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Short communication

Phytoremediation of lead using *Ipomoea aquatica* Forsk. in hydroponic solution

Laitonjam Bedabati Chanu, Abhik Gupta*

Department of Ecology and Environmental Science, Assam University, Silchar, 788011, Assam, India

HIGHLIGHTS

- *Ipomoea aquatica* primarily accumulates lead in its roots from solution.
- Excess lead is also sequestered in the basal part of its stem that changes colour.
- The apical unaffected part can be excised and regrown in lead-free water.
- The affected lower stem and roots with high lead burden can be removed and disposed.
- *I. aquatica* holds promise as a biomonitor and bioremediator of Pb.

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ABSTRACT

Ipomoea aquatica Forsk., an aquatic macrophyte, was assessed for its ability to accumulate lead (Pb) by exposing it to graded concentrations of this metal. Accumulation of Pb was the highest in root followed by that in stem and leaf with translocation factor (TF) values of less than unity. On the other hand, all bioconcentration factor (BCF) values in root, stem and leaf were greater than unity. Furthermore, exposure to Pb concentrations over about 20 mg L^{-1} induced colour changes in the basal portion of stem which had significantly higher Pb accumulation than that in the unaffected apical part. This resulted in sequestration of excess metal in affected stem tissue, which could take up Pb by the process of caulofiltration or shoot filtration, and served as a secondary reservoir of Pb in addition to the root. The apical parts contained less lead and could regrow roots from nodes and survive when kept in Pb-free medium. The ability of the plant to store Pb in its root and lower part of stem coupled with its ability to propagate by fragmentation through production of adventitious roots and lateral branches from nodes raises the possibility of utilizing *Ipomoea aquatica* for Pb phytoremediation from liquid effluent.

1. Introduction

Lead (Pb), a heavy metal, is a member of Group 14 (IVA) of the periodic table. Several properties of Pb such as its density, low melting point and resistance to corrosion makes it a commonly used metal in solders, paints, weights, ammunitions, leaded glass and storage batteries (Abadin et al., 2007). About 80% of the total use of lead is in lead-acid batteries. The growth in motorized vehicles, computers, telecommunications and solar panels in developing countries is accompanied by a rapid rise in the demand for lead batteries (URL-1). Since lead can be easily melted, recycling and local manufacture of car batteries are widespread (URL-2) and undertaken in small-scale industrial units and road-side repair shops. As efficient systems of treatment and disposal of wastewater from these units are lacking in most urban centres in developing countries, lead contamination of natural water bodies continues unabated. Hence, simple, cost-effective yet reasonably efficient treatment mechanisms need to be found for lead and other heavy metals. Phytoremediation can provide a viable option to address this problem. A wide variety of aquatic macrophytes have been studied for their potential in extracting Pb from contaminated sites. Significant uptake of Pb has been observed in *Wolffia arrhiza* (Piotrowska et al., 2010); *Sagittaria sagittifolia* and *Potamogeton crispus* (Hu et al., 2010); and *Leptodictyum riparium* (Basile et al., 2012); which have been identified as scavengers of Pb from

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Abbreviations: BCF, Bioconcentration factor; TF, Transfer factor.

* Corresponding author.

E-mail addresses: bedabatichanu@gmail.com (L. Bedabati Chanu), abhik.eco@gmail.com (A. Gupta).

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aqueous solution. The ability of aquatic plants such as *Hydrilla verticillata*, *Marsilea quadrifolia* and *Ipomoea aquatica* to accumulate high amount of heavy metals from wastewater as well as their tolerance to metals make them suitable choice for rehabilitation of areas contaminated with chemical wastes (Ahmad et al., 2011). Fawzy et al. (2012) studied the accumulation of Pb and some other heavy metals in six selected aquatic vascular plants from polluted sites of River Nile. All the examined plant species were found to be good accumulators among which *Ceratophyllum demersum* emerged as the most efficient species for phytoremediation. *Ipomoea aquatica* Forsk. has been shown to possess abilities to remove several metals such as Cd and Cr as well as nutrients like N and P from wastewater and reduce biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS) and chlorophyll ‘a’ (Hu et al., 2008; Wang et al., 2008; Chen et al., 2010). Khumanleima Chanu and Gupta (2014) found that this plant is a Cu accumulator, and although it primarily sequesters Cu in the roots, the lower part of the stem is also used for accumulation of excess Cu, thereby keeping Cu levels down in the apical parts. At higher ambient Cu concentrations, the lower stem becomes necrotic with significantly higher Cu concentrations than that in the apical parts, thereby protecting the latter from Cu toxicity. Baysa et al. (2006) found that the upper sections of stem contained far less Pb compared to that in the lower section, although they did not report necrosis of stem tissue. Göthberg et al. (2002) also found that the stem of plants collected from nine different sites in the greater Bangkok region of Thailand contained higher Pb concentrations in the bottom section, followed by the middle, with the least in the top segment. When *Ipomoea aquatica* was exposed to graded Pb concentrations in the laboratory, the highest accumulation was in root, followed by stem and then leaf, although possible differences between lower and upper stem were not reported (Göthberg et al., 2004). Thus the existing literature indicated the possibilities of *I. aquatica* as a Pb accumulator and phytoremediator, but did not establish the prospects on a firm ground. The aim of the present study was, therefore, to explore the Pb uptake capacity as well as the phytoremediation potential of this plant so that it could be used either singly or in combination with other hyperaccumulator species for Pb removal from wastewater. This plant commonly occurs in the study area as one of the ubiquitous amphibious macrophytes which can also trail into open water in the wetland ecosystems (Bordoloi, 2014; Purkayastha and Gupta, 2015). It is widely distributed in South and South East Asia and in parts of East Asia (Khumanleima Chanu and Gupta, 2014). It is also reported from Africa and United States of America, where it is considered to be an invasive species (Austin, 2007). *I. aquatica* is adapted to a wide range of habitats such as moist soil, inundated floodplains, ditches, ponds, canals and sluggish rivers (Prasad et al., 2008).

2. Materials and methods

2.1. Plant material and stock culture

Ipomoea aquatica Forsk. was collected from uncontaminated ponds of Irongmara area in Cachar district, Assam, India, and washed with tap water. Stock cultures were grown using the method adopted by Göthberg et al. (2004). The plants were grown in hydroponic tubs till new branches developed. These new branches were cut and planted in pots containing soil flooded with 50% Hoagland nutrient media. The pH of nutrient media was adjusted at a range of 5.8–6.2. A moderately acid regime (from pH 5.0 to 6.5) has been found to be suitable for most plants, and adjustment of pH is required if nutrient medium is prepared in dechlorinated tap water (Hoagland and Arnon, 1950). Further, low pH helps metals to remain in solution and available for absorption by plant roots (Odjegba and Fasidi, 2004).

2.2. Experimental methods

Healthy and fully grown shoots of similar height were cut from the same mother plant, washed with tap water and acclimatized in 50% Hoagland nutrient media for 1 week at 25–27 °C, 12 h light with an intensity of 100–120 μ mol^{-2} s^{-1} and 12 h dark periods. For bioaccumulation study, the plants were exposed to a solution of Pb $(NO_3)_2$ in 50% Hoagland nutrient media. The Pb concentrations were 0.63, 6.26, 11.26, 20.02, 35.03 and 62.56 mg L^{-1}. These nominal concentrations were fixed on the basis of earlier observations that Pb concentrations in river water reached upto 46.3 mg L^{-1} (Lokhande et al., 2011), while in soil it ranged from 2 to 293 mg kg^{-1} (Krishna and Govil, 2004; Parth et al., 2011) in heavily polluted industrial areas of India. KH_2PO_4 was not added to the nutrient medium to prevent Pb precipitation (Qiao et al., 2013). Control plants were also cultured in 50% Hoagland nutrient media without KH_2PO_4. The actual concentrations of Pb in the medium were measured by atomic absorption spectrometry (graphite furnace model – Analytik Jena Vario-6) at the beginning of the experiment, which was run for 15 days in plastic tubs with five replicates for each treatment and comparisons were made between Pb-treated and control plants. The plants were placed in the tubs by keeping them upright with the help of thermocol sheets having central apertures, so that only a small segment of stem bearing roots was dipped in the medium keeping the upper portion of stem along with leaves free from direct contact with the medium. Water loss due to evaporation or transpiration was compensated by renewal of solutions every week. At the end of the exposure period, exposed plants were dried in a hot air oven at 70 °C till constant weight.

2.3. Estimation of Pb accumulation

Metal content in plant samples were analyzed following standard methods (Gupta, 1996; Khumanleima Chanu and Gupta, 2014). At the end of 15 days of Pb treatment the plants were collected and washed twice with deionized water. The root, stem and leaf were separated, dried to constant weight at 60 ± 2 °C, and digested with concentrated reagent grade HNO_3. The affected portion of stem as well as the unaffected portion of stem, that is the segment which did not show any change in colour, were cut with a new stainless steel razor blade, and separately dried and digested. The residue was dissolved in distilled water and Pb content in the samples was determined with a graphite furnace atomic absorption spectrometer (graphite furnace model - Analytik Jena Vario-6). The final Pb concentration in the media in which the plants were grown was determined at the end of the experiment.

The bioaccumulation factor (BCF) and Pb transfer factor (TF) were calculated as follows:

BCF = Pb concentration in μg g^{-1} dry weight (dw) plant tissue/ Initial concentration of Pb in the medium (mg L^{-1}) (Ivanciuc et al., 2006; Ahmad et al., 2011)

TF = Pb concentration in stem or leaf (μg g^{-1} dw)/Pb concentration in root (μg g^{-1} dw) (Ahmad et al., 2011).

2.4. Statistical analysis

Statistical significance of differences among the data sets was tested with One-way ANOVA, with multiple comparisons made by Tukey tests. Significance of differences between affected and unaffected portions of stems were determined with *t*-test. All tests were done using SPSS 20 software for Windows. Log