Chemosphere 210 (2018) 495–502

Contents lists available at ScienceDirect

Chemosphere

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

Monitoring the stress resistance of *Pennisetum purpureum* in Pb (II) contaminated soil bioaugmented with *Enterobacter cloacae* as defence strategy

Anamika Das, Jabez W. Osborne*

School of BioSciences and Technology, Vellore Institute of Technology, Vellore, 632014 Tamil Nadu, India

HIGHLIGHTS

- Eco-friendly strategy for bioremediation of lead by living biosystems.
- Bacterial augmentation reduces lead toxicity on plants with enhanced lead uptake.
- Bioaugmentation increases protein and proline content of plants significantly.
- Low antioxidant enzymatic activities indicate reduced oxidative damage to plants.
- Significant role of bacteria in enhanced resistance of plants to lead toxicity.

ARTICLE INFO

Article history:
Received 23 April 2018
Received in revised form
14 June 2018
Accepted 9 July 2018
Available online 11 July 2018

Handling Editor: T. Cutright

Keywords:
Lead
Pennisetum purpureum
Enterobacter cloacae
Antioxidant enzymes
Oxidative damage

ABSTRACT

Lead (Pb) is reported to have negative effects on the biogeochemical behaviour of the plant growth. In recent years, the significance of rhizoremediation of heavy metals has been of great focus aiding in the development rates of plants under stressed conditions. The present study evaluated the physio-biochemical response of *Pennisetum purpureum* to different concentrations of Pb (II) viz., 0, 50, 100 and 150 mg kg^{-1} in the form of lead (II) nitrate. The pre-characterized PGPR strain, *Enterobacter cloacae* - KU598849 was used to augment the plants. After Pb exposure for 45 d, parameters such as plant growth, lead accumulation, H_2O_2 content, MDA content, protein, proline content and antioxidant enzymatic activities were quantified. Results illustrated that increasing Pb concentration reduced the early growth, metal accumulation, protein content and affected physio-biochemical changes by causing oxidative damage in plants. Upon augmentation of the bacterial inoculum, the plants significantly resisted the toxic effects of Pb. Increased Pb bioaccumulation pattern was recorded in roots than shoots, were highest uptake was found to be 72 mg kg^{-1} dry weight when exposed to 150 mg kg^{-1} Pb concentration. Lead supplementation increased the activities of malonylaldehyde (MDA), superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX) and catalase (CAT) in *P. purpureum*. Bacterial bio-augmentation resulted in the reduction of the oxidative stress aided with reduced antioxidant enzyme activities indicating the minimization of the damages under stress.

1. Introduction

Heavy metals threat is a widespread and an emerging area of concern for sustainability of agro-ecosystems and human well-being (Anjum et al., 2016). Certain metals such as Cu and Zn are essential nutrients for the growth of the plant at lower concentrations but are toxic at higher concentrations, hence acts as double-edged sword (Deng et al., 2016). There is an inevitability to increase the crop productivity by utilizing the heavy metal polluted soil to eliminate the risk of the heavy metals entering the food chain at higher concentrations (Gall et al., 2015).

Lead (Pb) has been identified as the second most hazardous heavy metal due to its potential toxicity worldwide. It is mostly used in paints, batteries, insecticides etc. and is extremely persistent and toxic (Giaccio et al., 2012). It has been reported to pollute most of the arable agricultural land across the globe (Manikandan

* Corresponding author.
E-mail addresses: dasanamika02@gmail.com (A. Das), jabez.vit@gmail.com (J.W. Osborne).

https://doi.org/10.1016/j.chemosphere.2018.07.050
0045-6535/

et al., 2015) and has a negative effect in plant growth by decreasing the absorption of essential nutrients and altering their physio-biochemical functions (Islam et al., 2008). The absorption of the nutrients in the plants is inhibited by increasing concentrations of lead as it binds to the ion transporters and makes them unavailable for plant uptake (Rodriguez-Hernandez et al., 2015). When exposed to higher concentrations of lead, it leads to decreased chlorophyll synthesis, imbalance in water translocation, morphological alterations and genotoxicity (Cenkci et al., 2010). Pb induces oxidative stress in plants by excessive production of reactive oxygen species (ROS) including superoxide anion (O^{2-}), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH) that oxidizes numerous biological proteins and can cause cell death (Sharma and Dubey, 2005; Clemens, 2001). Plants have a strong defense system which has the ability to quench ROS and free radicals providing protection to the plants under heavy metal stressed condition (Anjum et al., 2012). Osmoregulation is another phenomena in plants used to scavenge excess ROS (Anjum et al., 2012). There are several reports on variations in the activities of the antioxidants due to the toxicity of Pb (Mishra et al., 2006; Ali et al., 2014).

Application of resources of living origin for metal uptake has gained popularity in the past years. Plants are well documented for heavy metals uptake from contaminated soil and water (Prasad and Maiti, 2016; Chand et al., 2016). The major drawbacks for the plant to establish efficient phytoextraction property is slow growth, low biomass yield and unavailability of the nutrients in metal contaminated soil (Komarek et al., 2007). *Pennisetum purpureum* is a monocot perennial grass of the Poacea family, commonly known as Napier grass. The species has high biomass production and requires less water and nutrients (Khan et al., 2007). The plant has deep penetrating, fibrous root system with horizontal shoot growth, making it ideal for the study of higher metal concentration uptake (Lawal and Ologundudu, 2014). Inoculation of plants with microbes (bioaugmentation) has attained prominence for bioremediation of metal polluted soils. An effective lead resistant bacteria, *Enterobacter cloacae* was augmented in our study. The bioaugmentation process has been reported to reduce the toxic effects of the metals on the plant and promote the plant growth (Das et al., 2017). It possess the lead resistant gene, *pbrA* and has been reported to aid in the bioremoval of lead (Das et al., 2017). Therefore, the further investigation was carried out in the present study a) to find the impact of Pb on plants and its uptake, b) to study the changes in oxidative stress caused by higher Pb concentration, c) to monitor the activation or inhibition of enzymes of antioxidant defense system of the plants under Pb stress and d) to study the influence of *E. cloacae* (bacterial inoculation) in plants for all the above mentioned parameters.

2. Materials and methods

Pot-culture study was conducted in green-house at the Experimental Research Farm in Vellore Institute of Technology (12.9165 °N, 79.1325 °E), India. The region has a humid and subtropical climate characterized by hot weather with temperature ranging from 18.4 °C (65.1 °F) to 39.4 °C (102.9 °F) with 79–89% relative humidity.

2.1. Plant material and experimental procedure

Fresh plantlets of *P. purpureum* were collected from a farm near Brahmapuram, India (12.9657 °N, 79.1676 °E) and rinsed thoroughly with water to remove the surface dust particles and pruned to 5 cm of the root and shoot length prior to planting. The overall experimental flowchart is depicted in Fig. 1. The experiment was performed for 45 d in a randomized factorial design (2 × 2) under greenhouse conditions. The pot dimensions of 30 cm diameter and 20 cm height was used packed with 1 kg of garden soil which was collected from the nursery of Vellore Institute of Technology. It had a red soil texture with pH 5.9 (slightly acidic). The Pb content of the soil was found to be negligible. The complete physicochemical analysis of the soil used for the experimental set-up was performed at National Agro foundation, Chennai by Fertiliser Development and Consultation Organisation (FDCO). The complete details of the experimental soil and contaminated soil is provided in Table S1. The soil was autoclaved twice to eliminate all the native microbes. Each pot consisted of one plantlet and the experiments were performed in triplicates.

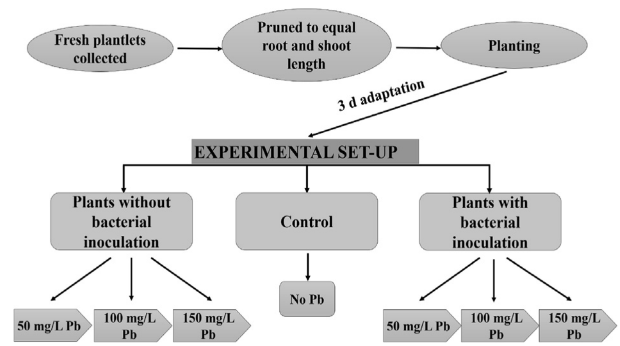

Fig. 1. Experimental flowchart for the pot-culture study.

2.2. Lead treatment and bacterial inoculation

After 3 d of adaptation, the soil was artificially contaminated with lead (II) nitrate which was dissolved in distilled water to make 50, 100 and 150 mg kg^{-1} concentrations in the soil. The pots without Pb supplementation were considered as control. The ability of the plant to tolerate Pb was evaluated by the changes in the physio-biochemical changes. For bioaugmentation, the bacterial strain *Enterobacter cloacae* (accession number- KU359261) was procured from TT-635 Biomolecules lab, Vellore Institute of Technology which was previously isolated from the Pb-contaminated sludge samples of Erode district and was proved to an effective lead resistant bacteria (LRB) (Das et al., 2017). The overnight grown bacterial culture with 0.5 OD having 1.0×10^{12} CFU/mL was used. Cell suspension of 10 mL was added to 1 kg of soil in the pots and mixed for uniform distribution. All the pots received equal volume of water. The bacterial bioaugmentation was provided in intervals to maintain a constant microbial count.

2.3. Growth parameters and Pb content

The plants were harvested at an equal interval of 15 d. The plants were carefully uprooted from the pots and were washed with deionized water. The impact of the treatments on the plant growth was determined by measuring the length of the root and shoot. The relative water content (RWC) of leaf was estimated as described by Araniti et al. (2017). The leaf discs were made from the leaves and incubated in 100 mL distilled water for 24 h. The turgid weight of the leaf samples were taken and the plant samples were dried at 60 °C for 3 d and its dry weight was recorded. The data was generated through the following equation (Eq. (1)).