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Accumulation efficiency, genotoxicity and antioxidant defense mechanisms in medicinal plant *Acalypha indica* L. under lead stress

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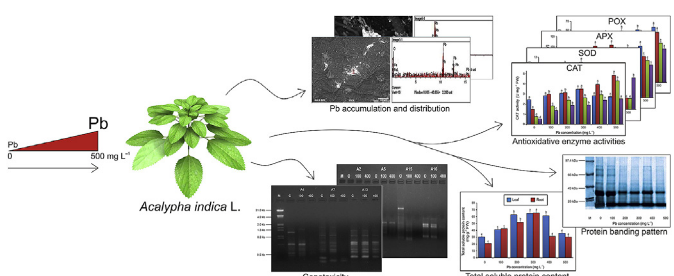
HIGHLIGHTS

- Hyperaccumulation of Pb by *Acalypha indica* was not affected up to 500 mg L^{-1} exposure.
- Pb exposure increased superoxide dismutase activity in both the leaf and root tissues.
- High levels of antioxidative defense enzymes evidenced the detoxification strategy.
- DNA changes showed the activation of molecular mechanism due to Pb genotoxicity.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study was designed to assess the physiological and biochemical changes in roots and shoots of the herb *Acalypha indica* grown under hydroponic conditions during exposure to lead (Pb) (100–500 mg L^{-1}) for 1–12 d. The accumulation of Pb by *A. indica* plants was found to be 121.6 and 17.5 mg g^{-1} dry weight (DW) in roots and shoots, respectively, when exposed to a Pb concentration of 500 mg L^{-1}. The presence of Pb ions in stem, root and leaf tissues was confirmed by scanning electron microscope (SEM) and Energy-dispersive X-ray spectroscopy (EDX) analyses. Concerning the activity of antioxidant enzymes, *viz.*, peroxidase (POX) catalase (CAT) and ascorbate peroxidase (APX), they were induced at various regimes during 5, 8 and 12 d of Pb exposure in both the leaves and roots than untreated controls. Lead treatment increased superoxide dismutase (SOD) activity in both the leaf and root tissues over control, irrespective of the duration of exposure. Anew, it was observed that Pb treatments induced variations in the number and intensity of protein bands. Random amplified polymorphic DNA (RAPD) results show that the Pb treatment caused genotoxicity on DNA molecules as evidenced by the amplification of new bands and the absence of normal DNA amplicons in treated plants. Results confirm

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RAPD-PCR
Toxicity

that *A. indica* is a Pb accumulator species, and the antioxidants might play a crucial role in the detoxification of Pb-induced toxic effects.

1. Introduction

The major health impacts from toxic contaminants such as hazardous metal ions, insecticides and radionuclides have become a serious problem in recent years (Favas et al., 2016). Among these toxic pollutants, heavy metals have shown to cause deleterious effects on agricultural crop productivity, while heavy metal toxicity has proven to be a major threat to human and animal health (Lamhamdi et al., 2011; Malar et al., 2014a,b). Nowadays various metal species are used in a variety of industrial products and unauthorized disposal of metal possessing wastes has led to the pollution of soils, sediments and ground waters. In recent years, most of the agricultural sites across the world comprising arable land are highly polluted with various heavy metals, including lead (Pb) (Manikandan et al., 2015; Venkatachalam et al., 2016). The necessity to increase the agricultural crop productivity may allow the use of heavy metal polluted land soils with a potential threat to food chain (Gall et al., 2015). There are several edible taxa belonging to *Brassica* genus that were characterized as hyperaccumulators of heavy metals. However, there is an urgent need to search for potential plant species which can be easily utilized in the phytoremediation (phytoextraction) of polluted sites (Cordeiro et al., 2016; Venkatachalam et al., 2016). These heavy metal-accumulating plants should have the following characteristics: (i) potential to (hyper)accumulate toxic heavy metals in plant biomass above the ground, (ii) capacity to tolerate higher level of toxic metal ions, (iii) rapid growth rate with more biomass productivity, (iv) quick acclimatization to cultivate in agricultural land throughout the year, and (v) harvesting plant biomass within a short period of time (Manikandan et al., 2016; Venkatachalam et al., 2016).

Lead has become a major ecological contaminant due to rapid growth of industries across the globe. Pollution due to Pb results from mining and smelting activities, paint, paper and pulp industries, gasoline and explosives, as well as from the disposal of municipal sewage sludge (Sharma and Dubey, 2005; Jayalakshmi and Venkatachalam, 2011). This is not an essential element for plant cell growth and development, but the plants can uptake and accumulates Pb when it is available in their environment, especially in contaminated soil and water bodies (Favas et al., 2013; Pratas et al., 2013; Wójcik et al., 2014; Chand et al., 2016; Prasad and Maiti, 2016). Lead exerts clastogenic and mutagenic effects on plants. Responses of plants to Pb metal exposure include decrease in chlorophyll synthesis, diminution in the uptake of minerals, water imbalances and changes in structure and permeability of cell membranes (Lamhamdi et al., 2011; Malar et al., 2014a), nucleic acids/chromatin (Zhang et al., 2007), genotoxicity, and alternations in DNA synthesis (Cenkci et al., 2010; Malar et al., 2014b).

Lead decreases the absorption and transport characteristics of essential nutrients in plants, such as Ca, Fe, Mg, Mn, P and Zn, by inhibiting the entry or binding of the ions to ion-transporters and making them practically unobtainable for uptake and movement from roots to leaves (Sharma and Dubey, 2005; Rodriguez-Hernandez et al., 2015). The exposure of plants to Pb induces oxidative stress due to the enhanced synthesis of reactive oxygen species (ROS) including the superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH) (Sharma and Dubey, 2005). These ROS are highly toxic and can induce damage to macromolecules, ultimately leading to programmed cell death. Lead not only affects the plant system but it can also enter into the food chain inducing health impacts to humans as well as animals (Gall et al., 2015). This can interact with proteins and DNA molecules and adversely impacts the reproductive, immune, central nervous systems, hampers the circulatory system causing O_2 absorption, causes variations in blood pressure levels and affects the developmental processes (Gall et al., 2015).

For the extraction of hazardous heavy metal ions from contaminated sites, conventional physico-chemical methods including adsorption, oxidation, reduction and precipitation have been successfully tested. However, all these technologies merely rely on simple mass transfer of the toxic heavy metal from one phase to another, and they are expensive and energy intensive. Recently, the establishment of plant-mediated phytoremediation approaches (particularly the phytoextraction technique) for the clean-up of contaminated sites or water bodies is considered as an environmentally friendly approach. Phytoextraction is simple, rapid and eco-friendly technique that uses plants to absorb, transfer and accumulate the contaminants from polluted soil and water bodies (Augustynowicz et al., 2014; Malar et al., 2014b; Sasmaz et al., 2015; Syukor et al., 2016).

Heavy metal hyperaccumulator plants can accumulate higher levels of metal ions into their aerial biomass. Plants pertaining to the *Brassicaceae*, *Euphorbiaceae*, *Asteraceae*, *Lamiaceae* and *Scrophulariaceae* families have been frequently identified to uptake heavy metals including Pb. However, the potential hyperaccumulator plants, namely *Sesbania drummondii* (Rydb.) Cory (Srivastava et al., 2007), *Eichhornia crassipes* (Mart.) Solms (Malar et al., 2014a) and *Sesbania grandiflora* (L.) Pers. (Malar et al., 2014b) have shown good capacities to Pb uptake. Recently, some of the plant species, including *Acalypha indica* L., were also shown to acquire different heavy metal ions such as Cd, Pb, Ni, Cr, Zn, Fe and Cu from polluted environments (Olowu et al., 2015). Plants that accumulate high level of metal ions have effective antioxidant defense strategies to protect themselves from heavy metal induced biotoxicity. To combat metal(loid) phytotoxicity, plants have various anti-oxidant defense enzymes including superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and guaiacol peroxidase (GPX) that play in scavenging excess ROS (Zhang et al., 2007; Begum et al., 2016; Han et al., 2016; Hattab et al., 2016; Sidhu et al., 2016).

The plant *Acalypha indica* is known to inherently contain rich sources of tannins, glycosides and flavonoids and phytochemical studies have proved its importance as a valuable medicinal plant. Due to its innumerable medicinal properties and therapeutic applications, this plant has a short life span (40 days) and it is presently being widely used in traditional medicines for various ailments such as antibacterial agent, anti-inflammatory, antifungal and wounds healing activity, cardiac disorders, biliousness, rheumatism, haemorrhages, ulcers, amenorrhoea, and for the treatment of various skin diseases (Jagatheeswari et al., 2013). In this study, the potential role of *A. indica* for hyperaccumulation and translocation of Pb content was investigated using a hydroponic system. This medicinal herb of the family *Euphorbiaceae* has been chosen for the following reasons: ubiquitous and terrestrial distribution, perennial in nature and there are scarce information regarding its