Cadmium phytoremediation potential of *Gnaphalium affine* D. Don and physiological response in metal tolerance

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**Abstract.** In this study, the plant growth, physiological responses and phytoremediation potential of the herbaceous plant *Gnaphalium affine* D. Don were evaluated in varying extents of Cd contaminated soil and 40 days cultivation. The results demonstrated that the plant had a high Cd tolerance of up to 300 mg·kg⁻¹ in which Cd content in roots and shoots reached a maximum of 733.9 and 404.6 mg·kg⁻¹ respectively. Cd exposure enhances the malondialdehyde (MDA) level and antioxidative response (SOD, POD and CAT) in shoots of *Gnaphalium affine* D. Don. The bioconcentration factor (BCF) values in all treatments were greater than 1 and the translocation factor (TF) values  were almost 2 at the high Cd concentration treatments. These findings indicated that *Gnaphalium affine* D. Don as a Cd-accumulator can be considered as a potential candidate for phytoremediation in Cd polluted sites.

1. **Introduction**  
Cadmium (Cd) contamination release by industry and other anthropogenic activities, as a serious environmental issue, affects many regions worldwide [1]. Cadmium exerts highly toxic to organisms even at low concentrations [2]. Additionally, Cd containing waste release into the environment can induce cytotoxicity and carcinogenicity due to its nonbiodegradable and environmental accumulation characteristics [3]. Hence, there is an urgent need for the remediation of Cd contaminated soil. Phytoremediation as a cost effective and sustainable remediation approach has been widely concerned by many scholars [4]. The ideal plants for phytoremediation should be rapid growth, large biomass, tolerant to high levels of heavy metals and efficient heavy metals absorption in shoots [5]. Therefore, autochthonous plants in the cadmium-polluted sites especially in the dominant species should be a potential plant for phytoremediation [6]. *Gnaphalium affine* D. Don, a pioneer herbaceous plant belonging to the *Asteraceae* family, is one of the common plants around mining area [7]. Previous studies show that the Cd concentrations in shoots of *Gnaphalium affine* D. Don almost 143 mg·kg⁻¹ indicate that the plant may be a Cd-hyperaccumulator [8]. Moreover, *Gnaphalium affine* D. Don exhibits rapid growth, easy cultivation and high tolerance to metals, which can be suitable to use it for phytoremediation [9]. However, there has been no detailed investigation about the physiological response mechanisms of the herb under heavy metals Cd stress. Oxidative damage of plants is the primary response to heavy metal stress. Cd induced plants overproduction of reactive oxygen species (ROS) like hydrogen peroxide, superoxide anion and hydroxyl radicals that cause cytotoxicity and damage to plants [10]. In order to survive, heavy metal-tolerant plants must have a series of defensive
mechanism such as superoxide dismutase or peroxidase to counteract ROS-induced oxidative stress[11].
Furthermore, the plant physiological response processes under heavy metal pressure should also not be
ignored. In the present study, we intend to elucidate the phytoextraction potential of *Gnaphalium affine*
*D. Don* in Cd-contaminated sites by evaluated its antioxidant system and physiological stress responses
exposed to high levels of cadmium.

2. Materials and methods

2.1. Tested soil sampling and *Gnaphalium affine* D. Don seedlings
The unpolluted soil (0–10 cm) was collected from a vegetable field of He Chi city, Guangxi Province,
China and sieved through a 5-mm diameter steel mesh and stored in the dark at 4°C until use. The main
physical characteristics of the soil were as follows: pH 6.5, organic matter 24.6 g·kg⁻¹, soluble salt 1.17
g·kg⁻¹, Ntot 0.82 g·kg⁻¹, Ptot 0.34 g·kg⁻¹, water holding capacity 25.7 (all w/w). The background
concentration of total Cd in soil was 0.94 mg·kg⁻¹. *G. affine* seedlings were purchased from a commercial
nursery in Zhejiang Province, China. Seedlings with a uniform height of approximately 4 cm were
chosen for the pot experiment.

2.2. Pot experimental design
Five treatments with Cd concentrations of 0 (control), 3, 10, 30, 300 mg·kg⁻¹ were designed, and each
treatment contained three replicates. Fifth plastic pot (9 cm diameter, 8 cm height) were prepared with
each containing contaminated soil equivalent to 800 g (dry weight) at 15% moisture content (dry weight),
and each pot was transplanted three seedlings. All pots were settled in a greenhouse and irrigated with
deionized water to hold the soil humidity at 50% water holding capacity during 40 days cultivation.

2.3. Determination of Plant Growth and Cd Content
All plants were sampled after 40 days of days of planting. Roots were lifted out of the soil and gently
rinsed several times with deionized water. Then, shoot lengths and root lengths in each plant were
measured. A part of fresh leaves was collected for detection of ROS and enzyme activity. Remaining
samples were separated into shoots and roots and dried at 70°C until its weight remained constant. Cd
contents in plants were determined by acid digestion of 2.0 g dry samples in microwave digestion system.
Subsequently, the Cd concentration in the digestion were determined using an ICP mass spectrometer
(7700, Agilent, USA).

2.4. Lipid peroxidation contents
Lipid peroxidation was determined by estimation of the MDA content in plant leaves by the
thiobarbituric acid (TBA) reaction [12]. Briefly, 0.5 g fresh leaf sample was ground into homogenate
with 5mL 0.1% trichloroacetic acid (TCA). After centrifuged at 10,000 g for 5 min, transfer of 2mL
supernatant to a new tube. Add 2mL 0.67% thiobarbituric acid (TBA) to the tube and heat it at 100°C
for 30 min. After cooling naturally, the supernatant were collected by centrifugation and measure it at
the wavelengths of 450, 532, and 600 nm. The concentration of MDA were calculated using the
following formula:
\[
\text{MDA (µmol·L}^{-1}) = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}
\]  

2.5. Determination of antioxidative enzymes activity
1.0 g of fresh leaves were homogenized in 3mL of 50 mmol·L⁻¹ phosphate buffer (pH 7.8) in an ice-
water bath. The homogenate was centrifuged at 10000 rpm for 10 min, and collect supernatant for the
further enzymatic assays. SOD activity was determined using the SOD activity kit (Nanjing Jiancheng
Biotech Inst, China), according to manufacturer’s instructions. The CAT activity was measured using
the catalase assay kit (Nanjing Jiancheng Biotech Inst, China). POD activity was measured according to
manufacturer’s protocol about peroxidase assay kit (Nanjing Jiancheng Biotech Inst, China).
3. Results and discussion

3.1. Effect of Cd on plant growth in Gnaphalium affine D. Don
During 40 days cultivation, visual toxicity symptoms such as necrosis or wilted were not observed in G. affine. Pot experimental suggest that G. affine is able to tolerate high levels of Cd stress. The growth characteristics of plants is an important index to evaluate the phytoremediation of contaminated soil [13]. The growth of G. affine exposed to Cd contaminated soil are indicated by shoot lengths and root lengths which have shown in Figure 1. The growth of both shoots and roots first showed an increasing trend and then decreased. Under 10 mg·kg⁻¹ cadmium stress, the shoot lengths and root lengths increased by 38.8% and 35.2% compared with the blank control, respectively. However, their lengths were significantly shortened in the high concentrations of cadmium (300 mg·kg⁻¹) treatment. The results indicate that Cd could promote G. affine growth at low concentrations but inhibits growth at high concentrations. Similar results also showed that the growth of other hyperaccumulator affected by Cd contaminated soil [10]. It can be explained by Hormesis effect which low toxicity bring an excitatory effect to stimulate plant growth [14].

![Figure 1. Effect of Cd on plant growth](image)

3.2. Cd distribution and accumulation characteristics in Gnaphalium affine D. Don
The Cd concentration in roots and shoots of G. affine plant after 40 days of exposure to different concentrations of CdCl₂ are shown in Figure 2. The Cd accumulation in shoots and roots of G. affine were enriched with the increasingly cadmium stress. At 300 mg·kg⁻¹ Cd treatment, Cd content in roots and shoots reached the maximum of 733.9 and 404.6 mg·kg⁻¹ respectively. Cd accumulation in shoots under high concentration treatments all exceeded 100 mg·kg⁻¹, which is a standard of Cd hyperaccumulators. There was no significant trend in the heavy metal accumulation when treated with Cd at low concentration. However, Cd concentration was far higher in shoots than in roots at high Cd concentration stress. Metal uptake by plant from bottom to top involves transport of metals across the plasma membrane of root cells, translocate of xylem tissues and sequestration of metals[15]. A good hyperaccumulator is allowed to have the characteristics of transporting heavy metals from roots to shoot. Therefore, bioconcentration factor (BCF) and translocation factor (TF) are two important indicators for exploring hyperaccumulator. In the present study, the BCF values for all treatments were greater than 1 and the TF values were almost 2 at the high Cd concentration treatments (Table 1). These results indicating Cd transport from root to shoot efficiently and G. affine is potentially employed to phytoremediation of Cd-contaminated soils.
Figure 2. Cd distribution and accumulation characteristics in *Gnaphalium affine* D. Don

Table 1. Bioconcentration factor (BCF), Translocation factor (TF) in *Gnaphalium affine* D. Don

| Cd concentration (mg·kg\(^{-1}\) dry weight soil) | BCF  \(\frac{C_{\text{shoot}}}{C_{\text{soil}}}\) | TF  \(\frac{C_{\text{shoot}}}{C_{\text{root}}}\) |
|-----------------------------------------------|----------------|----------------|
| 0                                            | 3.18           | 0.54           |
| 3                                            | 4.21           | 0.96           |
| 10                                           | 1.62           | 0.73           |
| 30                                           | 9.54           | 2.62           |
| 300                                          | 2.45           | 1.81           |

3.3. Effect of Cd on MDA in *Gnaphalium affine* D. Don

MDA of plant leaves was measured to evaluate the oxidative damage of membrane lipids in response to Cd stress. MDA content can reflect the harm to the plant under adverse environment. High Cd concentration will lead plants accumulate ROS which may cause membrane peroxidation and produce malondialdehyde (MDA) accumulation [16]. In the present study, MDA levels tended to raise with increasing soil Cd concentrations however it was not developing after the Cd levels in soil were up to 30 mg·kg\(^{-1}\) (Figure 3). Our results were consistent with the discoveries described of a herbaceous Cd-hyperaccumulator *Coronopus didymus* [17].
3.4. Effect of Cd on antioxidant enzyme activities in Gnaphalium affine D. Don

The detoxification capacities of plants to Cd toxicity were assessed by the shifts in SOD, POD and CAT enzyme activities in the leaves of plants exposed to Cd. Antioxidative enzymes like SOD, CAT and POD play an essential role in detoxify reactive or toxic intermediates generated due to heavy metal stress [18]. As compared with the controls, the activity of SOD, CAT and POD in shoots were significantly higher by 61.3%, 82.4% and 74.2% respectively at highest (300 mgꞏkg⁻¹) Cd concentration. In Figure 4, there is a clear trend that SOD, CAT and POD activities were increased with the Cd levels in the soil. The result was consistent with the tendency in MDA as mentioned above. Hence, the increasing anti-oxidative response detected in this work should be attributable to the ROS accumulation that ultimately led to the upregulation of antioxidant enzymes. These results were in line with previous research in Cd hyperaccumulators Thlaspi caerulescens [19]. Such effective anti-oxidative response might be one of the detoxification mechanisms to help Gnaphalium affine D. Don tolerate high concentrations of heavy metals.
4. Conclusions
In the present work, *G. affine* demonstrated a strong tolerance ability to Cd at physiological levels. Its BCF and TF were both greater than 1 at high levels of cadmium proved that Cd transport from root to shoot efficiently. *G. affine* displayed a strong antioxidative response (SOD, CAT and POD) with the elevated Cd levels indicated that it plays an essential role in suppression of ROS production. *G. affine* can be considered as a potential Cd accumulator for phytoremediation in contaminated sites.

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