Environmental Science and Pollution Research (2022) 29:52483–52492
https://doi.org/10.1007/s11356-022-19417-2

RESEARCH ARTICLE

Effect of indole-3-acetic acid supplementation on the physiology of *Lolium perenne* L. and microbial activity in cadmium-contaminated soil

Xiaoyang Xu[1] · Jihai Zhou[1] · Kun Chen[2] · Yang Wang[1] · Yanmei Ai[1] · Chenyang Zhang[1] · Shoubiao Zhou[1]

Received: 7 October 2021 / Accepted: 21 February 2022 / Published online: 8 March 2022

Abstract

Cadmium (Cd) pollution has led to a serious deterioration in soil quality, plant growth, and human health. Therefore, restoration of soil quality is imperative. Phytoremediation is inexpensive and yields acceptable outcomes. Phytoremediation involves interaction between plant physiology and microbial activity and has been widely used in the remediation of Cd-contaminated soil. In the present study, *Lolium perenne* L. (perennial ryegrass) was planted in Cd-spiked soil and indole-3-acetic acid (IAA) was used to explore the physiological and biochemical characteristics of ryegrass as well as soil enzyme activity to remove Cd. The present study provides a theoretical basis for the phytoremediation of Cd-contaminated soil. The study investigated the effect of 30-mg/kg Cd-spiked soil on ryegrass (C) and 30-mg/kg Cd-spiked soil on ryegrass treated with 10-mg/kg IAA (CI) compared with uncontaminated soil and ryegrass as the control. At the end of the experiment, the ryegrass biomass, total chlorophyll, superoxide dismutase (SOD) activity, and soil invertase activity in C group were decreased by 33.7%, 23.0%, 29.7%, and 18.3%, respectively, whereas the peroxidase (POD) activity and soil basal respiration increased by 17.1% and 87.9%, respectively, compared with the control. In the CI group, the biomass of ryegrass, chlorophyll content, SOD activity, sucrase activity, fluorescein diacetate (FDA) hydrolase activity, and Cd removal rates increased by 14.5%, 19.9%, 24.3%, 12.1%, 20.4%, and 15.1%, respectively, whereas the POD activity, soil basal respiration, and Cd residues in the soil declined by 8.0%, 15.0%, and 17.0%, respectively, compared with the C group. Therefore, exposure to exogenous IAA alleviated the Cd stress on ryegrass and soil microorganisms and improved Cd absorption by ryegrass from the contaminated soil.

Keywords Ryegrass · Cadmium pollution · IAA · Plant physiological characteristics · Soil microbial activity

Introduction

Soil is an integral part of the Earth's ecosystem. Soil quality is directly related to the development of human society (Pereira et al. 2018). Heavy metal pollution of soil in China is severe. According to the 2014 "National Soil Pollution Survey Bulletin," soil contamination with heavy metals in China involves primarily cadmium (Cd), mercury (Hg), lead (Pb), chromium (Cr), and copper (Cu). Cd pollution is the most serious challenge (Ministry of Land and Resources of China 2014). Cd pollution involves various types of land including cultivated areas, forests, and grasslands. Cd ranks at the top of inorganic pollutants accounting for 7% of all the surveyed points in an area of 6.3 million sq km. Severe Cd contamination was reported in 0.5%. According to Chinese soil environmental quality standards (GB 15618–2018 2018, GB 36600–2018 2018), the values of risk screening and intervention against Cd pollution in agricultural and

Responsible Editor: Kitae Baek

Xiaoyang Xu and Kun Chen contributed equally to this work.

✉ Jihai Zhou
zhoujihai2006@163.com

[1] Collaborative Innovation Center of Recovery and Reconstruction of Degraded Ecosystem in Wanjiang Basin Co-Founded By Anhui Province and Ministry of Education, School of Ecology and Environment, Anhui Normal University, Wuhu 241002, China

[2] Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210018, China

developmental lands were 0.3 mg/kg and 1.5 mg/kg, 20 mg/kg and 47 mg/kg, respectively. Soil Cd is mainly attributed to industrial production, sewage irrigation, and use of Cd-containing pesticides. When the soil Cd content reaches a certain level, it turns toxic to the terrestrial ecosystem, leading to soil degradation and reduced crop yield and quality. It also contributes to surface and groundwater pollution via runoff and leaching, thus deteriorating the hydrological environment, which may endanger human life and health via direct contact and the food chain (Song et al. 2018). Therefore, it is imperative to restore contaminated soil using inexpensive and effective techniques without damaging the soil structure.

Phytoremediation is an inexpensive and effective green technology. Phytoremediation involves the interaction between plant physiology and microbial activity and has been widely used in the remediation of Cd-contaminated soil. Ryegrass is a crucial gramineous forage grass extensively used in many countries because of its wide adaptability, strong tiller regeneration, rapid growth rate, and high yield. Previous studies reported normal growth of perennial ryegrass in Cd-contaminated soil and absorption of Cd from contaminated soil (Fang et al. 2017; Zhang et al. 2019) due to its higher tolerance to Cd (Li et al. 2018). Therefore, perennial ryegrass with high biomass can be used in remediation of soil contaminated by heavy metals.

Soil microorganisms dissolve or precipitate heavy metal ions in contaminated soil or change the form of heavy metals via organic acids or complexes released during metabolism for detoxification (Abatenh et al. 2017; Panigrahi et al. 2019; Ojuederie and Babalola 2017; Baoune et al. 2019). These microorganisms have been widely used in the study of heavy metal pollution and bioremediation.

Indole-3-acetic acid (IAA) is a hormone synthesized in plants. IAA affects plant growth, development, physiology, and biochemistry as well as promotes plant cell division and growth. Ran et al. (2020) reported that IAA induced Cd accumulation in the upper parts of *Solanum nigrum* by maintaining higher superoxide dismutase (SOD) activity in the leaves. The study of Chen (2020) showed that IAA alleviated the adverse effects of uranium and Cd on sunflowers by promoting photosynthesis, reducing the active oxygen and lipid peroxidation, and improving the antioxidant defense mechanisms. Studies have shown that the toxic effects of heavy metals such as Pb, Cu, and Cd on *Chlorella* are attenuated by antioxidant enzymes induced by IAA, indole butyric acid, and naphthalene acetic acid (Piotrowska-Niczyporuk et al. 2012).

In summary, the cultivation of ryegrass in Cd-contaminated soil and treatment with IAA effectively alleviate Cd toxicity in ryegrass, reduce soil microbial stress, and promote elimination of Cd from contaminated soil. To investigate the physiological and biochemical changes in ryegrass as well as the altered soil microbial activity, and their impact on Cd removal during the remediation of Cd-contaminated soil, we designed an indoor pot experiment using Cd-spiked soil to grow ryegrass in the presence of exogenous IAA. We provide a theoretical basis for the use of ryegrass and exogenous IAA in decontaminating soils containing Cd and provide practical guidance for improving the soil environment, ensuring food safety, and achieving sustainable development.

Materials and methods

Plants and soil

The test soil was collected from a soil layer of 0–20 cm at the Red Soil Research Institute, Jinxian County, Jiangxi Province, and was air-dried and sieved with a 2-mm sieve for indoor pot experiments. The soil pH was 4.35. The levels of organic matter, total nitrogen, total phosphorus, and available phosphorus in the soil were 7.24, 1.58, 0.96, and 0.13 g/kg, respectively. The Cd concentration in the soil was 0.196 mg/kg.

Ryegrass seeds were purchased from Suqian Horticulture Co., Ltd., Jiangsu Province. Sterilized seeds were obtained by soaking them for 3 min in a sterile flask containing added 5% sodium hypochlorite.

Experimental design

Three treatments were utilized in the present study: uncontaminated soil + ryegrass (Control), 30 mg/kg Cd-spiked soil + ryegrass (C), and 30 mg/kg Cd-spiked soil + 10 mg/kg IAA + ryegrass (CI). Basal fertilizer (N: P_2O_5: K_2O = 30: 15: 17.5) 0.125 g/kg was used according to the conventional standards designed for farmland fertilization. According to the Chinese quality control standards for soil environment and contaminated lands (GB36600-2018), a soil Cd concentration of 30 mg/kg was achieved using 2 g/L Cd^{2+}. We performed a 2-month indoor culture with 50% saturated moisture content under 12 h of light daily. Destructive sampling was performed on days 15, 30, and 60, and five replicates were randomly selected for each treatment.

Methods

Ryegrass was dried at 80 °C to a constant weight to obtain the biomass. The chlorophyll content was extracted with 95% ethanol (Palta 1990). A known amount of leaf tissue was homogenized with 95% ethanol using a blender. To remove the debris, the homogenate was filtered, and the sample was thoroughly mixed. A portion of the filtrate was obtained and the absorbance was read at 654 nm via spectrophotometry.

The SOD activity (Shafi et al. 2015) was measured by adding 50 µL enzyme extract to a reaction mixture (3 mL) containing 20 µM riboflavin, 750 µM NBT, 130 mM Dl-methionine, and 100 µM EDTA-Na_2 in 50 mM phosphate buffer. A unit of enzyme activity was defined as the amount of enzyme required for 50% inhibition of NBT reduction per min at 25 °C. The specific activity of SOD was calculated as previously described. The peroxidase (POD) activity was measured using the guaiacol colorimetric method (Wang and Huang 2015). Ryegrass leaves (500 mg) were homogenized with a mortar and pestle in phosphate buffer (50 mM, pH 7.8) and centrifuged at 6000 rpm for 10 min in 4 °C, followed by the addition of 1 mL of supernatant to the reaction mixture containing 1 mL of 1% guaiacol and 1 mL of 0.18% H_2O_2. The changes in the absorbance of the mixture were measured at 470 nm over 2 min in 30 s intervals. The soil basal respiration was measured via alkali absorption method (Xu et al. 2019). Soil (10 g) was incubated in Schott bottles at 25 °C in the dark for 24 h. A 20-mL open-top vial containing 10 mL of 0.05 M NaOH solution was used to trap the CO_2 released within the sealed Schott bottles. For each set, three blank Schott bottles (without soil) with NaOH were incubated and titrated as described above, and used as controls. Soil invertase activity was determined using 8% glucose solution as the substrate (Akhtar et al. 2018). A 5-g fresh soil sample was incubated with 15 mL substrate, 5 mL 0.2 M phosphate buffer (pH 5.5), and 5 drops of toluene for 24 h at 37 °C. After incubation, the mixture was filtered immediately and a 1-mL aliquot was reacted with 3 mL of 3, 5-dinitrylsalicylate in a volumetric flask, and heated for 5 min. The soil solution in the flask was measured using an ultraviolet spectrophotometer at 508 nm. The soil catalase activity was determined following the addition of 40 mL distilled water and 5 mL 0.3% H_2O_2 to 2 g fresh soil (Akhtar et al. 2018). The mixture was shaken for 20 min at 150 rpm and filtered immediately; the filtrate was titrated with 0.1 mol L^{-1} $KMnO_4$ in sulfuric acid. To measure FDA hydrolase activity (Wang et al. 2019), 1.0 g of soil was mixed with 15 mL phosphate buffer (pH 7.6) and 0.2 mL FDA substrate solution (1 mg mL^{-1}), followed by incubation at 30 °C for 2 h. The fluorescein released was extracted with 10 mL acetone and analyzed at 490 nm with a colorimeter. Cd was digested using an HCl-HNO_3-$HClO_4$-HF tetra-acid digestion system, and the Cd content was measured with an atomic absorption spectrophotometer (Long et al. 2013). The significance and correlation of the experimental data were analyzed using SPSS 19.0. Excel 2016 was used for statistical and graphical analysis of the data.

Results

Removal of Cd from contaminated soil

IAA addition to Cd contaminated soil enhanced the ability of ryegrass to remove Cd (Fig. 1). Following the experiment, the rates of Cd removal in C and CI groups were 10.8% and 25.9%, respectively. The Cd elimination rate in CI was 2.4-fold higher than in C ($p = 0.043$).

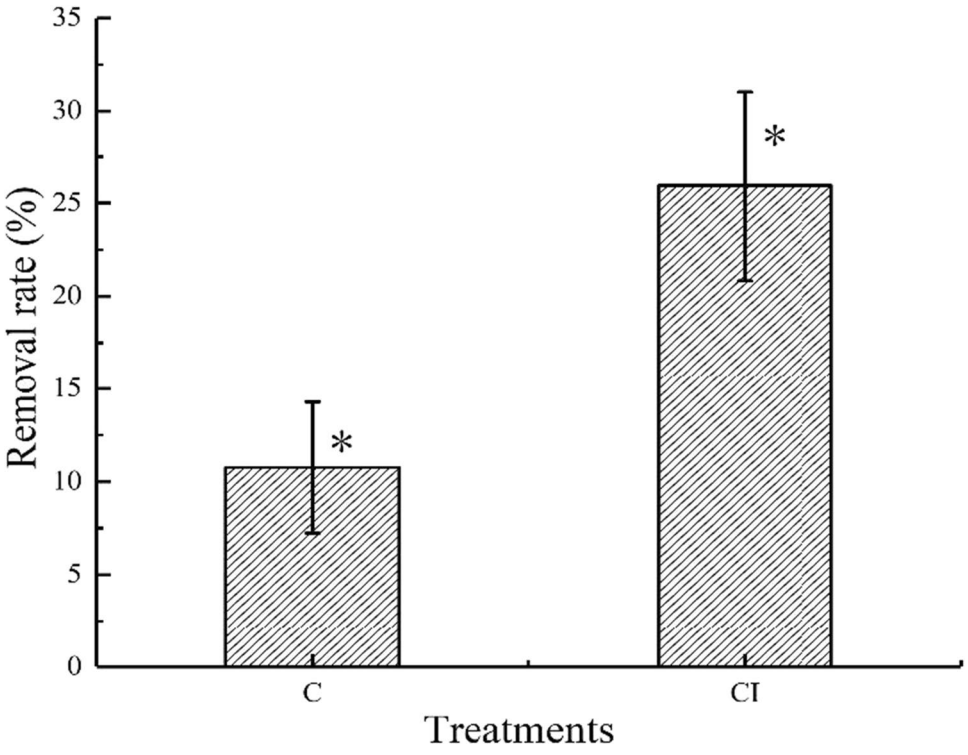

Fig. 1 Cd removal rates in the two experimental treatments. C: 30 mg/kg Cd-spiked soil + ryegrass; CI: 30 mg/kg Cd-spiked soil + 10 mg/kg IAA + ryegrass. The symbol * denotes statistically significant difference ($p < 0.05$) between the treatments

Effects of IAA addition on ryegrass growth, physiology, and biochemistry in Cd-contaminated soil

Changes in ryegrass biomass

Ryegrass biomass showed an upward trend with increasing cultivation time, with treatment-related differences (Table 1). On day 15, the underground, above-ground, and total ryegrass biomasses of the C group were significantly decreased by 35.9%, 55.8%, and 67.3%, respectively, compared with the control ($p < 0.05$). On day 30, the underground, above-ground, and total ryegrass biomasses of C plants were significantly lower than in control by 45.8%, 26.2%, and 33.5%, respectively. The above-ground biomass and the total biomass of CI plants were significantly higher than in treatment C by 23.3% and 26.3%, respectively. On day 60, the underground, above-ground, and total ryegrass biomasses of plants exposed to treatment C were significantly lower than that of the control by 45.4%, 30.9%, and 33.7%, respectively. The underground, above-ground, and total biomasses of plants in the CI group were higher than in C, however, with no significant differences observed.

Changes in chlorophyll content of ryegrass

The changes in chlorophyll content and chlorophyll a:b ratios during cultivation are shown in Table 2. The levels of chlorophyll a, total chlorophyll, and the chlorophyll a:b ratio following the three treatments increased first and then decreased with increasing cultivation time. The chlorophyll content in CI treatment was higher than in treatment C during the cultivation period. On day 15, the levels of chlorophyll a, chlorophyll b, and total chlorophyll in the CI group were 11.2%, 16.2%, and 12.6% higher than in treatment C, respectively. On day 30, the levels of chlorophyll a, chlorophyll b, and total chlorophyll in treatment C were significantly decreased by 28.9%, 21.6%, and 27.9%, respectively, compared with the control. The levels of chlorophyll a and total chlorophyll in the CI group were significantly higher

Table 1 Changes in ryegrass biomass (dry weight) under different treatments

Treatment code	Sampling time/d	Below-ground ($g \cdot pot^{-1}$)	Above-ground ($g \cdot pot^{-1}$)	Total biomass ($g \cdot pot^{-1}$)
Control		0.092 ± 0.004a	0.150 ± 0.035a	0.242 ± 0.079a
C	15	0.059 ± 0.006b	0.048 ± 0.006b	0.107 ± 0.022b
CI		0.060 ± 0.005b	0.049 ± 0.007b	0.110 ± 0.019b
Control		0.273 ± 0.027a	0.470 ± 0.031a	0.743 ± 0.117a
C	30	0.148 ± 0.007b	0.347 ± 0.018b	0.494 ± 0.047c
CI		0.196 ± 0.007b	0.428 ± 0.022a	0.624 ± 0.053b
Control		1.010 ± 0.103a	0.932 ± 0.050a	1.941 ± 0.228a
C	60	0.551 ± 0.030b	0.736 ± 0.061b	1.287 ± 0.188b
CI		0.698 ± 0.052b	0.785 ± 0.022b	1.473 ± 0.113b

Data represent mean ± standard deviation ($n = 5$). Different small letters in the same column represent statistically significant differences between treatments ($p < 0.05$)

Table 2 Changes in chlorophyll content of ryegrass exposed to different treatments

Treatment code	Sampling time/d	Chlorophyll a/($mg \cdot g^{-1}Fw$)	Chlorophyll b/($mg \cdot g^{-1}Fw$)	Total chlorophyll/($mg \cdot g^{-1}Fw$)	Chlorophyll a:b
Control		0.894 ± 0.045a	0.356 ± 0.018a	1.251 ± 0.064a	2.511 ± 0.026a
C	15	0.818 ± 0.016a	0.315 ± 0.008a	1.133 ± 0.024a	2.600 ± 0.028a
CI		0.910 ± 0.021a	0.366 ± 0.020a	1.276 ± 0.037a	2.511 ± 0.127a
Control		1.344 ± 0.041a	0.227 ± 0.021a	1.571 ± 0.057a	6.125 ± 0.575a
C	30	0.955 ± 0.010c	0.178 ± 0.009b	1.133 ± 0.016c	5.425 ± 0.286a
CI		1.114 ± 0.024b	0.174 ± 0.009b	1.288 ± 0.022b	6.464 ± 0.382a
Control		0.717 ± 0.062a	0.290 ± 0.026a	1.007 ± 0.878a	2.479 ± 0.037a
C	60	0.548 ± 0.042b	0.213 ± 0.015b	0.761 ± 0.058b	2.569 ± 0.067a
CI		0.681 ± 0.036ab	0.280 ± 0.017a	0.961 ± 0.051ab	2.431 ± 0.032a

Data represent mean ± standard deviation ($n = 5$). Different small letters in the same column denote statistically significant differences in treatment

than in C by 16.6% and 13.7%, respectively ($p < 0.05$). On day 60, the levels of chlorophyll a, chlorophyll b, and total chlorophyll following treatment C were significantly decreased by 23.6%, 26.6%, and 24.4%, respectively, compared with the control. The levels of chlorophyll a, chlorophyll b, and total chlorophyll in plants exposed to CI were higher than in treatment C, and chlorophyll b content was significantly higher than in treatment C by 29.1%. There were no significant treatment-related differences in chlorophyll a:b ratio during the cultivation period.

Changes in enzyme activity of ryegrass leaves

SOD activity in ryegrass leaves showed an overall downward trend with extended cultivation time (Fig. 2A). On day 15, the SOD activity of leaves in the C group was significantly lower than in the control by 19.7% ($p < 0.05$), and the SOD activity of leaves in the CI treatment group was significantly higher than in C by 16.6%. On day 30, the SOD activity of group C was significantly decreased by 39.6% compared with the control. The SOD activity of the CI group was significantly higher than in the C group by approximately 25.6%. On day 60, the SOD activity of group C was significantly lower than in the control by 29.7% and the SOD activity of the CI group was significantly higher than in C group by 24.3%.

During the cultivation period, the POD activity of the ryegrass leaves exposed to each treatment decreased and then increased (Fig. 2B). On day 15, the POD activity of the C group was significantly higher than that of the control, and the POD activity of the CI group was significantly lower than that of the C group by 21.5%. On day 30, the POD activity of the C group was significantly higher than that of the control by 64.3%, and the POD activity of the CI group was significantly lower than that of the C group by 34.8%. On day 60, the POD activity of each group was significantly increased; the POD activity of the C group was significantly higher than that of the control by 17.1%, and the POD activity of the CI group was lower than that of the C group, with no significant treatment-related differences.

Effect of IAA addition on soil microbial activity in Cd-contaminated soil

Changes in soil basal respiration

The soil basal respiration of each group during the incubation period showed an initial decline, followed by a rise and then a further decline (Fig. 3). On day 15, the soil basal respiration of the CI group was significantly higher than in the C group by 140.4%. On day 30, the soil basal respiration of group C was significantly higher than in the control by 47.7%, with no significant treatment-related differences. On day 60, the soil basal respiration of group C was significantly higher than that of the control by approximately 87.9%, and the soil basal respiration of group CI was significantly lower than that of group C by 15.0%.

Changes in soil enzyme activity

During the cultivation, the soil invertase activity showed a decline, followed by an increase (Fig. 4A). On day 15, the invertase activity of group C was significantly lower than in the control by 12.5%, whereas the invertase activity of group CI was significantly higher than that of the control and group C by 7.2% and 22.4%, respectively. On day 60, the invertase activity of the C group was significantly lower than that of the control by 18.3%, and the invertase activity of group CI was 12.1% higher than that of group C.

Increase in cultivation time led to an upward trend in soil catalase activity of each treatment group (Fig. 4B). On day 15, the soil catalase activity of group C was significantly lower than that of the control and the catalase activity of group CI was significantly higher than that of group C by 15.8%. Otherwise, there were no significant treatment-related differences.

The soil FDA hydrolase activity of each group increased and then decreased over time (Fig. 4C). On day 15, the FDA hydrolase activity of group C was higher than that of the control by 5.9%. On day 30, the FDA hydrolase activity of group C was significantly higher than that of the control and

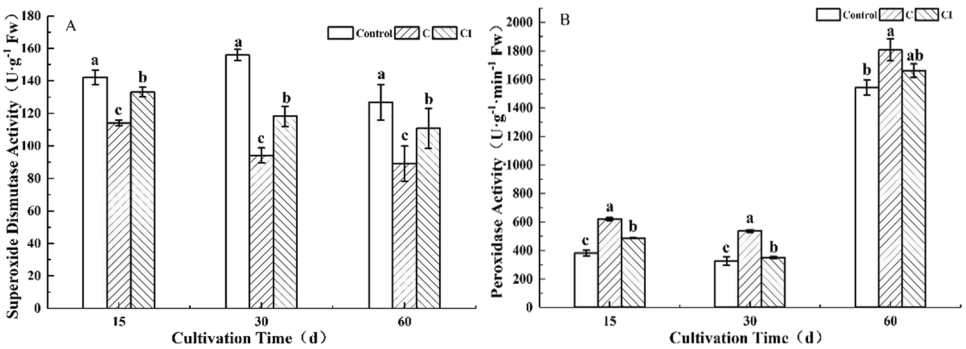

Fig. 2 Dynamic changes in SOD (**A**) and POD (**B**) activity of ryegrass following different treatments. Control: uncontaminated soil + ryegrass; C: 30 mg/kg Cd-spiked soil + ryegrass; CI: 30 mg/kg Cd-spiked soil + 10 mg/kg IAA + ryegrass. The different letters denote significant treatment-related differences at $p < 0.05$

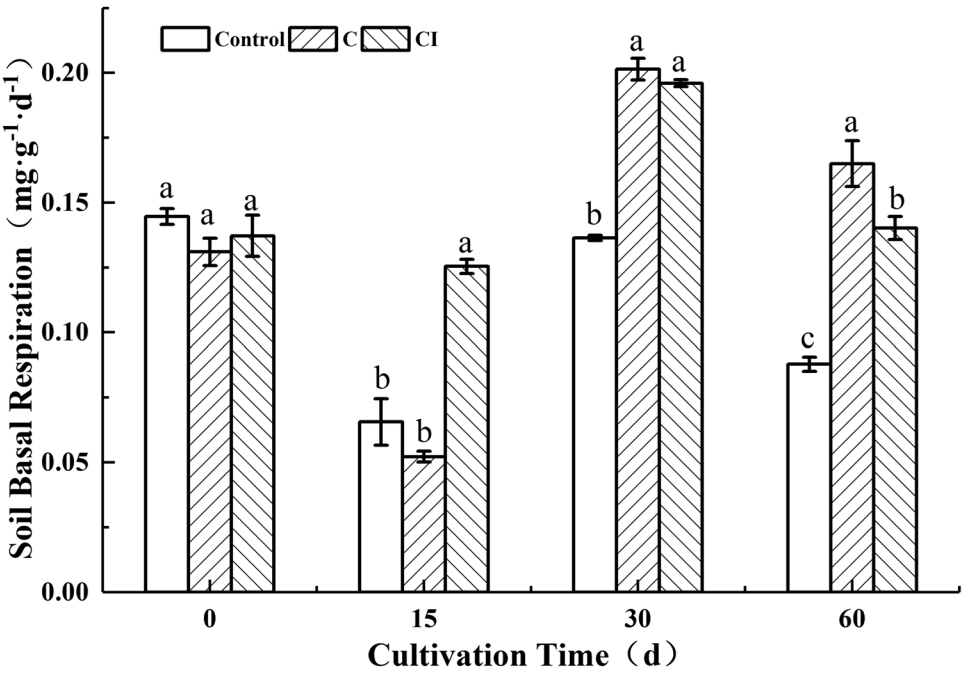

Fig. 3 Dynamic changes in soil basal respiration under different treatments. Control: uncontaminated soil + ryegrass; C: 30 mg/kg Cd-spiked soil + ryegrass; CI: 30 mg/kg Cd-spiked soil + 10 mg/kg IAA + ryegrass. The different letters denote significant treatment-related differences ($p < 0.05$)

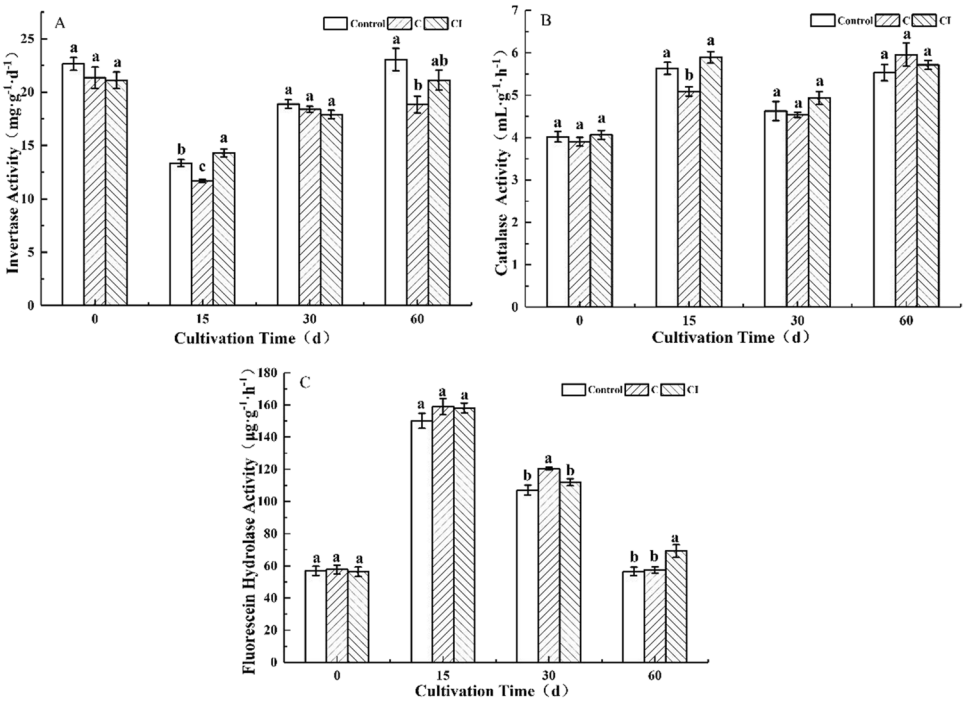

Fig. 4 Dynamic changes in soil invertase (**A**), soil catalase (**B**), and soil FDA hydrolase activity (**C**) following exposure to different treatments. Control: uncontaminated soil + ryegrass; C: 30 mg/kg Cd-spiked soil + ryegrass; CI: 30 mg/kg Cd-spiked soil + 10 mg/kg IAA + ryegrass. The different letters show a significant difference at $p < 0.05$ between the treatments

experimental group CI by 12.4% and 7.6%. On day 60, the FDA hydrolase activity of group CI was significantly higher than in group C by 20.4%.

Correlation between soil Cd removal and ryegrass physiology and biochemistry and soil microbial activity

The results of the correlation analysis of different factors are presented in Table 3. The rate of Cd removal was significantly negatively correlated with soil invertase activity ($p < 0.01$). Ryegrass biomass was significantly positively

Table 3 Cd removal rate associated with physiological and biochemical parameters of ryegrass and soil microbial activity

Factor	Removal rate	Biomass	Total chlorophyll	SOD	POD	Soil basal respiration	Soil catalase	Soil invertase	Soil FDA hydrolase
Removal rate	1.000	−0.265	−0.109	0.264	−0.048	−0.314	0.229	−0.397*	0.251
Biomass		1.000	−0.707**	−0.602**	0.854**	0.363*	0.292	0.819**	−0.954**
Total chlorophyll			1.000	0.565**	−0.867**	−0.006	−0.367*	−0.473**	0.728**
SOD				1.000	−0.525**	−0.506**	0.160	−0.495**	0.700**
POD					1.000	−0.006	0.569**	0.520**	−0.826**
Soil basal respiration						1.000	−0.254	0.690**	−0.471**
Soil catalase							1.000	0.047	−0.292
Soil invertase								1.000	−0.812**
Soil FDA hydrolase									1.000

**Correlation is significant at $p < 0.01$; *correlation is significant at $p < 0.05$ (two-tailed)

correlated with soil basal respiration, POD activity, and soil invertase activity ($p < 0.01$). Biomass was negatively correlated with total chlorophyll, SOD, and soil FDA hydrolase activity ($p < 0.01$). Total chlorophyll was positively correlated with SOD and soil FDA hydrolase activity ($p < 0.01$). Total chlorophyll was negatively correlated with POD, soil catalase, and soil invertase activity. SOD activity was positively correlated with soil FDA hydrolase activity ($p < 0.01$) and negatively correlated with POD activity, soil basal respiration, and soil invertase activity ($p < 0.01$). POD activity was positively correlated with soil catalase and soil invertase activity ($p < 0.01$), and significantly negatively correlated with soil FDA hydrolase activity ($p < 0.01$). Soil basal respiration was positively correlated with soil invertase activity ($p < 0.01$) and negatively correlated with soil FDA hydrolase activity ($p < 0.01$). Soil invertase activity was negatively correlated with soil FDA hydrolase activity ($p < 0.01$).

Discussion

Effect of ryegrass and IAA on soil Cd removal

Ryegrass has received substantial attention in restoring soils polluted with Cd because of its high tolerance to Cd and large biomass (He et al. 2020). Studies have shown that the higher the Cd content in the soil, the higher the available Cd content, and the greater the toxicity to ryegrass (Shi et al. 2020). A high concentration of Cd also inhibits Cd absorption in the soil by ryegrass (Li et al. 2017). In the present study, the CI group showed a decrease in soil Cd content and increased Cd loss from the soil compared with the C group. Chen's study of *Brassica juncea* L. yielded similar results (Chen et al. 2020a, b). These similarities might be attributed to increased affinity of the ryegrass inner membrane transporter for Cd following IAA treatment, thereby resulting in increased Cd absorption (Ji et al. 2020). IAA also promotes the secretion of organic acids, which induces Cd^{2+} transport from underground to the surface through ryegrass conduits (ryegrass roots), thereby improving the Cd absorption capacity of ryegrass (Wang et al. 2017).

Effects of Cd pollution and IAA addition on ryegrass growth and physiological and biochemical characteristics

Biomass is an important indicator of plant tolerance. Increased plant biomass in polluted environments leads to higher resistance. High levels of Cd pollution can seriously damage plants and significantly reduce plant biomass (Jian et al. 2015). Long-term Cd pollution inhibits plant growth and leads to biomass decline and plant death (Luo et al. 2016). In the present study, the biomass of ryegrass spiked with Cd in each period was lower than that of the control, thereby inhibiting ryegrass growth. The addition of 10 mg/kg of exogenous IAA (group CI) increased the biomass of ryegrass and enhanced its tolerance to heavy metals such as Cd compared with group C. This finding might be attributed to IAA, which reduces the negative effects of Cd on the ryegrass root length, volume, and surface area, and thereby promotes growth, similar to the results of Faessler's study involving sunflowers (Faessler et al. 2010).

Previous studies have shown that Cd stress negatively impacted chlorophyll content, which was gradually decreased with increasing soil Cd concentration (Han et al. 2018). Cd stress causes yellowing or death of plant leaves and decreases the chlorophyll content of ryegrass in the presence of elevated Cd concentrations (Chen et al. 2018). The chlorophyll content of group C was affected

by Cd pollution and declined with increasing cultivation time. The entry of additional divalent Cd^{2+} ions may have a negative effect on photosystem II, reducing the electron transfer rate, and inhibiting the formation of oxygen complexes (Ivanov and Kosobryukhov. 2020). In addition, exposure to Cd damages the pigment synthesis and structure, resulting in a decreased chlorophyll content, as reported by Jian et al. (2015). Exogenous IAA increases the chlorophyll content by promoting the growth of ryegrass and alleviates the toxic effects of Cd on chloroplasts, similar to the study of *Trigonella foenum-graecum* L. by Bashri and Prasad (2015).

SOD and POD are two common protective enzymes in plants and are highly sensitive to changes in the external environment (Feng et al. 2017). SOD activity decreased with increasing cultivation time in the present study. Because of prolonged Cd stress, ryegrass produces an abundance of reactive oxygen species, which trigger oxidative stress (Dube et al. 2009). During oxidative stress, a large amount of peroxide is produced, which disrupts cellular homeostasis, thereby inhibiting SOD activity, as reported by Huang et al. (2017) investigating the changes in the antioxidant enzyme system of *Althaea rosea* Cavan. under Pb stress. The POD activity increased with increasing incubation time, which eliminated H_2O_2 from plant cells as a protective mechanism. In the present study, IAA enhanced SOD activity by alleviating the oxidative damage in ryegrass induced by Cd stress, which is consistent with prior work by Khalid and Aftab (2020). Thus, the decrease in POD activity may be attributed to a decrease in H_2O_2 content in ryegrass induced by IAA via other mechanisms.

Effects of Cd pollution and IAA addition on soil microbial activity

Soil basal respiration is an important parameter for studying soil microbial activity. The present study showed that the soil basal respiration of ryegrass treated for Cd contamination was higher than that of the control during the middle and late cultivation stages. These findings might be related to the need for higher levels of energy for survival of microorganisms in the soil exposed to pollution, thus promoting their metabolic activity (Zheng et al. 2019). The soil basal respiration of the CI group decreased during the later cultivation period probably due to the growth of ryegrass induced by IAA, which facilitated the colonization of soil microorganisms on the ryegrass root surface, thereby alleviating Cd stress (Spaepen et al. 2007).

The effect of pollutants on soil microbial activity is reflected by the effects on soil enzyme activity, which is a strong indicator of soil microbial activity (Liu et al. 2018). The soil invertase activity first decreased and then increased with increasing pollution time, which is consistent with the findings of Xiang et al. (2012). The invertase activity of group C was lower than in the control, which might be explained by the inhibition of the activity of soil microorganisms by Cd stress, thereby inhibiting invertase activity. The catalase activity increased with increasing pollution time. Under Cd stress, the soil might have accumulated a large quantity of H_2O_2 that triggered an increase in catalase activity to counter the oxidative stress. FDA hydrolase is one of the most important biological indicators reflecting changes in soil microbial activity and quality (Swisher and Carroll 1980), and can be used to monitor the overall enzyme activity of soil microorganisms (Adam et al. 2001). During the cultivation period, the FDA hydrolase activity of group C was higher than that of the control, which might be attributed to Cd-induced synthesis of microbial enzymes to maintain normal life of microorganisms. Exogenous IAA attenuates the effects of Cd on soil enzyme activity by increasing the root biomass of ryegrass. The increased root system of ryegrass provides additional space for survival of soil microorganisms in the rhizosphere (Li et al. 2016). The root exudates of ryegrass provide further nutrients for soil microorganisms, thereby increasing the biomass and activity of soil microorganisms.

Conclusion

Cd stress decreased chlorophyll content, biomass, and POD activity of ryegrass and increased SOD activity. The addition of exogenous IAA increased the biomass, chlorophyll content, and catalase activity of ryegrass in Cd-contaminated soil and reduced SOD activity. Exposure to exogenous IAA alleviated the adverse effects of Cd on ryegrass and facilitated its growth and development.

Cd pollution increases soil basal respiration and invertase and catalase activity, and decreases FDA hydrolase activity. The addition of exogenous IAA decreases the soil basal respiration and increases FDA hydrolase activity, thereby enhancing the survival of soil microorganisms. Exogenous IAA directly or indirectly attenuated the Cd stress on ryegrass and improved its ability to absorb and elimate Cd.

Acknowledgements This work was sponsored by the University Synergy Innovation Program of Anhui Province (GXXT-2020-075), the Science and Technology Key Project of Jiangxi Provincial Department of Education (GJJ180921), and the National Natural Science Foundation of China (31460149). We thank the three anonymous reviewers whose constructive comments have improved this manuscript greatly.

Author contribution JHZ and SBZ proposed the study concept and design. YW, YMA, and CYZ conducted the preparation and treatment of test materials. JHZ, XYX, and KC designed and performed the experiment, analyzed the data, and wrote the manuscript. JHZ and SBZ revised the article. All authors read and approved the final manuscript.

Data availability All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Abatenh E, Gizaw B, Tsegaye Z, et al. (2017) The role of microorganisms in bioremediation-a review. Open J Environ Biol 2(1): 038–046. https://doi.org/10.17352/ojeb

Adam G, Duncan H (2001) Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils[J]. Soil Biol Biochem 33(7–8):943–951. https://doi.org/10.1016/S0038-0717(00)00244-3

Akhtar K, Wang WY, Ren JX et al (2018) Changes in soil enzymes, soil properties, and maize crop productivity under wheat straw mulching in Guanzhong, China[J]. Soil Tillage Res 182:94–102. https://doi.org/10.1016/j.still.2018.05.007Get

Baoune H, Aparicio JD, Pucci G et al (2019) Bioremediation of petroleum-contaminated soils using Streptomyces sp Hlh1[J]. J Soils Sediments 19(5):2222–2230. https://doi.org/10.1007/s11368-019-02259-w

Bashri G, Prasad SM (2015) Indole acetic acid modulates changes in growth, chlorophyll a fluorescence and antioxidant potential of Trigonella foenum-graecum L. grown under cadmium stress[J]. Acta Physiologiae Plantarum 37(3):49. https://doi.org/10.1007/s11738-014-1745-z

Chen L, Hu WF, Long C et al (2020) Exogenous plant growth regulator alleviate the adverse effects of U and Cd stress in sunflower (Helianthus annuus L.) and improve the efficacy of U and Cd remediation[J]. Chemosphere 262:127809. https://doi.org/10.1016/j.chemosphere.2020.127809

Chen L, Long C, Wang D et al (2020) Phytoremediation of cadmium (Cd) and uranium (U) contaminated soils by Brassica juncea L enhanced with exogenous application of plant growth regulators[J]. Chemosphere 242:125112. https://doi.org/10.1016/j.chemosphere.2019.125112

Chen W, Dong Y, Hu G et al (2018) Effects of exogenous nitric oxide on cadmium toxicity and antioxidative system in perennial ryegrass[J]. J Soil Sci Plant Nutr 18(1):129–143. https://doi.org/10.4067/S0718-95162018005000601

Dube BK, Sinha P, Shukla K et al (2009) Involvement of excess cadmium on oxidative stress and other physiological parameters of eggplant[J]. J Plant Nutr 32(6):996–1004. https://doi.org/10.1080/01904160902872206

Faessler E, Evangelou MW, Robinson RH et al (2010) Effects of indole-3-acetic acid (IAA) on sunflower growth and heavy metal uptake in combination with ethylene diamine disuccinic acid (EDDS)[J]. Chemosphere 80(8):901–907. https://doi.org/10.1016/j.chemosphere.2010.04.077

Fang ZG, Hu Z, Zhao H et al (2017) Screening for cadmium tolerance of 21 cultivars from Italian ryegrass (*Lolium multiflorum* Lam) during germination[J]. Grassland Sci 63(1):36–45. https://doi.org/10.1111/grs.12138

Feng K, Zheng QS, Yu JH et al (2017) The characteristics of superoxide dismutase (SOD) in evolutions and its research in plant resistance[J]. Molecular Plant Breeding 15(11):4498–4505 (in Chinese)

GB 15618–2018 (2018) Soil environmental quality-risk control standard for soil contamination of agricultural land[S]. http://www.mee.gov.cn/ywgz/fgbz/bz/bzwb/trhj/201807/t20180703_446029.shtml

GB 36600–2018 (2018) Soil environmental quality-Risk control standard for soil contamination of development land[S]. http://www.mee.gov.cn/ywgz/fgbz/bz/bzwb/trhj/201807/t20180703_446027.shtml

Han SJ, Li XN, Amombo N et al (2018) Cadmium tolerance of perennial ryegrass induced by Aspergillus aculeatus[J]. Front Microbiol 9:1579. https://doi.org/10.3389/fmicb.2018.01579

He S, Li Y, Guo H et al (2020) Combined effect of ryegrass and Hyphomicrobium sp GHH on the remediation of EE2-Cd co-contaminated soil[J]. J Soils Sediments 20(3–4):1–10. https://doi.org/10.1007/s11368-019-02358-8

Huang YP, Zu LH, Shen GS et al (2017) Effects of lead stress on accumulation ability and antioxidant enzyme activities of Althaea rosea Cavan[J]. J Agro-Environ Sci 36(9):1746–1752

Ivanov A.A., Kosobryukhov A.A. (2020) Ecophysiology of plants under cadmium toxicity: photosynthetic and physiological responses. In: Hasanuzzaman M. (eds) Plant ecophysiology and adaptation under climate change: mechanisms and perspectives I[M]. Springer: Singapore, 430–465. https://doi.org/10.1007/978-981-15-2156-0_15

Ji S, Gao L, Chen W et al (2020) Urea application enhances cadmium uptake and accumulation in Italian ryegrass[J]. Environ Sci Pollut Res 27(3):34421–34433. https://doi.org/10.1007/s11356-020-09691-3

Jian MF, Yang YP, Yu HP et al (2015) Influences of different cadmium concentration stress on chlorophyll and its photosynthetic fluorescence characteristics of ramie (Boehmeria nivea)[J]. Plant Physiol J 51(8):1331–1338 (in Chinese)

Khalid A, Aftab F (2020) Effect of exogenous application of IAA and GA3 on growth, protein content, and antioxidant enzymes of Solanum tuberosum L. grown in vitro under salt stress[J]. Vitro Cell Dev Biol Plant 56(3):377–389. https://doi.org/10.1007/s11627-019-10047-x

Li W, Wang D, Hu F et al (2016) Exogenous IAA treatment enhances phytoremediation of soil contaminated with phenanthrene by promoting soil enzyme activity and increasing microbial biomass[J]. Environ Sci Pollut Res 23(11):10656–10664. https://doi.org/10.1007/s11356-016-6170-y

Li X, Wang X, Du SJ et al (2017) Effect of different plants combinations on cadmium uptake grown in a mixed matrix of coal gangue weathered material and soil[J]. Chin J Agrometeorol 38(12):787–794. https://doi.org/10.3969/j.issn.1000-6362.2017.12.005 (in Chinese)

Li YH, Qin YL, Xu WH et al (2018) Differences of Cd uptake and expression of MT family genes and NRAMP2 in two varieties of ryegrasses[J]. Environ Sci Pollut Res Int 26(14):13738–13745. https://doi.org/10.1007/s11356-018-2649-z

Liu SS, Fu JP, Cai XD et al (2018) Effect of heavy metals pollution on ecological characteristics of soil microbes: a review[J]. Ecol Environ Sci 27(6):1173–1178 (in Chinese)

Long JH, Tan J, Wu YJ et al (2013) A comparative study on the detection of heavy metal in soil with different digestion methods[J]. Environ Monit China 29(1):123–126 (in Chinese)

Luo J, Qi SH, Gu XWS et al (2016) Ecological risk assessment of EDTA-assisted phytoremediation of Cd under different cultivation systems[J]. Bull Environ Contam Toxicol 96(2):259–264. https://doi.org/10.1007/s00128-015-1678-2

Ministry of Land and Resources of China. (2014) Report on the national general survey of soil contamination[EB/OL]. (in Chinese) http://www.gov.cn/foot/site1/20140417/782bcb88840814ba158d01.pdf.

Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review[J]. Int J Environ Res Public Health 14(12):1504. https://doi.org/10.3390/ijerph14121504

Palta JP (1990) Leaf chlorophyll content[J]. Remote Sens Rev 5(1):207–213. https://doi.org/10.1080/02757259009532129

Panigrahi S, Velraj P, Rao TS (2019) Functional microbial diversity in contaminated environment and application in bioremediation[J]. Microbial Diversity in the Genomic Era, 359–385. https://doi.org/10.1016/B978-0-12-814849-5.00021-6

Pereira P, Bogunovic I, Munoz-Rojas M et al (2018) Soil ecosystem services, sustainability, valuation and management[J]. Curr Opin Environ Sci Health 5(1):7–13. https://doi.org/10.1016/j.coesh.2017.12.003

Piotrowska-Niczyporuk A, Bajguz A, Zambrzycka E et al (2012) Phytohormones as regulators of heavy metal biosorption and toxicity in green alga *Chlorella vulgaris* (Chlorophyceae)[J]. Plant Physiol Biochem 52:52–65. https://doi.org/10.1016/j.plaphy.2011.11.009

Ran J, Zheng W, Wang H et al (2020) Indole-3-acetic acid promotes cadmium (Cd) accumulation in a Cd hyperaccumulator and a non-hyperaccumulator by different physiological responses[J]. Ecotoxicol Environ Saf 191:110213. https://doi.org/10.1016/j.ecoenv.2020.110213

Shafi A, Chauhan R, Gill T et al (2015) Expression of SOD and APX genes positively regulates secondary cell wall biosynthesis and promotes plant growth and yield in Arabidopsis under salt stress[J]. Plant Mol Biol 87:615–631. https://doi.org/10.1007/s11103-015-0301-6

Shi GY, Yan YJ, Yu ZQ et al (2020) Modification-bioremediation of copper, lead, and cadmium-contaminated soil by combined ryegrass (Lolium multiflorum Lam) and Pseudomonas aeruginosa treatment[J]. Environ Sci Pollut Res 27(5):37668–37676. https://doi.org/10.1007/s11356-020-09846-2

Song B, Yang ZJ, Zhang YX et al (2018) Accumulation of Cd and its risks in the soils of the Xijiang river drainage basin in Guangxi[J]. Environ Sci 39(4):1888–1900. https://doi.org/10.13227/j.hjkx.201707238 (in Chinese)

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling[J]. FEMS Microbiol Rev 31(4):425–448. https://doi.org/10.1111/j.1574-6976.2007.00072.x

Swisher R, Carroll GC (1980) Fluorescein diacetate hydrolysis as an estimator of microbial biomass on coniferous needle surfaces[J]. Microb Ecol 6(3):217–226. https://doi.org/10.1007/BF02010387

Wang ST, Dong Q, Wang ZL (2017) Differential effects of citric acid on cadmium uptake and accumulation between tall fescue and Kentucky bluegrass[J]. Ecotoxicol Environ Saf 145:200–206. https://doi.org/10.1016/j.ecoenv.2017.07.034

Wang XK, Huang JL. (2015). Principles and techniques of plant physiological biochemical experiment[M]. Higher Education Press, Beijing. 131 and 284–286 (in Chinese)

Wang ZQ, Tian HX, Tan XP et al (2019) Long-term as contamination alters soil enzyme functional stability in response to additional heat disturbance[J]. Chemosphere 229:471–480. https://doi.org/10.1016/j.chemosphere.2019.05.055

Xiang B, Wang B, Xu WH et al (2012) Effects of Cd, Zn, Cu, and Pb on soil microbial biomass and enzyme activities[J]. Biotic Res 34(3):4–8 (in Chinese)

Xu YL, Seshadri B, Bolan N et al (2019) Microbial functional diversity and carbon use feedback in soils as affected by heavy metals[J]. Environ Int 125:478–488. https://doi.org/10.1016/j.envint.2019.01.071

Zhang J, Yang N, Geng Y et al (2019) Effects of the combined pollution of cadmium, lead and zinc on the phytoextraction efficiency of ryegrass (Lolium perenne L.)[J]. RSC Adv 9(36):20603–20611. https://doi.org/10.1039/C9RA01986C

Zheng L, Li Y, Shang W et al (2019) The inhibitory effect of cadmium and/or mercury on soil enzyme activity, basal respiration, and microbial community structure in coal mine-affected agricultural soil[J]. Ann Microbiol 69(602):849–859. https://doi.org/10.1007/s13213-019-01478-3