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Research article

Evaluation of uranium removal by *Hydrilla verticillata* (L.f.) Royle from low level nuclear waste under laboratory conditions

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ABSTRACT

The present study evaluated uranium (U) removal ability and tolerance to low level nuclear waste (LLNW) of an aquatic weed *Hydrilla verticillata*. Plants were screened for growth in 10%–50% waste treatments up to 3 d. Treatments of 20% and 50% waste imposed increasing toxicity with duration assessed in terms of change in fresh weight and in the levels of photosynthetic pigments and thiobarbituric acid-reactive substances. U concentration, however, did not show a progressive increase and was about 42 μg g^{-1} dw from 20% to 50% waste at 3 d. This suggested that a saturation stage was reached with respect to U removal due to increasing toxicity. However, in another experiment with 10% waste and 10% waste+10 ppm U treatments, plants showed an increase in U concentration with the maximum level approaching 426 μg g^{-1} dw at 3 d without showing any toxicity as compared to that at 20% and 50% waste treatments. Hence, plants possessed significant potential to take up U and toxicity of LLNW limited their U removal ability. This implies that the use of *Hydrilla* plants for U removal from LLNW is feasible at low concentrations and would require repeated harvesting at short intervals.

1. Introduction

Uranium (U) is found distributed in both aquatic and terrestrial environment. It has the maximum abundance among various naturally present actinides. The average concentration of U in the earth's crust is about 2.5 mg kg^{-1} (in a range of 1–4 mg kg^{-1}) (Gavrilescu et al., 2009). However, due to realization of U application in nuclear industry, anthropogenic activities from mining, reprocessing, to disposal have posed a risk and therefore, U pollution has become an important environmental concern throughout the world.

Uranium can gain entry into crop plants and hence reach to humans through the food chain. Since, U is not biologically functional in humans and animals, its entry can cause adverse effects on their health (Anke et al., 2009). The biochemical toxicity of U is higher (about six times) in comparison to its radioactivity. Although plants suffer from U toxicity, attempts to find out suitable remediators of U have been successful with the identification of various plants, mosses (Duquène et al., 2009; Misson et al., 2009; Shtangeeva, 2010; Soudek et al., 2011), bacteria, algae, and fungi (Suzuki et al., 2003; Merroun and Selenska-Pobell, 2008; Bhat et al., 2008; Acharya et al., 2009). Among plants, both aquatic (*Phragmites australis* (Cav.) Trin. ex Steud., *Scirpus lineatus* Michx., *Sagittaria latifolia* Willd., *Callitriche stagnalis* Scop. and *Fontinalis antipyretica* Hewd) and terrestrial (*Impatiens capensis* Meerb., *Cyperus esculentus* L. and *Solidago speciosa* Nutt.) have been found to show significant accumulation of U (Caldwell et al., 2012; Favas et al., 2014). Various studies conducted till date on a number of plants like *Helianthus annuus* L., *Raphnus sativus* L., etc. indicated that age of the plant and its characteristics play significant role in its U accumulation ability (Singh et al., 2005). Uranium is stored mostly in roots (Straczek et al., 2010). When U accumulation reaches beyond a certain limit for a specific plant, toxic effects of U are seen in growth, biomass and seed production (Sheppard et al., 2005; Panda et al., 2001) and in various genotoxic and oxidative stress parameters (Panda et al., 2001; Vandenhove et al., 2006; Srivastava et al., 2010; Vanhoudt et al., 2011a,b). Although the mechanisms of U uptake are yet unknown, studies correlating the uptake of U with other plant nutrients like Ni, Fe, Ca and Mg (Caldwell et al., 2012) suggest that U mimics other ions and gains entry through "divalent ion transporters". Accordingly, nutrient (e.g. Fe)-deficient conditions result in increased U accumulation owing to lesser competitive effects (Viehweger and Geipel, 2010). The concentration of

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phosphate in the medium is another major regulator of U uptake by plants as phosphate levels affect chemical forms of U and hence, U bioavailability (Ebbs et al., 1998; Rufyikiri et al., 2006; Mkandawire et al., 2007).

Considering the impacts of U on plant, animal and human health, the need exists to remediate the sites contaminated with U and other radionuclide. The use of plants to clean up contaminated soil and waters is gaining attention due to economical aspects of the approach. Various plants have been tested for U accumulation both at the laboratory scale and at the field scale; for example *Brassica juncea* (L.) Czern., *Helianthus annuus* L., *Chenopodium album* L. var. *album* lambsquarters, *Lolium perenne* L. (Eapen et al., 2003; Vandenhove and Van Hees, 2004; Singh et al., 2005; Vera Tomé et al., 2009). In our earlier study, we tested the U accumulation ability of an aquatic weed plant *Hydrilla verticillata*. We found it to be potential accumulator of U and suggested that it can be a promising candidate for U phytoremediation (Srivastava et al., 2010). However, in earlier study, U solutions were prepared in distilled water since presence of phosphate in Hoagland medium was found to decrease U accumulation by the *Hydrilla* plants. In view of results of earlier study, this work was planned to analyze the U removal ability and tolerance to low level nuclear waste (LLNW), which contains U and other salts too, to evaluate the feasibility of using them for on field phytofiltration purposes.

2. Material and methods

2.1. Plant material and treatment conditions

Hydrilla verticillata (L.f.) Royle plants were collected from cultures established in the field. All experiments were set up in triplicate. Fresh plant biomass used for each replicate was 1 g L^{-1}. Initial experiments were conducted to standardize the concentration of LLNW and plants were exposed to 20%–100% waste (data not shown) and then to 10%–50% waste treatment (pH 6.6) under laboratory conditions (temp. 25 ± 2 °C, light intensity of 115 μmol m^{-2} s^{-1}, 14 h light/10 h dark photoperiod). Waste was collected from LLNW treatment plant. Initial activity in the LLNW was 100 kBq L^{-1} (gross β, γ) and 100 kBq L^{-1} (gross α) with U concentration being less than 5 mg L^{-1}. The pH of the solution was 6.6. Sodium was the major constituent in the waste having concentration of 125 mg L^{-1}. The total dissolved salt of the waste solution was less than 0.05% (w/v). Plants were subjected to LLNW for a period of 3 d. In further experiments, 10% waste was supplemented with 10 ppm U (pH 5.0; using the salt $UO_2(NO_3)_2.6H_2O$; Merck; Germany) and grown for 3 d. Treatments containing no waste or U, kept with each set of experiment, served as control. Growth and other parameters were analyzed at 1, 2 and 3 d after thorough cleaning of plants with double distilled water.

2.2. Estimation of uranium

Estimation of U was done by arsenazo(III) method (Shumate et al., 1978) by measuring the absorbance at 650 nm as described previously (Srivastava et al., 2010).

2.3. Assay of photosynthetic pigments, lipid peroxidation and total soluble proteins

For the estimation of photosynthetic pigments, acetone-extracted plant material was used (Lichtenthaler and Buschmann, 2001a) and the absorbance of supernatant was read at 470, 647 and 663 nm. The levels of chlorophylls and the sum of carotenoids (xanthophylls and carotenes) were calculated according to the equations given by Lichtenthaler and Buschmann (2001b). Lipid peroxidation was determined by the estimation of the thiobarbituric acid-reactive substances (TBARS; ε of 155 mM^{-1} cm^{-1}) content following Hodges et al. (1999). The level of total soluble proteins was estimated following Lowry et al. (1951).

2.4. Assay of antioxidant enzyme (SOD and GPX) activities

Enzyme extraction was done at 4 °C. Samples were homogenized in 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 1 mM ASC and 1% polyvinylpyrrolidone (w/v). The homogenates were centrifuged at 15 000 × *g* for 15 min and the supernatants were stored in separate aliquots at −80 °C, prior to enzyme analyses. The protein content in the supernatant was measured according to Lowry et al. (1951). The activities of superoxide dismutase (SOD; EC 1.15.1.1) and guaiacol peroxidase (GPX; EC 1.11.1.7) were assayed following Beauchamp and Fridovich (1971) and Hemeda and Klein (1990), respectively as described previously (Srivastava et al., 2006).

2.5. Statistical analysis

The experiments were carried out in a randomized block design. One–way analysis of variance (ANOVA) was done on all the data to confirm the variability of data and validity of results. Duncan's multiple range test (DMRT) was performed to determine the significant difference between treatments. Regression analysis was also performed to assess the relationship between U accumulation and response of parameters analyzed.

3. Results

Plants were initially exposed to 10%, 20% and 50% LLNW for 3 d. In 10% waste, U concentration increased with duration (maximum 14 μg g^{-1} dw at 3 d), while at 20% and 50% treatments, U concentration increased till 2 d and afterwards, there was no significant increase (maximum 42 μg g^{-1} dw at 3 d) (Fig. 1). Toxicity of waste was evaluated in terms of percent change in fresh weight. Control plants showed positive growth and gained weight, whereas plants exposed to LLNW showed a loss in weight. Plants showed progressive toxicity in all waste treatments; however growth at 10%

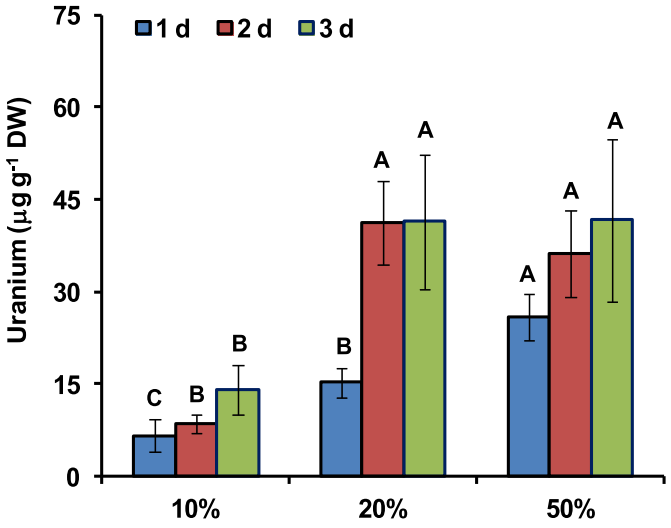

Fig. 1. Accumulation of uranium (μg g^{-1} dw) by *Hydrilla verticillata* from low level nuclear waste during 3 d exposure. All values are means of triplicates±S.D. ANOVA significant at $p \leq 0.01$. Different letters indicate significantly different values at a particular duration (DMRT, $p \leq 0.05$).