

EFFECTS OF DIFFERENT EXOGENOUS SELENIUM ON ENZYME ACTIVITIES AND MICROORGANISMS IN ARSENIC- CONTAMINATED SOIL

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Abstract. The environmental behavior of arsenic (As) in soil is closely related to the composition of the soil microbial community and soil enzyme activity. In this paper, the effects of inorganic selenium (Se(IV), Se(VI)) and organic Se (Se-Cys, Se-Met) on enzyme activity and microbial community composition in As-contaminated soil were studied by a pot experiment under greenhouse conditions. The results showed that the application of inorganic Se and organic Se can significantly affect the activities of urease, catalase, invertase, fluorescein diacetate (FDA) hydrolase and dehydrogenase in As-contaminated soil. The total phospholipid fatty acid (PLFA) microbial content in the soil with organic Se treatments was higher than that in the control group (CK), while the inorganic Se treatments was opposite. Organic Se promoted the growth of microorganisms in the soil, while the inorganic Se treatments was opposite. From the perspective of the distribution and community structure of microorganisms, organic Se treatment enriches the microbial diversity and increases the content of gram-positive bacteria (G⁺), gram-negative bacteria (G⁻), and fungi, while the inorganic Se treatment group shows the opposite trend. This study can provide a reference for in-depth exploration of the mechanism of Se and microbial metabolism of As and its application in the treatment of As pollution.

Keywords: *Se, As, Soil enzyme, Microbial community structure*

Introduction

Arsenic (As) pollution in farmland soil poses a serious threat to the sustainable development of modern agriculture and the quality and safety of agricultural products, and it can spread to the human body through the food chain, thereby posing a serious threat to human health. The restoration and safe use of As-contaminated farmland soil has become a key issue that urgently needs to be solved in agricultural and environmental fields around the world (Dong et al., 2020). Soil enzymes are the general term for a class of protein compounds in the soil that can catalyze biologically exclusive organisms. They participate in various biochemical processes in the soil and are an important part of the soil ecosystem. These enzymes play a role in biocatalysis in the soil, promote the metabolism of organic matter, provide nutrients to plants, and play a pivotal role in the soil ecosystem. Soil enzyme activity roughly reflects the relative

intensity of soil biochemical processes (Moscatelli et al., 2018) and can be used as one of the early warning indicators to distinguish the degradation of soil ecosystems under stressful environments (Moscatelli et al., 2018). To date, approximately 60 kinds of enzymes have been identified. Among them, urease, dehydrogenase, fluorescein diacetate (FDA) hydrolase, invertase, phosphatase, etc. are usually used as indicators of the impact of Se and As on soil quality (Bhattacharyya et al., 2008). For example, Wang et al. (2016) simulated the effects of As pollution and long-term As pollution in mining areas on soil enzyme activities and confirmed that soil enzymes were more sensitive and accurate pollution evaluation indicators. Research has shown that enzymatic activity is very effective for understanding the negative effects of heavy metals on soil. By measuring the activity of dehydrogenase and catalase, they were considered indicators of soil overall and respiration activity, and valuable information about soil fertility status can be obtained (Samuel et al., 2012). Lyubun et al. (2013) conducted studies on the effects of As on soil enzyme activities and the bioavailability of As extracted from five field crops. The results showed that plant growth increased the activity of soil dehydrogenase by 2.4 times and 2.5 times, respectively, by 3 times for ryegrass and sudangrass, and 5.2 times by spring oilseed relative to the contaminated but unplanted control soil. The activity of soil peroxidase increased by 33% with the increase in ryegrass and rape, while the activity of soil phosphatase was directly related to residual As. Mondal et al. (2015) carried out the seasonal variation characteristics of soil enzymes (amylase, invertase, cellulase, urease) in As-contaminated areas, and the study of soil enzyme activity was helpful to evaluate the impact of As on soil biochemical quality.

Microorganisms in the soil play an important role in the migration and transformation of As. The quantity, population and activity intensity of soil microorganisms change with changes in soil quality and environment. The use of the number of microorganisms and the composition of the community structure was one of the important ways to identify the quality and fertility of the soil. Soil microbial community composition and soil enzyme activity affect the bioavailability of soil As, which is a research hotspot in soil As pollution control methods. The microorganisms in the soil ecosystem are divided according to their morphological characteristics and are mainly divided into three groups: actinomycetes, fungi and bacteria (Lin et al., 2012). Studies have shown that As is the main driving factor for the reduction of soil functional gene diversity, and rhizosphere bacteria play an important role in the process of centipede grass absorption and hyperaccumulation of soil As (Xiong et al., 2010). As pollution reduces metabolic diversity, there was a strong correlation between the level of As pollution in rhizosphere soil and the distribution of functional genes. It was reported that the metabolic potential and diversity of microorganisms along the depth in As-contaminated soil were significantly reduced, and the community structure was significantly different (Xiong et al., 2012). Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) showed that the bacterial community composition of As-contaminated soil was different from that of control soil. Soil fungi and Proteobacteria showed tolerance to As, while the tolerance of other flora decreased (Lorenz et al., 2006). Turpeinen et al. (2004) suggested that microorganisms can respond to soil metal pollution and maintain metabolic activity by changing the community structure and resistance selection.

Selenium (Se) is one of the essential trace elements for the human body. It has important biological functions and can prevent diseases and improve health. In

agriculture, applying the appropriate amount of Se fertilizer can improve soil quality and increase crop yield and stress resistance (Ramkissoon et al., 2019; Tremblay et al., 2014).

At present, there are few reports on the influence of exogenous Se, especially organic Se, on soil microorganisms. In addition to physical and chemical processes, microorganisms also play an important role in the regulation and transformation of Se and As and can metabolize Se through a variety of methods and pathways (Michael et al., 2020). It was reported that the metabolism of Se by microorganisms mainly includes the transport, reduction, oxidation, assimilation and methylation of Se (Michael et al., 2020). Microorganisms can convert inorganic Se into nano-Se (Lampis et al., 2014), reduce it to selenoprotein through assimilation, and convert it into Se methyl selenide through methylation (Lampis et al., 2014). It was reported that the metabolic mechanism of As by microorganisms was mainly divided into the following three types: As methylation, As reduction and As oxidation (Sodhi et al., 2019). Owing to Se antagonism to As (Feng et al., 2021), so exploring the metabolic mechanism of microorganisms to As is extremely important for the treatment of As pollution due to Se antagonism to As. Research on the regulation of Se on enzyme activity and microbial community composition in As-contaminated soil is lacking. The aim of this study was to carry out a comparative study on the effect of inorganic Se and organic Se on the enzyme activity and microbial community in As-contaminated soil. The findings in this study could provide novel understanding about the microbial regulation of Se on As and could provide a research basis for the treatment of As-contaminated soil.

Materials and methods

Soil cultivation test

The test soil was collected from a vegetable field in the suburbs of Nanchang City in China. A multi-point sampling method was used to collect soil samples from a depth of 0–20 cm. After the samples air dried, they were passed through a 2 mm sieve. The tested soil had the following physical and chemical properties: the pH (soil:water = 1:2.5) was 4.9; the organic matter content was 16.22 g kg⁻¹; the alkaline hydrolysable nitrogen (N) was 137.83 mg kg⁻¹; the available phosphorus (P) was 5.61 mg kg⁻¹; the available potassium (K) was 67.32 mg kg⁻¹; the As content was 11.32 mg kg⁻¹; and the Se content was 0.53 mg kg⁻¹. The soil cultivation experiment was carried out in a greenhouse according to a previously published method (Hu et al., 2020). In short, 30 mg As kg⁻¹ with sodium arsenite was added to each pot in the soil to simulate As-contaminated soil. The pot was 21 cm in diameter and 18 cm in height. The exogenous inorganic Se added to the soil was sodium selenite and sodium selenate, and the exogenous organic Se added was yeast selenium (Se-Y) and malt selenium (Se-M). The detailed test treatment was as follows: (1) 30 mg As kg⁻¹ (control group [CK]); (2) 30 mg As kg⁻¹ + 1 mg Se kg⁻¹ (1Se(IV)); (3) 30 mg As kg⁻¹ + 3 mg Se kg⁻¹ (3 Se(IV)); (4) 30 mg As kg⁻¹ + 6 mg Se kg⁻¹ (6Se(IV)); (5) 30 mg As kg⁻¹ + 12 mg Se kg⁻¹ (12Se(IV)); (6) 30 mg As kg⁻¹ + 24 mg Se kg⁻¹ (24Se(IV)); (7) 30 mg As kg⁻¹ + 1 mg Se kg⁻¹ (1Se(VI)); (8) 30 mg As kg⁻¹ + 3 mg Se kg⁻¹ (3Se(VI)); (9) 30 mg As kg⁻¹ + 6 mg Se kg⁻¹ (6Se(VI)); (10) 30 mg As kg⁻¹ + 12 mg Se kg⁻¹ (12Se(VI)); (11) 30 mg As kg⁻¹ + 24 mg Se kg⁻¹ (24Se(VI)); (12) 30 mg As kg⁻¹ + 1 mg Se kg⁻¹ (1Se-Y); (13) 30 mg As kg⁻¹ + 3 mg Se kg⁻¹ (3Se-Y); (14) 30 mg As kg⁻¹ + 6 mg Se kg⁻¹ (6Se-Y); (15) 30 mg As kg⁻¹ + 12 mg Se kg⁻¹ (12Se-Y); (16) 30 mg As kg⁻¹ + 24 mg Se kg⁻¹ (24Se-Y); (17)

30 mg As kg⁻¹ + 1 mg Se kg⁻¹ (1Se-M); (18) 30 mg As kg⁻¹ + 3 mg Se kg⁻¹ (3Se-M); (19) 30 mg As kg⁻¹ + 6 mg Se kg⁻¹ (6Se-M); (20) 30 mg As kg⁻¹ + 12 mg Se kg⁻¹ (12Se-M) and (21) 30 mg As kg⁻¹ + 24 mg Se kg⁻¹ (24Se-M). Each treatment was replicated three times and the treatments were arranged in random blocks. The indoor temperature was set to 23 °C during the day and 18 °C at night. The soil moisture content was maintained at 60–80% of the maximum value. After 50 days of equilibration, 100 g of topsoil in the pot was taken for experimental determination. Among them, 50 g of fresh soil was used for soil enzyme analysis, and the other 50 g of soil was freeze-dried for determination of soil PLFA. sodium selenite, sodium selenate, sodium arsenite were analytically pure from Sinopharm Reagent Company (China). Se-Y (containing 2,000 mg kg⁻¹ Se) and Se-M (containing 1,600 mg Se kg⁻¹), which have been analyzed and verified, were purchased from Nanchang Industrial Biotechnology Co., LTD in Nanchang, China.

Determination of soil enzymes

The activities of urease, catalase, sucrase, FDA hydrolase and dehydrogenase were measured according to the procedures presented in Zhang et al. (2015b). The soil urease activity was determined by the phenol active sodium-sodium hypochlorite colorimetric method, while sucrase activity was determined by the 3,5-dinitrosalicylic acid colorimetric method. The urease and sucrase activities were expressed as mg g⁻¹ d⁻¹ and mg glucose g⁻¹ d⁻¹, respectively. Catalase (ml g⁻¹ h⁻¹) activity was determined by potassium permanganate titration, while FDA hydrolase (μg g⁻¹ h⁻¹) activity was determined by fluorescein colorimetry. The dehydrogenase (μg g⁻¹ h⁻¹) activity was measured by the TTC colorimetric method.

Determination of phospholipid fatty acids

The determination and calculation of phospholipid fatty acid (PLFA) in soil were completely determined by the method described by Shen et al. (2019). In brief, after phospholipid extraction, SPE extraction, fatty acid separation, methylation and cleaning, the samples were identified by gas chromatography (7890A, Agilent Technologies, USA) fitted with a MIDI Sherlocks Microbial Identification System (Version 4.5, MIDI, USA). The different PLFA microbial components are shown below. The sum of 16:0 10-methyl, 17:0 10-methyl and 18:0 10-methyl represented the actinomycetes, and the sum of 18:2 w6c, 16:1 w5c, 18:3 w6c and 20:4 w6c was identified as the fungus. The sum of 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso and 17:0 anteiso were identified as gram-positive bacteria, and the sum of 16:1 w7c, 17:0 cyclo w7c, 18:1 w7c and 19:0 cyclo w7c represented gram-negative bacteria.

Statistical analysis

All data are expressed as the mean and standard deviation (SD) with three repetitions, and diagrams were made using Origin 9.1 (OriginLab, USA). Statistical analysis and correlation analysis were carried out using SPSS 19.0 (IBM, USA). Two-sided p values < 0.05 were considered statistically significant. A normal distribution test and a homogeneity of variance test were performed prior to one-way analysis of variance (ANOVA), which was used to assess the variability of the data sets and validity of the results. The Shapiro–Wilks method was used for the normal distribution test.

Results and discussion

Activity characteristics of soil enzymes

Urease is an amidase that promotes the hydrolysis of enzymatic bonds in organic molecules (Javadi et al., 2018) and is closely related to the action of organic matter and microorganisms in the soil. The activity of urease in the soil is shown in *Figure 1A*. Compared with the CK, the soil enzyme activity of the Se(IV) and Se(VI) treatment groups both showed a slight increase and then a decrease. The application of the two kinds of organic Se significantly activated urease activity, the overall trend was increasing, and the urease activity of each Se application level was higher than that of the corresponding CK and inorganic Se treatment groups. At the levels of 3Se, 6Se and 12Se, the urease activity of the Se-M group was higher than that of the Se-Y group, while at the 24Se level, the urease activity of the Se-Y group was higher than that of the Se-M group, reaching the highest value among all treatment groups. Catalase can promote the decomposition of excessive hydrogen peroxide in organisms, thereby preventing damage and poisoning. Its activity is related to the content of soil organic matter and the number of microorganisms (Xiong et al., 2013).

In this study, the inorganic Se treatment group showed a significant inhibitory effect on catalase, and each Se level group was lower than the CK ($P < 0.05$) (*Figure 1B*). The catalase activity of the Se-Y and Se-M treatment groups at the 24Se level was higher than that of the CK group, and other Se levels were lower than that of the CK group ($P < 0.05$). With the increase in the Se level, the catalase activity of the inorganic Se group and the organic Se group showed a trend of first decreasing and then increasing. Sucrase is called invertase because its enzymatic substrate is sucrose, which can characterize soil fertility and microbial activity (Zhang et al., 2015a). The sucrase activity of each treatment group was not the same (*Figure 1C*). Both the Se(IV) and Se(VI) treatment groups showed a tendency to increase first and then decrease, while the Se-Y and Se-M treatment groups both showed a gradually increasing trend. This may be due to the complex biochemical interaction with As and other substances in the soil under the cumulative effect of this Se level, causing the sudden activation of enzyme activity. It has been reported that the complex biochemical interaction from was caused by complexing agents, which can act as carriers for trace elements in soil solution (Violante et al., 2010). So, the toxicity of metals can be reduced by complexation. The interaction between as and se is a key factor to understand their transport, environmental fate and related toxicological effects in soil plant systems (Ali et al., 2021). As and Se induce cytotoxicity and genotoxicity through the generation of reactive oxygen species (ROS). In this study, the four Se sources have different chemical compositions and have their own chemical properties, they produce different results.

Soil FDA hydrolase can be hydrolyzed by soil microorganisms, such as fungi, bacteria, algae, etc., resulting in changes in enzyme activity (Tao et al., 2021). As shown in *Figure 1D*, the FDA hydrolase activity of the Se(IV) and Se(VI) (except for the 24Se level) treatment groups was lower than that of the CK. The enzyme activities of the Se-Y and Se-M groups at high Se levels (6Se, 12Se and 24Se) were significantly higher than those of the CK group ($P < 0.05$) and showed a trend of gradual increase. It was reported in the literature that As-contaminated soil can reduce the activity of FDA hydrolase (Ghosh et al., 2004). In this study, after applying exogenous Se to As-contaminated soil, it interacted with As and aroused changes in soil microorganisms, increasing the activity of FDA hydrolase.

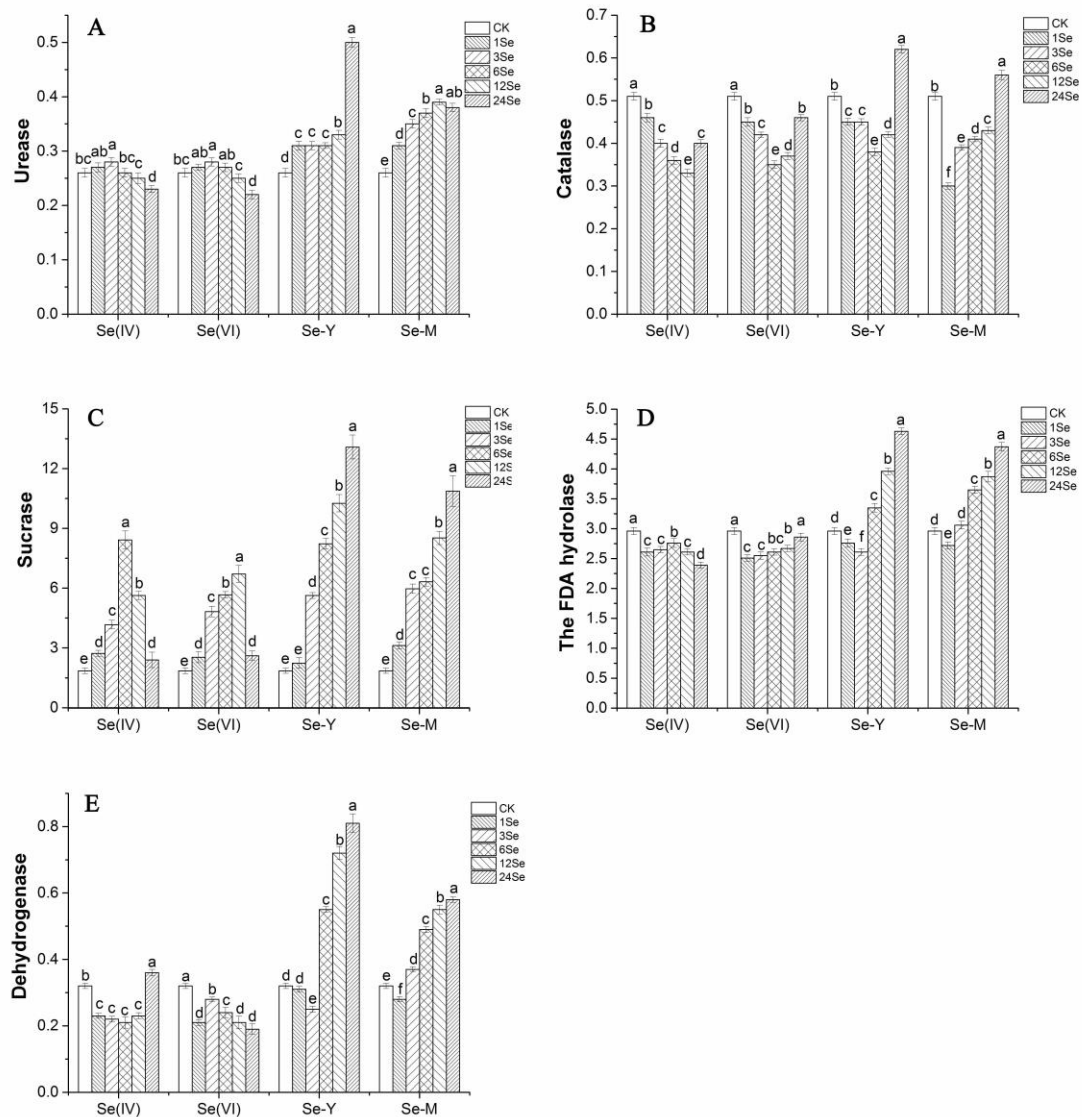


Figure 1. Enzyme activity in soil after adding different Se. (A) urease ($\text{mg g}^{-1} \text{d}^{-1}$). (B) catalase ($\text{ml g}^{-1} \text{h}^{-1}$), (C) sucrase ($\text{mg g}^{-1} \text{d}^{-1}$). (D) the FDA hydrolase ($\mu\text{g g}^{-1} \text{h}^{-1}$). (E) dehydrogenase ($\mu\text{g g}^{-1} \text{h}^{-1}$). All data are expressed as mean \pm SD ($n = 3$); the letters in each column indicate the significant differences at a level of 0.05

Dehydrogenase is an enzyme that can promote the oxidation–reduction reaction of organic substances. It belongs to the oxidoreductase system and can reflect the metabolism of soil microorganisms. As shown in *Figure 1E*, the 24Se level of soil dehydrogenase after applying Se(IV) was significantly higher than that of the CK ($P < 0.05$), and other Se levels were significantly lower than the CK ($P < 0.05$). After applying Se(VI), the dehydrogenase activity of each treatment level was significantly lower than that of the CK ($P < 0.05$). When the 3Se level reached a maximum value, it showed a trend of increasing first and then decreasing. The dehydrogenase activity of the Se-Y group showed a trend of first decreasing and then increasing, while the Se-M group showed a gradually increasing trend. The two organic Se treatment groups had significantly higher high Se levels than the CK group ($P < 0.05$).

Studies have shown that the dehydrogenase activity of soil polluted by As-containing tailings was related to total As and total water-soluble As (As(III) + As(V)), which could be used to evaluate the effect of tailings dispersion on the influence of soil microbial oxidation ability (Fernández et al., 2005). The results of this study showed that, compared with the CK, the effects of organic or inorganic Se treatment on dehydrogenase were the opposite. Inorganic Se treatments had a certain inhibitory effect on soil dehydrogenase, while organic Se treatment had a certain stimulating effect on soil dehydrogenase. This may be due to the different properties of inorganic Se and organic Se, which has been reported that the bioavailability of Se in soil was determined by the form of Se and soil organic components (Li, et al., 2017). So Se has different effects on As and microbes in soil, leading to different enzyme responses. Under the action of organic Se, soil microorganisms may enhance their metabolic activities, resulting in an increase in soil microbial biomass and enhancing the repair of biofilms. This in turn causes changes in enzyme activity. The activity of various soil enzymes is an important manifestation of the biological properties of soil. There have been many studies on the effect of exogenous Se addition on the activity of soil enzymes. For example, Wu et al. (2010) and Fan et al. (2015) studied the effects of exogenous inorganic Se on soil enzyme activities. Shi et al. (2018) studied the dynamic response of soil enzymes to exogenous organic Se and inorganic Se. The effect of Se on soil enzyme activity varies according to the type of enzyme. For example, Yang et al. (2010) found that soil catalase and urease were more sensitive to Se, while amylase was not sensitive to Se. Similarly, Yang et al. (2017) studied the effects of Se on the soil urease, invertase and acid phosphatase activities of different varieties of tea plants. However, when soil Se was contaminated, it had a certain inhibitory effect on the activities of soil catalase and urease. Since urease was most inhibited by Se and there was a significant correlation between its inhibition rate and soil Se content, the urease inhibition rate can often be used as a biological indicator of Se ecological risk assessment (Lin et al., 2005). Studies have shown that low concentrations of Se have varying degrees of activation effects on the activities of soil catalase, dehydrogenase, urease and alkaline phosphatase, while high concentrations of Se have a certain inhibitory effect on the activities of four soil enzymes (Wu et al., 2010). Inorganic Se of different valences has different responses to soil enzyme activities. Wu et al. (2010) found that there was a significant negative correlation between the concentration of exogenous Se(VI) and Se(IV) and soil urease activity ($P < 0.01$). Through correlation analysis and stepwise regression analysis, it was found that Se(VI) and Se(IV) have inhibitory effects on soil invertase activity and urease activity, and the two valences of Se applied to the soil were mainly manifested in water-soluble Se on soil enzyme activity. In addition, compared with inorganic Se, organic Se was more conducive to the growth of soil microorganisms, improved soil enzyme activity and promoted the circulation of N, P and C nutrients in the soil ecosystem (Shi et al., 2018). This was consistent with our study results. In our study, compared with Se(IV) and Se(VI), both Se-Y and Se-M increased the activities of urease and dehydrogenase to varying degrees. The role of Se in plant antioxidant stress showed that Se induces a mechanism to protect photosynthesis from damage by slightly changing the sensitivity of photosynthetic cell membranes. Generally, the antioxidant effect of Se is related to an increase in glutathione peroxidase (GSH-Px) activity (Pilarczyk et al., 2001), thereby increasing the scavenging ability of hydrogen peroxide and improving the ability of plants to resist stress. In addition, reports

indicated that added exogenous Se can promote the maintenance of antioxidant capacity by inducing more glutathione and nonprotein thiols (Srivastava et al., 2009). As an inducer, low concentrations of Se can upregulate defense and stress resistance genes and downregulate related growth genes. Exogenous pollutants in the soil, such as heavy metals, As and Se, may have different mechanisms for soil enzymes: binding with enzyme-substrate complexes; binding with enzyme active centres; and chemical reactions with substrates (Karaca et al., 2010).

Community structure characteristics of soil microorganisms

Phospholipid fatty acid (PLFA) composition monomer type statistics

As shown in *Table 1*, a total of 33 PLFA monomers were detected in the control soil. After the addition of Se(IV), the number of PLFA monomers detected in the soil at each Se application level was lower than that of the CK, showing a decreasing trend. Twenty-eight and 27 types of PLFAs were detected in the soil with 12Se and 24Se levels, respectively, which indicates that the addition of sulphite can inhibit the types of microorganisms in the soil. After the application of Se(VI), the number of monomers detected in the low-Se level soil was lower than that of the CK. In the 24Se high Se level group, 50 PLFA monomers were detected, which increased sharply. It can be seen from the data that the number of microorganisms has an increasing trend.

After the application of Se-Y, the number of monomers in the Se treatments was higher than that in the CK. Compared with the Se(VI) group, except for the 24Se level, the number of monomers at the other Se levels was higher than that in the Se(VI) group. After adding Se-M, the number of PLFA monomers detected in the soil was significantly higher than that of the CK. Under the 6Se level, a total of 51 monomers were detected, reaching the highest level of all treatments. From the above analysis, it is evident that the application of organic Se is more beneficial to increase the types of PLFAs in the soil than the application of inorganic Se. Se-M was significantly richer than the PLFA species in the Se-Y and inorganic Se groups.

Total PLFA was the sum of the individual numbers of various microorganisms ($n = 3$). By analyzing the PLFAs that have been detected in the soil, we found that gram-positive bacteria (G^+) and gram-negative bacteria (G^-) were the most distributed PLFAs in the soil. The sum of the two accounts for more than 63% of the total phospholipid fat, and the species were more abundant than other types. One species of arbuscular mycorrhizal fungus (16:1 w5c) and another species (18:2 w6c) were detected in all soils. Arbuscular mycorrhizal fungi can form a mutually beneficial symbiosis with plants, help plants resist adverse stress and have important ecological research significance. This probably suggested that when Se and As were added to the soil, they affected the composition and structure of microorganisms in the soil. The addition of different forms of Se to As-contaminated soil resulted in different numbers and communities of microorganisms in the soil.

Total PLFA content and microbial community distribution characteristics

PLFAs have the exclusive specificity of microorganisms and can be used as characteristic fatty acids of microorganisms. Therefore, PLFAs can be used to characterize soil microbial biomass and its diversity. The soil microorganisms identified in the soil in this study were divided into G^+ , G^- , actinomycetes, fungi and anaerobic microorganisms. Among them, G^+ , G^- , actinomycetes and fungi were detected in

different Se-treated soils and control soils. According to reference reports (Shen et al., 2019; Liu et al., 2016) experimental results in this article, the following PLFAs were selected as specific microbial markers. Among them, there were three kinds of actinomycetes, six kinds of G^+ , four kinds of G^- , four kinds of fungi and a total of 17 kinds of PLFA monomers.

Table 1. The number of PLFA species detected in the soil after adding different exogenous Se

Treatment	Se levels	Actinomycetes	G^+	G^-	Fungus	Anaerophyte	Total PLFA
CK	0Se	5	11	10	7	0	33
Se(IV)	1Se	5	10	8	4	0	27
	3Se	5	12	9	6	0	32
	6Se	5	10	8	4	0	27
	12Se	5	11	8	4	0	28
	24Se	5	10	8	4	0	27
Se(VI)	1Se	5	10	9	4	0	28
	3Se	5	9	8	4	0	26
	6Se	5	10	10	5	0	30
	12Se	4	12	9	4	0	29
	24Se	7	17	16	7	3	50
Se-Y	1Se	5	12	9	6	0	32
	3Se	5	12	11	7	0	35
	6Se	6	11	10	4	2	33
	12Se	5	12	11	9	0	37
	24Se	6	14	10	10	1	41
Se-M	1Se	6	14	11	8	2	41
	3Se	5	12	14	6	1	38
	6Se	5	15	20	10	1	51
	12Se	5	15	12	9	3	44
	24Se	6	15	17	8	2	48

As shown in *Figure 2*, the total PLFA content in the control soil was 28.82 nmol g⁻¹. In the Se(IV) treatments, the total PLFA content in the soil was lower than that in the CK. In the 6Se, 12Se and 24Se treatments, the PLFA contents were 19.88, 16.17 and 13.09 nmol g⁻¹, respectively, showing a significant decrease. The PLFA content of the Se treatment group showed a gradual increase in Se(VI) treatments, but they were all lower than the CK. When the Se concentration was 24 mg kg⁻¹, the PLFA microbial content rose to 25.77 nmol g⁻¹. The PLFA content in the soil showed a gradually increasing trend in the Se-Y treatments. It was lower than the CK in the 1Se, 3Se and 6Se treatments, and higher than the CK in the 12Se and 24Se treatments. The PLFA contents were 39.31 and 48.32 nmol g⁻¹, respectively. The PLFA content showed a gradual increase in the Se-M treatment. The PLFA contents in the 1Se and 3Se treatments were 19.18 and 26.84 nmol g⁻¹, respectively, which were lower than that in the CK. The levels of PLFAs at the 6Se, 12Se and 24Se levels were higher than those of the CK, and their contents were 29.46, 34.44 and 40.19 nmol g⁻¹, respectively. It can be

seen from the above results that the addition of inorganic Se sources reduces the total PLFA microbial content in the soil, and the addition of organic Se sources increases the total PLFA microbial content in the soil.

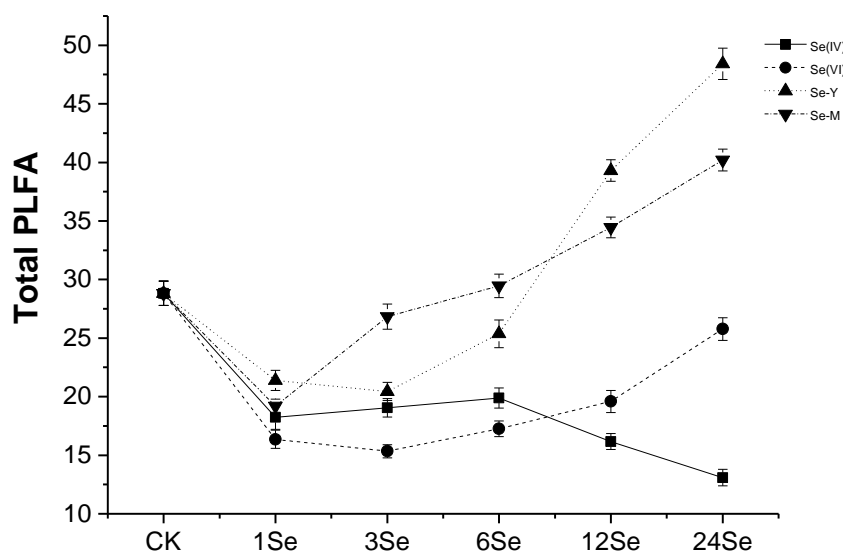


Figure 2. Total PLFAs content in soil after adding different Se sources (nmol g^{-1}). All data are expressed as mean \pm SD, $n = 3$); variance bars represent significant differences ($p < 0.05$)

As shown in *Figure 3*, the G^+ content of the soil in the CK was 5.95 nmol g^{-1} . In the Se(IV) and Se(VI) treatments, the G^+ content in the soil was lower than that of the CK. The addition of inorganic Se sources reduces the G^+ content in the soil. With the increase in the concentration of Se(IV), the G^+ content of each Se treatment showed a decreasing trend, while the Se(VI) group showed an increasing trend. In the Se-Y and Se-M treatments, the G^+ content in the soil showed an increasing trend as the Se level increased. In the low-Se group, the G^+ content was lower than that in the CK group, and in the high-Se group, the G^+ content was higher than that in the control. In the 24Se treatment, the soil G^+ after adding Se-Y and Se-M was 8.56 and 7.80 nmol g^{-1} , respectively. This shows that the application of organic Se increases the number and communities of microorganisms that produce resistance to As stress in the soil, and the ecosystem is readjusted to adapt to the environment and gain stress resistance. The G^- content of the soil in the CK was 6.76 nmol g^{-1} . After the addition of inorganic Se, its value was lower than that of the CK, and its size and G^+ content were consistent. In the Se(IV) treatment group, the Se level gradually decreased with the addition of Se, while in the Se(VI) group, the opposite was true. In the low Se group (1Se, 3Se, 6Se), the Se(IV) group was higher than the Se(VI) group, and in the high Se group (12Se, 24Se), the opposite was true.

The soil G^- content in the organic Se group showed inconsistent results with that in the inorganic Se group. In the Se-Y and Se-M groups, the soil G^- level was lower than the CK at low Se application and higher than the CK at high Se application, and both showed an increasing trend with the increase of Se level. Under the Se levels of 1Se, 3Se, 6Se, 12Se and 24Se, the soil G^- contents of the Se-Y group and the Se-M group were 3.48 , 4.18 , 5.58 , 10.84 and $12.62 \text{ nmol g}^{-1}$ and 3.49 , 5.93 and 6.99 , respectively, and 8.51 and 8.95

nmol g^{-1} . It can be seen from the above that the G^- content in Se-Y at the low Se level is lower than that in Se-M, while at the high Se level, the opposite was true.

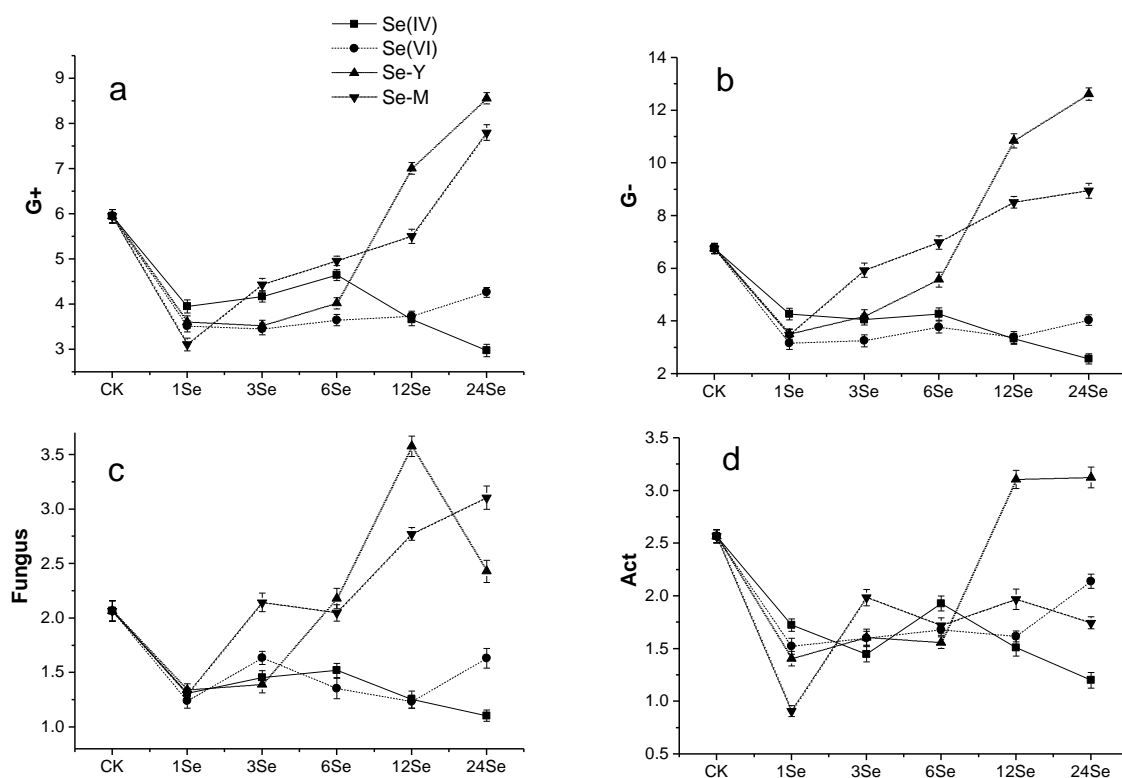


Figure 3. The contents of different microbial communities in soil after adding different Se sources (nmol g^{-1}). All data are expressed as mean \pm SD, $n = 3$; variance bars represent significant differences ($p < 0.05$)

The fungal content in the soil of the CK was 2.07 nmol g^{-1} . The content of fungi in the soil in the inorganic Se group was lower than that in the CK group. In the organic Se group, the content of fungi with high Se levels was higher than that in the CK. As the organic Se level increased, the microbial content in the soil increased.

The content of actinomycetes in the soil of the CK was 2.57 nmol g^{-1} . After applying inorganic Se and Se-M, the content of soil actinomycetes at each Se level was lower than that of the CK. The addition of Se reduces the content of actinomycetes in the soil, and the response of actinomycetes to Se is more obvious. After applying Se-Y, the level of Se applied at 1, 3, 6Se was significantly lower than that of the CK, and the level of Se applied at 12 and 24Se was significantly higher than that of the CK. The contents of actinomycetes were 3.11 and 3.12 nmol g^{-1} , respectively.

The G^+/G^- value of the soil in the CK was 0.880, and the G^+/G^- value of the inorganic Se treatment group was higher than that of the CK. As shown in *Figure 4*, the F/B value in the CK was 0.163. The average F/B values of the Se(IV), Se(VI), Se-Y and Se-M treatment groups were 0.177, 0.197, 0.183 and 0.192, respectively, which were higher than those of the CK.

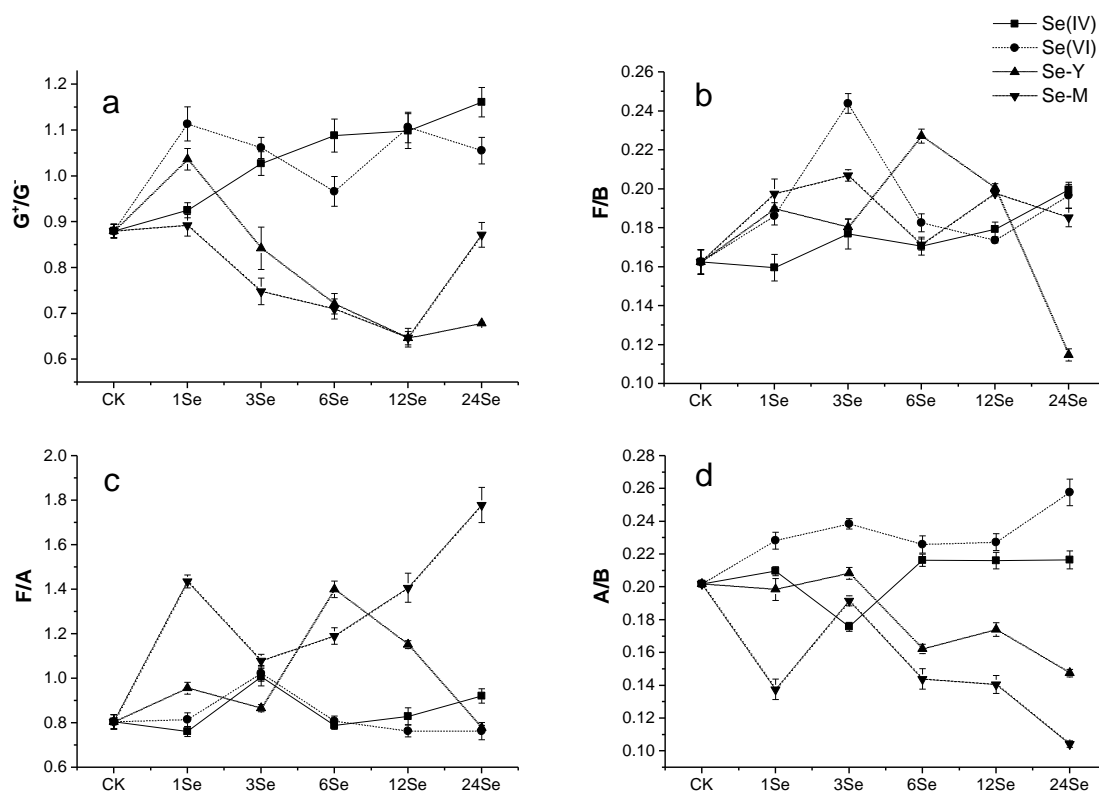


Figure 4. The ratio of F/B, G^+/G^- , A/B, F/A in soil after adding different Se sources. All data are expressed as mean \pm SD, $n = 3$; variance bars represent significant differences ($p < 0.05$)

The addition of exogenous Se will increase the F/B value in the soil. With increasing Se application level, the F/B value of the Se(IV) treatment showed a gradually increasing trend, and the F/B value of the other three Se treatments showed a trend of first increasing and then decreasing. The F/A value in the CK was 0.805, and the average F/A values of the Se(IV), Se(VI), Se-Y and Se-M treatment groups were 0.861, 0.834, 1.030 and 1.379, respectively, all of which were higher than that of CK. This indicates that the application of exogenous Se can increase the F/A value of the soil.

The A/B value in the CK was 0.202, and the average A/B values of the Se(IV), Se(VI), Se-Y and Se-M treatment groups were 0.207, 0.236, 0.178 and 0.144, respectively. The A/B value of the inorganic Se group was greater than that of the CK, while the A/B value of the organic Se group was smaller than that of the CK.

Soil microorganisms are involved in various biochemical processes in the soil and have a positive effect on the conversion of soil organic matter and nutrients and the formation of soil fertility. The quantity distribution and structural characteristics of soil microorganisms are not only related to the ecological conditions of the soil but also affected by exogenous soil pollutants. This may be due to heavy metal pollutants or exogenous substances (such as As and Se) entering the soil and plants; they will participate in related biochemical actions with microorganisms, causing changes in soil enzyme activity (Wang et al., 2020), changing soil microbial biomass and communities (Turpeinen et al., 2014) and stimulating changes in antioxidant enzymes in the plant, thereby changing the environmental quality of the soil and the growth and yield of crops.

Other studies have shown that as the level of Se application increases (1-30 mg kg⁻¹), the number of bacteria and fungi and actinomycetes in the soil shows a trend of first increasing and then decreasing (Fan et al., 2015). However, under different Se treatment conditions, the levels of the maximum number of various microorganisms are not the same (Fan et al., 2015). Research has suggested that the application of an appropriate concentration (5-10 mg kg⁻¹) of inorganic Se fertilizer can promote an increase in soil bacteria and fungi and actinomycetes. High concentrations (30 mg kg⁻¹ or higher) of Se reduce the number of bacteria and fungi and actinomycetes in the soil (Fan et al., 2015). It was reported that microorganisms can change the form of As in the soil and that bacteria in the soil can promote the conversion of As(V) to As(III) (Jomova et al., 2011). Inorganic As may be methylated into less toxic organic forms of As, such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Jomova et al., 2011). Zhang et al. (2018) used *Trichoderma aculeatus* (SM-12F1) and ferrihydrite to repair As-contaminated soil. The results showed that compared with the CK, the total PLFA, G⁺, G⁻, actinomycetes, bacteria and fungi PLFAs in the repaired soil increased by 114%, 68%, 276%, 292%, 133% and 626%, respectively. Sun et al. (2015) studied the distribution characteristics of microbial diversity in As-contaminated soil in a realgar mining area, and the results showed that the content of each fungus was in the order of bacteria > fungi > actinomycetes. Among them, bacteria accounted for 71.54%-80.66% of the total microorganisms. In our study, the changes in the number of microorganisms under different Se treatments were different. Organic Se can increase G⁺ and G⁻ in the soil, while inorganic Se can reduce G⁺ and G⁻ in the soil.

Correlation analysis

There was a significant positive correlation between soil enzyme activity and most microorganisms, which indicates that the two have good consistency in response to exogenous Se (Tables 2, 3, 4 and 5). In the inorganic Se and organic Se treatment groups, FDA hydrolase was also significantly positively correlated with G⁻ and G⁺, which was consistent with the results of other studies (Ma et al., 2010). The above results show that the application of Se to As-contaminated soil significantly affects the activity of soil enzymes and the changes in the structure of the microbial community. It was reported in the literature (Pal et al., 2009) that urease activity was significantly related to the level of As pollution in the soil, and the number of microorganisms and soil enzymes can reflect the characteristic level of soil As pollution.

Through the biochemical action of microorganisms on Se-containing soil, the absorption of Se by plants can be strengthened, which is conducive to the production of Se-rich food (Paulraj and Kumar, 2016). Fan et al. (2015) studied the relationship between the application amount of exogenous Se in different valences and the soil enzyme activity and the number of microorganisms in tobacco-growing soil and found that the amount of Se(VI) and the soil invertase activity, urease activity, catalase activity and fungi. They found that there was a negative correlation between the number and the number of actinomycetes and a positive correlation with the soil neutral phosphatase activity and the number of bacteria. The amount of Se(IV) was negatively correlated with soil invertase activity, urease activity, neutral phosphatase activity and the number of bacteria and fungi and positively correlated with soil catalase activity and the number of actinomycetes. The regression analysis between soil enzyme activity and the number of soil microorganisms also showed that the number of soil microorganisms

was negatively correlated with soil neutral phosphatase activity, urease activity and catalase activity but had no significant effect on soil invertase activity (Fan et al., 2015).

Table 2. Correlation among soil As, soil enzymes and microorganisms after selenite addition (n = 15)

	Urease	Sucrase	Catalase	Dehydrogenase	The FDA hydrolase	Actinomyces	G ⁺	G ⁻	Fungus
Urease	1								
Sucrase	.173	1							
Catalase	.341	-.662**	1						
Dehydrogenase	-.703**	-.570*	.168	1					
The FDA hydrolase	.744**	.761**	-.163	-.804**	1				
Actinomyces	.504*	.678**	-.014	-.731**	.830**	1			
G ⁺	.720**	.713**	-.085	-.849**	.911**	.847**	1		
G ⁻	.858**	.414	.254	-.829**	.831**	.824**	.915**	1	
Fungus	.754**	.670**	-.069	-.760**	.886**	.710**	.951**	.862**	1

**Significant correlation at the 0.01 level. *Significant correlation at the 0.05 level

Table 3. Correlation among soil As, soil enzymes and microorganisms after selenate addition (n = 15)

	Urease	Sucrase	Catalase	Dehydrogenase	The FDA hydrolase	Actinomyces	G ⁺	G ⁻	Fungus
Urease	1								
Sucrase	.304	1							
Catalase	-.296	-.884**	1						
Dehydrogenase	.804**	.383	-.248	1					
The FDA hydrolase	-.763**	-.105	.224	-.417	1				
Actinomyces	-.769**	-.358	.390	-.398	.934**	1			
G ⁺	-.834**	-.270	.325	-.563*	.919**	.914**	1		
G ⁻	-.548*	-.092	.025	-.291	.733**	.780**	.831**	1	
Fungus	-.220	-.295	.427	.282	.370	.561*	.418	.489*	1

**Significant correlation at the 0.01 level. *Significant correlation at the 0.05 level

In this study, there was a significant positive correlation between soil microorganisms and urease activity in the Se(IV) treatments (Table 2). In the Se(VI) treatments, soil microorganisms were also negatively correlated with urease activity and positively correlated with catalase (Table 3). After adding two kinds of organic Se, there was a significant positive correlation between soil microorganisms and most enzymes. Many studies have analyzed and summarized the effects of heavy metals on the community structure of soil microorganisms, the physiological and biochemical effects of soil microorganisms, different levels of heavy metal pollution, heavy metal compound pollution and the combined effects of soil physical and chemical properties and heavy metals on soil enzyme activities (Subrahmanyam et al., 2016; Xian et al., 2015). However, there are few studies on the relationship between soil enzyme activity and microorganisms at present, and the mechanism or mechanisms of its action are not very clear. The addition amount of the four kinds of exogenous Se showed a good curve fitting relationship with the soil PLFA microbial biomass (Figure 5), and the correlation coefficient was above 0.95. As the amount of Se in the Se(IV) treatment group

increased, the microbial biomass in the soil showed a trend of first increasing and then decreasing, while the other three Se sources all showed a gradual increase in microbial biomass with the increase in the Se application rate. This shows that compared with other types of exogenous Se, inorganic selenite has a significant inhibitory effect on soil microorganisms. This may be due to the inconsistent chemical and biological functions of different types of exogenous Se, resulting in different effects on soil microorganisms (Guo et al., 2021).

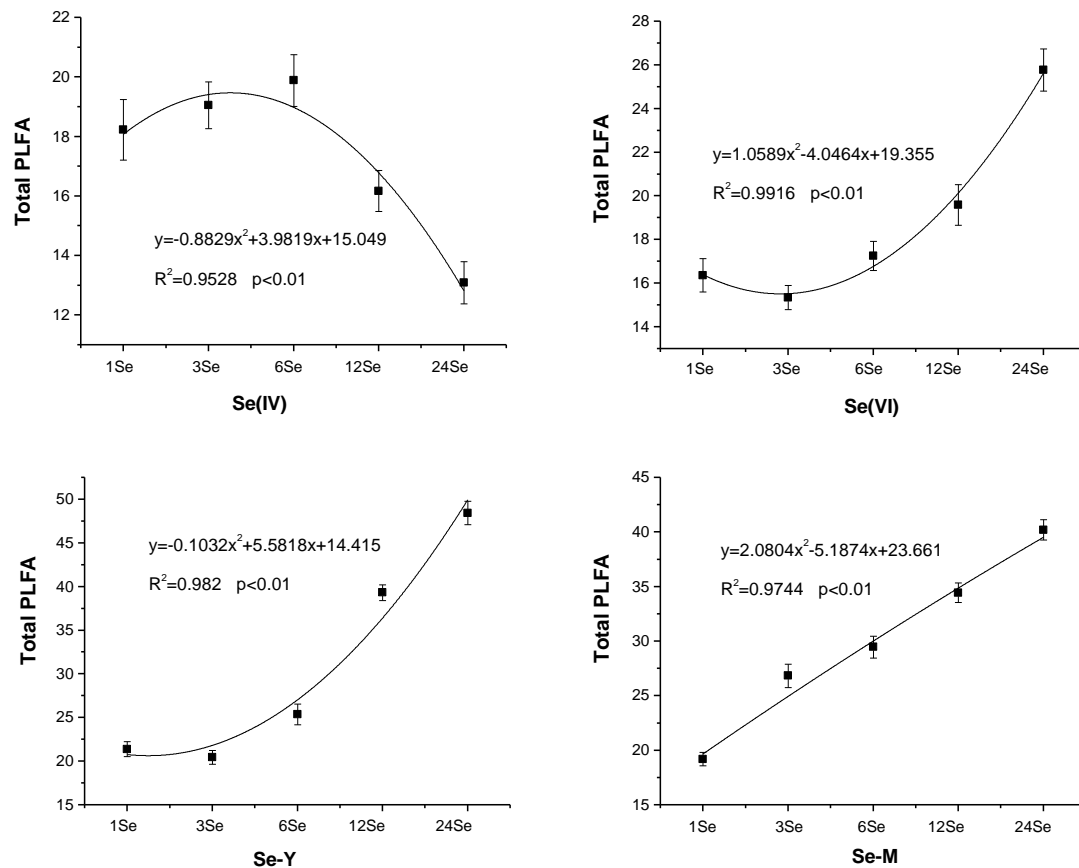


Figure 5. Relationship between the amount of different Se sources and the microbial content of PLFA

Table 4. Correlation among soil As, soil enzymes and microorganisms after Se-Y addition ($n = 15$)

	Urease	Sucrase	Catalase	Dehydrogenase	The FDA hydrolase	Actinomyces	G ⁺	G ⁻	Fungus
Urease	1								
Sucrase	.745**	1							
Catalase	.941**	.516*	1						
Dehydrogenase	.702**	.922**	.452*	1					
The FDA hydrolase	.826**	.928**	.619**	.978**	1				
Actinomyces	.693**	.857**	.551*	.878**	.901**	1			
G ⁺	.841**	.887**	.698**	.919**	.965**	.965**	1		
G ⁻	.780**	.928**	.603**	.945**	.969**	.977**	.988**	1	
Fungus	.249	.728**	.004	.822**	.730**	.824**	.716**	.793**	1

**Significant correlation at the 0.01 level. *Significant correlation at the 0.05 level

Table 5. Correlation among soil As, soil enzymes and microorganisms after Se-M addition (n = 15)

	Urease	Sucrase	Catalase	Dehydrogenase	The FDA hydrolase	Actinomyces	G ⁺	G ⁻	Fungus
Urease	1								
Sucrase	.867**	1							
Catalase	.778**	.967**	1						
Dehydrogenase	.946**	.927**	.880**	1					
The FDA hydrolase	.881**	.957**	.940**	.974**	1				
Actinomyces	.812**	.642**	.572*	.651**	.553*	1			
G ⁺	.778**	.971**	.996**	.893**	.954**	.534*	1		
G ⁻	.962**	.953**	.896**	.978**	.956**	.753**	.898**	1	
Fungus	.892**	.986**	.931**	.916**	.922**	.727**	.932**	.963**	1

**Significant correlation at the 0.01 level. *Significant correlation at the 0.05 level

Conclusions

This research carried out the regulation of Se on microbial and enzyme activities in As-contaminated soil. Research has shown that the application of inorganic Se and organic Se can significantly affect the activities of dehydrogenase, urease, FDA hydrolase, invertase and catalase in As-contaminated soil. The application of organic Se could increase the total amount of microorganisms in As-contaminated soil and enrich the microbial community structure, while inorganic Se could have the opposite effect. There was a significant positive correlation between various microorganisms and enzyme activities in the soil. This study also showed that the regulation of As in soil by Se was closely related to soil microorganisms and enzyme activities. Follow-up work should be conducted to study the mechanism of Se and microorganisms in the treatment of soil As pollution.

List containing the detected species and their classification

Soil enzymes: urease, catalase, sucrase, FDA hydrolase and dehydrogenase.

Soil microorganisms: actinomycetes, fungus, gram-positive bacteria (G⁺), gram-negative bacteria (G⁻).

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