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Activity of Earthworm in Latosol Under Simulated Acid Rain Stress

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Abstract Acid rain is still an issue of environmental concerns. This study investigated the impacts of simulated acid rain (SAR) upon earthworm activity from the Latosol (acidic red soil). Laboratory experiment was performed by leaching the soil columns grown with earthworms (*Eisenia fetida*) at the SAR pH levels ranged from 2.0 to 6.5 over a 34-day period. Results showed that earthworms tended to escape from the soil and eventually died for the SAR at pH = 2.0 as a result of acid toxicity. The catalase activity in the earthworms decreased with the SAR pH levels, whereas the superoxide dismutases activity in the earthworms showed a fluctuate pattern: decreasing from pH 6.5 to 5.0 and increasing from pH 5.0 to 4.0. Results implied that the growth of earthworms was retarded at the SAR pH $\leq$ 3.0.

Keywords Acid rain · Enzyme activity · Earthworm · Latosol

Acid rain is one of the serious ecological and environmental problems in some countries around the world (Galloway 1995; Tao and Feng 2000). The typical values of acid rain resulted from anthropogenic emissions range from pH 3.5 to 5.0 (Menz and Seip 2004). Acid rain can kill fishes and aquatic plants. Acid rain with SO_2, NO_x, and NH_3 could react with organic compounds and contribute to ozone (O_3) depletion. Ozone and acid rain may also damage materials (Menz and Seip 2004). About 40 % of world arable lands and about 70 % of world non-arable lands are acidic, which are mainly distributed in tropic, sub-tropic, and temperate areas (Kochian 1995).

Natural rain has pH > 5.6 that is slightly acidic. However, rainwater may be more acidic because of the natural emissions of sulfur dioxide (SO_2), nitrogen oxides (NO_x), or organic acids. The major sources for acid rain in China are SO_2 and NO_x. Acid rain in China covers the areas of Yangtze River to the South, Qinghai-Tibet Plateau to the East, and in Sichuan Basin. Wang et al. (2011) reported that large area of acid rain with pH below 4.5 (with a range from 3.41 to 4.53) occurred in Southern China in recent years. Based on the environmental protection and monitoring agencies in Guangdong Province in South China, about 50 % of the rainfalls are acid rains and the direct economical loss due to acid rains for Guangdong Province is estimated to be $ 4.4 billion US dollars (Zhang et al. 2007). In general, acid rain is a serious problem in China (Bian and Yu 1992; Larssen and Carmichael 2000) and is a particular concern in Southern China because most of the soils in that part of China are acidic.

Acid rain in the soil can mobilize base cations, decompose organic compound, and change mineral structure. The H^+ ion in the acidic water displaces the cations from their binding sites, reduces the cation exchange capacity, and increases the concentrations of these cations in the soil solution (Brady 1984; Liu et al. 1990). Acid rain can increase the weathering of silicate minerals in soils, which leads to a change of mineral structure and reduces soil fertility. Zhang et al. (2007) investigated the impacts of simulated acid rain (SAR) on cation leaching from the Latosol in South China. These authors found that a linear increase in effluent K^+ concentration exists at the SAR

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with pH < 3.0, while an exponential decrease in effluent Na^+ concentration is observed at all levels of the SAR pH. 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.

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. Earthworms are very sensitive to anthropogenic contaminants and have been used as an indicator soil animal for estimating soil pollution (Nahmani and Rossi 2003; Li and Cheng 2006). Earthworms are often less abundant in soils with low pH. However, it is not known if earthworms physiologically cannot tolerate low pH or if low pH soils lack nutrients (e.g., calcium) that are necessary for earthworm survival (Hauxwell et al. 1992).

The purpose of this study was to ascertain the impacts of SAR upon earthworm activity in Latosol (acidic red soil) in South China. Our specific objectives were to: (1) evaluate the activities of catalase (CAT) and superoxide dismutases (SOD) as well as the content of protein in the earthworms from the Latosol as impacted by the SAR with different pH levels; and (2) estimate the rates of earthworm survival and growth in the Latosol by the SAR with different pH levels.

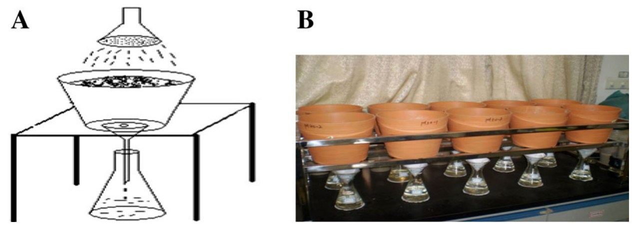

Fig. 1 A schematic diagram showing the SAR leaching experimental setup (**a**) and a photo (**b**) taken during the experiment

Materials and Methods

In South China, acid rain primarily consists of H_2SO_4 and HNO_3 with a ratio of 4–1 (Zhang et al. 2012). In order to have the SAR reflecting the natural conditions in south China, the stock acid solution was prepared by using this ratio. The working solutions of acid rain with pH 2.0, 3.0, 4.0, 5.0, and 6.5 (CK or control) were prepared in volumetric flasks diluting the stock solution with deionized (DI) water. It should be noted that the lowest acid rain pH currently observed in China is above 3.0. However, we expected this value to be continuously decreased as the air pollution grows in China. Based on this projection, a lowest pH has been selected at 2.0 in this study to cover the lowest possible situation which would occur in China.

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 (CEC) of 6.69 mol kg^{-1}, and a base saturation (BS) 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+} measured with ICP-AES (Varian 710-ES) (Sparks 1996). Analytical grade sulfuric acid (H_2SO_4) and nitric acid (HNO_3) were purchased as standard catalog items from commercial supplier (Guangzhou Chemical Reagent Manufacture Plant, China). The earthworms (*Eisenia fetida*) were purchased from Organization for Economic Co-operation and Development Inc. from Jiang Men City, Guangdong Province, China.

A pot with a top inner diameter of 19 cm and a bottom inner diameter of 14 cm was used to contain an 18 cm long soil column (Fig. 1). A 3,000 g air-dried soil, passed through a 1-mm sieve and mixed thoroughly, was then poured into the pot in a 2-cm increment and stirred to prevent layering. The pot was tapped to settle the soil to a bulk density of 1.6 g cm^{-3}. A hole with a diameter of 3.5 cm was drilled at the bottom center of the pot to collect effluent (Fig. 1). Prior to and after filling the pot, a piece of plastic filer and two pieces of paper filters were placed at both ends of the pot to prevent the leakage of the soil. A total of five pot leaching treatments (i.e., one pH level for each treatment) with four replications were carried out in this study. Prior to the leaching experiments, the pot for each treatment were saturated with DI water for 24 h and a total of 30 empty-stomach earthworms were introduced into each treatment. The earthworms in each treatment were feed with 15 g of cow waste every week to avoid starvation.

To reflect the natural rainfall conditions, an intermittent influent application method was employed. That is, a 250-mL influent of the SAR was slowly sprayed to the top of the column every 24 h. The leaching experiments were performed for 34 days with a total of 8,500 mL influent of the SAR. This volume of the SAR was equivalent to three-year acid rain in South China. The soil solution pH was measured with pH meter (pH Meter (CM-230), Qingdao Lead International Company Ltd., China). The laboratory experiment was conducted during the period from December 10, 2009 to January 12, 2010.

After the pot leaching experiment was terminated, the number of earthworms were counted and weighed. Three earthworms from each treatment were randomly selected for analyzing the protein content with Bradford assay (Bradford 1976). The homogenates were prepared by adding nine times physiological saline solution to the earthworm tissues based on the ratio of weight to volume of earthworms, which resulted in 10 % tissue homogenates. The catalase (CAT, EC: 1.11.1.6) activity was assayed in a reaction mixture (3 mL) containing 100 mM sodium phosphate buffer pH 6.8 (2 mL), 30 mM H_2O_2 (0.5 mL) and 0.5 mL enzyme extract according to the procedure of Lei et al. (1993), whereas the activity of superoxide dismutase (SOD, EC: 1.15.1.1) was measured in a reaction mixture by mixing 1.110 mL of 50 mM phosphate buffer (pH 7.4), 0.075 mL of 20 mM L-methionine, 0.040 mL of 1 % (v/v) Triton X-100, 0.075 mL of 10 mM hydroxylamine hydrochloride and 0.1 mL of 50 µM EDTA according to the procedure of Das et al. (2000). The growth rate of earthworms was then calculated by:

$$G = \left(\frac{W - W_o}{W}\right) 100\,\% \qquad (1)$$

where G is the growth rate of the earthworms, W is the weight of earthworms at the end of the experiments, and W_o is the weight of earthworm before the experiments. Correlation analysis and comparisons of the differences of earthworm numbers and weights as well as their protein content and the SOD and CAT activities were performed using Duncan's post hoc test with SAS 8.1. The differences among treatments are statistically significant at $p = 0.05$.

Results and Discussion

There was no obvious adverse impact of the SAR upon earthworms and the survival rate of the earthworms was 100 % over a 34-day leaching experiment for all of different pH levels (or treatments) except for pH 2.0. For the pH levels ranged from 3.0 to 6.5, the activities of the earthworms such as wriggle with losing soil and waste production at the soil surface were normal. In contrast, for the SAR pH 2.0, some earthworms moved to the soil surface and tended to escape from the soil columns, and their wriggle activity was very weak after 7 days of the leaching experiments. From day 7 to 14 for pH 2.0 treatment, the liquids from the bodies of the earthworms leaked out and the earthworms gradually died at the soil surface. At the end of the leaching experiment, all of the earthworms for the pH 2.0 treatment were dead. This occurred because the acid toxicity to earthworms due to a prolong leaching with low SAR. Additionally, the low pH SAR increased Al concentration in the soil (Zhang et al. 2013) resulting in additional toxicity to the earthworms.

Figure 2 shows the rate of earthworm growth under the influence of the SAR for different pH levels. This rate increased as the acid rain pH decreased from 6.5 (CK) to 4.0. The rate was about 1.7 % at pH 6.5 and about 7.8 % at pH 4.0. The latter was about 4.5 times greater than the former. However, as the SAR pH decreased to 3.0 and below, a negative growth rate was found. Result further confirmed that when the SAR pH level was equal or below pH 3.0, the growth of the earthworms was retarded. Attempt to locate published literature for comparison with our findings was not successful as very little effort has been devoted to investigating the impacts of acid rain on earthworm growth and survival rates.

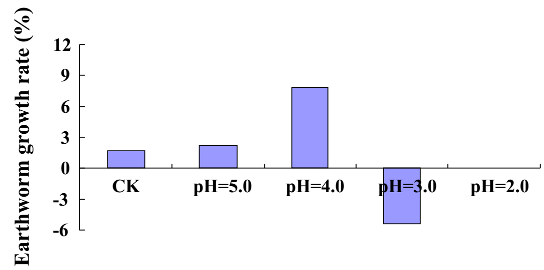

Fig. 2 Rate of earthworm growth under the influence of the SAR at different pH levels. *CK* denotes the control treatment at pH 6.5

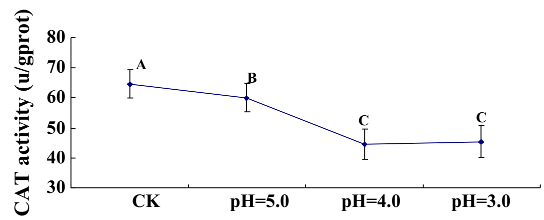

Fig. 3 Catalase (CAT) activity of the earthworms under the influence of the simulated acid rain at different pH levels. *CK* denotes the control treatment at pH 6.5. The *error bars* are average standard deviations and the *same letters* for the *bars* mean no statistical difference at $p = 0.05$

Catalase 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 greatest 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 the SAR upon the CAT activity of the earthworms at four different pH levels are shown in Fig. 3. The CAT activity in the earthworm decreased significantly with pH from 6.5 to 4.0 and there was no statistical difference in CAT activity between pH 4.0 and 3.0. The decrease in CAT activity would result in accumulation of H_2O_2 in the animal body and injure the structure of cell membrane (Vitoria et al. 2001). Results suggest that acid rain is an unfavorable condition for such enzyme.

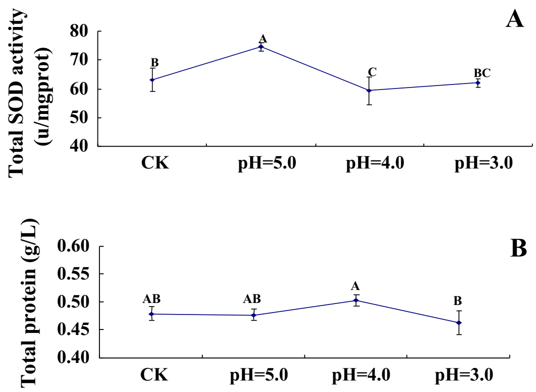

Fig. 4 **a** Superoxide dismutases (SOD) activity of the earthworms under the influence of the simulated acid rain at different pH levels. **b** Total protein content of the earthworms under the influence of the simulated acid rain at different pH levels. *CK* denotes control treatment at pH 6.5. The *error bars* are average standard deviations and the *same letters* for the *bars* mean no statistical difference at $p = 0.05$

Superoxide dismutases 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 1967). Unlike the case of CAT, the impact of the SAR on the activity of SOD in the earthworms showed a fluctuate pattern: increasing from pH 6.5 to 5.0 and decreasing from pH 5.0 to 4.0 (Fig. 4a).

There was a significant difference ($p < 0.05$) in protein content in the earthworms between pH 3.0 and 4.0 treatments and no difference between the pH 5.0 and CK treatments (Fig. 4b). The protein content in the earthworm was highest (0.48 g/L) at pH 4.0 and lowest (0.46 g/L) at pH 3.0. Results implied that the growth of earthworms was retarded at pH 3.0 or below due to the lower protein content.

The imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage is the process of oxidant stress (Betteridge 2000). The CAT and SOD are two important biomarkers of oxidative stress. Results showed that the activities of these two biomarkers were low at pH = 4.0 although the exact reasons remain to be investigated.

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