Chemosphere 175 (2017) 85–96

Contents lists available at ScienceDirect

Chemosphere

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

Phytoextraction of contaminated urban soils by *Panicum virgatum* L. enhanced with application of a plant growth regulator (BAP) and citric acid

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HIGHLIGHTS

- Application of plant growth regulator benzylaminopurine (BAP) increased dry mass (DM) production (48%) of *P. virgatum*.
- Chemical application of benomyl (B), citric acid (C) and PGR (H) increased lead (Pb) concentration 955% in plants foliage.
- Total phytoextracted Pb was significantly highest for plants treated with the combined chemical application of B + C and B + C + H.
- Soil acidification increased significantly concentrations of aluminum (Al) and iron (Fe) in plants foliage.

ARTICLE INFO

Article history:
Received 4 October 2016
Received in revised form
5 January 2017
Accepted 3 February 2017
Available online 4 February 2017

Handling Editor: T Cutright

Keywords:
Biomass production
Lead
Phytoextraction
Plant growth regulator
Soil acidification
Switchgrass

ABSTRACT

Lead (Pb) contamination in soil represents a threat to human health. Phytoextraction has gained attention as a potential alternative to traditional remediation methods because of lower cost and minimal soil disruption. The North American native switchgrass (*Panicum virgatum* L.) was targeted due to its ability to produce high biomass and grow across a variety of ecozones. In this study switchgrass was chemically enhanced with applications of the soil-fungicide benomyl, chelates (EDTA and citric acid), and PGR to optimize phytoextraction of Pb and zinc (Zn) from contaminated urban soils in Atlanta, GA.

Exogenous application of two plant hormones was compared in multiple concentrations to determine effects on switchgrass growth: indole-3-acetic acid (IAA), and Gibberellic Acid (GA_3), and one PGR benzylaminopurine (BAP), The PGR BAP (1.0 μM) was found to generate a 48% increase in biomass compared to Control plants.

Chemical application of citric acid, EDTA, benomyl, and BAP were tested separately and in combination in a pot experiment in an environmentally controlled greenhouse to determine the efficacy of phtyoextraction by switchgrass. Soil acidification by citric acid application resulted in highest level of aluminum (Al) and iron (Fe) in plants foliage resulting in severe phytotoxic effects. Total Pb phytoextraction was significantly highest in plants treated with combined chemical application of B + C and B + C + H.

Suppression of AMF activities by benomyl application significantly increased concentrations of Al and Fe in roots. Application of benomyl reduced AMF colonization but was also shown to dramatically increase levels of septa fungi infection as compared to Control plants.
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1. Introduction

Heavy metal contamination of urban soils has produced a legacy of persistent chronic exposure of generations of urban children to dangerous levels of contaminants (Lanphear et al., 2005; Filippelli and Laidlaw, 2010). Lead (Pb) contamination of soil in excess of

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http://dx.doi.org/10.1016/j.chemosphere.2017.02.022
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200 mg kg^{-1} is common in many urban areas and represents a serious threat to human health (Ragaini et al., 1977; Ettler et al., 2004; Centers for Disease Control & Prevention CDC, 2010). Once in the soil, Pb is extremely persistent through time and available to become re-suspended, resulting in a long-term exposure hazard for infants and children (Laidlaw and Taylor, 2011; Zahran et al., 2013; Mielke et al., 2016).

Lead has been commonly used in many industrial applications and as tetraethyllead ($(CH_3CH_2)_4Pb$), a former additive in automotive gasoline and current additive to some aviation fuel, making it one of the most widespread heavy metal pollutants in soil (Lagerwerff and Specht, 1970; Gulson et al., 1995; Mielke and Reagan, 1998). In addition, Pb contamination in soil is derived from Pb-arsenate as pesticide, Pb-mining and recycling activities, Pb-bullets in shooting ranges and also as impurities in mineral fertilizers (Dudka and Adriano, 1997; Peryea, 1998; Schooley et al., 2009; Huo et al., 2007; Zhuang et al., 2009; Chrastný et al., 2010; Al-Sabbak et al., 2012; Greipsson et al., 2013; Zahran et al., 2013; Nacke et al., 2013; Johnson et al., 2015).

Health effects of chronic exposure to low level of Pb include cognitive impairments, such as IQ deficits, attention-related behavioral problems and early-onset of Alzheimer's disease, as well as cardiovascular, immunological and adverse endocrine effects (Bellinger et al., 2003; Canfield et al., 2003; Lanphear et al., 2005; Tellez-Rojo et al., 2006; Bellinger, 2008; Jusko et al., 2008; Wu et al., 2008; Nigg et al., 2010; Bakulski et al., 2012; Sawalha et al., 2013). In addition, the adverse effect of maternal exposure to Pb can result in DNA methylation changes and multigenerational epigenetic inheritance in humans (Sen et al., 2015).

Several remediation options are available for Pb-contaminated soils. Phytoextraction is a preferred soil remediation technique for moderately contaminated sites because of low cost and reduced environmental impact through decreased soil disturbance (Cunningham and Berti, 2000; McGrath and Zhao, 2003; Leung et al., 2013; Ali et al., 2013; Sheoran et al., 2016). Phytoextraction utilizes plants to extract metals and chemical pollutants from soil by translocation of the pollutant into harvestable plant tissue (Cunningham and Berti, 2000; Peer et al., 2006). Phytoextraction of Pb by plants alone is not yet feasible on an industrial scale because of slow uptake and incorporation of Pb into plant's foliage (Huang et al., 1997; Van Nevel et al., 2007). Phytoextraction is usually a lengthy process and remediation of soil with 1000 mg kg^{-1} of Pb could take up to 30 years to reach an acceptable level (Huang et al., 1997). Slow Pb uptake is caused by many factors including low solubility of Pb in soil and presence of symbiotic soil microbes such as arbuscular mycorrhizal fungi (AMF) (Leung et al., 2013). Phytoremediation of contaminated soils using high biomass biofuel crop such as switchgrass (*Panicum virgatum* L.) has recently been emphasized (Van Ginneken et al., 2007; Oh et al., 2013; Balsamo et al., 2015). Phytoextraction capability of fast growing and high biomass producing plants can be optimized through addition of chemicals such as ethylenediaminetetraacetic acid (EDTA) or natural acids which increase Pb mobility by forming soluble EDTA-Pb complexes. These compounds acts as ligands that are able to grasp transition metals between two or more donor atoms, thereby increasing the uptake of metals such as Pb that normally occur bound to soil particles or as stable salts (Huang et al., 1997; Bhargava et al., 2012). Previous studies have shown that optimal phytoextraction of Pb occurs with applications of 1.0 mmol EDTA kg^{-1} soil (Hovsepyan and Greipsson, 2005; Perry et al., 2012), Use of EDTA in phytoextraction is of concern because of the risk of groundwater contamination and natural acids have been considered as a replacement chelate (Bolan et al., 2014). Citric acid has been proposed as a possible alternative because, unlike EDTA, it is readily broken down by soil microbes, has a short persistence time of approximately one week in soil, and therefore has a lower risk of groundwater contamination (Freitas et al., 2013). Citric acid application can however, have adverse effects by inducing hydrogen toxicity resulting in severe oxidative stress causing chlorosis and necrosis of plants (Kidd and Proctor, 2001). Previous studies have shown that Pb phytoextraction is maximized when soil pH is lowered to 4.0–4.5; the concentration of citric acid required depends on initial soil pH (Dong et al., 1999). Lowering soil acidity to pH 4.0–4.5 for maximum Pb mobility may also solubilize other metals to toxic levels, such as aluminum (Al), present in the soil; aluminum toxicity results in severe discoloration, interference with chlorophyll synthesis, stunted growth, and high mortality rate (Kochian, 1995; Dong et al., 1999; Geebelen et al., 2002). Use of citric acid as a replacement for EDTA has shown limited success in previous studies (Chen et al., 2003; Cui et al., 2013; Sinhal et al., 2010). Cui et al., 2013 indicated that soil acidification is not feasible for effective phytoextraction and citric acid must be supplemented with EDTA. However, Freitas et al. (2013) indicated that citric acid can be effectively used as a chelate without supplemental application of EDTA. This study investigates the effect of citric acid on Pb uptake of switchgrass with and without supplemental applications of EDTA.

Soil microbes such as AMF must be considered in phytoextraction using switchgrass since they can enhance uptake of macronutrients (mainly phosphorus (P) and metals such as zinc (Zn) and magnesium (Mg) and reduce uptake of iron (Fe) and aluminum (Al) (Clark, 2002). AMF can influence metal uptake through down-regulation of plant genes encoding for metal transporters (MT2 and Nramp1) by preventing metal transportation from root cells into vascular xylem and excessive metal (including Pb) uptake into plant foliage (Ouziad et al., 2005). AMF are also thought to up-regulate expression of several plant genes coding for proteins (including metallothionein) presumably involved in heavy metal tolerance of plants (Lanfranco et al., 2002) and increase phytochelatin (peptides) synthase gene (PCS1) expression (Xu et al., 2014). Phytochelatins are thought to be critical for transporting and sequestering Pb into plant vacuoles to minimize interferences with cellular processes (Xu et al., 2014). AMF were also found to produce a glycoprotein (glomalin) that sequesters toxic metals (Gonzalez-Chavez et al., 2004). Previous studies indicated that improved Pb uptake and translocation occurs after application of the fungicide benomyl (Hovsepyan and Greipsson, 2004). Benomyl is known to provide temporary suppression of AMF activities (Kahiluoto et al., 2000). However, benomyl application can also result in decreased phosphorus (P) uptake of plants, decreased biomass production, and chlorosis (Paul et al., 1989; Hovsepyan and Greipsson, 2004; Perry et al., 2012; Johnson et al., 2015).

Exogenous application of plant hormones has been successfully used on crops in phytoextraction (Cabello-Conejo et al., 2013; Bulak et al., 2014). Plant hormones and plant growth regulators (PGR) can affect plant biomass and stress responses related to metal toxicity (Sayed, 1999; Tassi et al., 2008; He et al., 2013; Bulak et al., 2014). Recent studies have shown that foliar application of plant hormones such as gibberellic acid (GA_3) and indole-3-acetic acid (IAA) have improved phytoextraction of Pb by corn (*Zea mays*) (Hadi et al., 2010) and alfalfa (*Medicago sativa*) (López et al., 2005). In both cases dual application of plant hormone and EDTA resulted in synergistic effects on Pb accumulation in plant's foliage (Hadi et al., 2010; López et al., 2005). Dual application of IAA and EDTA increased phytoextraction of Pb into harvestable biomass by 2800% over control plants (López et al., 2005). Foliar application of a PGR, benzylaminopurine (BAP) resulted in increased biomass in switchgrass (Burris et al., 2009).

The main aim of this study was to examine the effects of exogenous plant hormone or PGR application on biomass

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