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Effects of root exudates of Vetiver on physicochemical properties and fractionation of heavy metals in tannery effluent contaminated soil

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ABSTRACT

A range of organic substances, referred to as root exudates can be released by plant roots either actively or passively which are the notable source for microbial growth in the root zone and also involved in nutrient cycling and mineralisation of organics thus they could possibly influence the degradation of contaminants in the rhizosphere. The key components of the root exudates of vetiver are total carbohydrates, reducing sugars, total phenolics, flavonoids, total alkaloids, organic acids and total proteins. The organic acids are found in significantly higher amount under heavy metal stress conditions. On addition of this root exudates manually for a period of 60 days yielded in alteration in the physico-chemical properties of the soil and fractionation of chromium and lead – the representative contaminants present in the experimental soil which was collected from the tannery effluent polluted areas of Vellore, Tamil Nadu. The pH and organic carbon content in the soil is increased while the electrical conductivity got decreased. The root exudates application possibly increased the bioavailability of the heavy metals besides reducing the residual form which paves the way for easier remediation of such metals since their availability got increased. The positive effect of root exudates of vetiver provided a new idea to remediate the contaminated soils.

Key words: *Vetiver, Root exudates, Chromium, Lead, bioavailability*

Introduction

Pollutants in soils are present in varying concentration and composition (Zhang *et al*., 2014). Amongst all the contaminants in the soil heavy metals are regarded as major contributors to soil and water pollution (Sun *et al.,* 2011). Heavy metals are becoming increasingly popular topics of discussion due to its toxicological significance in ecosystems (Huang *et al.*, 2011). Scientists, decision-makers, and the general public are concerned about the hazardous consequences that soil heavy metals have on plants, animals, and people as pollutants move through the water and food chains. A helpful indicator is the measurement of the soil's total heavy metal concentration (Zhang *et al.,* 2009). However, it is unable to

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offer enough details regarding the availability and toxic effects of heavy metals. Heavy metals' mobility, bioavailability, and ecotoxicity were largely correlated with their forms (Chen *et al*., 2008). Among the various sources of heavy metal pollution in the environment, tannery industries does play a major role. The tanneries in north India are concentrated along the Ganga's banks, but in Tamil Nadu, they are concentrated in Palar (Sheik *et al.* , 2021).

Tamil Nadu being the largest leather producer holding 45% production having a cluster of about 1120 tanneries in and around Vellore, Dindigul, erode and some other districts (Mohamed and Aneez, 2002). More than 3000 industrial units in Tamil Nadu have been classified as highly polluting, producing approximately 6 lakh litres of effluent per day. In Tamil Nadu the districts of Vellore, Erode, and Dindigul, tanning industry wastes are estimated to have degraded over 50000 ha of productive agricultural land (Saha *et al.,* 2017).

Heavy metals which are predominantly present in their effluent exist in a variety of forms, including residual, oxidizable, reducible, and water-soluble forms. In these forms, the water-soluble and acid-soluble forms are bioavailable while the residual form is unavailable to both plants and microbes. Consequently, it is necessary to take into account the study of soil heavy metal speciation on soil heavy metals pollution (Kartal *et al*., 2006). BCR is frequently used to examine various types of heavy metals that have been proposed (Bhattacharyya *et al.* ,2008). This method employs a number of selective agents to dissolve the various heavy metal forms in soil, leading to a more accurate evaluation of the environmental impact (Guevara-Riba *et al*., 2004).

A variety of organic substances, known as REs, such as carbohydrates, OAs, and amino acids, can be actively or passively released by plant roots. These REs play an important role in nutrient transformation, decomposition, and the mineralization of organic compounds. As a result, they may be a valuable resource for fast-growing microorganisms and change the species composition in the rhizosphere, which can lead to the biodegradation of pollutants. Thus in this study chromium and lead have been taken as representative metal contaminants. The main aim of this paper is to identify the components of the root exudates of *Chrysopogan zizanioides* under heavy metal stress and impacts of these root exudates on the fraction distribution of these heavy metals in the soil.

Materials and Methods

Soil collection and analysis

The experimental soils were collected from tannery effluent contaminated areas of Vellore. The collected soil is shade dried and sieved with 2 mm sieve and then analysed for various physico-chemical parameters and the heavy metals. The pertinent physical and chemical parameters of the experimental area are presented in Table 1. All the analytical works were carried out in the Department of Environmental Sciences, Tamil Nadu Agricultural University, Coimbatore.

Table 1. Initial physico-chemical parameters of the experimental soil

S.No	Parameters	Composition
1	pH	7.18
2	Electrical conductivity (dS m^{-1})	0.36
3	Organic carbon (%)	0.66
4	Chromium (mg/kg)	128
5	Lead (mg/kg)	96

Root exudates collection and extraction

To collect the root exudates, a funnel flask set up consisting of a conical flask of 500 ml capacity over which a funnel of 150 mm diameter with stem end covered with aluminium foil with pin sized pores was placed. The funnel was filled with glass beads till the neck and soil (300 g) was added till it fills the $3/4^{th}$ capacity of the funnel. Vetiver saplings were planted in each set up and watered regularly. The root exudates collected are to be poured to the soil in incubation cups for further studies.

For extraction of root exudates solvent extraction method using ethyl acetate. Then solvent was evaporated and the residue was dissolved in 1ml of ethyl acetate and analysed using GC – MS.

Incubation experiment

The soil collected from the tannery polluted site were stir dried, sieved and sterilized to remove the indigenous microbes. The 400 g of sterilized soil is then filled in each incubation cups. Three replications for each treatment are kept. Root exudates collected is added in 2 days interval. Apart from this, the cups were maintained with 50% moisture.

Speciation of heavy metals

To determine the relative quantity of distinct Cr species, a stepwise extraction approach was used. The extractants employed in the first few phases of the sequential extraction tend to target certain constituents of the adsorbent structure, while those utilised in the last step are far less specific, more forceful, and destructive (Beckett, 1989).

Statistical analysis

The experiments were performed in triplicates. All the data were analysed using Microsoft excel and SPSS software.

Results and Discussion

Characterisation of root exudates extract

The composition of chemical compounds that are found in the GC – MS analysis of RE are total carbohydrates, reducing sugars, total pholics, flavonoids, total alkaloids and total proteins.

The majority of the Total carbohydrates content obtained is made up by the mucilaginous substances (HMW) produced by the root tissues in REs, but sugars make up a small fraction of the total LMW. These carbohydrates change the activity and population of microbes, which in turn affects the bioavailability of HM in soil. They serve as the carbon supply for microbes.

In the presence of HM, plant roots secrete more proteins, which suggests important biological processes, enzymatic alterations, and modifications to the defence mechanisms involved in lead chemical uptake and transformation.

There have been reports of plants containing more reducing sugar after being exposed to HM (Shah *et al*., 2017). When *Tagetes erecta* L. was grown in soil that had been treated with cadmium (18 mg kg^{-1}), the scientists obtained a 47.95% rise of the reducing sugar content.

Various earlier investigations have found that roots exposed to heavy metal stress secrete more phenolic acids (Irtelli and Navari-Izzo, 2006). The phenolic acids found in REs are frequently possess redox characteristics that serve as antioxidants. It is well known that the presence of hydroxyl groups is what neutralises the free radicals produced by sudden environmental stress. The reduced properties of phenolic compounds, which enable them to serve as metal chelators, absorb, and neutralise free radicals, are primarily responsible for their antioxidant activity.

The amount of isoflavonoids in Alfalfa roots increased when they were exposed to 1 mM $CuCl_2$, according to a prior work by Kumar and Pandey (2013). Plants contain a variety of secondary metabolites, including flavonoids, flavones, condensed tannins, and flavanols. Free radicals produced by oxidative stress can be scavenged by flavonoids (Kumar and Pandey, 2013).

Chemotactic impacts of root exudates on soil

By chelating, reducing, and acidifying the insoluble heavy metal component in soil, REs had an impact on the form of the metals there. Numerous studies have shown that when plants are exposed to specific toxic and dangerous substances, the composition as well as concentration of REs change, and the production and buildup of part components of REs increases (Kim *et al*., 2010). This is the plant's stress response to the toxic and dangerous substances, which modifies the rhizosphere microenvironment by continuing to increase REs, making the rhizosphere microenvironment favourable for the decomposition of harmful substances (Maria *et al.,* 2014). The changes in the physicochemical properties like pH, EC and organic carbon in the soil by the addition of root exudates over a period of 60 days is portrayed in Figure 1 and 2.

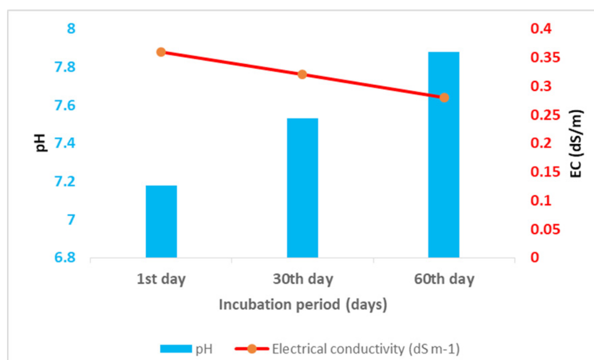

Fig. 1. Influence of Vetiver root exudates on pH and EC of the contaminated soil

Addition of root exudates to the experimental soil slightly increased the pH of the soil from 7.18 (1st day) to 7.88 (60th day) while it gradually decreased the EC of the soil from 0.36 dSm^{-1} (1st day) to 0.28 dSm^{-1} (60th day). The similar kinds of results were obtained by Jiang *et al.* (2022) where with the addition of root exudates the pH increased from 5.3 to 6.6 in 30 days. This may be due to the organic acids

Fig. 2. Influence of Vetiver root exudates on organic carbon content of the contaminated soil

(oxalic acid, citric acid etc.,) which are present in the added root exudates. The low molecular weighed organic acids in the root exudates plays a major role in altering the physico-chemical properties of the soil (Jiang *et al.*, 2022).

The organic carbon in the soil was increased from 0.66% (1st day) to 0.69% (60th day). This may be due to the multiple soluble organic substances that are excreted by roots to cause the rhizosphere to have a "priming effect" that speeds up the turnover of soil organic carbon (SOC) (Kuzyakov *et al.,* 2000). The definition of this "priming effect" is strong, typically short-term variations with in turnover of native SOC probably caused by relatively mild treatments of the soil (Kuzyakov *et al.,* 2000). It has been discovered that these changes are highly reliant on the elemental composition of the compounds that the roots excrete. The main organic compounds responsible for this priming effect may be the oxalic acid, glucose, acetic acid etc., where a much stronger priming effect would be caused by the oxalic acid (Wen *et al*., 2022).

Influence of root exudates on the fractionation and bioavailability of heavy metals

Following the continuous extraction procedure the heavy metals which were divided into several fractions for both chromium and lead ranged from low to high as: water soluble fraction >exchangeable fraction > residual fraction > organic fraction > organic plus Al/Fe bound fraction. The water soluble fraction of chromium initially accounted for about 67.7%. The exchangeable fraction of chromium accounts to 10.86%. The organic and organic plus Al/Fe bound fraction accounted to about 4.08% and 3.08% respectively. The residual fraction was found to be predominant and it accounted to 13.8%. However the amount of water soluble fraction after 60 days of treating with root exudates increased to 71.7% and the amount of the exchangeable fraction also increased to 11.16%. The organic and organic plus Al/Fe bound fraction of chromium were also increased slightly to 6.08% and 4.18% respectively whereas the residual fraction is found to be decreased to about 6.47%.

The fractionation for lead initially provided with 59.8% of water soluble fraction, 9.32% of exchangeable fraction, 7.02% of organic fraction, 5.83% of organic plus Al/Fe bound fraction and 18.03% of residual fraction. Except the residual fraction of lead all the other fractions were found to be increased progressively when treated with root exudates. The water soluble, exchangeable, organic and organic plus Al/Fe bound fraction were increased to 65.44%, 10.89%, 8.91% and 7.32% respectively. Besides, the residual fraction was reduced to a greater extent of 7.44%.

Figure 3 and 4 show the trend that was obtained regarding the fractionation of both chromium and lead where the bioavailable fractions (water soluble fraction and exchangeable fraction) were found to be increasing to an extent when treated with root exudates of vetiver whereas the residual fraction is found to be decreased for both.

Heavy metal ions in soils can be trapped in a variety of fractions, such as metal adsorb to clays, metal trapped within the matrix of soil minerals, and metal trapped in hydrous oxides as well as organic matter (Reichman and Parker, 2002). The content of the bioavailable portion is clearly increasing, which suggests that REs might make heavy metals more bioavailable. At acidic or reducing circumstances, the metals bonded to minerals like carbonates or oxide can be liberated. As a result, root-induced variations in pH and EC can have an impact on the bioavailability (Marschner and Ro¨mheld, 1996).

REs, particularly OAs in REs, may alter the properties of the soil and increase the bioavailability of this heavy metal contaminants, which may aid in the removal of these pollutants from the rhizosphere. Mono, di, and the tri-carboxylic acids with unsaturated carbon & hydroxyl groups are examples of OAs found in REs. Di and tricarboxylic aliphatic acid concentrations in soil were found to range from less than 50 µmol L^{-1} to as high as 650 µmol L^{-1}in general (Hou *et al*., 2015).

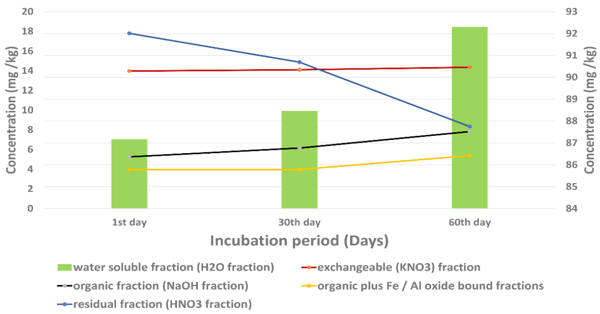

Fig. 3. Impacts of Vetiver root exudates on different fractions of chromium

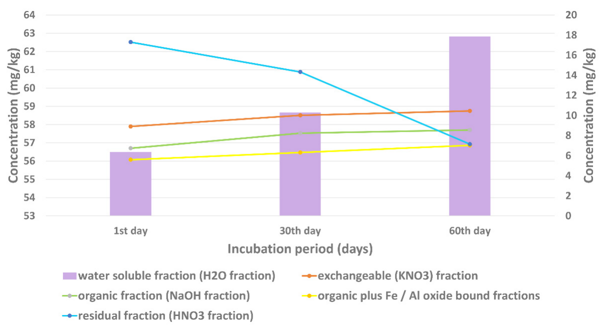

Fig. 4. Impacts of Vetiver root exudates on different fractions of Lead

Oxalic acid, citric acid, formic acid, acetic acid, malic acid, succinic acid, maleic acid, lactic acid, aconitic acid and fumaric acids are natural OAs that are produced by soil-dwelling microorganisms, plant and animal wastes, and REs. Therefore, it is more likely that metal breakdown by OAs will constitute a mobile metal component that is accessible to biota (Labanowski *et al*., 2008).The exchangeable, carbonate, and reducible portions of heavy metals can be removed by washing techniques used with chelating OAs. There are still questions about the best option for a large-scale application despite the fact that several chelating substances, such as citric and tartaric acid, have been studied for mobilising heavy metals.

Conclusion

According to the results of photochemical studies, the root exudates of *Chrysopogan zizanioides* contain a variety of bioactive phytoconstituents, including phenolic, flavonoid, alkaloid, protein, and carbohydrates. The root exudates play a crucial in the phyto degradation of contaminants in the rhizospheric area in a complicated pollution package which incorporates the plants and microorganisms (Joner *et al*., 2002). From the study conducted with the heavy metal contaminated soil, the root exudates possibly increased bioavailability of the heavy metals to about 4 – 10% and also affected the physical and chemical properties of the soil. By increasing the bioavailability of metals, they make it simple for the hyperaccumulator plants for easy remediation. The favourable effects of root exudates on chromium and lead availability in this study offer a novel strategy for remediating soil contaminated with heavy metals by adding root exudates.

Abbreviations

OAs	-	Organic acids
REs	-	Root exudates
Cr	-	Chromium
Pb	-	Lead
BCR	-	European Community Bureau of Reference
GC-MS	-	Gas chromatography-mass spectrometry
HM	–	Heavy Metals
HMW	–	High Molecular weight
LMW	–	Low Molecular Weight

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