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RESEARCH ARTICLE

The phytotoxicities of agricultural soil samples from a coal gangue stacking area to several maize cultivars (*Zea mays* L.)

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Abstract
In Shanxi, a major energy province in China, environmental pollution caused by coal gangue accumulation is becoming an increasingly serious problem. In addition, crops are the first trophic level in the human food chain, and the security and production of crops are closely related to human well-being. The objective of this study was to estimate the phytotoxicities of agricultural soil samples contaminated by coal gangue accumulation using maize (*Zea mays* L.) as a model organism. Finally, a tolerant maize cultivar was screened for coal gangue stacking areas. Seven cultivars of maize seeds were treated with agricultural soil leachate around the coal gangue stacking area at various concentrations of 0, 1:27, 1:9, 1:3, and 1:1. The results revealed that the agricultural soil leachate treatment could inhibit seed germination and the growth of roots and shoots and that the soil leachate-induced phytotoxicities were cultivar-dependent. At the same exposure concentration, tolerant maize cultivar displayed lower toxicity symptoms than sensitive maize cultivar in terms of growth inhibition, oxidative damage, and DNA damage. Stronger activities of antioxidant enzymes were observed in the tolerant maize cultivar than in the sensitive maize cultivar, indicating that the difference between cultivars in antioxidant capacity is one reason for the difference in plant tolerance. Our study provides experimental evidence for the ecological risk assessment of soil and the selection of maize cultivars with high environmental pollutant tolerance for use in coal gangue stacking areas.

Keywords *Zea mays* L. · Coal gangue stacking area · Agricultural soil samples · Phytotoxicity

Introduction

The National Development and Reform Commission of China (NDRCC) estimated that as of 2011, China had accumulated 4.5 billion metric tons of coal gangue nationwide around coal fields, with an annual increase of approximately 659 million tons of coal gangue (NDRCC 2012). Coal gangue accumulation are prone to spontaneous combustion during stacking, through atmospheric precipitation leaching processes and strong wind erosion, producing coal gangue dust (Jiang et al. 2014) and discharging harmful heavy metals, radioactive elements, polycyclic aromatic hydrocarbons (PAHs), and other organic pollutants into the downstream areas. These environmental pollutants may permeate the deep soil and contaminate groundwater through leaching, causing ecological problems and presenting potential health risks to local residents (Fan et al. 2013; Gonzalez Canibano et al. 1990). It is estimated that in Shanxi Province, a major energy province of China, the coal stockpiles of the province exceed approximately 600 billion tons, representing approximately 30% of the total stores of the country. Excellent coal types and good coal mining conditions have enabled Shanxi coal resources to be exploited and to be utilized on large scales. As a result, large amounts of coal gangue are not effectively recycled and reused and thus

Highlights
• Agricultural soil leachate inhibits maize seed germination and seedling growth.
• The phytotoxicities of agricultural soil leachate are cultivar-dependent.
• O_2^- and H_2O_2 radicals lead to oxidative damage and contribute to maize growth delay.
• ROS scavenging ability leads to differences in tolerance of different maize cultivars.

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pile up on land in open air. The environmental pollution caused by coal gangue accumulation in Shanxi Province and in China is becoming increasingly severe.

Several studies have shown that a variety of environmental pollutants can be detected in the soil and groundwater around coal mines (Bhuiyan et al. 2010; Zhang et al. 2016). Plants can absorb chemical contaminants from soil solutions and soil-air and accumulate them in roots or transport them through the xylem to the above-ground parts, which can lead to phytotoxicity (Doucette et al. 2010). It was reported that 71% of wheat grains in the Xuzhou coal mine area in eastern China were contaminated with Pb, Cd, Cu, Zn, As, and Cr because of excessive mining activities, thereby posing high metal-associated health risks via food ingestion to local residents (Maqbool et al. 2019). The transfer of Pb, Cd, Cr, Fe, Zn, Cu, and Co from soil and water around the coal mining areas in the Allahabad district of Northern India to local common crops has been reported, including cereals (rice, wheat, and maize) and vegetables (spinach and potato) (Rai et al. 2015). The Pb content of rice (0.38 mg/kg) in the vicinity of coal gangue piles in Guqiao Coal Mine, China, was found to exceed the maximal permissible limit of 0.2 mg/kg (Wang et al. 2013). Moreover, PAHs can enter crops via foliar and soil-root uptake (Shi et al. 2017; Tao et al. 2009). In a previous study, 15 PAHs (at 221–432 μg/kg) were detected in the tissues of winter wheat collected from coal combustion areas (Tian et al. 2018). Residents living in coal mine areas will directly or indirectly contact environmental pollutants through the consumption of vegetables and cereals contaminated with these compounds, which may have long-term adverse health effects.

As noted previously, heavy metals and PAHs are commonly found in coal gangue-accumulated soils (Fan et al. 2013) and have been shown to have toxic effects on plants. These chemical contaminants stimulate the excessive production of reactive oxygen species (ROS), which is regarded as an important toxicity response (Ahammed et al. 2012; Emamverdian et al. 2015). Excess ROS production can have a variety of toxic effects, such as lipid peroxidation, protein cleavage, and DNA damage (Unyayar et al. 2006). Furthermore, plants under oxidative stress invoke the antioxidant defense system, including the activation of enzymes and nonenzymatic mechanisms, to scavenge ROS and to avoid oxidative damage (Gill and Tuteja 2010; Unyayar et al. 2006). The variation in the toxic effects of oxidative stress among different plant cultivars might be attributed to their different ROS scavenging capacities (Cho and Seo 2005; Quan et al. 2016). A previous study found that the phytotoxicity of BDE-209 to rice varied among cultivars, that the antioxidant-related enzyme activity of tolerant rice cultivars was higher than that of sensitive cultivars, and that the tolerant cultivars accumulated more L-tryptophan and L-valine to mitigate oxidative stress than did the sensitive cultivars (Li et al. 2018).

Maize (*Zea mays* L.) is a crop widely planted in northern China with high economic value and application prospects. Maize is extremely sensitive to a wide range of pollutants and is often used as a model plant in the study of the ecotoxicological effects of environmental pollutants (Tanyolaç et al. 2007; Wang et al. 2007). In the present study, we used maize as the model organism and assessed the possible toxic effects of agricultural soil samples from coal gangue stacking areas. Through the tolerance indices (TIs) of each morphological trait in seedlings of seven maize cultivars, maize cultivars tolerant and sensitive to agricultural soil were screened. The possible tolerance mechanisms of these two maize cultivars were investigated by analyzing oxidative damage, DNA damage, and antioxidant capacity in maize seedling roots. Since changes in antioxidant capacity may affect the yield and quality of crops, this study can provide a useful reference for crop cultivation in areas contaminated by coal gangue accumulation.

Materials and methods

Study area and soil sample collection

In this study, a coal gangue hill of the Xinjing coal mine in Yangquan City was selected as the study area. Yangquan City is located in the eastern part of Shanxi Province, at 37°52′14.33″N, 113°27′47.90″E, which is the key coal production base in Shanxi Province. Agricultural soil samples were collected approximately 600 m distant from the coal gangue stacking area. The agricultural soil sample was composed of three subsample sites, the surface soil was removed, the topsoil samples (0–20 cm) were collected, and then the three subsamples were mixed thoroughly to obtain a representative soil sample. Packaged soil samples were moved to the laboratory and kept under natural drying conditions for 2 weeks. The agricultural soil samples were sifted through a 100-mesh sieve to obtain particle sizes of less than 0.15 mm in diameter. Following the preprocessing of samples, soil samples were stored at 4 °C for further analysis.

Soil leachate preparation

A 100-g agricultural soil sample was weighed, a 100-mL ultrapure water (1 g/mL) was added, and the sample was vigorously shaken for 3 days. Then, the mixture was precipitated overnight and centrifuged at 3000 rpm for 10 min to obtain soil leachate and stored in a PTFE (polytetrafluoroethylene) gasket brown jar at 4 °C until subsequent toxicological studies.

Seeds and germination

Seven main cultivars of maize seeds cultivated in Shanxi Province were purchased from the Shanxi High-Tech Agricultural Technology Market (Shanxi, China). Maize seeds were surface-sterilized with 3% (v/v) H_2O_2 solution for 30 min, washed with ultrapure water, and then soaked in ultrapure water for 48 h in the dark. Thirty soaked seeds were transferred into individual glass Petri dishes and treated with increasing concentrations of 0, 1:27, 1:9, 1:3, and 1:1 ($V_{soil\ leachate}$: $V_{ultrapure\ water}$) soil leachate exposure solutions. Ultrapure water was used for the control groups and diluted soil leachate. Further, 10 mL of the exposure solution was pipetted into each Petri dish. The seeds were grown in a plant growth chamber at 25 °C in the dark, and the exposure solution was renewed with fresh solution daily. Ten days later, we calculated the germination rates.

Growth inhibition of seedlings

The impact of coal gangue stacking on maize seedling growth was tested after germination. For each treatment, six germinated seedlings that grew uniformly were relocated to a 100-mL beaker with 50 mL of exposure solution for 5 days. Each beaker was wrapped with aluminum foil and parafilm to prevent volatilization and photolysis. The experiments were held in a plant growth chamber (14 h, 25 °C, day; 10 h, 22 °C, night, and a relative humidity of 60%). The exposure solutions were renewed with fresh solutions daily. After 15 days of exposure, the roots and shoots of maize seedlings were washed thoroughly with ultrapure water. Photographs were taken of maize seedling roots of each treatment group under a stereomicroscope (OLYMPUS, SZX2-ILLT, Japan). The root and shoot lengths and biomass (fresh weight of six maize seedlings) were measured. The roots and shoots of maize seedlings were separately harvested. For each cultivar of maize, the relative values obtained by dividing the apparent morphological indices (length, biomass, and germination rate) of agricultural soil leachate treatment groups by the control groups were used as the tolerance indices (TIs) (Wilkins 1957). The TIs were used to screen tolerant and sensitive cultivars for further experiments.

Since the roots are the target tissues that directly contact soil environmental pollutants, the roots are the first organ to feel adverse stress and the first organ to respond to stress (Hossain et al. 2012; Huang et al. 2010), we performed the following experiments on maize roots. Fresh roots were used to analyze the ROS concentration and DNA damage. The remaining roots were frozen in liquid nitrogen instantly and stored at -80 °C for other parameter detection.

Oxidative damage assay

After 15 days of exposure, the fresh root fragments were pestled in a mortar with ice-cold phosphate buffer saline (PBS, 100 mmol/L, pH = 7–7.4) (g/ml = 1:9). After centrifugation at 3000 g for 15 min at 4 °C, the supernatant was collected for further analysis. The ROS contents were measured by using fluorescent probes 2,7-dichlorofluorescein diacetate (DCFH-DA) (Nanjing Jiancheng Biochemistry Co. Ltd, China). DCFH-DA was added into the collected root supernatant at a ratio of 1:200. Following incubation in the dark for 30 min at 37 °C, the fluorescence intensity was measured with a Thermo Scientific Varioskan Flash reader (Thermo Fisher Scientific, USA) at 485 nm excitation and 530 nm emission wavelength. The protein content of the collected supernatant was determined by the Bradford method. The results were expressed as FLU/mg prot. The malondialdehyde (MDA) content was determined following the thiobarbituric acid (TBA) test and measured by the testing kits (Nanjing Jiancheng Biochemistry Co. Ltd, China). The results were expressed as nmol/mg prot. The content of phosphorylated histone H2AX (γ-H2AX) was analyzed with an enzyme-linked immunosorbent assay (ELISA) kit (Meimian Biotechnology Co. Ltd, China). ELISAs were based on a Thermo Scientific Varioskan Flash reader at 450 nm, and the results were expressed as ng/g FW (fresh weight). The specific experimental operations were based on the manufacturer's instructions for the testing kits.

Antioxidant enzyme activities

Enzyme extracts for superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) assays were prepared as described above. The activities of SOD, POD, and CAT in root samples were measured by testing kits (Nanjing Jiancheng Biochemistry Co. Ltd, China), and the methods were mainly performed according to the manufacturer's instructions. In the present study, we used a Thermo Scientific Varioskan Flash reader to measure all absorbance. The results were expressed as U/mg prot.

Statistical analysis

Statistics were performed using SPSS 23.0, and figure generation was conducted using Origin 9.1. All data are presented as the mean ± standard error (SE) and evaluated via one-way ANOVA followed by an LSD test to analyze the mean differences between treated groups and the control. Significant differences were established at $p < 0.05$.

Results

Phytotoxicities of agricultural soil samples from a coal gangue stacking area

After the culture period, the toxic effects of agricultural soil samples from a coal gangue stacking area on seedling growth in seven maize cultivars were examined based on the lengths and biomass of roots and shoots and germination rate at various exposure concentrations of soil leachate (Fig. 1). The results showed that agricultural soil from the coal gangue stacking area had toxic effects on the seedling growth of seven maize cultivars, inhibited the growth of seedlings, and had both concentration and cultivar dependence.

Screening of tolerant and sensitive maize cultivars

As shown in Fig. 1, the different cultivars demonstrated differences in growth performance as the concentration of exposed soil leachate changed. When the concentration of the exposed soil leachate was 1:1, the growth of most cultivars was inhibited compared with that under the control treatment, indicating that the concentration of 1:1 was too high to observe differences in the maize cultivars. When the concentration of the exposed soil leachate was 1:27 or 1:9, it had no marked effect on the growth of some maize cultivars, indicating that this concentration was too low to screen the maize cultivars. In the 1:3 treatment group, the growth of some maize cultivars was inhibited relative to the growth in the control treatment, whereas that of the others was promoted, and significant differences in morphology among the cultivars were apparent. Therefore, we selected the 1:3 concentration group as the optimal concentration for screening tolerant and sensitive cultivars. According to the TI in the 1:3 treatment group, DF30 (abbreviated as DF) was identified as the most tolerant cultivar, and XY335 (abbreviated as XY) was identified as the most sensitive cultivar (Fig. 2). Therefore, these two cultivars were selected for further analysis.

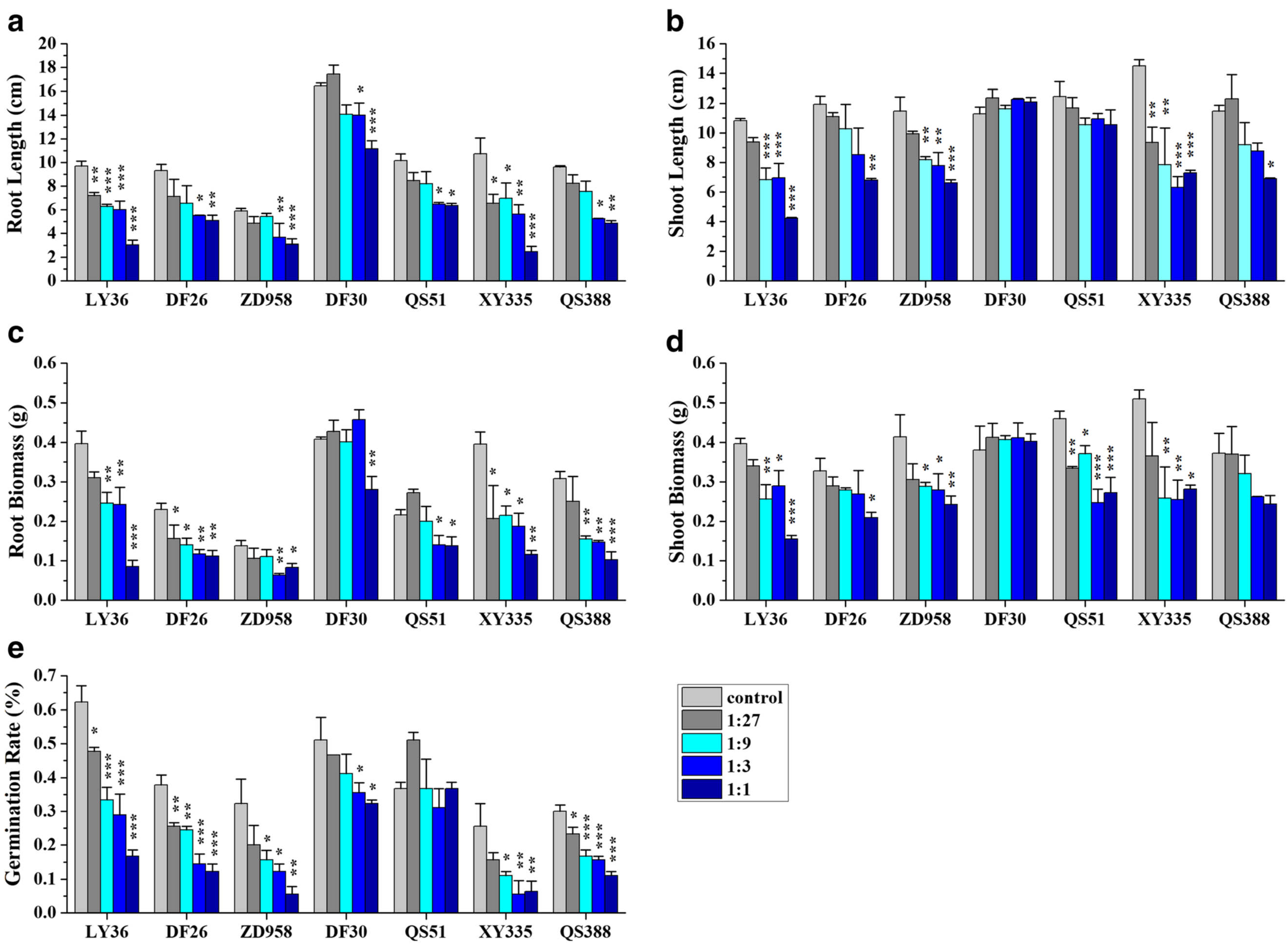

Fig. 1 Effects of agricultural soil leachate on the morphological traits of seedlings of seven maize cultivars. **a** Root length, **b** shoot length, **c** root biomass, **d** shoot biomass, and **e** germination rate. The results are the mean ± SE of triplicates with 6 seedlings each. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ versus the control group

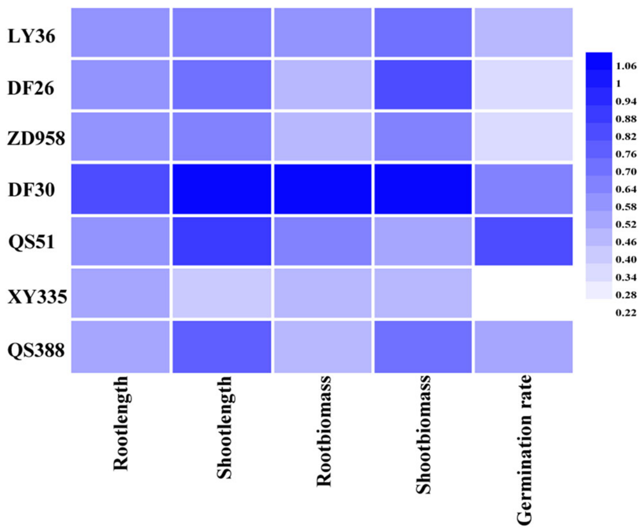

Fig. 2 Heatmap generated by the analysis of tolerance indices (TI) at 1:3 agricultural soil leachate treatment of seven maize cultivars

For the tolerant cultivar DF, in all treatment groups, shoot length and shoot biomass were promoted rather than inhibited, and no significant differences among treatments were observed ($p > 0.05$) (Fig. 1). The root biomass of DF was increased at lower concentrations of agricultural soil leachate (1:27, 1:9, and 1:3) and decreased at the highest concentration (1:1). DF root biomass was reduced by 0.31-fold in the 1:1 treatment group (relative to the control level). The root length of DF was significantly reduced relative to the control value by 0.15- and 0.32-fold, and the germination rate was reduced by 0.30-fold and 0.37-fold at higher concentrations (1:3 and 1:1, respectively). For the sensitive cultivar XY, significant inhibition of root length, shoot length, root biomass, shoot biomass, and germination rate was observed at the lower doses of agricultural soil leachate. For XY, the root length was significantly reduced by 0.39-fold, 0.35-fold, 0.48-fold, and 0.77-fold relative to the control value, respectively, after treatment with 1:27, 1:9, 1:3, and 1:1 soil leachate. The shoot length was significantly reduced by 0.36-fold, 0.46-fold, 0.57-fold, and 0.50-fold compared to the control. The root biomass of XY significantly decreased relative to that of the control biomass by 0.48-fold, 0.46-fold, 0.53-fold, and 0.71-fold when treated with concentrations of 1:27, 1:9, 1:3, and 1:1 of agricultural soil leachate, respectively. Similarly, when treated with concentrations of 1:9, 1:3, and 1:1 of agricultural soil leachate, shoot biomass decreased by 0.49-fold, 0.50-fold, and 0.45-fold, respectively, and the germination rate decreased by 0.57-fold, 0.78-fold, and 0.75-fold, respectively, relative to the control. The tolerant cultivar, DF, only exhibited significant inhibition of seeding growth at the higher concentrations (the 1:3 and 1:1 treatments). However, for the sensitive cultivar, XY, significant inhibition was observed under low concentrations of agricultural soil leachate.

Effects of agricultural soil leachate on root growth

After 15 days of treatment, visible morphological changes in root growth were observed (Fig. 3). For the tolerant cultivar DF and the sensitive cultivar XY, morphological toxicity symptoms, both concentration- and cultivar-dependent, were observed under exposure to agricultural soil leachate. As the concentration increased, the main and lateral roots became shorter, and the number of lateral roots was reduced. In addition, DF showed stronger tolerance to agricultural soil leachate than XY.

Effects of agricultural soil leachate on oxidative damage and DNA damage

Figure 4 shows the changes in ROS, MDA, and γ-H2AX contents. ROS are considered the most important indicators of plant oxidative stress (Choudhury et al. 2017). The present study demonstrated that agricultural soil leachate from a coal gangue stacking area stimulated ROS generation in maize roots (Fig. 4a). As the exposure concentration of agricultural soil leachate increased, the ROS content in the two maize cultivars increased significantly. The ROS contents of XY were increased to greater extents than those of DF. For example, in the 1:1 treatment group, the ROS contents of DF and XY were significantly upregulated by 0.98-fold and 1.29-fold, respectively, relative to those of the control groups. The results suggested that the accumulation of ROS in the sensitive cultivar was higher than that in the tolerant cultivar.

Agricultural soil leachate at various concentrations caused oxidative damage to maize seedlings, damaging principal macromolecules, such as lipids and DNA, at the molecular level. The MDA contents in the two cultivars were generally enhanced as the concentration of agricultural soil leachate increased (Fig. 4b). Specifically, in DF, MDA content in roots did not significantly differ between the control and 1:27, 1:9, or 1:3 treatments but was significantly increased by 0.93-fold at the highest dose of 1:1, relative to the control level. For XY, under 1:9, 1:3, and 1:1 treatment groups, MDA content was significantly elevated by 0.71-fold, 1.09-fold, and 3.44-fold relative to the control level.

In DF, the γ-H2AX level was increased by 0.26-fold in the 1:1 treatment group. In XY, the γ-H2AX level showed significant differences between the control treatment and the 1:3 and 1:1 treatment groups, increasing by 0.53-fold and 0.72-fold, respectively, relative to the control value (Fig. 4c).

Fig. 3 Effects of agricultural soil leachate on the root morphology of **a** DF and **b** XY seedlings

Effects of agricultural soil leachate on antioxidative enzyme activities

The ROS in maize roots induced by agricultural soil leachate were eliminated by the plant antioxidant enzyme system, which involves SOD, CAT, and POD. The three antioxidant enzymes in the two maize cultivars varied with the concentration of agricultural soil leachate (Fig. 5a–c). In the roots of DF, the SOD activity in the exposure groups increased with increasing concentrations of agricultural soil leachate. The activity of SOD reached a maximum at a concentration of 1:1, which was upregulated by 2.62-fold relative to the control activity. However, in XY, SOD activity first increased and then decreased with increasing leachate concentration, with the highest level observed in the 1:9 group, which was upregulated by 0.15-fold of the control value although the difference was not significant. In XY, as the leachate concentration increased, SOD activity began to gradually decrease at 1:3 and was reduced by 0.13-fold compared to the control level at 1:1. SOD activity increased to a greater extent in DF than in XY, which further indicated the higher tolerance of DF than of XY.

CAT activity in the roots of the two cultivars increased with increasing concentrations of agricultural soil leachate. Relative to the corresponding control activities, the activities in the 1:1 group were significantly increased by 1.78-fold in DF but not in XY.

In DF, POD activity demonstrated an increasing trend with increasing concentrations of agricultural soil leachate. For the treatment concentrations of 1:9–1:1, POD activity

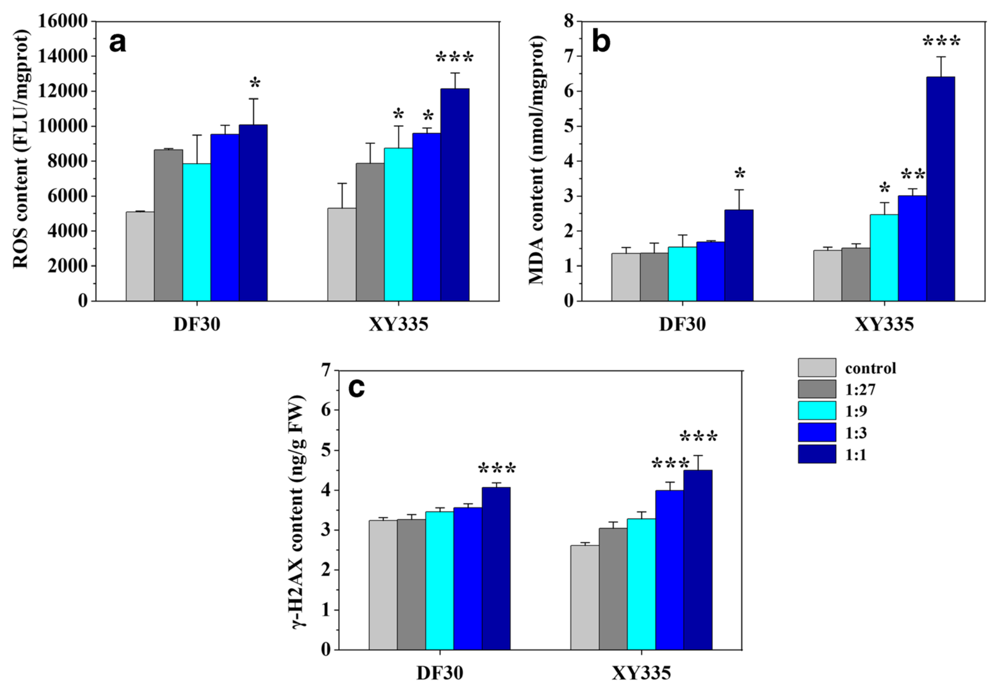

Fig. 4 Oxidative damage and DNA damage in maize roots of two cultivars caused by agricultural soil leachate. **a** ROS content, **b** MDA content, and **c** γ-H2AX content. The results are the mean ± SE of six replicate samples. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus the control group

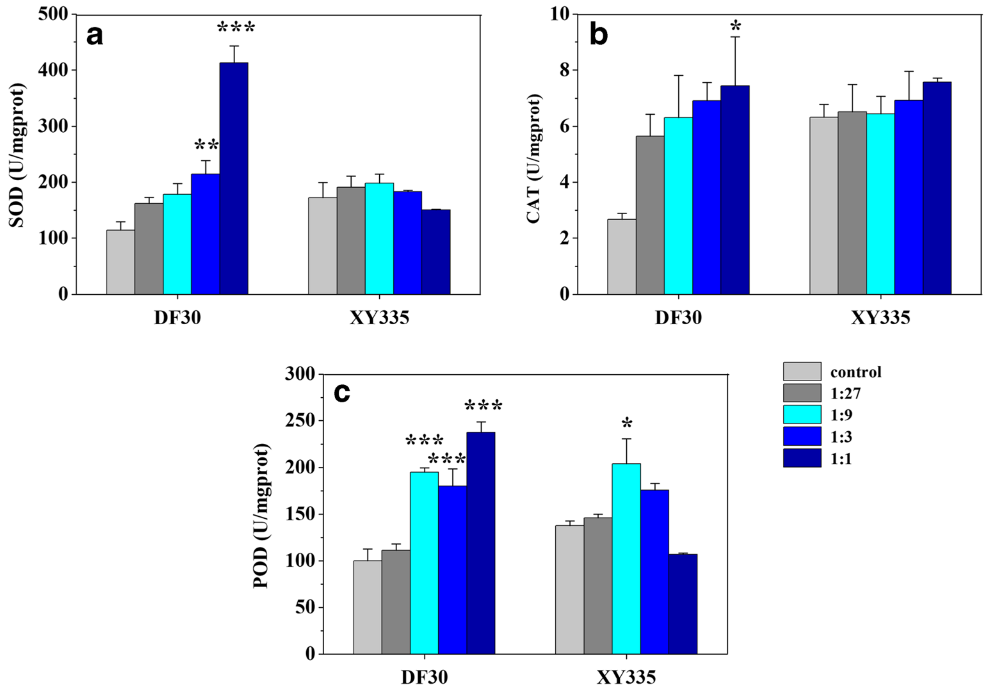

Fig. 5 Antioxidant enzyme activities in maize roots of two cultivars exposed to agricultural soil leachate. **a** SOD activity, **b** CAT activity, and **c** POD activity. The results are the mean ± SE of six replicate samples. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus the control group

increased by 0.95-fold to 1.38-fold relative to the control activity. In XY, POD activity showed an increasing trend at 1:27 and 1:9, and POD activity was significantly upregulated by 0.48-fold relative to the control activity in the 1:9 group. In the 1:3 group, POD activity tended to decrease but remained higher than the control level. At the highest concentration (i.e., in the 1:1 group), POD activity was reduced by 0.22-fold relative to the control activity, but the difference was not significant.

Discussion

Shanxi Province is a coal energy base. The development of long-term, high-intensity coal mining and coal gangue stacking has caused great damage to the environment in the region. However, little is known about the phytotoxicities of agricultural soil samples from a coal gangue stacking area. Moreover, maize is one of the most important crops worldwide. As maize is an important source of human food and animal feed, the security and production of maize in coal gangue stacking areas are closely related to human well-being (Kong et al. 2011). The findings of this study have particular relevance for ecological risk and agricultural production in coal gangue stacking areas.

The growth status of seeds during germination directly affects the growth and biomass yield of the plant. To date, several indicators of seedling growth have been used to study the ecotoxicological effects of heavy metals, PAHs, and other pollutants (Song et al. 2002a; Song et al. 2002b). The analyses of morphological indicators, including plant height, root length, biomass, and germination rate in maize, revealed that agricultural soil leachate had inhibiting effects on the seedling growth of seven maize cultivars and that the inhibiting effect became stronger as the concentration of soil leachate increased. The growth of the tolerant cultivar (DF) showed a pattern of low concentration promotion and high concentration inhibition under soil leachate exposure, whereas the sensitive cultivar (XY) showed inhibition at a low concentration (1:27). This cultivar difference can be attributed to a difference between cultivars in ROS scavenging capacity (Cho and Seo 2005; Quan et al. 2016).

The effects of soil leachate on the structure of maize roots were observed by stereomicroscopy. A previous study of five soybean cultivars found that root morphological traits were potentially the main factors determining cultivar differences in Cd tolerance. The tolerant soybean cultivars had the most extensive root systems at the young seedling stage, whereas the sensitive cultivars had the fewest roots (Wang et al. 2016). Consistent with a previous study, our study revealed that higher concentrations of soil leachate inhibit lateral root development in XY. In addition, the roots of DF show greater tolerance to agricultural soil leachate than those of XY.

The different toxic effects observed between the two maize cultivars may be attributed to differences in their ROS contents after exposure to agricultural soil leachate. A previous study showed that under pollutant stress, ROS accumulation in roots was significantly higher in sensitive plants than in tolerant plants (Cho and Seo 2005). Li et al. (Li et al. 2018) demonstrated that pollutant-sensitive rice exhibited a higher accumulation of ROS than tolerant rice. Our results are consistent with a previous study showing that the higher ROS levels in XY than in DF suggest that the sensitive seedlings experienced greater oxidative stress after soil leachate exposure. The mechanism underlying the toxicity of soil leachate to maize was mainly a reactive mechanism, and the toxic effects were positively correlated with ROS content.

Under pollutant stress, plant cells from various tissues increase their production of ROS, which, if not removed in time, have ecotoxicological effects, such as lipid peroxidation and enzyme inactivation (Winston 1991). To clarify the toxic effects of soil leachate in the two cultivars, including inhibited seedling growth, the MDA and γ-H2AX contents of maize roots after soil leachate exposure were determined. MDA is the final product of lipid peroxidation during oxidative damage, and its content indirectly indicates the level of free radicals in an organism (Qiao et al. 2019). In the present study, soil leachate stimulated greater increases in MDA content in XY than in DF. This result indicated that the damage at the molecular level to XY was more severe than that to DF. Previous studies have demonstrated that in plants treated with heavy metals, tolerant plants have lower levels of lipid peroxidation than sensitive plants (Cho and Seo 2005).

Additionally, γ-H2AX content is a molecular marker of DNA double-strand breaks and thus a useful indicator of genotoxicity; in the present study, γ-H2AX was investigated to estimate the degree of DNA damage in the two cultivars (Dickey et al. 2009). When DNA double-strand breaks (DBSs) occur, γ-H2AX can be aggregated at the DSB site, and the measured content is proportional to the number of DBSs (Toyooka et al. 2017; Xu et al. 2015). A previous study reported that the formation of γ-H2AX "foci" at DSBs in plants was induced by PAHs (Toyooka and Ibuki 2006). In the present study, the yield of γ-H2AX foci indicated that the soil leachate induced greater genotoxicity in XY than in DF (Fig. 4c).

To combat oxidative damage, organisms employ well-developed antioxidant defense systems to scavenge ROS and to resist the toxicity of pollutants (Qiao et al. 2019; Shao et al. 2007). SOD, POD, and CAT play key roles in scavenging ROS. SOD is the first line of the antioxidant defense system, which catalyzes O_2^- to H_2O_2 (Qin et al. 2011; Xie et al. 2013). POD and CAT then convert SOD-catalyzed H_2O_2 into H_2O and O_2 (Monteiro et al. 2012). In the present study, agricultural soil leachate exposure elevated the SOD activities in the roots of the tolerant cultivar DF in a dose-dependent manner, indicating that agricultural soil leachate can stimulate SOD activity and thus influence the scavenging of ROS. In contrast, in the sensitive cultivar XY, SOD activities were inhibited under the high concentration of leachate. The inhibition of SOD activity may occur when the content of ROS exceeds the scavenging capacity of SOD itself (Gill et al. 2015). The results seem to indicate that DF is highly tolerant to soil leachate stress.

The elevated SOD activity in the maize seedlings indicated that the contaminants in the soil leachate induced SOD to catalyze O_2^- to H_2O_2 and led to H_2O_2 accumulation (Sang et al. 2010). Excess H_2O_2 is toxic and must be eliminated by converting it into H_2O in subsequent reactions. Protecting plants from damage by H_2O_2 requires the induction of multiple antioxidants (Malar et al. 2016). Under these conditions, CAT is stimulated to scavenge H_2O_2 (Li et al. 2008). In the present study, CAT activity in the two maize cultivars increased with increasing concentrations of soil leachate and was significantly increased in DF, whereas no marked oxidative stress was observed in XY.

POD is ubiquitously distributed in the plant kingdom and is one of the main enzymes that eliminates active oxygen species (AOS) under stress (Malar et al. 2016). It has been reported that the activity of POD is strongly related to plant tolerance, and the underlying mechanism may involve the removal of toxic peroxides to regulate oxidative stress (Sang et al. 2010). In this study, when the soil leachate concentration was 1:9, POD activity was highest in XY. However, with increasing soil leachate concentration, the H_2O_2 levels increased, eventually exceeding the scavenging capacity of POD. As a result, POD activity was inhibited, and oxidative damage occurred. In contrast, the enzyme activity of DF was enhanced at even the highest soil leachate concentration (1:1). Our results imply that DF better protects against oxidant damage.

The present study demonstrated that the agricultural soil leachate around coal gangue stacking area could induce oxidative stress and accumulate excessive ROS in maize roots. Excessive accumulation of ROS will lead to various oxidation reactions of unsaturated fatty acids in cell membrane, and ROS could cause lipid peroxidation and DNA damage by reacting with biomacromolecules in plants (Mittler et al. 2004; Yun et al. 2019) and finally induced maize seedlings' growth delay. To combat the oxidative damage, the antioxidant defense system was activated to scavenge the ROS (Shao et al. 2007). The greater increase in POD activity than in CAT activity in both cultivars suggests that SOD and POD are the main enzymes involved in ROS scavenging in maize roots. A possible pathway is the catalysis of O_2^- by SOD to produce H_2O_2, which is then catalyzed by POD to produce H_2O. The results of this study demonstrated that differences between tolerant and sensitive maize cultivars in antioxidant ability are one reason for those in toxicity effects among different maize cultivars.

Conclusions

This study confirmed the potential phytotoxicity of the inhibited seed germination and seedling growth of various maize cultivars after exposure to agricultural soil samples around coal gangue stacking areas. DF30 showed more tolerance, and that such tolerance was associated with the stronger activity of the antioxidant enzymes while the less MDA accumulated and DNA damage. The current results demonstrated that DF30 is a highly tolerant crop cultivar and can be considered a promising alternative cultivar in gangue stacking areas.

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Availability of data and materials The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions G. L. conceived and designed the experiments. N. S. provided much of the work for the revision of the manuscript. F. Y. drafted the manuscript and conducted the experiments. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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