Ecotoxicology and Environmental Safety 80 (2012) 393–400

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Model evaluation of plant metal content and biomass yield for the phytoextraction of heavy metals by switchgrass

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ARTICLE INFO

Article history:
Received 9 February 2012
Received in revised form
7 April 2012
Accepted 10 April 2012
Available online 25 April 2012

Keywords:
Heavy metal
Phytotoxicity
Phytoremediation
Panicum virgatum L.
High-biomass plant

ABSTRACT

To better understand the ability of switchgrass (*Panicum virgatum* L.), a perennial grass often relegated to marginal agricultural areas with minimal inputs, to remove cadmium, chromium, and zinc by phytoextraction from contaminated sites, the relationship between plant metal content and biomass yield is expressed in different models to predict the amount of metals switchgrass can extract. These models are reliable in assessing the use of switchgrass for phytoremediation of heavy-metal-contaminated sites. In the present study, linear and exponential decay models are more suitable for presenting the relationship between plant cadmium and dry weight. The maximum extractions of cadmium using switchgrass, as predicted by the linear and exponential decay models, approached 40 and 34 $\mu g\ pot^{-1}$, respectively. The log normal model was superior in predicting the relationship between plant chromium and dry weight. The predicted maximum extraction of chromium by switchgrass was about 56 $\mu g\ pot^{-1}$. In addition, the exponential decay and log normal models were better than the linear model in predicting the relationship between plant zinc and dry weight. The maximum extractions of zinc by switchgrass, as predicted by the exponential decay and log normal models, were about 358 and 254 $\mu g\ pot^{-1}$, respectively. To meet the maximum removal of Cd, Cr, and Zn, one can adopt the optimal timing of harvest as plant Cd, Cr, and Zn approach 450 and 526 $mg\ kg^{-1}$, 266 $mg\ kg^{-1}$, and 3022 and 5000 $mg\ kg^{-1}$, respectively. Due to the well-known agronomic characteristics of cultivation and the high biomass production of switchgrass, it is practicable to use switchgrass for the phytoextraction of heavy metals in situ.

1. Introduction

Phytoextraction, developed to remove heavy metals from contaminated sites using plants, has drawn a lot of attention due to its cost-effectiveness and good environmental effect (Alkaorta et al., 2004). A plant that produces high biomass, is easy to harvest, and has high capacity as well as high tolerance for heavy metal uptake that is readily accumulated in the tissues harvested is preferred for phytoextraction (Nanda-Kumar et al., 1995). The review papers (Clemens et al., 2002; Padmavathiamma and Li, 2007; Vamerali et al., 2010) have summarized the plant species to accumulate heavy metals with their subsequent use in phytoextraction. However, some studies on heavy metal accumulation have shown that the dry weight of plant tissues decreases as metal concentrations in plant tissues increase (Gardea-Torresdey et al., 2005; Scheirs et al., 2006; Tang et al., 2009). This indicates that the accumulation of heavy metals in plants would result in toxicity and a reduction in biomass yield (Lindblom et al., 2006). Several studies have investigated the reduction of biomass production due to harsh metal contamination (Shen et al., 2002; Gardea-Torresdey et al., 2004; Shiyab et al., 2009). Biomass reduction related to metal toxicity has been used as an index for assessing the metal tolerance of plants (Mei et al., 2002). So far, there has been little effort to assess the effect of biomass reduction on the performance of phytoextraction (Megateli et al., 2009; Khellaf and Zerdaoui, 2010; Juang et al., 2011).

A quantitative conceptual model can be used to express the amount of phytoextraction (w), which is shown as

$$w = xm, \tag{1}$$

where x is the plant metal content and m represents the dry weight of biomass. The x value indicates the accumulation of metals by plants, and m shows biomass yield in response to metal accumulation. The w value reveals metal removal by harvesting plant tissues. Maxted et al. (2007) used Eq. (1) to measure the amount of phytoextractable cadmium (Cd) removed by *Thlaspi caerulescens*. Liang et al. (2009) also defined metal removal by *T. caerulescens*, *Arabidopsis halleri*, *Nicotiana tabacum*, and *Brassica juncea* in Eq. (1). Previous studies focused only on phytoextraction

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http://dx.doi.org/10.1016/j.ecoenv.2012.04.011

under low-level contamination and given plant growth without retardation. This, however, was not enough to certainly determine the performance of metal extraction by plants in a real contaminated site. In an early study, Brennan and Shelley (1999) emphasized that plant growth is a key component in predicting phytoextraction of heavy metals. The results of the sensitivity analyses show that effective root mass and maximum shoot dry mass were moderately to highly sensitive to modeling phytoextraction. This suggests that one should be careful when using the assumption of plant growth without retardation in modeling phytoextraction of heavy metals. In recent studies by Guala et al. (2010, 2011), a kinetic model was proposed to predict the inhibition of plant growth due to heavy metal concentrations. While the reduction of biomass due to high heavy-metal concentrations in soil was considered, the maximum accumulations of heavy metals in the harvestable parts of plants could be estimated by using the proposed kinetic model. Because the model relates the dynamics of the uptake of heavy metals by plants to the heavy metal deposed in soil, the model parameters are complex and have to be adjusted by the plant species and the soil properties. Its applicability, however, should be tested by a series of further experimental results. In our previous study (Juang et al., 2011), a simple kinetics model without taking soil characteristics into account was used to assess the phytoaccumulation and phytotoxicity of metals. The results also suggest a compromise between metal accumulation and biomass production to predict the phytoextraction of heavy metals for the selection of potential plant species.

Switchgrass (*Panicum virgatum* L.), a perennial grass, is often relegated to marginal agricultural areas with minimal inputs and yields the highest biomass among native grasses in North America. It was adapted for nearly all of the USA east of the Rocky Mountains and much of eastern Canada as a feedstock for bioenergy production (Casler and Boe, 2003). It was also planted along stream banks and riparian lands for phytoremediation of petroleum contaminants (Euliss et al., 2008) and used as a filter strip to prevent soil nutrient loss into runoff water (Sanderson et al., 2001). Because of the well-known agronomic characteristics of cultivating switchgrass (i.e., fertilization, irrigation, soil pH optima, plant density, biomass field-drying, and harvest schedule), switchgrass has been used for the phytoextraction of metals. In early studies, switchgrass was used for the phytoremediation of ^{137}Cs- and ^{90}Sr-contaminated soil (Entry and Watrud, 1998) and of chromium (Cr)-contaminated soil (Shahandeh and Hossner, 2000). Reed et al. (1999, 2002) reported that switchgrass was tolerant of Cd when grown in a sand culture and could accumulate Cd at a low soil pH. Chen et al. (2011) suggested that Alamo is the better cultivar of switchgrass suited for the phytoextraction of Cd from contaminated soil. However, in previous studies involving switchgrass, the effect of the reduction of biomass on the performance of phytoextraction accompanying high metal concentrations in plants was not determined.

In this study, the relationship between plant metal concentration and dry weight was taken into account in predicting metal removal by harvesting plant tissues. According to Eq. (1), dry weight (m) was defined as a decreasing function $f(x)$ of plant metal concentration that illustrated the conflict between biomass production and metal accumulation. Predicting the amount w of metal extracted by plants was thus a compromise between metal accumulation and biomass production. In addition, the chronic toxicity endpoints, no-observed-adverse-level (NOAEL) and lowest-observed-adverse-level (LOAEL), were used to illustrate the effects of a significant decrease in dry weight on the metal accumulation by plants. The objectives of the present study were (i) to examine the metal accumulation and biomass production of switchgrass influenced by different heavy metal exposures in hydroponic experiments, (ii) to model the relationships between plant metal concentration and the dry weight of switchgrass, and (iii) to predict the extractions of different metals by switchgrass using models of the relationship between plant metal content and biomass yield.

2. Materials and methods

2.1. Hydroponic experiment

A lowland eco-type of switchgrass, Alamo, was used in the experiments. Alamo cultivar was reported to have the highest yield of biomass in a given environment. The seeds of Alamo switchgrass were soaked in deionized water until they germinated. After 3 weeks of growth, the Alamo switchgrass seedlings were in the morphological development stage, with three leaves completely emerged and the plant about 8 cm high. Then, they were transplanted in 1.5 L polypropylene pots filled with 1.2 L of 25% strength modified Hoagland solution (Greger and Löfstedt, 2004). The solution's pH was controlled at about 5.5 ± 0.5. A full-strength Hoagland solution contains 1 mM KH_2PO_4, 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 92 μM H_3BO_3, 1.5 μM $ZnSO_4$, 0.6 μM $CuSO_4$, 0.2 μM MoO_3, 18 μM $MnSO_4$, and 3.6 μM $FeSO_4$ (Scheirs et al., 2006). To compare switchgrass with other plant species, the exposure levels of Cd, Cr, and Zn were set taking into consideration the previous studies and reports that investigated other plants' tolerance of Cd, Cr, and Zn exposures and the phytoaccumulation of Cd, Cr, and Zn (Mei et al., 2002; Gardea-Torresdey et al., 2004; Pedler et al., 2004; Arduini et al., 2006; Cosio et al., 2006; Tandy et al., 2006). Five cadmium treatments (i.e., 0, 0.4, 4, 8, and 20 μM Cd as $CdCl_2$ added to the nutrient solution), four chromium treatments (i.e., 0, 10, 20, and 40 μM Cr as $K_2Cr_2O_7$ added to the nutrient solution), and five zinc treatments (i.e., 0, 7.5,75, 225, and 450 μM Zn as $ZnSO_4$ added to the nutrient solution) were applied, respectively. Three seedlings were placed in one pot containing the nutrient solution for a 1 week acclimation. Then, the seedlings were exposed to the metal-treated solutions for 30 days. Three pots were used as duplicates for each metal treatment. The potted plants were grown in a greenhouse under natural light while keeping the intensity at about 600 μE m^{-2} s^{-1}, where the photoperiod was at about 16/8 h day/night cycle. The mean temperature throughout the vegetation period was 30 °C, within the range of 25–35 °C. The mean relative humidity was 75%, within the range of 65–85%. The shoots and roots of the switchgrass plants were harvested and thoroughly washed with deionized water. The samples were oven-dried at 65 °C for 72 h. The dry weight of the harvested tissues (the shoots and roots pooled together) for each potted plant was recorded. Then, each plant sample of 0.2 g was placed in a digestion tube with 5 mL concentrated H_2SO_4 mixed thoroughly and allowed to sit overnight. The digestion tubes were placed in a heating block set at 300 °C for 4 h. 2 mL of 30% H_2O_2 was added to each digestion tube after cooling. They were heated again at 150 °C for about 2 h and cooled down to room temperature. Upon complete digestion of the plant sample, the sample solution was diluted to 50 mL, and the Cd, Cr, and Zn concentrations of each sample were determined on a flame atomic absorption spectrophotometer (PerkinElmer AAnalyst 200). In addition, the chemicals used in this study were all of reagent grade and purchased from Merck Ltd.

2.2. Statistical analysis and curve model fitting

Analysis of variance (ANOVA) was used to determine the effects of metal treatments on plant metal concentration and dry weight. The generalized linear model (GLM) procedure was carried out to perform ANOVA (SAS Institute Inc., 1985), and a multiple comparison of the treatment means was conducted with Fisher's protected least significant difference (LSD) at the 0.05 level. The treatment plant metal concentration and dry weight means were used to examine metal accumulation and biomass production in switchgrass under different metal exposure concentrations. NOAEL (defined as the highest exposure concentration producing no adverse effect significantly different from the control) and LOAEL (defined as the lowest exposure concentration producing adverse effects significantly different from the control) values based on the dry weight of harvested switchgrass plants were determined using the LSD procedure.

Three decreasing functions $f(x)$ frequently used for dose–response relationship modeling (Schabenberger et al., 1999; Faust and Christians, 2000; Santos et al., 2007) were adopted in this study. These are linear (L), exponential decay (ED), and log normal (LN), whch are respectively expressed as follows:

$$f(x)_{\mathrm{L}} = a - bx, \quad (2)$$

$$f(x)_{\mathrm{ED}} = a\exp(-bx) \quad (3)$$

$$f(x)_{\mathrm{LN}} = a\exp\{-0.5b^{-2}[\ln(x/c)]^2\}. \quad (4)$$

where a, b, and c are the model parameters. $f(x)$ indicates the dry weight corresponding to the plant metal concentration of x. The parameters (a, b, and c) were obtained by using the linear and nonlinear fitting procedures (SAS Institute Inc., 1985).