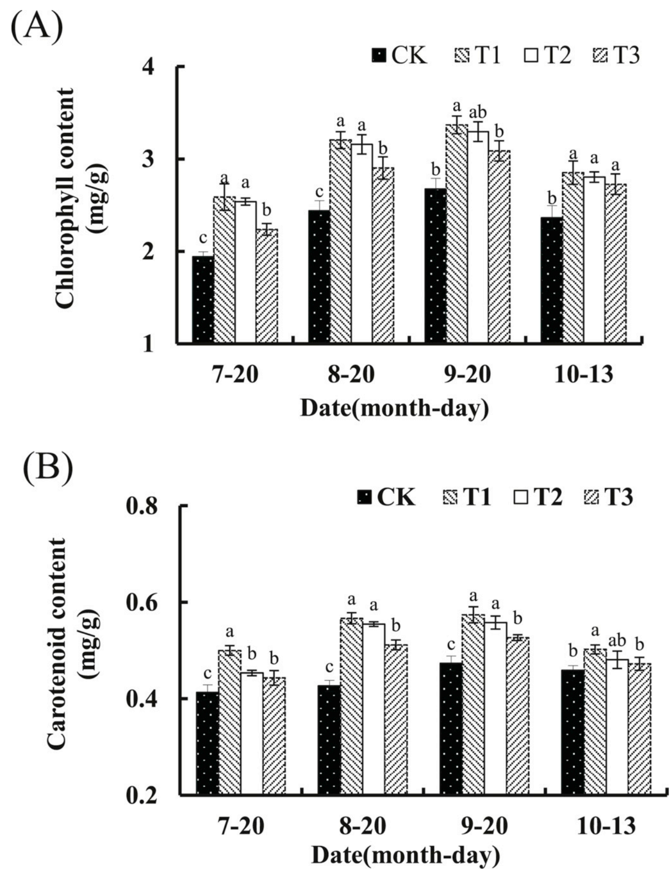

Fig. 4. Effect of soil conditioner on pigment content of ginger leaves at different growth stage under Pb stress. (A)Chlorophyll content of CK,T1,T2,T3 on 7–20,8-20,9–20,10-13. (B) Carotenoid content of CK,T1,T2,T3 on 7–20,8-20,9–20,10-13. Error bars stand for the standard errors. Mean values followed by the same letter are not significantly different at P = 0.05.

65.63%–82.41%, followed by the residual state, and then the ethanol-extracted state.

The proportion of Pb in the root system was significantly higher in the treatments than in CK. Residual-state Pb in CK, T1, T2, and T3 accounted for 12.92, 31.30, 26.35 and 20.65% of total Pb, respectively. The results showed that Pb adsorption and precipitation in the root system were promoted by all the soil conditioners. These conditioners also promoted the conversion of Pb^{2+} into residual-Pb, thus reducing the toxic effect of Pb and improving the growth status of ginger plants (Fig. 7).

4. Discussion

Excess Pb can lead to poisoning and environmental pollution. In plants, Pb negatively affects morphology, growth, and photosynthesis, and suppresses enzyme activities, leading to changes in membrane permeability and mineral nutrition (Guo et al., 2018). This heavy metal has various direct and indirect effects on plant growth and metabolism, accompanied by visible symptoms such as stunted growth, smaller leaves, and inhibited root development (Muramoto, 1989). Previous study showed that Pb blocked primary root growth and decreased branching in maize seedlings (Okant and Kaya, 2019). In banana, Pb toxicity negatively affected the yield of seedlings, and the roots showed heavy metal toxicity symptoms including blackening, stunting, and a brittle texture (Li et al., 2012). In onion, Pb inhibited root growth and blocked DNA synthesis, which restricted the normal progression of the cell cycle (Jiang et al., 2014). In the present study, root growth at the seedling stage of ginger was inhibited by excess Pb (Table 2). In addition, leaf edges gradually curled and yellowed and the leaf thickness decreased. New leaves lost their green color, which could be related to the accumulation of Pb that inhibited chlorophyll synthesis and damaged chloroplast structure and function (Fig. 4).

Excess Pb can also affect the activity of antioxidant enzymes and the ROS scavenging system in plants (Li et al., 2019). The formation of ROS induces a crucial protective mechanism, which can serve as a biomarker of Pb tolerance. Excess Pb can induce oxidative stress by over-

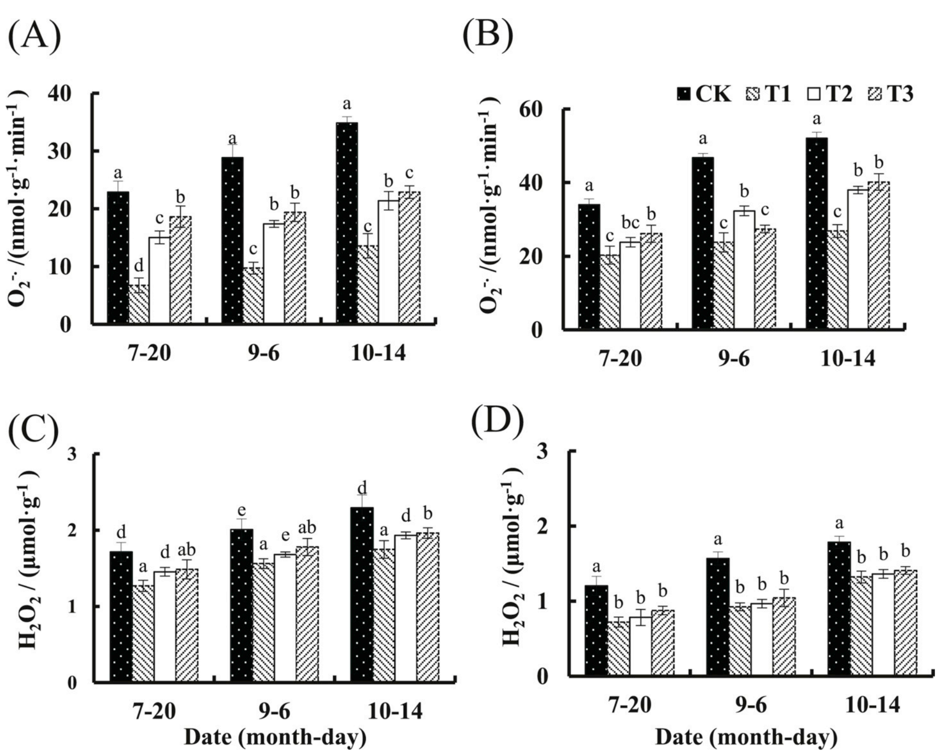

Fig. 5. Effect of soil conditioner on reactive oxygen in ginger leaves and roots. (A) $O^{2-\cdot}$ (Superoxide anion) of ginger leaves in CKT1. T2,T3 on 7–20,9-6,10–14. (B) $O^{2-\cdot}$. (Superoxide anion) of ginger roots in CK, T1. T2,T3 on 7–20,9-6,10–14. (C) H_2O_2 of ginger leaves in CK,T1. T2,T3 on 7–20,9-6,10–14. (D) H_2O_2 of ginger roots in CK,T1. T2,T3 on 7–20,9-6,10–14. Values followed with the same letter was not significant at P = 0.05. Error bars stand for the standard errors.

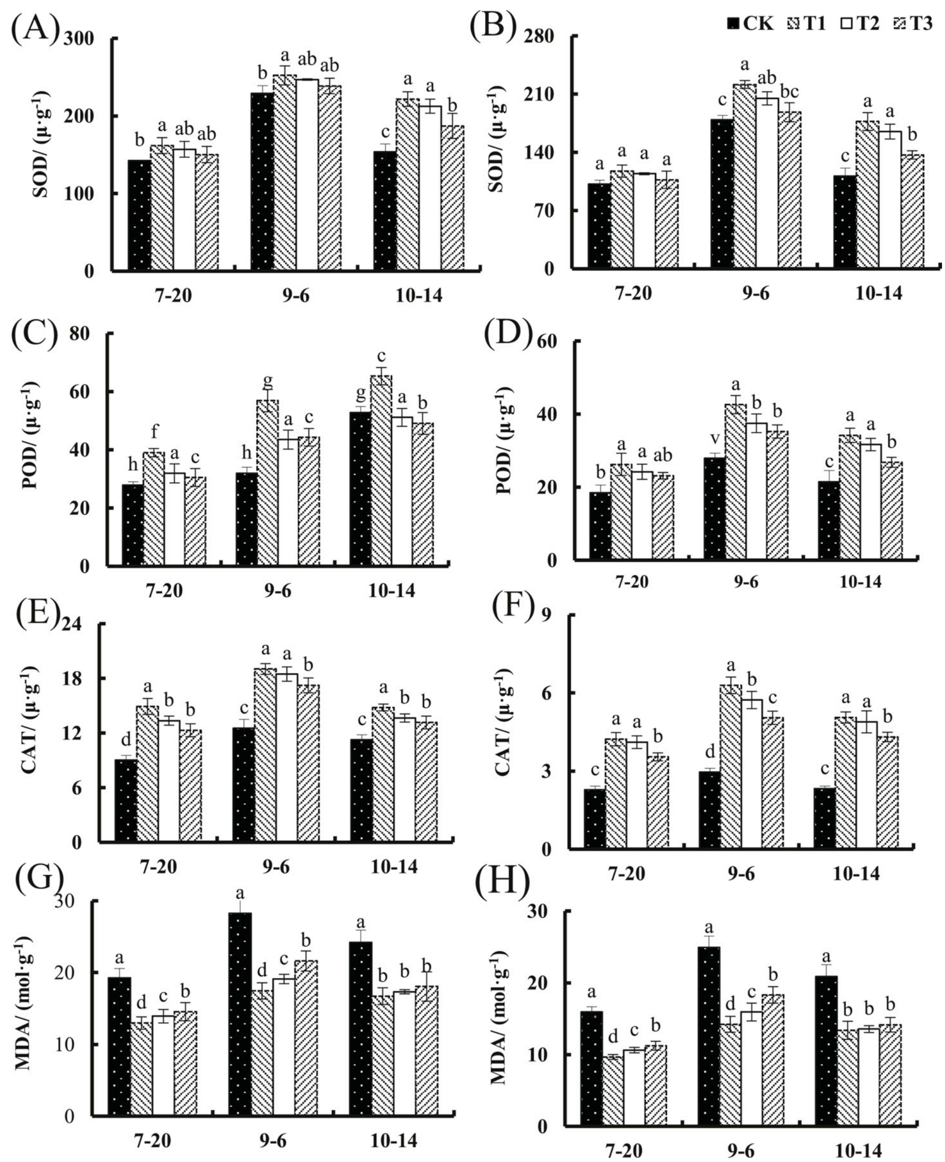

Fig. 6. Effect of soil conditioner on antioxidant enzyme activities in ginger leaves and roots. (A) SOD activity of ginger leaves and ginger roots (B) in CK,T1. T2,T3 on 7–20,9-6,10–14. (C)POD activity of ginger leaves and ginger roots (D) in CK,T1. T2,T3 on 7–20,9-6,10–14. (E) CAT activity of ginger leaves and ginger roots (F) in CK,T1. T2,T3 on 7–20,9-6,10–14. (G) MDA content of ginger leaves and ginger roots (H) in CK,T1. T2,T3 on 7–20,9-6,10–14. Values followed with the same letter was not significant at $P = 0.05$. Error bars stand for the standard errors.

Table 7
Effect of soil conditioner on the content of mineral element in ginger under Pb stress.

Organ	Treatment	Ca (mg·g^{-1})	Mg (mg·g^{-1})	Fe (mg·kg^{-1})	Mn (mg·kg^{-1})	Cu (mg·kg^{-1})	Zn (mg·kg^{-1})
Root	CK	14.28 ± 0.28d	7.14 ± 0.14d	1765.53 ± 34.86c	96.75 ± 1.91 ab	33.16 ± 0.65a	92.37 ± 1.82 ab
	T1	19.58 ± 0.39a	11.32 ± 0.22a	2064.57 ± 40.76a	93.28 ± 1.84b	29.34 ± 0.58b	87.46 ± 1.73b
	T2	17.49 ± 0.35b	10.07 ± 0.20b	1933.48 ± 38.17b	92.66 ± 1.83b	29.95 ± 0.59b	89.53 ± 1.77b
	T3	15.33 ± 0.30c	9.26 ± 0.18c	1910.76 ± 37.72b	102.54 ± 2.02a	31.28 ± 0.62 ab	96.75 ± 1.91a
Stem	CK	5.56 ± 0.11d	3.28 ± 0.06b	186.35 ± 3.68b	71.86 ± 1.42b	6.59 ± 0.13b	96.54 ± 1.91b
	T1	6.37 ± 0.13a	3.95 ± 0.08a	210.24 ± 4.15a	76.33 ± 1.51 ab	7.26 ± 0.14a	104.42 ± 2.06a
	T2	6.04 ± 0.12b	3.66 ± 0.07 ab	197.95 ± 3.91 ab	75.48 ± 1.49b	7.05 ± 0.14 ab	98.70 ± 1.95 ab
	T3	5.78 ± 0.11c	3.59 ± 0.07 ab	203.42 ± 4.02 ab	80.51 ± 1.59a	6.73 ± 0.13 ab	102.35 ± 2.02 ab
Leaves	CK	6.82 ± 0.13d	7.13 ± 0.14d	457.71 ± 9.04c	305.44 ± 6.03b	8.36 ± 0.17d	58.63 ± 1.16c
	T1	7.74 ± 0.15a	8.69 ± 0.17a	482.36 ± 9.52a	315.82 ± 6.24 ab	8.77 ± 0.17b	63.38 ± 1.25b
	T2	7.45 ± 015b	7.86 ± 0.16c	472.11 ± 9.32b	323.49 ± 6.39a	8.45 ± 0.17c	60.92 ± 1.20bc
	T3	7.31 ± 0.14c	8.25 ± 0.16b	466.58 ± 9.21b	317.70 ± 6.27a	9.23 ± 0.18a	66.81 ± 1.32a
Rhizome	CK	1.68 ± 0.03c	3.72 ± 0.07b	249.55 ± 4.93b	165.11 ± 3.26b	7.21 ± 0.14b	30.05 ± 0.59b
	T1	1.94 ± 0.04a	4.25 ± 0.08a	261.08 ± 5.15a	168.83 ± 3.33 ab	7.74 ± 0.15 ab	31.06 ± 0.61 ab
	T2	1.83 ± 0.04 ab	4.22 ± 0.08a	254.56 ± 5.03 ab	177.15 ± 3.50a	7.98 ± 0.16 ab	32.15 ± 0.63 ab
	T3	1.77 ± 0.04bc	3.91 ± 0.08b	255.79 ± 5.05 ab	177.68 ± 3.51a	8.31 ± 0.16a	32.88 ± 0.65a

Note: Values followed with the same letter was not significant at $P = 0.05$.

Table 8
Effect of soil conditioner on root activity of ginger under Pb stress.

	Seedling stage (07–15)	Tillering stage (08–15)	Rhizome expanstage (09–22)	Harvest stage (10–14)
CK	65.84 ± 3.86b	76.31 ± 2.28c	53.62 ± 4.57c	29.57 ± 1.97c
T1(Si)	80.92 ± 5.21a	110.39 ± 4.26a	82.17 ± 3.17a	67.36 ± 2.28a
T2(Se)	77.35 ± 2.19a	103.45 ± 3.98a	74.69 ± 3.67b	59.76 ± 2.69b
T3(Mi)	72.18 ± 3.37b	91.10 ± 5.20b	69.01 ± 3.23b	54.01 ± 3.36b

Note: Values followed with the same letter was not significant at P = 0.05.

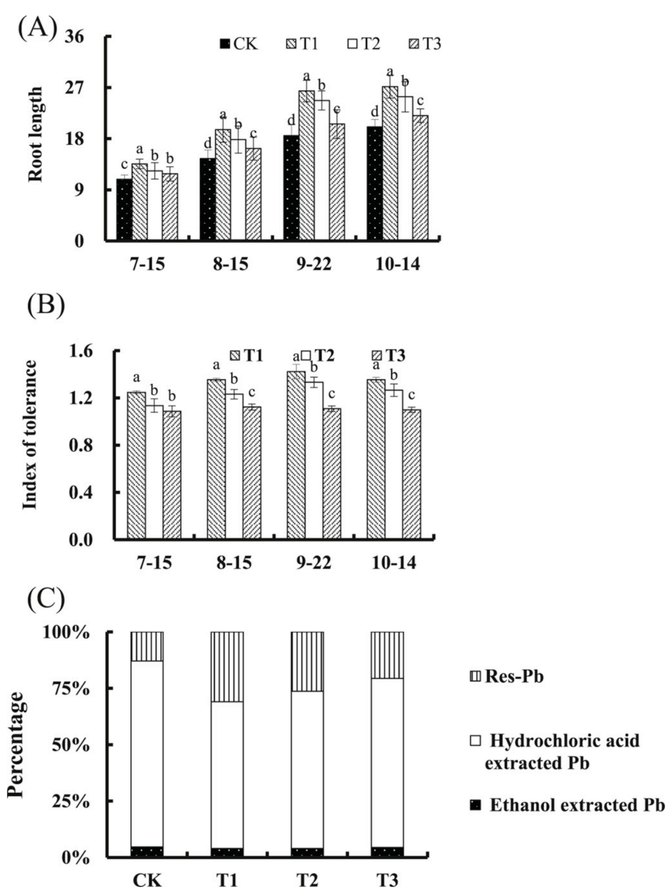

Fig. 7. Effect of soil conditioner on root length and tolerance index of root in ginger under Pb stress. (A) Root length in CK,T1,T2,T3 on 7–15,8-15,9–22,10-14. (B) Indeex of tolerance in CK,T1,T2,T3 on 7–15,8-15,9–22,10-14. (C) Percentage of Res-Pb,Hydrochloric acid extracted Pb, Ethanol extracted Pb. Values followed with the same letter was not significant at *P* = 0.05. Error bars stand for the standard errors.

production of ROS such as H_2O_2, hydroxyl radicals (•OH), and O^{2-} (Reddy et al., 2005). Free radicals and H_2O_2 are known to cause membrane damage, which is correlated with lipid peroxidation. Catalase can decompose H_2O_2 into H_2O and O_2 (Yan et al., 2018). In *Sedum alfredii*, excess Pb induced H_2O_2 accumulation and lipid peroxidation, and caused dramatic changes in antioxidant enzyme activity (Liu et al., 2008). In Pb-stressed *Nymphoides peltatum*, the MDA content and O^{2-} generation rate gradually increased, while the H_2O_2 content and the activity of guaiacol POD increased alternately (Qiao et al., 2013). Various soil conditioners can alleviate the negative effects of heavy metals pollution by regulating antioxidant enzyme activity and ROS production. In *Brassica rapa* seedlings, Cd toxicity was alleviated by Se via its ability to regulate the antioxidant system and ROS production (Filek et al., 2008). In the red seaweed *Gracilaria dura*, Se and spermine alleviated Cd toxicity by regulating the antioxidant system and DNA methylation (Kumar et al., 2012). In the present study, SOD, POD and CAT activity increased after adding Si, Se, and microorganism fertilizer as soil conditioners. All the soil conditioners resulted in reduced accumulation of O^{2-} and H_2O_2, thus helping to maintain the balance of free radical metabolism in ginger and reduce Pb toxicity.

There are various ways to alleviate the effects of heavy metals pollution. In the present study, Si was the most effective soil conditioner, followed by Se, and then the microorganism fertilizer. As an important element for plant growth, Si plays a role in mechanical support and forms an effective physical barrier against heavy metals, thus improving plant tolerance to heavy metals (Adrees et al., 2015; Liang et al., 2015). In a previous study, addition of Si was shown to increase plant height, total root length, leaf number, and leaf area under Pb, Cd, and Zn stresses (Keller et al., 2015). There is an antagonistic effect between Se and heavy metals, which alleviates heavy metals toxicity (Khan et al., 2015). In this study, the use of Se as a soil conditioner alleviated the heavy metals toxicity symptoms of ginger. Thus, Se reduced the toxicity of Pb and increased the Pb resistance of ginger. In rice, Si alleviated low-level Cd toxicity by enhancing light-use efficiency (Lin et al., 2016). Microorganism fertilizer has been shown to promote the production of a variety of low molecular weight organic acids, such as formic acid, acetic acid, malic acid, and citric acid (Yu et al., 2018). These organic acids participate in the process of soil formation, promote mineral dissolution, improve the physical and chemical properties of soil, and promote the nutrient absorption rate. In the rice rhizosphere, the microbial biomass decreased significantly with increasing soil Pb content (Zeng et al., 2006). This may have been due to the effect of Pb on cell metabolism and microbial function, or to a change in microbial genetic diversity caused by Pb stress (Wang et al., 2011). In this study, the addition of microorganism fertilizer allowed ginger to grow normally under Pb stress, possibly because of enhanced microbial metabolic activity that reduced the inhibitory effect of Pb on microbial function. Other substances that alleviate heavy metals pollution include EDTA, which was shown to alleviate the Pb-induced decrease in root growth in *S. alfredii* to a certain extent (Liu et al., 2008).

5. Conclusion

Soil conditioners alleviated the negative effects of Pb stress on ginger plants: Si was the most effective, followed by Se, and then the microorganism fertilizer. The rhizome fresh weight in T1, T2, and T3 was increased by 96.06, 85.81 and 41.58%, respectively, compared with CK. Soluble sugar, soluble protein, and free amino acid ranked from highest to lowest as follows: T3 > T2 > T1 > CK. The Pb contents in organs of ginger under Pb stress were reduced by all treatments, with the largest decrease in T1, followed by T2, and a smaller decrease in T3. The accumulation of Pb in organs was lower in all treatments than in CK. In the rhizome, Pb accumulation was lowest in T1, followed by T2, and then T3. However, the chlorophyll and carotenoid contents in leaves, and root activity, root length, and the tolerance index, were higher in the treatments than in CK. The reactive oxygen species content in ginger leaves and roots was significantly lower in all treatments than in CK. Overall, our results suggest that the three soil conditioners could alleviate the negative effects of Pb stress on ginger plants.

Declaration of interest statement

We declared that the paper has no conflict of interest.

Author contributions

Data curation: Zijing Chen, Jiamin Xu,Yue Xu.
Investigation: Jiamin Xu,Bili Cao.
Resources: Kun Xu.
Software: KaiWang
Writing – original draft: Zijing Chen.
Writing – review & editing: Kun Xu.

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