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Selecting tolerant grass seedlings and analyzing the possibility for using aged refuse as sward soil

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ABSTRACT

In order to test the possibility for recycling use of aged refuse as sward soil, the study determined the responses of *Lolium perenne* L. (perennial ryegrass), *Festuca arundinacea* (tall fescue), and *Poa annua* (annual bluegrass) to its leaching. The growth of three seedlings was significantly inhibited after treatment, especially for longer treatment duration and higher concentration leaching; however, with the better growth and chlorophyll content for shorter time and lower concentration, tall fescue was more tolerant to the stress. Afterwards, several physiological responses of tall fescue were determined. For shorter treatment duration, antioxidant enzyme activities remained unchanged, and no obvious oxidative damage was observed. Prolonging exposure time, lipid peroxidation and protein oxidation occurred after treatment of higher concentration leaching, accompanying by changes of antioxidant status. It implicates that it is possible for using aged refuse as sward soil, and the critical point focused on selecting tolerant grass and controlling exposure condition.

1. Introduction

For many countries, sanitary landfill continues to be an affordable and environmentally acceptable method of solid waste disposal (Lei et al., 2007; Reddy et al., 2009). In China, currently, more than 80% of municipal solid waste has been deposited into landfills without any pretreatment, and landfills are projected to accept approximately 70% of all municipal solid waste in the next 20 years (Lou et al., 2009). However, most of the landfills have reached their design capacity (Chen et al., 2009), with a larger amount of solid waste generation resulting from greater economic prosperity and a larger urban population (Agdag, 2009; Narayana, 2009). Therefore, in order to actualize continuable use of landfill, it is an obligatory and optimal approach to recycle landfilled refuses.

After about 8–10 years of placement, the most refuse in landfills becomes aged or stabilized, except for some non-degradable matter, such as stones, glass bottles, plastic film, and rubber, and is referred to aged refuse (Chai et al., 2007b; Li et al., 2008c). Currently, researches have concentrated on utilizing the characteristics of huge surface proportion and abundance of microbes in aged refuse to design bio-filters to treat landfill leachate and various wastewaters (Shao and Zhang, 2002; Wang and Zhao, 2004; Zhao and Shao, 2004; Zhao et al., 2006; Chai et al., 2007a). Also, the excavated aged refuse could be used as daily cover material, processed constructing material, and nutrimental soil (Zhang and Zhao, 2004). Especially, because aged refuse included abundant dissolved organic carbon compounds and nutrient composition for plant growth (Zhao et al., 2006), it is suggested that aged refuse could be used as vegetated soil. The success of this recycling option, however, is critically dependent on whether plant system could be tolerant to its environmental stress from higher heavy metals contained in aged refuse (Li et al., 2008b).

It is reported that grass could be used for the phytoremediation of multiple heavy metals. For example, *Lolium perenne* L. (perennial ryegrass), *Festuca arundinacea* (tall fescue) and *Poa annua* (annual bluegrass), due to accumulating heavy metals, could grow on the heavy metal-polluted soil (Duo et al., 2006; Li et al., 2008a; Wen and Fu, 2008).

Perennial ryegrass, tall fescue and annual bluegrass are three kinds of familiar grass seedlings in the north of China. Currently, it consumes plentiful farming soil for sward production every year in China. When taking the sward, it will shovel off 3–5 cm soil, which must influence the next production. Commonly, after taken the sward for three times or more, the residual soil will not be suitable to plant any kind of crop, and results in a severe obsolescence of farming soil (Hu et al., 2002). For a country with a lacking soil resource, it is not a wise choice to produce the sward in this traditional way.

To find the solution for the recycling use of aged refuse and severe obsolescence of farming soil from sward production, more attention was paid to whether aged refuse could be used as vegetable soil to produce the sward by selecting tolerant grass

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seedlings. Therefore, the present study was conducted to identify tolerant grass to aged refuse by determining their growth and chlorophyll content; and then we produced some data clarifying the tolerant characteristics by analyzing the physiological responses under the aged refuse-induced stress, including oxidative stress and antioxidant capacity.

2. Materials and methods

2.1. Preparation of aged refuse leaching

The aged refuse was excavated from Xingou Soild Waste Landfill in Taiyuan of China, which had been filled for more than 13 years. After screened, the proportion ($d_p < 900\ \mu m$) was chosen to use.

Above aged refuse sample was mixed with distilled water (1:1, V/M). The mixture was stirred for 3 h, placed for 24 h at room temperature, and then centrifuged at 3000*g* for 15 min. The supernatant portion was collected and diluted to 20%, 50%, 80%, and 100% with distilled water.

2.2. Analysis of characteristics of aged refuse and its leaching

To compare with ordinary lawn soil, several basic properties of aged refuse were determined: pH, electrical conductivity (EC) by the method recorded by Xi et al. (1996); humus, effective nitrogen (Effective N), and effective phosphorus (Effective P) according to the methodology described by Bao (2005).

Then the following parameters of its leaching were systematically measured: pH, EC, chemical oxygen demand (COD_{Cr}), biochemical oxygen demand (BOD_5), total nitrogen (Total N) and total phosphorus (Total P) by the method published by Xi et al. (1996). The levels of total chromium (Total Cr), zinc (Zn), manganese (Mn) and iron (Fe) analyzed by atomic absorption spectrophotometry.

2.3. Plant materials and leaching sample treatment

Three kinds of familiar grass seedlings (perennial ryegrass, tall fescue and annual bluegrass), supplied by Institute of Agriculture Science in Shanxi Province, were selected as the test plants. Dry seeds were soaked for 48 h in distilled water and allowed to germinate on moist filter paper.

When reached about 1.0 cm in shoot length, the seedlings, which were placed in the culture dishes with a diameter of 12 cm and two layers of filters, exposed to the leaching samples of different concentrations, and the negative control was exposed to distilled water for the same period. The water samples of 15 mL were added to each filter every 12 h. All the experimental groups were maintained in an incubator at $25 \pm 1\ ^{\circ}C$ with a dark/light cycle.

2.4. Test of growth

All the seedlings were treated for 7 d with leaching samples and distilled water control in the incubator, and the length of root and shoot from 30 seedlings in each treatment were randomly measured.

2.5. Measurement of chlorophyll content

Chlorophyll content was determined by the method reported by Bao (2005). Briefly, fresh leaf sample was pulverized with distilled water, and the homogenate was extracted by 80% acetone. Absorbance of the supernatant was measured at 663 and 645 nm using a Spectrophotometer (U3010), and chlorophyll content was expressed as mg g^{-1} fresh weight (Fw).

2.6. Determination of physiological responses of the tolerant grass seedling

After the tolerant grass to be chosen, several below physiological responses of it were determined. Because the grass treated by the refuse leaching could grow normally relatively within 7 d, then it would tend to droop after that, and 13 d later, get worse. Hence, in the present study, we treated 7 d as shorter treatment duration, and 13 d as longer time exposure.

2.6.1. Estimation of lipid peroxidation and protein oxidation

The level of lipid peroxidation was estimated by measuring the concentration of malondialdehyde (MDA), a common product and a sensitive diagnostic index of lipid peroxidation (Janero, 1990;El-Moshaty et al., 1993). Fresh leaf sample was ground in 5% trichloroacetic acid (TCA, W/V), and the homogenate was centrifuged at 4000*g* for 10 min. The supernatant portion was added to an equal volume of 0.6% thiobarbituric acid (TBA, W/V). The mixture was incubated at 100 °C for 10 min, and then centrifuged at 3000*g* for 10 min after cooling. The absorbance of the supernatant was measured at 532 nm, and lipid peroxidation level was expressed as μmol g^{-1} Fw.

Protein carbonyl (PCO) content, the most general indicator of protein oxidative damage, was tested by 2,4dinitrophenylhydrazine (DNPH) spectrophotometry (Levine et al., 1990). Briefly, fresh leaf sample was crushed in phosphate buffer (0.05 M, pH 7.0) on ice, and then the homogenate was centrifuged at 10,000*g* for 5 min at 4 °C. After the whole DNPHreaction, the reactive solution was centrifuged at 10,000*g* for 15 min at 4 °C once again. At last, the absorbance of the supernatant was measured at 370 nm, and PCO content was expressed as nmol mg^{-1} protein.

2.6.2. Measurement of activities of antioxidant enzymes

Superoxide dismutases (SOD) activity was measured by Nitro Bluetetrazolium (NBT) spectrophotometry (Zhu et al., 1990). Fresh leaf sample was homogenized in phosphate buffer (0.05 M, pH 7.8), and the homogenate was centrifuged at 1000*g* for 20 min at 4 °C. The supernatant was added to the reaction mixture, containing 130 mM DL-Methionine (Met), 750 μM NBT, 100 μM $EDTANa_2$, and 20 μM lactoflavin, for determining the absorbance at 560 nm. One unit of SOD was defined as the amount of the enzyme that produced a 50% inhibition of NBT reduction, and the activity was expressed as U g^{-1} Fw.

Catalase (CAT) activity was assayed according to the method of Gao (2006) with some modifications: the use of polyvinylpyrrolidone (PVP) instead of Tris–HCl, adjustment of the concentration of H_2O_2 and centrifugal speed. Fresh leaf sample was ground in phosphate buffer (0.2 M, pH 7.8) containing 1% PVP. The homogenate was centrifuged at 4000*g* for 15 min at 4 °C, and the supernatant was used for the enzyme assay. CAT activity was examined by measuring the decrease of absorbance at 240 nm in a reaction mixture, containing 0.3 ml H_2O_2 (0.1 M) and 0.1 ml extract, and the result was expressed as U $min^{-1}\ g^{-1}$ Fw.

Peroxidase (POD) activity was determined using guaiacol method (Maehly, 1955). Fresh leaf sample was ground in phosphate buffer (0.1 M, pH 6.0) on ice, and the homogenate was centrifuged at 4000*g* for 15 min at 4 °C. Then the reaction mixture, which included 5 mM guaiacol as donor and 6.7 mM H_2O_2 as substrate, was added to the supernatant. The rate of change in absorbance of the reactive solution at 470 nm was measured, and the activity was expressed as $\Delta A_{470}\ min^{-1}\ g^{-1}$ Fw.

2.7. Statistical analysis of data

Experiments were repeated for three times, and all values were expressed as mean ± SE. The statistical difference (0.05, 0.01 or 0.001) among the negative control and a series of treated groups was analyzed by oneway analysis of variance (ANOVA), using Origin 7.0 software package.

3. Results

3.1. Properties of aged refuse and its leaching

Basic properties of the aged refuse and ordinary lawn soil were measured and shown in Table 1. Compared with ordinary lawn soil, the aged refuse contained more humus, effective N and P (1.36-fold of ordinary lawn soil for humus; 2.25- and 3.13-fold of ordinary lawn soil for effective N and P, respectively). On the other hand, EC value in aged refuse reached 15-fold of ordinary lawn soil. The results suggested that the aged refuse not only contained excessive EC, which was adverse to plant system, but also included abundant humus, effective N and P, which provided sufficient nutrition for plant growth.

3.2. Selection of tolerant grass seedling to aged refuse

3.2.1. Growth of grass seedlings

Tested leaching sample caused obvious variations on the growth of three grass seedlings in time- and concentration-dependent manners. As indicated in Fig. 1, growth inhibition of shoot augmented with the increase of leaching concentration, and reached peak values after the treatment of 100% sample (8.2% of control for perennial ryegrass, $n=3$, $P < 0.001$; 18.6% of control for tall fescue, $n=3$, $P < 0.001$; and 19.8% of control for annual bluegrass, $n=3$, $P < 0.001$ in 7-day-old seedlings). After the first day of the treatment, perennial ryegrass and annual bluegrass presented significant growth inhibition, but not tall fescue.