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RESEARCH ARTICLE

Responses of earthworm to aluminum toxicity in latosol

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Abstract Excess aluminum (Al) in soils due to acid rain leaching is toxic to water resources and harmful to soil organisms and plants. This study investigated adverse impacts of Al levels upon earthworms (*Eisenia fetida*) from the latosol (acidic red soil). Laboratory experiments were performed to examine the survival and avoidance of earthworms from high Al concentrations and investigate the response of earthworms upon Al toxicity at seven different Al concentrations that ranged from 0 to 300 mg kg^{-1} over a 28-day period. Our study showed that the rate of the earthworm survival was 100 % within the first 7 days and decreased as time elapsed, especially for the Al concentrations at 200 and 300 mg kg^{-1}. A very good linear correlation existed between the earthworm avoidance and the soil Al concentration. There was no Al toxicity to earthworms with the Al concentration ≤50 mg kg^{-1}, and the toxicity started with the Al concentration ≥100 mg kg^{-1}. Low Al concentration (i.e., <50 mg kg^{-1}) enhanced the growth of the earthworms, while high Al concentration (>100 mg kg^{-1}) retarded the growth of the earthworms. The weight of earthworms and the uptake of Al by earthworms increased with the Al concentrations from 0 to 50 mg kg^{-1} and decreased with the Al concentrations from 50 to 300 mg kg^{-1}. The protein content in the earthworms decreased with the Al concentrations from 0 to 100 mg kg^{-1} and increased from 100 to 300 mg kg^{-1}. In contrast, the catalase (CAT) and superoxide dismutase (SOD) activities in the earthworms increased with the Al concentrations from 0 to 100 mg kg^{-1} and decreased from 100 to 300 mg kg^{-1}. The highest CAT and SOD activities and lowest protein content were found at the Al concentration of 100 mg kg^{-1}. Results suggest that a high level of Al content in latosol was harmful to earthworms.

Keywords Al toxicity · Earthworm · Latosol

Introduction

Aluminum (Al) is the most abundant metal in the earth's crust, and it accounts for about 7 % of the mass (by weight) of the earth surface. There are multiple forms of Al such as water-soluble Al, exchangeable Al, and organic Al in the soils, and most of them are not directly harmful to plants and organisms (Brady 1984). Aluminum is usually bound to the negatively charged surface of soil particles. Under the influence of acid rain, Al can be displaced by H^+ ion and activated in the soil (Brady 1984; Liu et al. 1990). The excess Al in the soil is toxic to water resources and harmful to soil organisms and plants (Kochian 1995; Menz and Seip 2004). Acid rain contains an unnatural acidity and is one of the serious ecological and environmental problems around the world besides global warming and ozone damage (Galloway 1995). The typical pH values of acid rain resulted from anthropogenic emissions that range from 3.5 to 5.0 (Menz and Seip 2004). Recently, Zhang et al. (2007) investigated the impacts of simulated acid rain (SAR) on cation leaching from the latosol in south China. A linear increase in effluent K^+ concentration is found at the SAR pH <3.0,

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whereas an exponential decrease in effluent Na^+ concentration was observed at all levels of the SAR pH. In general, leaching of Ca^{2+} and Mg^{2+} from the latosol increases as the SAR pH decreases. Ling et al. (2007) reported that about 34, 46, 20, and 77 % of the original exchangeable soil Ca^{2+}, Mg^{2+}, K^+, and Na^+, respectively, are leached out by the SAR at pH 2.5 after 21 days. These studies have provided insightful information on the impacts of the SAR upon soil cations and nutrients. However, no effort has been devoted to investigate the toxic impacts of Al upon soil animals such as earthworms under the influence of the acid rain.

Earthworms are very important soil animals that aerate the soil with their burrowing action and enrich the soil with their waste products. They enhance soil nutrient cycling, the activity of other beneficial soil organisms, and soil physical properties (Nahmani and Rossi 2003). Earthworms are very sensitive to anthropogenic contaminants and have been widely used as an indicator animal for estimating soil pollution (Nahmani and Rossi 2003). Responses of earthworms to soils contaminated with organics and metals are important indicators for assessing soil ecological toxicity. Spurgeon et al. (2005) studied earthworm responses to Cd and Cu under fluctuating environmental conditions. These authors found that both metals significantly influence the earthworm reproduction, compromise lysosomal membrane stability, and induce *MT-2* gene expression in the outdoor system. However, a thorough literature search reveals that little effort has been devoted to investigating the toxicity of Al to earthworms in the soil.

There are two kinds of toxicity testing with earthworms while performing soil ecological assessment: one is the acute toxicity testing and the other is the chronic toxicity testing (Foetide 1991; Abdul et al. 1996). The purpose of this study was to ascertain the impacts of Al levels on earthworms in latosol (acidic red soil) with chronic testing. Our specific objectives were to (1) investigate survival and avoidance rates of earthworms at different Al concentrations and (2) assess chronic toxicity of Al upon earthworms in latosol. Latosol arises in the tropical rainforest biome where high temperature and high precipitation occur throughout the year. Climatic conditions that permit the extensive chemical weathering lead to the development of a deep soil profile (20 to 30 m). This soil has a loose structure and suffers from rapid erosion during heavy rainfall, which results in leaching of cations such as Al and Ca, nutrients, and metals. Leaching of Al is anticipated to harm soil animals and plants.

Materials and methods

Materials and reagents

Top 20-cm latosol collected from a forest garden located on the campus of South China Agricultural University, Guangdong Province, China was used for the experiments. This soil had pH 4.3 with an organic matter content of 44.95 $g kg^{-1}$, a cation exchange capacity of 6.69 mmol kg^{-1}, and a base saturation of 12.9 %. The initial soil cation contents were 0.374, 3.209, 0.067, 0.049, 0.679, and 0.0681 cmol kg^{-1}, respectively, for H^+, Al^{+3}, K^+, Na^+, Ca^{2+}, and Mg^{2+}. Analytical grade sulfuric acid (H_2SO_4) and nitric acid (HNO_3) were purchased as standard catalog items from a commercial supplier (Guangzhou Chemical Reagent Manufacture Inc., China).

The earthworms (*Eisenia fetida*) were purchased from the Organization for Economic Co-operation and Development Inc. in Jiangmen City, Guangdong Province, China. These earthworms were fed by the cow wastes and peats with a ratio of 1:1 in the artificial climate boxes (RXZ-300B, Ning Bo Southeast Equipment Inc., China). The active and sexual matured earthworms with bright body color and weights that ranged 300 to 500 mg were selected for the experiments. These earthworms were washed, their stomata were emptied, and their body surface was dried prior to the experiments. The working solutions with Al concentrations of 0, 12.5, 25, 50, 100, 200, and 300 mg kg^{-1} (dry soil) were prepared by diluting the analytical $AlCl_3$ with deionized water. These concentrations were used to make Al-treated soils. Each Al concentration represented one experimental treatment.

Earthworm avoidance testing

A plastic container with 18-cm width, 27-cm length, and 8-cm height was separated into two equal compartments A and B by inserting a solid plastic board. The compartment A was filled with 500 g dried natural soil, whereas the compartment B was filled with 500 g dried and Al-treated soil. The solid plastic board was removed after the container was filled with the soils. A total of 15 empty-stomata earthworms were introduced into the interface between the compartments A and B of the container, which was wrapped with a cheesecloth to prevent the earthworms to escape. The container was then placed in an artificial climate box at a temperature of 20°C, a relative humidity of 7 %, and a light intensity of 333 lx with an intermittent light cycle (i.e., 12-h light and 12-h dark). After 48 h of the experiment, the solid plastic board was again inserted into the container to separate the two compartments and the number of earthworms in each compartment was counted. The earthworms that were cut into segments by the solid plastic board in each compartment were counted as 1/2 earthworm regardless of their lengths. There were a total of seven treatments (i.e., seven different Al concentrations) with five replications for each treatment.

Earthworm chronic toxicity testing

The soil and Al concentrations used for the chronic toxicity testing were the same as those used in "Materials and reagents." There were a total of seven treatments (one Al concentration for a treatment) with four replications for each treatment. A plastic cylindrical cup with a top inner diameter of 12 cm and a bottom inner diameter of 9 cm was used to contain a 13-cm long soil column. After a 5,000-g air-dried and Al-treated soil was poured and a total of 10 empty-stomata earthworms were introduced into the cup, the cup was wrapped with a cheesecloth to prevent the earthworm to escape. The soil in the cup was wetted to field capacity, and the cup was then placed in the artificial climate box with the same ambient conditions as described in "Earthworm avoidance testing." Similar procedures were used by Abdul et al. (1996) and Saint-denis et al. (2000). The experiment was conducted for 28 days. The water content in the cup was kept constant by adding water weekly, and the earthworms in the cup were fed with 5 g dried cow waste each week (Kokta 1992).

The earthworms in the cup were temporally taken away, washed, counted, and weighed at days of 7, 14, and 21. At the end of the experiment, the earthworms in the cup were removed, washed, counted, and weighed. After the stomata were emptied, the earthworms were oven-dried at 100°C for 24 h, grounded, and weighed (Sun et al. 2007). The dried samples were used to analyze Al contents with ICP-OES (Varian 710-ES), protein contents with Bradford assay (Bradford 1976), the superoxide dismutase (SOD) contents with the procedures reported by Xie et al. (1988), and the catalase (CAT) contents with the procedures reported by Lei et al. (1993). Correlation analysis and comparisons of the differences of earthworm numbers and weights as well as their protein, SOD, and CAT contents were performed using DUNCAN statistics with SAS 8.1.

Results and discussion

Impact of Al on earthworm survival and avoidance rate

Impacts of different Al concentrations upon earthworm survival rate at days 7, 14, 21, and 28 are given in Fig. 1 (α=0.01, p<0.001). The earthworm activities such as losing soil and producing wastes were observed in all of the treatments, and most of the earthworms survived at the end of the experiment. The earthworm survival rate was 100 % within the first 7 days, but this rate decreased as time elapsed, especially for the treatments with Al concentrations at 200 and 300 mg kg^{-1} (Fig. 1). For example, the earthworm survival rate was 80 % in 14 days, 70 % in 21 days, and 60 % in 28 days for the treatment with the Al concentration of 300 mg kg^{-1}. Figure 1 further revealed that there was no Al toxicity to earthworms when the soil Al concentration was less or equal to 50 mg kg^{-1}. However, the toxicity was observed when the soil Al concentration was greater or equal to 100 mg kg^{-1}.

Figure 2 showed the avoidance rate as a function of Al concentrations applied to the soil during the avoidance testing. The avoidance rate was calculated using the following equation (Monica et al. 2005):

$$\mathrm{AR}(\%) = 100 \times (C - T)/N \quad (1)$$

where AR is the avoidance rate (in percent), C is the number of earthworms in the natural soil, T is the number of earthworms in the Al-treated soil, and N is the total number of earthworms introduced into the soils during the avoidance testing. When the AR is positive and larger than 80 %, the earthworm avoidance is highly significant (Monica et al. 2005). The earthworm avoidance rate increased as the soil Al concentration increased with a very good linear correlation (R^2=0.896). The earthworm avoidance rate was 25 % when the soil Al concentration was about 100 mg kg^{-1}, while the earthworm

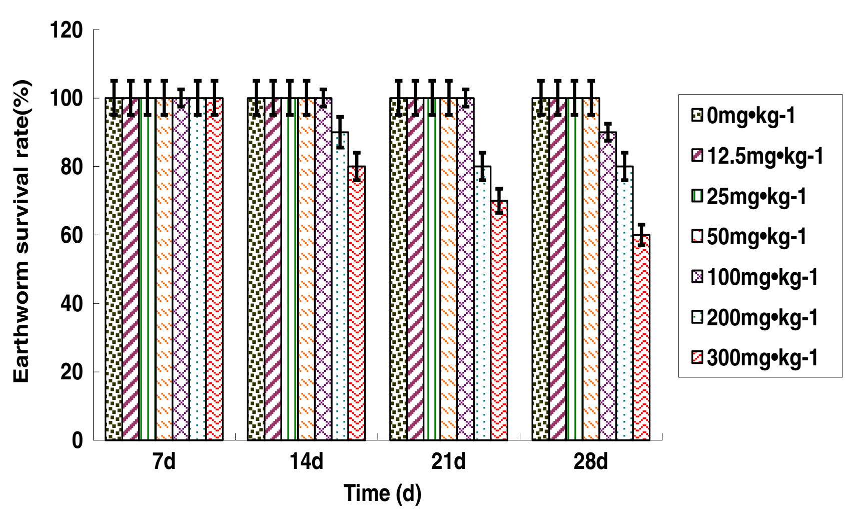


Fig. 1 Impact of Al concentrations on earthworm survival rate

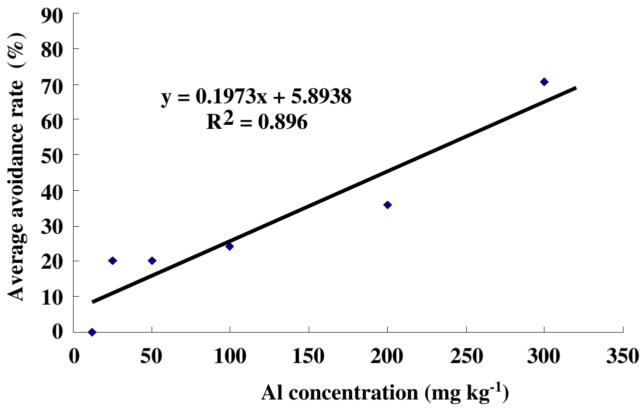


Fig. 2 Relationship between the average earthworm avoidance rate and the soil Al concentration

avoidance rate was 75 % when the soil Al concentration was about 300 mg kg^{-1}. A three-time increase in soil Al concentration increases the earthworm avoidance rate by 50 %.

Impact of Al level on earthworm growth

There were no differences in earthworm weights from 0 to 7 days of the experiment, and two distinct patterns of earthworm weights started to develop after 7 days (Fig. 3). For the treatments with the soil Al concentrations from 0 to 50 mg kg^{-1}, the weight of the earthworms increased from 7 to 28 days, whereas for the treatments with the soil Al concentrations from 100 to 300 mg kg^{-1}, the weight of the earthworms decreased at the same period (i.e., from 7 to 28 days). For example, the weight of the earthworms was 415 mg in 7 days and was 440 mg in 28 days for the treatment with the soil Al concentration of 50 mg kg^{-1}. There was about 6 % increase in the weight of the earthworms within 3 weeks for this treatment. All of the differences were statistically significant with α=0.01 and p<0.0001. Results revealed that the growth of earthworms was not affected by Al level ≤50 mg kg^{-1}. In contrast, the weight of the earthworms was 415 mg in 7 days and was 375 mg in 28 days for the treatment with the soil Al concentration of 300 mg kg^{-1}. There was about a 10 % decrease in the weight of the earthworms within 3 weeks for this treatment. Overall, a 6-fold (i.e., 300/50 mg kg^{-1}) increase in Al concentration had resulted in about 1.2-fold (i.e., 440/375 mg) decrease in the weight of the earthworms at the end of the experiment. Results indicated that low Al concentrations (i.e., from 0 to 50 mg kg^{-1}) enhanced the growth of the earthworms, while high Al concentrations (i.e., from 100 to 300 mg kg^{-1}) retarded the growth of the earthworms.

Uptake of Al by earthworm

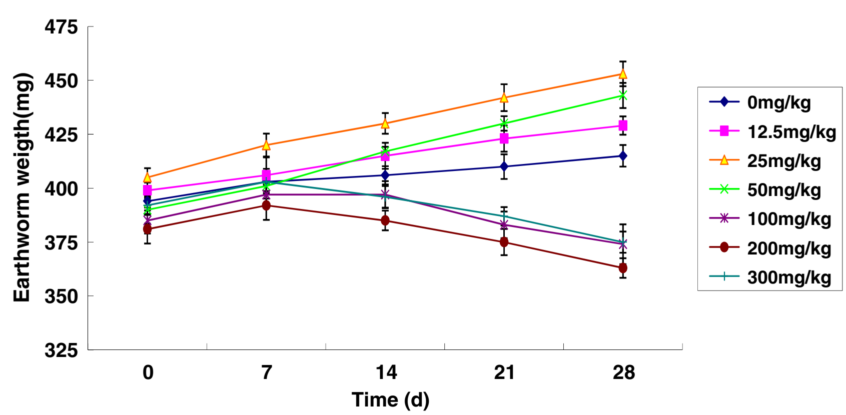
Figure 4 shows the uptake of Al by the earthworms at different Al concentrations. This figure was constructed using the following equation:

$$R = \left(\frac{M_a^{Al} - M_b^{Al}}{M_e}\right)100 \qquad (2)$$

where R is the uptake of Al by earthworms (in percent), M is the mass (in milligram), and the subscribers a, b, and e denote, respectively, after the experiment, before the experiment, and earthworm. All of the differences were statistically significant with α=0.01 and p<0.0001.

Uptake of Al from the soil increased as the Al concentration increased from 0 to 50 mg kg^{-1} and decreased as the Al concentration increased from 50 to 300 mg kg^{-1}. The maximum uptake of Al was 0.225 % when the Al concentration was 50 mg kg^{-1}. The pattern of Al uptake was similar to the pattern of Al growth, i.e., the weight of earthworms and the uptake of Al by earthworms increased with the Al concentration from 0 to 50 mg kg^{-1} and decreased with the Al concentration from 50 to 300 mg kg^{-1}. As the weight of earthworm increased, more Al from the soil was taken up by the earthworms. Kou et al. (2008) and Li et al.

Fig. 3 The weight of the earthworms as a function of time at different Al concentrations



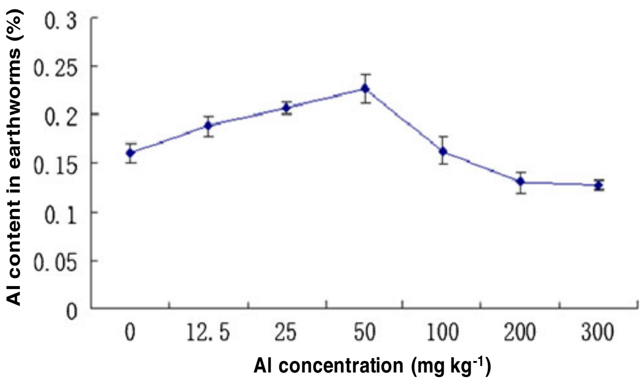


Fig. 4 Al contents in the earthworms as a function of Al concentrations

(2009) reported that earthworm can hyperaccumulate Pb and Cu from soils, while Sun et al. (2008) found that earthworm does not hyperaccumulate Al. Our finding agreed with this latter conclusion as the maximum percentage increase in Al uptake by earthworms from the soil was only 0.225 %.

Impact of Al toxicity on protein content and enzyme activity

The protein content in the earthworms for the control treatment (i.e., 0 mg kg^{-1}) was much higher than those for the other treatments (Fig. 5). All of the differences were statistically significant with α=0.01 and p<0.001. For example, the protein content was 0.63 gL^{-1} for the control treatment and was 0.475 gL^{-1} for the treatment with the Al concentration of 300 mg kg^{-1}. The former was 1.3-fold higher than the latter. Although the exact reason for this phenomenon remains unknown, a possible explanation would be that the production of protein in earthworms was retarded due to Al toxicity (Sun et al. 2008). The decrease in protein contents due to pesticide and Cu toxicity were reported by Davies et al. (2003) and Tai et al. (2008). There were no significant differences in protein contents among the treatments with Al concentrations at 12.5, 25, 50, and 200 mg kg^{-1} but were significant among the treatments with Al concentrations at 0, 100, 300 mg kg^{-1}. The protein content was highest (0.63 gL^{-1}) with the Al concentration at 0 mg kg^{-1} and lowest (0.425 gL^{-1}) with the Al concentration at 100 mg kg^{-1}.

CAT is a common enzyme found in nearly all living organisms, which are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover numbers among all of the enzymes; one molecule of CAT can convert millions of molecules of hydrogen peroxide to water and oxygen per second (Chelikani et al. 2004). Impacts of Al upon the CAT activity of the earthworms at seven different treatments (or Al concentrations) are shown in Fig. 6. The CAT activity in the earthworms increased with Al concentrations from 0 to 100 mg kg^{-1} and decreased with Al concentrations from 100 to 300 mg kg^{-1}, which was opposite to the case of protein production as shown in Fig. 5. It is very interested to note that the highest CAT activity (85 U g $protein^{-1}$) and lowest protein content (0.41 gL^{-1}, Fig. 5) were found at the Al concentration of 100 mg kg^{-1}. Further study is, therefore, warranted to investigate the relationship between CAT and protein production at this concentration level. Overall, the CAT activity in the earthworms increased with Al concentration as compared to that of the control treatment. Similar result was obtained by Liang et al. (2007).

SOD are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. They are an important antioxidant defense in nearly all cells exposed to oxygen (Brewer 1976). Similar to the case of CAT, the impact of Al on SOD activity in the earthworms increased with the Al concentrations from 0 to 100 mg kg^{-1} and

Fig. 5 Protein contents in the earthworms as a function of Al concentrations

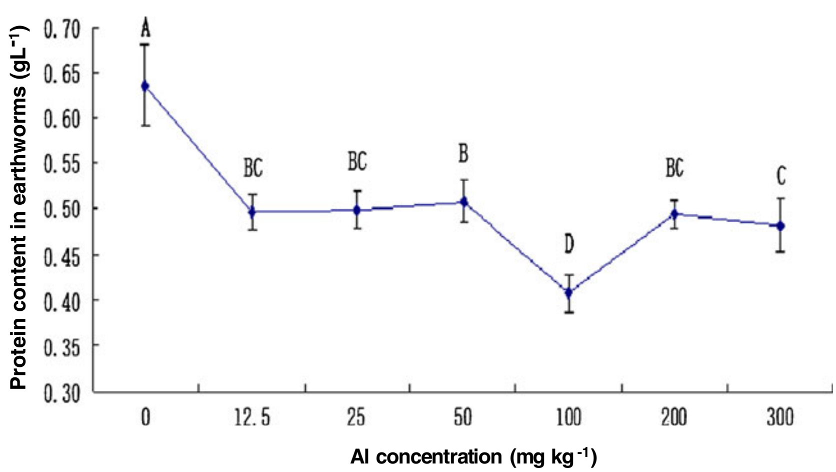
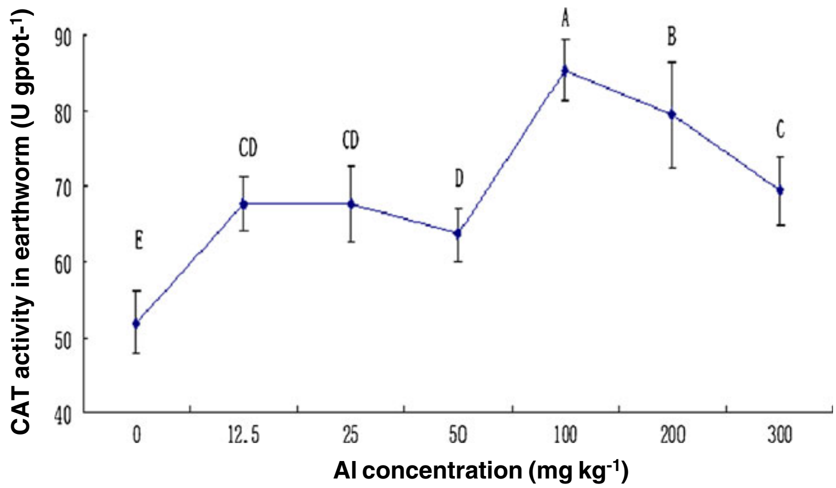


Fig. 6 CAT activities in the earthworms as a function of Al concentrations



decreased from 100 to 300 mg kg^{-1} (Fig. 7). Sun et al. (2007) argued that the SOD activity in the earthworms increases with a moderate environmental stress and decreases with a severe environmental stress. Overall, the SOD activity in the earthworms increased with Al concentration.

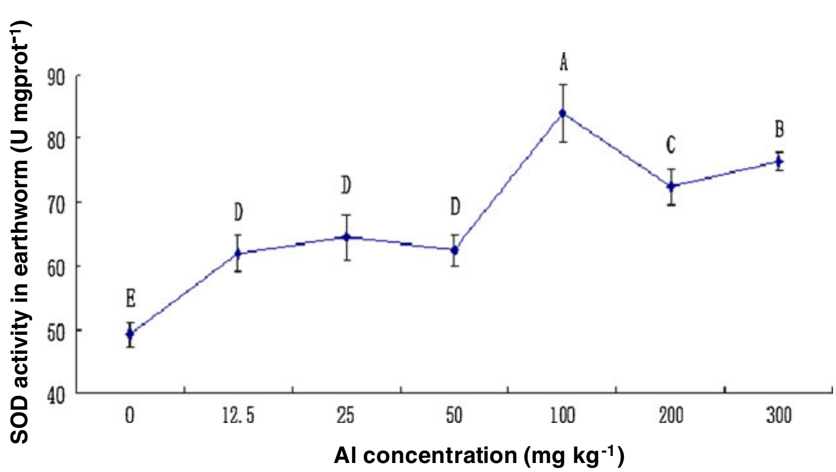
Summary and conclusions

Laboratory experiments were performed to examine the avoidance and response of the earthworms in latosol due to Al toxicity at seven different Al concentrations that ranged from 0 to 300 mg kg^{-1} over a 28-day period. The earthworm survival rate was 100 % within the first 7 days, but this rate decreased as time elapsed, especially for the treatments with Al concentrations at 200 and 300 mg kg^{-1}. There was no Al toxicity to earthworms when the soil Al concentration was ≤50 mg kg^{-1}. The toxicity occurred when the soil Al concentration was greater or equal to 100 mg kg^{-1}.

Low Al concentration (i.e., <50 mg kg^{-1}) enhanced the growth of the earthworms, while high Al concentrations (i.e., >100 to 300 mg kg^{-1}) retarded the growth of the earthworms. Uptake of Al from the soil increased as the Al concentration increased from 0 to 50 mg kg^{-1} and decreased as the Al concentration increased from 50 to 300 mg kg^{-1}. We also found that earthworms did not hyperaccumulate Al.

The protein content in the earthworms for the control treatment was much higher than the other treatments. The CAT and SOD activities in the earthworms increased with Al concentrations from 0 to 100 mg kg^{-1} and decreased with Al concentrations from 100 to 300 mg kg^{-1}. It is very interested to note that the highest CAT and SOD activities and lowest protein content were found at the Al concentration of 100 mg kg^{-1}.

Fig. 7 SOD activities in the earthworms as a function of Al concentrations



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