1 Introduction

With the rapid worldwide agricultural development, the issues of heavy metal pollution have been taken into paramount considerations. Heavy metal pollution exerts severe toxic effects on potable water, and leads to soil erosion and food crop contamination, thus directly posing a severe threat to food security and human health [1–6]. The ions of such heavy metal elements as zinc, copper, manganese, cadmium, mercury, and lead exert great unfavorable effects on plant growth, especially on the growth of seedlings at the early developmental stages [7–19]. Due to their toxicities, heavy metal elements bring about severe adverse effects on reactive oxygen species (ROS) metabolism, on growth conditions and on physiologically and biochemically developmental process of plants [20, 21].

Under various kinds of heavy metal stresses, many plants experience disorders in their metabolism process, and such disorders often directly exert disadvantageous effects on plant growth and development, which even causes death of a whole plant [22, 23]. Therefore, it is of urgent importance to pay more attentions to the issues of plant damages caused by heavy metal toxicities.

As heavy metal elements, zinc (Zn), copper (Cu), manganese (Mn), cadmium (Cd), mercury (Hg), and lead (Pb) exert certain adverse effects on plant growth and development through the form of divalent cations. A myriad of researches [24–27] have shown that heavy metal ions exerted considerably negative effects in lowering the photosynthesis rate through damaging photosystems. The toxic effect of heavy metals on plant growth and development is commonly known. Inhibition of growth limited photosynthesis and respiration. Inhibited biosynthesis of chlorophyll and carotenoids and reduced phosphorylation are most frequently observed symptoms of metal toxicity.

Such elements as Zn, Cu, Mn are considered as micro-nutritional elements needed for plant growth and development. Zinc serves as an essential micro-nutrition for plants, functioning as an indispensable catalytic component of over 300 enzymes, including...
Cu/Zn superoxide dismutase (SOD) [7, 9]. Copper is a trace element necessary for plant nutrition, it plays an important role in many physiological processes. However, it can become toxic to plants at high concentrations [27, 28]. Manganese (Mn) represents an essential plant nutrient acting as the key part of prosthetic groups in important physiological and biochemical processes, including photosynthesis. Moreover, Mn also involves in the process of ROS scavenging in mitochondria through serving as a cofactor in a Mn-containing SOD (Mn-SOD) [29, 30].

Cadmium (Cd) is a non-essential element in plants, recognized as one of the most potentially hazardous of all metal pollutants because it shows phytotoxicity, even at low doses, causing perturbations in various plant processes, such as development of chloroplasts, photosynthesis, biological nitrogen fixation, sulfate assimilation, and respiration [31, 32]. As a highly reactive heavy metal, cadmium (Cd) can be found in low concentrations in the environment and shows a high affinity towards many bio-molecules [33, 34], thus interfering with various physiological processes that are indispensable for plant growth and development such as photosynthesis, respiration, and transpiration [35–37]. As highly toxic heavy metal elements, mercury (Hg) and lead (Pb) exerted certain unfavorable effects on many physiological and biochemical processes in plants, causing reduced photosynthesis rates and lowered plants’ resistances or tolerances to many adverse biotic and abiotic stresses.

Activities of catalase (CAT) and SOD have long been considered as important physiological indexes for determining plants’ resistances or tolerances to both biotic and abiotic stresses exerted by non-optimal factors [38, 39]. Results from many researches [31–34, 39–44] indicated that CAT and SOD activities can be affected by many non-optimal factors for plant growth and development. Therefore, under abiotic stresses, such as those exerted by heavy metal ions, changes in CAT and SOD activities can reflect plants’ physiological and biochemical processes in response to these stresses.

As a sort of organic acid, salicylic acid (SA) is believed to exert alleviative effects on plants growing under heavy metal ion-stressed environment. As a novel kind of endogenous plant hormone, SA exerts beneficial physiological effects on plants. SA can promote florescence process, improve plants disease-resistance, reduce transpiration and inhibit ethylene synthesis. And under ozone stress and osmotic stress, SA can induce the generation of some stress proteins. The results of numerous researches showed that SA could alleviate the stress effects on many heavy metal elements on a variety of crops [20–23].

A increasing number of experimental researches also indicated that SA can induce plants’ resistances to biotic stresses caused by fungi, bacteria, and viruses, and certain plants’ resistances (or tolerances) to abiotic stresses resulted from heavy metals, ozone, UV radiation, low temperature, heat shock, water deficit, salt, and so forth [20–23, 43–48]. In addition, it has been also indicated in many recent researches [20–23] that SA may enhance resistances or tolerances of many plants to abiotic stresses through involving in SA-mediated cellular signal transduction pathways, which induces a cascade of biochemical and physiological processes in plants, while both the physiological and molecular mechanisms for such pathways are still largely unknown.

Many researches concerning the physiological and molecular mechanisms for cereal crops’ responses to stresses exerted by heavy metal ions paid more attentions to wheat, maize, oat, and rice and attached less important to such an economically promising crop as barley [27, 40–42]. Therefore, in this paper, we aimed to study the physiological mechanism for the changes of CAT and SOD activities in malting barley seedling leaves under different concentrations of different heavy metal ion treatments and SA’s alleviative effects on these treatments, so as to provide certain theoretical references for the studies of wheat growth in response to unfavorable heavy metal polluted environments, and for the breeding practices of malting barley for the selection of malting barley cultivars with high tolerances or resistances to different heavy metal ions.

2 Materials and methods

2.1 Plant Material

A malting barley genotype (named Ganpi No.4) was kindly provided by Gansu Provincial Academy of Agricultural Sciences, Lanzhou, Gansu Province, P. R. China.

2.2 Seed Sterilization and Germination

Seeds of the selected barley genotype were surface-sterilized through utilizing 1% NaClO for 20 min, then washed by flushing water and placed into darkness for 24 h germination.

2.3 Hydroponic Cultivation of Seedlings and Zn^{2+}, Cu^{2+}, Mn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} Treatments

The germinated barley seedlings were planted into vermiculite-covered white ceramic plates for initial stage of seedling growth, and the plates were placed into an incubator with a set illumination time of 12 h/day and a temperature of around 25°C. At single-leave stage, the seedlings of different genotypes were placed into plastic pots with the addition of half-strength Hoagland solutions for further cultivation. At three-leave stage, the leaves of cultivated seedlings were taken and treated under different concentrations of Zn^{2+}, Cu^{2+}, Mn^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} for 7 days, respectively. The treatments for each kind of heavy metals were set as T0 (CK, with no addition of any heavy metal ions), T_{Zn1} (0.1 mM Zn^{2+}), T_{Zn2} (0.2 mM Zn^{2+}), T_{Zn3} (0.5 mM Zn^{2+}), T_{Zn4} (1 mM Zn^{2+}), T_{Zn5} (0.1 mM Zn^{2+} + 2 mM SA), T_{Zn6} (0.2 mM Zn^{2+} + 2 mM SA), T_{Zn7} (0.5 mM Zn^{2+} + 2 mM SA), T_{Zn8} (1 mM Zn^{2+} + 2 mM SA), T_{Cu1} (0.1 mM Cu^{2+}), T_{Cu2} (0.2 mM Cu^{2+}), T_{Cu3} (0.5 mM Cu^{2+}), T_{Cu4} (1 mM Cu^{2+}), T_{Cu5} (0.1 mM Cu^{2+} + 2 mM SA), T_{Cu6} (0.2 mM Cu^{2+} + 2 mM SA), T_{Cu7} (0.5 mM Cu^{2+} + 2 mM SA), T_{Cu8} (1 mM Cu^{2+} + 2 mM SA), T_{Mn1} (0.1 mM Mn^{2+}), T_{Mn2} (0.2 mM Mn^{2+}), T_{Mn3} (0.5 mM Mn^{2+}), T_{Mn4} (1 mM Mn^{2+}), T_{Mn5} (0.1 mM Mn^{2+} + 2 mM SA), T_{Mn6} (0.2 mM Mn^{2+} + 2 mM SA), T_{Mn7} (0.5 mM Mn^{2+} + 2 mM SA), T_{Mn8} (1 mM Mn^{2+} + 2 mM SA), T_{Hg1} (0.1 mM Hg^{2+}), T_{Hg2} (0.2 mM Hg^{2+}), T_{Hg3} (0.5 mM Hg^{2+}), T_{Hg4} (1 mM Hg^{2+}), T_{Hg5} (0.1 mM Hg^{2+} + 2 mM SA), T_{Hg6} (0.2 mM Hg^{2+} + 2 mM SA), T_{Hg7} (0.5 mM Hg^{2+} + 2 mM SA), T_{Hg8} (1 mM Hg^{2+} + 2 mM SA), T_{Pb1} (0.1 mM Pb^{2+}), T_{Pb2} (0.2 mM Pb^{2+}), T_{Pb3} (0.5 mM Pb^{2+}), T_{Pb4} (1 mM Pb^{2+}), T_{Pb5} (0.1 mM Pb^{2+} + 2 mM SA), T_{Pb6} (0.2 mM Pb^{2+} + 2 mM SA), T_{Pb7} (0.5 mM Pb^{2+} + 2 mM SA), T_{Pb8} (1 mM Pb^{2+} + 2 mM SA), respectively. All the above-mentioned treatments were conducted through the addition of ZnCl_2, CuCl_2, MnCl_2, CdCl_2, HgCl_2, PbCl_2.
respectively, whilst Zn$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, and Pb$^{2+}$ concentrations were calculated and recorded as pure Zn, Cu, Mn, Cd, Hg, and Pb element concentrations, respectively.

### 2.4 Detection of CAT activity

CAT activity was measured according to the method of Wei et al. [40] with a slight modification. Seedling leaves of the selected barley genotype were taken, 0.1 g for each measurement. Enzymatic solution with a volume of 100 μL was added with 3 mL PBS solution with a concentration of 50 mM (pH 7). The mixed solution was incubated at 25°C for 5 min. Then the reaction of measurement was initiated with the addition of 6 μM H$_2$O$_2$. At the wavelength of 240 nm, time scanning was conducted for 2 min and every 20 s was adopted as time interval. A unit of CAT activity was calculated as reference to the method described by Shao et al. [41], and presented as U/g FW.

### 2.5 Detection of SOD activity

SOD activity was measured in reference to the method of nitroblue tetrazolium (NBT) photochemical reduction described by Wei et al. [40] and Shao et al. [41] with little modification. Seedling leaves of the selected barley genotype were taken, 0.1 g for each measurement. PBS reactive solution (pH 7, containing 13 mM methionine, 75 mM NBT, and 0.1 mM Na$_2$EDTA) was added with 50 μL enzymatic solution and the measurement reaction was initiated through addition of 2 μM riboflavin. The mixed solution was illuminated at 25°C for 5 min and the absorbance values at wavelength of 560 nm were measured using UV-spectrophotometer, and the un-illuminated solution was utilized as blank. A unit of SOD activity was calculated as enzyme demand for 50% NBT reductive inhibition, and exhibited as U/g FW.

### 2.6 Statistical Analysis

The results presented in this paper are the mean values ±SE (standard errors) obtained from at least three replicates. Significant differences between the treated (under Zn$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Pb$^{2+}$ treatments and under SA-alleviated treatments, respectively) and control group of barley seedlings were determined using ANOVA test ($p < 0.05$ and $p < 0.01$). Data analysis was conducted through the utilization of SPSS 18.0 Software, through which regression analysis was implemented and the fitting equations for each treatment was acquired.

### 3 Result and discussion

#### 3.1 The alleviative effects of SA on CAT and SOD activities in barley seedling leaves treated by different Zn$^{2+}$ treatments

The measurement results of CAT and SOD activities in barley seedling leaves treated by different Zn$^{2+}$ treatments and SA-alleviated Zn$^{2+}$ treatments are shown in Figs. 1 and 2. From Fig. 1, it can be apparently seen that both CAT activities and Zn$^{2+}$ concentrations, and CAT activities and SA-alleviated Zn$^{2+}$ concentrations showed nonlinear relationships ($R^2 = 0.8941$ and 0.9052, respectively, $p < 0.05$). According to Fig. 1a, under Zn$^{2+}$ treatments, the value of CAT activity witnessed a general trend of decline, albeit such value was greater at T$_{Zn6}$ (0.2 mM Zn$^{2+}$) than at T$_{Zn1}$ (0.1 mM Zn$^{2+}$) treatment. And all CAT activity values at Zn treatments were less than that at CK, showing that different Zn$^{2+}$ treatments exerted negative effects to CAT activity in barley leaves during seedling growth. At T$_{Zn4}$ (1 mM Zn$^{2+}$) treatment, the minimum CAT activity value occurred, indicating that compared with all other Zn treatments, T$_{Zn4}$ exerted the most apparent effects in inhibiting CAT activity in seedling leaves. According to Fig. 1b, under Zn$^{2+}$-SA combined treatments, the value of CAT activity also experienced a general trend of decline, albeit such value was greater at T$_{Zn6}$ (0.2 mM Zn$^{2+}$ + 2 mM SA) than at T$_{Zn5}$ (0.1 mM Zn$^{2+}$ + 2 mM SA) treatment. Comparing Fig. 1a with Fig. 1b, it can be obviously seen that CAT activity values were significantly greater at T$_{Zn5}$, T$_{Zn6}$, T$_{Zn7}$, and T$_{Zn8}$ than at T$_{Zn1}$, T$_{Zn2}$, T$_{Zn3}$, and T$_{Zn4}$, respectively ($p < 0.05$), while at T$_{Zn4}$, T$_{Zn5}$, T$_{Zn6}$, T$_{Zn7}$, and T$_{Zn8}$ treatments exerted the most apparent effects in inhibiting CAT activity in seedling leaves. According to Fig. 1b, under Zn$^{2+}$-SA combined treatments, the value of CAT activity reached a minimum at T$_{Zn4}$ ($y = -2.5x^3 + 25x^2 - 94.5x + 455.4$, $R^2 = 0.9025$). Comparing Fig. 1a with Fig. 1b, it can be obviously seen that CAT activity values were significantly greater at T$_{Zn5}$, T$_{Zn6}$, T$_{Zn7}$, and T$_{Zn8}$ than at T$_{Zn1}$, T$_{Zn2}$, T$_{Zn3}$, and T$_{Zn4}$, respectively ($p < 0.05$), while at T$_{Zn4}$, T$_{Zn5}$, T$_{Zn6}$, T$_{Zn7}$, and T$_{Zn8}$ treatments exerted the most apparent effects in inhibiting CAT activity in seedling leaves. According to Fig. 1b, under Zn$^{2+}$-SA combined treatments, the value of CAT activity reached a minimum at T$_{Zn4}$ ($y = -2.5x^3 + 25x^2 - 94.5x + 455.4$, $R^2 = 0.9025$). Comparing Fig. 1a with Fig. 1b, it can be obviously seen that CAT activity values were significantly greater at T$_{Zn5}$, T$_{Zn6}$, T$_{Zn7}$, and T$_{Zn8}$ than at T$_{Zn1}$, T$_{Zn2}$, T$_{Zn3}$, and T$_{Zn4}$, respectively ($p < 0.05$)

![Figure 1. Effects of Zn treatments and SA–Zn combined treatments on CAT activities in seedling leaves ($p < 0.05$).](image)

![Figure 2. Effects of Zn treatments and SA–Zn combined treatments on SOD activities in seedling leaves ($p < 0.05$).](image)
demonstrating that SA exerted certain alleviative effects in enhancing CAT activity in seedling leaves stressed by different Zn\textsuperscript{2+} treatments.

From Fig. 2, it can be obviously observed that both SOD activities and Zn\textsuperscript{2+} concentrations, and SOD activities and SA-alleviated Zn\textsuperscript{2+} concentrations exhibited nonlinear relationships ($R^2 = 0.9542$ and 0.8106, respectively, $p < 0.05$). According to Fig. 2a, under Zn\textsuperscript{2+} treatment, the maximum value of SOD activity occurred at $T_{Zn3}$ treatment and amounted to 205 U\textsuperscript{g-1} FW, which was significantly higher than such value at CK ($p < 0.05$), showing that $T_{Zn3}$ treatment exerted certain effects in improving SOD activity. SOD activity was slightly higher than at $T_{Zn2}$ and $T_{Zn4}$ treatments, respectively ($p < 0.05$), and such value was extremely significantly greater at $T_{Zn5}$, $T_{Zn6}$, and $T_{Zn8}$ treatments than at CK, indicating that Zn\textsuperscript{2+} concentrations $>0.1$ mM exerted limited effects in influencing SOD activity. According to Fig. 2b, under Zn\textsuperscript{2+}-SA combined treatment, the value of SOD activity were significantly greater at $T_{Zn5}$, $T_{Zn6}$, and $T_{Zn8}$ treatments than at CK ($p < 0.05$), and such value was extremely significantly greater at $T_{Zn7}$ treatment than at CK ($p < 0.01$), demonstrating that SA exerted certain effects in improving SOD activity and such effects were most effective for seedling leaves stressed by $T_{Zn3}$ treatment. Comparing Fig. 2a with Fig. 2b, it can be apparently observed that SOD activity at $T_{Zn5}$ was slightly higher than at $T_{Zn1}$, SOD activities at $T_{Zn6}$ and $T_{Zn8}$ treatments were significantly higher than at $T_{Zn2}$ and $T_{Zn4}$ treatments, respectively ($p < 0.05$), and SOD activity at $T_{Zn7}$ was extremely significantly greater than at $T_{Zn3}$ ($p < 0.01$), showing that SA exerted certain effects in mitigating Zn\textsuperscript{2+} stresses, and such alleviative effects of SA were most effective for Zn\textsuperscript{2+} concentration of 0.5 mM.

### 3.2 The alleviative effects of SA on CAT and SOD activities in barley seedling leaves treated by different Cu\textsuperscript{2+} treatments

The measurement results of CAT and SOD activities in barley seedling leaves treated by different Cu\textsuperscript{2+} treatments and SA-alleviated Cu\textsuperscript{2+} treatments are shown in Figs. 3 and 4. From Fig. 3, it can be apparently seen that both CAT activities and Cu\textsuperscript{2+} concentrations, and CAT activities and SA-alleviated Cu\textsuperscript{2+} concentrations showed nonlinear relationships ($R^2 = 0.9883$ and 0.9995, respectively, $p < 0.05$). According to Fig. 3a, under Cu\textsuperscript{2+} treatments, the value of CAT activity increased with increasing Cu\textsuperscript{2+} concentrations, and such effects were most effective for seedling leaves stressed by higher Cu\textsuperscript{2+} concentrations. Comparing Fig. 3a with b, it can be clearly observed that the differences between SOD activities under $T_{Cu5}$ and $T_{Cu6}$, and under $T_{Cu7}$ and $T_{Cu8}$ were not significant, while the differences between SOD activities under $T_{Cu3}$ and $T_{Cu7}$, and under $T_{Cu4}$ and $T_{Cu8}$ amounted to the level of CAT activity experienced a general trend of decline, showing that Cu\textsuperscript{2+} treatments exerted certain inhibitive effects on CAT activity. CAT activity values were significantly greater at $T_{Cu1}$ and $T_{Cu2}$ treatments than at CK ($p < 0.05$) and were extremely significantly higher at $T_{Cu3}$ and $T_{Cu4}$ treatments than at CK ($p < 0.01$), indicating that higher Cu\textsuperscript{2+} concentrations (0.5 and 1 mM) exerted more striking inhibitory effects on CAT activity in barley seedling leaves than lower Cu\textsuperscript{2+} concentrations (0.1 and 0.2 mM) did. The minimum CAT activity value occurred at $T_{Cu4}$ treatment, indicating that the highest Cu\textsuperscript{2+} concentration exerted the strongest inhibitory effects on CAT activity. According to Fig. 3b, under Cu\textsuperscript{2+}-SA combined treatment the value of CAT activity also witnessed a general trend of decline. Such a trend indicated that SA exerted less alleviative effects on seedling leaves stressed by higher Cu\textsuperscript{2+} concentrations than those stressed by lower Cu\textsuperscript{2+} concentrations. Comparing Fig. 3a with Fig. 3b, it can be apparently seen that there occurred no significant differences between $T_{Cu5}$ and $T_{Cu6}$, between $T_{Cu6}$ and $T_{Cu2}$ between $T_{Cu7}$ and $T_{Cu3}$, and between $T_{Cu8}$ and $T_{Cu4}$, demonstrating that SA exerted merely slight effects in alleviating CAT activity.

From Fig. 4, it can be obviously observed that both SOD activities and Cu\textsuperscript{2+} concentrations, and SOD activities and SA-alleviated Cu\textsuperscript{2+} concentrations showed nonlinear relationships ($R^2 = 0.9462$ and 0.8705, respectively, $p < 0.05$). According to Fig. 4a, from CK to $T_{Cu2}$, the value of SOD activity experienced a trend of gradual increase, while from $T_{Cu2}$ to $T_{Cu4}$ such value witnessed a trend of gradual decrease, demonstrating that lower Cu\textsuperscript{2+} concentrations (0.1 and 0.2 mM) exerted activating effects on SOD activity while higher Cu\textsuperscript{2+} concentrations (0.5 and 1 mM) exerted inhibiting effects on SOD activity. At $T_{Cu2}$ the maximum value of SOD activity occurred, indicating that Cu\textsuperscript{2+} concentration of 0.2 mM exerted certain effects in enhancing SOD activity. According to Fig. 4b, from CK to $T_{Cu2}$, the value of SOD activity witnessed a trend of gradual increase, while from $T_{Cu2}$ to $T_{Cu4}$ such value experienced a trend of gradual decrease, demonstrating that SA exerted no effects in changing the trend for SOD activity under Cu\textsuperscript{2+} treatments. Comparing Fig. 4a with b, it can be clearly seen that the differences between SOD activities under $T_{Cu1}$ and $T_{Cu5}$, and under $T_{Cu2}$ and $T_{Cu6}$ were not significant, while the differences between SOD activities under $T_{Cu3}$ and $T_{Cu7}$, and under $T_{Cu4}$ and $T_{Cu8}$ amounted to the level of...
of significance ($p < 0.05$), showing that SA exerted slight effects in alleviating SOD activity under lower Cu$^{2+}$ concentrations (0.1 and 0.2 mM) while exerted significant effects under higher Cu$^{2+}$ concentrations (0.5 and 1 mM).

### 3.3 The alleviative effects of SA on CAT and SOD activities in barley seedling leaves treated by different Mn$^{2+}$ treatments

The measurement results of CAT and SOD activities in barley seedling leaves treated by different Mn$^{2+}$ treatments and SA-alleviated Mn$^{2+}$ treatments are shown in Figs. 5 and 6. From Fig. 5, it can be clearly observed that both CAT activities and Mn$^{2+}$ concentrations, and CAT activities and SA-alleviated Mn$^{2+}$ concentrations exhibited nonlinear relationships ($R^2 = 0.9983$ and 0.9939, respectively, $p < 0.05$). According to Fig. 5a, under Mn$^{2+}$ treatment, the value of CAT activity witnessed a general trend of gradual decrease, and such values at all Mn$^{2+}$ treatments were smaller than that at CK, indicating that Mn$^{2+}$ treatments exerted certain inhibitive effects on CAT activity. According to Fig. 5b, under SA-alleviated Mn$^{2+}$ treatment, the value of CAT activity also experienced a general trend of gradual decrease, and such values at all Mn$^{2+}$ treatments were slightly smaller than that at CK, showing that SA-alleviated Mn$^{2+}$ treatment exerted limited inhibitive effects on CAT activity. Comparing Fig. 5a with b, it can be clearly observed that CAT activities were merely slightly greater at TMn3 and TMn5 treatments than at TmCn1 and TmCn3 treatments, respectively, while significantly greater at TMn4 treatment than at TmCn4 treatment ($p < 0.05$), indicating that SA exerted significant alleviative effects on CAT activity merely at the highest Mn$^{2+}$ concentration (1 mM Mn$^{2+}$).

From Fig. 6, it can be clearly observed that both CAT activities and Mn$^{2+}$ concentrations, and SOD activities and SA-alleviated Mn$^{2+}$ concentrations exhibited nonlinear relationships ($R^2 = 0.9923$ and 0.7795, respectively, $p < 0.05$). According to Fig. 6a, the value of SOD activity witnessed a trend of increase from CK to TMn2 treatment, and then experienced a tendency of decrease from TMn2 to TMn4 treatment, indicating that lower Mn$^{2+}$ concentrations (0.1 and 0.2 mM) exerted certain enhancing effects on SOD activity while higher Mn$^{2+}$ concentrations (0.5 and 1 mM) exerted certain inhibiting effects on SOD activity. According to Fig. 6b, SOD activity value experienced a trend of increase from CK to TMn6 treatment, and then witnessed a trend of decrease from TMn6 to TMn8 treatment, demonstrating that SA-alleviated Mn$^{2+}$ treatments exerted certain effects on SOD activity, which were similar to those exerted by Mn$^{2+}$ treatments. Comparing Fig. 6a with b, it can be clearly observed that SOD activity values were lower at TMn6 and TMn8 than at TMn1 and TMn3, respectively, and such values were higher at TMn4 and TMn6 than at TMn2 and TMn8, respectively, showing that SA exerted merely limited effects in changing SOD activity under Mn$^{2+}$ treatments.

### 3.4 The alleviative effects of SA on CAT and SOD activities in barley seedling leaves treated by different Cd$^{2+}$ treatments

The measurement results of CAT and SOD activities in barley seedling leaves treated by different Cd$^{2+}$ treatments and SA-alleviated Cd$^{2+}$ treatments are shown in Figs. 7 and 8. From Fig. 7, it can be clearly observed that both CAT activities and Cd$^{2+}$ concentrations, and CAT activities and SA-alleviated Cd$^{2+}$ concentrations exhibited nonlinear relationships ($R^2 = 0.9974$ and 0.9999, respectively, $p < 0.05$). According to Fig. 7a, under Cd$^{2+}$ treatments, the value of CAT activity witnessed a tendency of gradually rapid decrease, and such values were significantly lower at TCd1 and TCd2 than at CK ($p < 0.05$), and extremely significantly lower at TCd3 and TCd4 than at CK ($p < 0.01$), showing that Cd$^{2+}$ treatments exerted certain inhibitive effects on CAT activity in barley seedling leaves. According to Fig. 7b, under SA-alleviated Cd$^{2+}$ treatments, CAT activity value also experienced a trend of gradually rapid decrease, and such values were merely slightly lower at TCd3 than at CK, significantly lower at TCd6 than at CK, and extremely significantly lower at TCd7 and TCd8 than at CK ($p < 0.01$), suggesting that SA-alleviated Cd$^{2+}$ treatments exerted similar effects on CAT activity as Cd$^{2+}$ treatments did, albeit there occurred no significant difference between CAT activity at TCd5 and such value at CK. Comparing Fig. 7a with b, it can be apparently observed that there were no significant differ-
treatment, suggesting that SA-alleviated Cd$^{2+}$ treatments exerted similar effects on SOD activity as Cd$^{2+}$ treatments did. Comparing Fig. 8a with b, it can be clearly observed that there occurred no significant differences between SOD activities at TCd8 and TCd2, at TCd6 and TCd2, at TCd7 and TCd2, and at TCd8 and TCd4, indicating that SA exerted limited effects in changing SOD activity at Cd$^{2+}$ treatments.

### 3.5 The alleviative effects of SA on CAT and SOD activities in barley seedling leaves treated by different Hg$^{2+}$ treatments

The measurement results of CAT and SOD activities in barley seedling leaves treated by different Hg$^{2+}$ treatments and SA-alleviated Hg$^{2+}$ treatments were shown in Figs. 9 and 10. From Fig. 9, it can be apparently seen that both CAT and SOD activities and CAT activities and SA-alleviated Hg$^{2+}$ concentrations showed nonlinear relationships ($R^2 = 0.9936$ and 0.9974, respectively, $p < 0.05$). According to Fig. 9a, under Hg$^{2+}$ treatments, the value of CAT activity witnessed a tendency of gradual decrease and such values were significantly lower at THg5 and THg6 than at CK ($p < 0.05$), and extremely significantly lower at THg3 and THg4 than at CK ($p < 0.01$), showing that Hg$^{2+}$ treatments exerted certain degrees of inhibitive effects on CAT activity in barley seedling leaves. According to Fig. 9b, under SA-alleviated Hg$^{2+}$ treatments, the value of CAT activity also experienced a tendency of gradual decrease, while such values were slightly lower at THg6 and THg7 than at CK and extremely significantly lower at THg3 and THg4 than at CK ($p < 0.01$), showing that SA-alleviated Hg$^{2+}$ treatments exerted slightly different effects on CAT activity from what Hg$^{2+}$ treatments did. Comparing Fig. 9a with b, it can be apparently observed that values of CAT activity were slightly higher at THg5 and THg6 than at THg3 and THg4, respectively, while such values were significantly greater at THg5 and THg6 than at THg3 and THg4 ($p < 0.05$), respectively, demonstrating that SA exerted limited effects in influencing CAT activity at lower concentrations of Hg$^{2+}$ (0.1 and 0.2 mM), while SA exerted certain effects in

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**Figure 7.** Effects of Cd treatments and SA–Cd combined treatments on CAT activities in seedling leaves ($p < 0.05$).

**Figure 8.** Effects of Cd treatments and SA–Cd combined treatments on SOD activities in seedling leaves ($p < 0.05$).

**Figure 9.** Effects of Hg treatments and SA–Hg combined treatments on CAT activities in seedling leaves ($p < 0.05$).

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alleviating CAT activity at higher concentrations of Hg\textsuperscript{2+} (0.5 and 1 mM).

From Fig. 10, it can be clearly observed that both SOD activities and Hg\textsuperscript{2+} concentrations, and SOD activities and SA-alleviated Hg\textsuperscript{2+} concentrations showed nonlinear relationships ($R^2 = 0.9994$ and 0.999, respectively, $p < 0.05$). According to Fig. 10a, under Hg\textsuperscript{2+} treatments, the value of SOD activity experienced a tendency of increase from CK to THg1 treatment, and then witnessed a trend of gradual decrease from THg2 to THg4, and SOD values were significantly high at THg5 and THg6 than at CK ($p < 0.05$), whilst SOD values were slightly lower at THg1 and THg2 than at CK, demonstrating that different Hg\textsuperscript{2+} treatments exerted different effects on SOD activity in promoting SOD activity, while higher concentrations of Hg\textsuperscript{2+} (0.5 and 1 mM) exerted slight effects in repressing SOD activity. According to Fig. 10b, under SA-alleviated Hg\textsuperscript{2+} treatments, the value of SOD activity witnessed a tendency of increase from CK to THg5 treatment, and then experienced a trend of gradual decrease from THg6 to THg8 treatment, and SOD values were significantly high at THg5 and THg6 than at CK ($p < 0.05$), whilst SOD values were slightly lower at THg1 and THg2 that at CK, demonstrating that different Hg\textsuperscript{2+} treatments exerted different effects on SOD activity, namely lower concentrations of Hg\textsuperscript{2+} (0.1 and 0.2 mM) exerted significant effects in promoting SOD activity, while higher concentrations of Hg\textsuperscript{2+} (0.5 and 1 mM) exerted slight effects in repressing SOD activity.

According to Fig. 10a, under Hg\textsuperscript{2+} treatments, the value of SOD activity exhibited a tendency of decrease from CK to THg1 treatment, and then experienced a trend of gradual decrease from THg2 to THg4, and SOD values were significantly high at THg5 and THg6 than at CK ($p < 0.05$), whilst SOD values were slightly lower at THg1 and THg2 than at CK, demonstrating that different Hg\textsuperscript{2+} treatments exerted different effects on SOD activity in promoting SOD activity, while higher concentrations of Hg\textsuperscript{2+} (0.5 and 1 mM) exerted slight effects in repressing SOD activity. According to Fig. 10b, under SA-alleviated Hg\textsuperscript{2+} treatments, the value of SOD activity witnessed a tendency of increase from CK to THg5 treatment, and then experienced a trend of gradual decrease from THg6 to THg8 treatment, and SOD values were significantly high at THg5 and THg6 than at CK ($p < 0.05$), whilst SOD values were slightly lower at THg1 and THg2 than at CK, demonstrating that different concentrations of Pb\textsuperscript{2+} treatments exerted different effects in repressing SOD activity. According to Fig. 10b, under SA-alleviated Hg\textsuperscript{2+} treatments, the value of SOD activity exhibited a tendency of decrease from CK to THg1 treatment, and then experienced a trend of gradual decrease from THg2 to THg4, and SOD values were significantly high at THg5 and THg6 than at CK ($p < 0.05$), whilst SOD values were slightly lower at THg1 and THg2 than at CK, demonstrating that different Pb\textsuperscript{2+} concentrations showed nonlinear relationships ($R^2 = 0.8719$ and 0.8657, respectively, $p < 0.05$). According to Fig. 11a, under Pb\textsuperscript{2+} treatment, the value of CAT activity exhibited a tendency of decrease from CK to TPb1 treatment, presented a trend of increase from TPb3 to TPb4 treatment, and then showed a tendency of gradual decrease from TPb5 to TPb8 treatment, showing that different concentrations of Pb\textsuperscript{2+} treatments exerted different effects on CAT activity in seedling leaves of malting barley. The values of CAT activity were significantly ($p < 0.05$) and slightly lower at TPb3 and TPb4 than at CK, respectively, whilst such values were extremely significantly lower at TPb3 and TPb4 that at CK ($p < 0.01$), demonstrating that the minimum Pb\textsuperscript{2+} concentration (0.1 mM) exerted significant effects, 0.2 mM Pb\textsuperscript{2+} concentra-

### 3.6 The alleviative effects of SA on CAT and SOD activities in barley seedling leaves treated by different Pb\textsuperscript{2+} treatments

The measurement results of CAT and SOD activities in barley seedling leaves treated by different Pb\textsuperscript{2+} treatments and SA-alleviated Pb\textsuperscript{2+} treatments are shown in Figs. 11 and 12. From Fig. 11, it can be apparently seen that both CAT activities and Pb\textsuperscript{2+} concentrations, and CAT activities and SA-alleviated Pb\textsuperscript{2+} concentrations showed nonlinear relationships ($R^2 = 0.8719$ and 0.8657, respectively, $p < 0.05$). According to Fig. 11a, under Pb\textsuperscript{2+} treatment, the value of CAT activity exhibited a tendency of decrease from CK to TPb1 treatment, presented a trend of increase from TPb3 to TPb4 treatment, and then showed a tendency of gradual decrease from TPb5 to TPb8 treatment, showing that different concentrations of Pb\textsuperscript{2+} treatments exerted different effects on CAT activity in seedling leaves of malting barley. The values of CAT activity were significantly ($p < 0.05$) and slightly lower at TPb3 and TPb4 than at CK, respectively, whilst such values were extremely significantly lower at TPb3 and TPb4 that at CK ($p < 0.01$), demonstrating that the minimum Pb\textsuperscript{2+} concentration (0.1 mM) exerted significant effects, 0.2 mM Pb\textsuperscript{2+} concentra-

### Figure 10. Effects of Hg treatments and SA–Hg combined treatments on SOD activities in seedling leaves ($p < 0.05$).

### Figure 11. Effects of Pb treatments and SA–Pb combined treatments on CAT activities in seedling leaves ($p < 0.05$).

### Figure 12. Effects of Pb treatments and SA–Pb combined treatments on SOD activities in seedling leaves ($p < 0.05$).
tration exerted significant effects, whilst higher Pb\(^{2+}\) concentrations (0.5 and 1 mM) exerted extremely significant effects in lowering CAT activity (\(p < 0.01\)). According to Fig. 11b, under SA-alleviated Pb\(^{2+}\) treatments, CAT activity values exhibited a tendency of decrease from CK to TPb5 treatment, then showed a tendency of increase from TPb5 to TPb6 treatment, and finally presented a trend of decrease from TPb6 to TPb8 treatment, indicating that different concentrations of Pb\(^{2+}\) treatments exerted different effects on CAT activity in seedling leaves of malting barley. The value of CAT activity was significantly (\(p < 0.05\)) and slightly lower at TPb5 and TPb6 treatments than at CK, respectively, whilst such values were extremely significantly lower at TPb5 and TPb6 treatments than at CK (\(p < 0.01\)), demonstrating that SA-alleviated Pb\(^{2+}\) treatments exerted generally similar effects on CAT activity as what Pb\(^{2+}\) treatment did. Comparing Fig. 11a with b, it can be apparently seen that CAT activities at TPb5, TPb6, and TPb8 were slightly higher than at TPb1, TPb2, and TPb3 respectively, while such value was significantly higher at TPb8 than at TPb4, showing that SA exerted certain effects in promoting CAT activity under Pb\(^{2+}\) treatments.

From Fig. 12, it can be clearly observed that both SOD activities and Pb\(^{2+}\) concentrations, and SOD activities and SA-alleviated Pb\(^{2+}\) concentrations showed nonlinear relationships (R\(^2\) = 0.9882 and 0.9697, respectively, \(p < 0.05\)). According to Fig. 12a, the values of SOD activity showed a tendency of increase from CK to TPb5 treatment, and then presented a trend of decrease from TPb5 to TPb4 treatment. The values of SOD activity were significantly higher at TPb5 and TPb6 than at CK (\(p < 0.05\)), while such values were slightly lower at TPb3 and TPb4 than at CK, showing that lower concentrations of Pb\(^{2+}\) (0.1 and 0.2 mM) exerted significant (\(p < 0.05\)) effects in elevating SOD activity while higher concentrations of Pb\(^{2+}\) (0.5 and 1 mM) exerted certain effects in suppressing SOD activity. According to Fig. 12b, the values of SOD activity experienced a tendency of increase from CK to TPb5 treatment, and then witnessed a trend of decrease from TPb5 to TPb4 treatment. The values of SOD activity were significantly higher at TPb5 and TPb6 treatments than at CK (\(p < 0.05\)), slightly higher at TPb7 than at CK and slightly lower at TPb8 than at CK, indicating that SA-alleviated Pb\(^{2+}\) treatment exerted quite different effects on SOD activity from what Pb\(^{2+}\) treatments did. Comparing Fig. 12a with b, it can be clearly seen that there occurred no significant differences between SOD activities at TPb4 and TPb7, and at TPb4 and TPb8, because SOD activities were significantly higher at TPb7 and TPb8 than at TPb3 and TPb4 (\(p < 0.05\)), demonstrating that SA exerted limited alleviative effects on SOD activities at lower Pb\(^{2+}\) concentrations (0.1 and 0.2 mM), whilst exerted significant alleviative effects on SOD activities at higher Pb\(^{2+}\) concentrations (0.5 and 1 mM).

4 Concluding remarks

With the rapid worldwide development of beer industry, malting barley has long been considered as an important crop with huge economic potential [1–11, 36–42]. However, many barley-growing areas have suffered from soil erosion and water pollution caused by heavy metal ions emitted by worldwide industrial production. Heavy metal pollution has been long held as an urgent issue in worldwide industrial development and agricultural production due to its toxic and damaging effects on potable water and food security [1–3]. In addition, the toxic effects of heavy metal ions can be passed and amplified through food chains by a cascade of physiological and biochemical processes in plants and animals [4–6]. It has been shown in many researches that such heavy metal ions as Zn\(^{2+}\), Cu\(^{2+}\), Mn\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\), and Pb\(^{2+}\) exerted certain adverse and damaging effects on plant growth [7–19, 43–48]. The results of this paper also indicated that Zn\(^{2+}\), Cu\(^{2+}\), Mn\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\), and Pb\(^{2+}\) exerted certain effects on barley seedling growth in terms of the changes in CAT and SOD activities, while the trends of such changes exerted by different ions were somewhat different [12–19].

In recent years, SA has been found to exert some positive effects on the improvement of plants’ resistances to some abiotic stresses, such as salt, drought, cold, and heat stresses [20–23]. Since the discovery of SA’s protective roles in safeguarding pathogen-infected plants, a myriad of researches have been conducted in SA-induced both locally and systematically acquired resistances of plants [7–15, 20–23]. In this paper, CAT and SOD activities in barley seedling leaves were measured under various concentrations of different heavy metal ion treatments and under different SA-alleviated treatments so as to reveal the physiological mechanism of barley seedlings’ responses to different heavy metal stresses, and of SA’s alleviative effects on these stresses. Our results showed that among the six kinds of divalent cations mentioned in this paper, namely Zn\(^{2+}\), Cu\(^{2+}\), Mn\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\), and Pb\(^{2+}\), Mn\(^{2+}\) exerted less effects in inhibiting CAT activities, while other kinds of ions exerted relatively significant effects in repressing CAT activities (\(p < 0.05\)), and CAT activities were severely repressed under Cd\(^{2+}\), Hg\(^{2+}\), and Pb\(^{2+}\) stresses. Lower concentrations of Zn\(^{2+}\), Cu\(^{2+}\), and Mn\(^{2+}\) merely exerted limited effects in suppressing SOD activities, while higher concentrations of Zn\(^{2+}\), Cu\(^{2+}\), and Mn\(^{2+}\) led to certain inhibitive effects on SOD activities, and SOD activities were also repressed under Cd\(^{2+}\), Hg\(^{2+}\), and Pb\(^{2+}\) stresses. And different heavy metal ions brought about generally similar changing trends in CAT activities except such changing trends under Zn\(^{2+}\) and Pb\(^{2+}\) stresses, and led to different changing trends in SOD activities [31–38, 43, 44]. And it was also indicated in our research that SA exerted certain alleviative effects in enhancing CAT and SOD activities under heavy metal stresses, indicating that SA can be applied as a kind of useful biochemical agent in elevating plant resistances or tolerances to heavy metal pollution [42].

Seedling leaves play important parts in seedling growth with the company of seedling root system. Leave cells are the sites for photosynthesis, which is directly related to plant growth and development. Therefore, CAT and SOD activities in seedling leaves can be indirectly considered as important physiological indexes concerning photosynthetic rates. Although changes in chlorophyll contents and indexes for photosynthesis still need to be measured in further experiments, changes of CAT and SOD activities in seedling leaves can also be applied as biomarkers and indexes for seedling growth and development under heavy ion stresses and SA’s alleviative effects. Apart from seedling leaves, root systems also play an important role in seedling growth and development. In addition, root systems serve as important sites for the hyper-accumulation of toxic heavy metal ions [10–16, 20–26, 42] in plants. Therefore, the changes of CAT and SOD activities in seedling root under different heavy metal ion treatments need to be further experimented. In addition, the mechanisms for the changes of many other physiological indexes concerning barley seedlings’ tolerances or resistances to heavy metal pollution need to be further studied and clarified. Finally, the differences among different malting barley varieties’ responses to heavy metal ions also need to be demonstrated and clarified in further studies.

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References
Potential Health Risk in Metropolitan Region of Northern China, Water Air Soil Pollut. 2007, 184, 105–126.


