RESEARCH ARTICLE

Visualization of Cr (VI) Reduction in Vetiver (*Vetiveria zizanioides*) Rhizosphere

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ABSTRACT

The present study aimed to visualize the chromium reduction in the rhizosphere of vetiver. Vetiver was grown in rhizoboxes supplemented with metal solutions such as Ni, Pb, Zn, and Cr(III) with sand as a medium for 30 days. At the end of the experiments, it was observed that Cr(III) (40.0 mg kg^{-1}) was accumulated more in the roots than the shoots compared to Ni (14.5 mg kg^{-1}), Pb (12.0 mg kg^{-1}) and Zn (11.6 mg kg^{-1}). But on the contrary, Zn is accumulated more (58.5 mg kg^{-1}) in shoots than in the roots compared to Ni (23.1), Pb (19.3), and Cr(III) (21.9). In order to validate this, root exudate activity of Vetiver was observed visually as a change in pH using bromocresol purple as an indicator. More root acidification was triggered by Pb and Cr(III). The rhizofiltration of Cr(III) to roots can be attributed to the enhanced root acidification and hence more accumulation. The Cr(VI) reduction was also visualized around vetiver roots using DPC as an indicator. This method is test verified and can be used as a quick method to screen plants for their root exudation potential for metal mobilization or filtration onto roots.

Keywords: *Heavy metals; Vetiver; Phytoremediation; Root exudation activity*

INTRODUCTION

Heavy metal contamination has become one of the most persistent environmental issues in recent decades. Rhizofiltration is primarily used to remediate extracted groundwater, surface water, and wastewater with low contaminant concentrations (Raskin and Ensley, 2000). It can be used for the removal of lead, cadmium, copper, nickel, zinc, and chromium, which are primarily retained within the roots (Moosavi and Seghatoleslami, 2013).

Vetiver has a massive and deep root system, tolerant to extreme climatic variations such as prolonged drought, flood, submergence, fire, frost, and heat waves. Vetiver was able to remove 77% Nickel (Mudhiriza *et al*., 2015), 606 mg/L of Pb and 23,285 mg/L of Zn (Aksorn and Chitsomboon, 2013). *Vetiveria zizanioides* (Linn) and *Vetiveria nemoralis* can remove 89% and 86% of chromium from chromium solution in 10 days of hydraulic retention time in free surface constructed wetlands.

MATERIAL AND METHODS

Visualization of the Cr(VI) reduction was carried out around the roots of vetiver using Diphenyl carbazide (DPC) as an indicator. Similar to the procedure outlined using bromocresol purple; DPC was used as an indicator in this experiment. The vetiver plants were grown for 24 hours in water (control) and Cr(VI) at pH 7.0 of the desired concentration. It is then washed in deionized water and transferred to the chamber of rhizobox. To this, the agar suspension containing DPC was added. The suspension was prepared by adding a drop of DPC in one litre of water, neutral pH was adjusted and maintained, and agar was added at the rate of 9 grams per litre of the solution. It is then melted and cooled in the microwave oven at three and half minutes intervals. After adding the agar suspension, the changes in colour around the roots were recorded.

RESULTS AND DISCUSSION

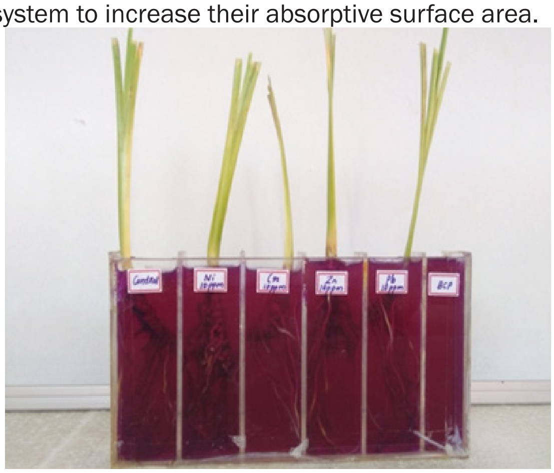
Rhizosphere acidification by metals in vetiver

Rhizosphere region differs in many ways compared to bulk soil in terms of microbial diversity, root exudation, organic acid pattern, and nutrient dynamics. Compared to bulk soil, the rhizosphere soil has a pH drop of 2 units based on the root exudation pattern or increases with N. In order to study the rhizosphere effect i.e., root acidification pattern, the experiment was done using a mini rhizobox with six compartments. The dimension of rhizobox is 20 X 15 X 5 cm and is fabricated using an acrylic sheet. Root exudation creates acidity around the roots and bromocresol purple was used as an indicator to visualize the rhizosphere effect. The vetiver plants were grown for 24 hours in water (control) and metal solution (50 mg/L) of Ni, Pb, Zn, and Cr (VI) at pH 7.0. After acclimatization, the roots were washed in deionized water and transferred to the chamber of rhizobox. To this, the agar suspension containing bromocresol purple was added.

Visualization of Cr(VI) reduction in vetiver rhizosphere.

The rhizosphere effect on biotransformation of Cr(VI) to Cr(III) is proven in many plants, including hyperaccumulators. The root acidification coupled with microbial enzymology aids the biotransformation process. DPC indicator is pink in the presence of Cr(VI) and colorless in the absence of Cr(VI). Vetiver roots acclimatized to 25, 50, 75, and 100 mgL^{-1} of Cr (VI) (Fig.1). Cr(VI) transformation around roots after 4 hours which was clearly visible only after 12 hours. After 24 hours, the area of transformation increased, indicating that vetiver has the potential to convert toxic Cr(VI) to Cr(III) . The results confirm that this method can be used for screening plants for heavy metal accumulation and rhizoremediation studies.

Screening studies using root exudate activity is a proven method to quantify the role of organic acids in rhizofiltration/hyperaccumulation of metals. Our preliminary investigation confirmed that vetiver roots trigger organic acid exudation, causing acidification around roots (Vignesh Kumar *et al.*, 2016). Our study reveals that this quick method can be used to screen plants chosen for remediation. Vetiver roots exposed to heavy metals showed more rhizosphere acidification compared to control. Similar studies were widely used to visualize the rhizosphere acidification triggered in the presence of nutrients. Yang *et al*. (2007) showed that limited phosphorous supply to the plant causes dramatic changes in the morphological and architectural changes in their root system to increase their absorptive surface area.



0^{th} hour

after 20 minutes

after 24 hours

(Column 5 - Control (plants grown in water), Column 6-DPC and Column 1, 2, 3, 4 – Plants grown in 25, 50, 75 and 100mg L^{-1} of Cr (VI)

Figure 1. Root acidification as triggered by heavy metals

0^{th} hour

after 20 minutes

after 24 hours

(Column 5 - Control (plants grown in water), Column 6-DPC and Column1, 2, 3, 4 – Plants grown in 25, 50, 75 and 100mg L^{-1} of Cr (VI))

Figure 2. Visualisation of Cr (VI) reduction in vetiver rhizosphere

CONCLUSION

Remediation of metals and wastewaters using vetiver is more promising due to its profuse rooting behavior and organic exudation. Cr(III) was highly accumulated in the roots of vetiver with a concentration of 40 mg kg^{-1} followed by Ni, Pb, and Zn with a concentration of 14.5 mg kg^{-1}, 12.0 mg kg^{-1} and 11.6 mg kg^{-1}. Root exudation was more pronounced in metal acclimatized vetiver roots compared to control (water). After 12 hours, root acidification was completely visible in Cr and Zn. Results reveal that vetiver plants acclimatized to Cr (VI) showed quick Cr (VI) transformation around the roots compared to control (water-grown vetiver). This technique can be used for screening plants based on their root exudation pattern for rhizofiltration or phytoaccumulation.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

Authors should ensure that they have written and submit only entirely original works, and if they have used the work and/or words of others, that this has been appropriately cited. Plagiarism in all its forms constitutes unethical publishing behavior and is unacceptable.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail; saraparwinbanu.k@tnau.ac.in

Author contributions

Idea conceptualization – Kamaludeen Sara PB, Experiments – Karthikeyan Ganesan, Maria Luke, Guidance – Kamaludeen Sara PB, Suganya K, Writing original draft – Karthikeyan Ganesan, Maria Luke, Writing, reviewing & editing - Karthikeyan Ganesan, Kamaludeen Sara PB, Suganya K

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