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SHORT COMMUNICATION

Root Exudates of *Cyperus alternifolius* in Partial Hydroponic Condition under Heavy Metal Stress

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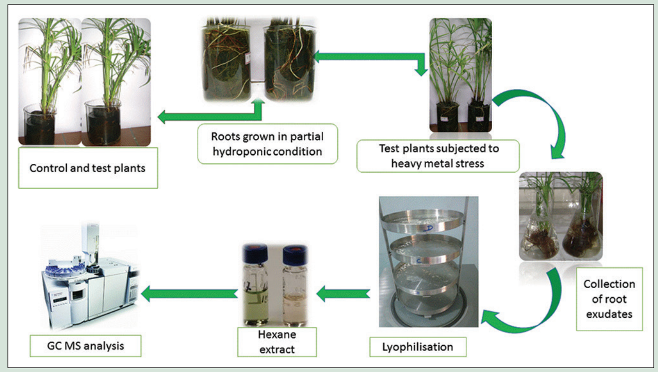
ABSTRACT

Secondary metabolites play a vital role in the treatment of various ailments as well as in phytoremediation. The link between secondary metabolites and phytoremediation needs exploration. Hitherto, no information is available regarding the phytochemical components that exist in the root exudates of *Cyperus alternifolius*. This study was designed to determine the phytocomponents in the root exudates of *C. alternifolius* under heavy metal stress. *C. alternifolius* was grown by a novel technique in partial hydroponic conditions and imperiled to a mixture of heavy metals (Cd, Cu, Cr, Ni, Zn, Pb, and Fe) at different concentrations. The root exudates were collected, freeze-dried, redissolved and reconstituted in hexane and analyzed in gas chromatography–mass spectrometry using JEOL GCMATE II in SAIF IIT-Madras. The analysis revealed that the profile of phytochemicals in root exudates is diverse with biological properties. Few phytochemicals found in the root exudates are not cited earlier in any literature. The composition and percentage of phytochemicals could not be correlated to heavy metal concentration. Phytochemical composition decreased with an increase in heavy metal concentration. Control plant released more phytochemicals than the plants under heavy metal stress. From the results, it is evident that root exudates of *C. alternifolius* contain various bioactive components. Further research can be extended to evaluate the pharmaceutical importance of the species and explore its role in phytoremediation of heavy metals.

Key words: *Cyperus alternifolius*, gas chromatography–mass spectrometry analysis, heavy metals, phytochemicals, root exudates

SUMMARY

- The control and test plants are grown under partial hydroponic condition. Test plants are subjected to heavy metal stress, root exudates were collected from control and test plants, freeze dried, constituted in hexane and subjected to GC-MS analysis.



Abbreviations Used: GC-MS: Gas chromatography-Mass spectrometry.

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INTRODUCTION

Root exudates are plant metabolites of low and high molecular weight molecules that are secreted by root surfaces precise for each plant species and depends on the biotic and abiotic environment. Biotic stress includes bacteria, parasites, virus, fungi, insects, and weeds. Abiotic stress includes nonliving factors. Anthropogenic activities have led to the accumulation of heavy metals globally. Heavy metals belong to a group of nonbiodegradable, persistent inorganic chemical constituents with atomic mass above 20 and density higher than 5 g/cm^3. It possesses cytotoxic, genotoxic, and mutagenic effects on humans, animals, and plants through food chains, soil, potable water, aquifers, irrigation, and surrounding atmosphere.[1-4]

Literature survey reveals that exudates include sugars, amino acids, fatty acids, organic acids, peptides, enzymes, vitamins, and nucleotides that account for 20%–40% of root carbon. The exudates provide bounteous energy and carbon for microbes in the rhizosphere. Some of the elements affecting root exudation are plant species, stress factor, root age, temperature, light, and microorganism.[5] Unprecedented bioaccumulation and biomagnification of heavy metals in the environment are a threat for all living organisms including plants. Plants require certain essential heavy metals for its normal growth. However, in excess amounts, these metals cause deleterious effects on plants.[6] Environment is habitually contaminated with heavy metals such as cadmium, copper, zinc, lead, nickel, and iron. Root exudates help in the uptake of toxic heavy metals by increasing the solubility of metal ions, thus enhancing its accumulation in plants.[7] Phytometallophores (organic ligands) are important class of compounds within root exudates which are associated with metal chelation.[8]

Root exudates play a vital role in the process of phytoremediation and are an emerging green and *in situ* remediation technology. Root exudates can react with heavy metals by influencing its mobility and phytoavailability[9-11] and also help plant to absorb and accumulate contaminants from soil.[12,13] An important strategy to avoid building up of toxic heavy metals in plants is exudation of complexing agents into the rhizosphere. Organic compounds released from the roots have an adaptive mechanism by which the plant can alter its microenvironment and subsequently affect nutrient availability in the rhizosphere.[14] Phytocomponents in the root exudates are involved in increased tolerance to heavy metals.[15]

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A single plant species exposed to different concentrations of heavy metal mixture has not been reported yet. *Cyperus alternifolius* is one among the prominent wetland plants used for the treatment of contaminated water. It is a wetland plant with rapid root growth and is capable of accumulation of heavy metals, thus playing a vital role in phytoremediation. Root rhizosphere plays a vital role in phytoremediation. An aspect related to the secretion of root exudates in response to heavy metal stress was premeditated. The major goal was to investigate whether plant metabolite patterns change quantitatively under heavy metal stress and is there a relationship between exudation pattern and stress.

MATERIALS AND METHODS

Plant saplings

Healthy plants were purchased from Venkateswara Nursery, Injambakkam, Chennai and identified as *C. alternifolius* in the Department of Centre for Advanced Studies in Botany University of Madras, Chennai.

Chemicals

All the chemicals used in the experiment are of analytical grade. The solvents were purchased from Merck.

Experimental setup

Plants grown in hydroponic condition are not representative of natural habitat. Hence, a novel technique of partial hydroponic condition was set up for easy collection of root exudates representing natural habitat. Plants were placed in polythene covers filled with soil. The covers were pierced to create holes on all sides and placed in a plastic mug containing water which was renewed with water whenever essential. The setup was left undisturbed for a period of 45 days. Through the holes, the roots started emerging out. The roots were grown to a length of 10 cm and above.

Heavy metal mixture in this context means mixture of copper, cadmium, chromium, lead, zinc, nickel, and iron. Five parts per million of each heavy metal in the mixture was accurately weighed and dissolved in deionized water. Plants with flourishing roots were placed in a beaker containing deionized water spiked with 5 ppm of heavy metal mixture. Similarly, 10 ppm and 20 ppm of heavy metals were also used in this study. A control was set up correspondingly, in which plants with flourishing roots were placed in a beaker containing deionized water devoid of heavy metals. The setup was left undisturbed for a period of 5 days. At the end of 5th day, root exudate was collected from plants by placing it in deionized water for a period of 8 h. The collected root exudate was freeze-dried and redissolved in 10 mL of cold methanol. The extract was blown to dryness and reconstituted in 1 mL of n-hexane and analyzed in gas chromatography–mass spectrometry (GC-MS).

Gas chromatography–mass spectrometer analysis

The hexane extract was analyzed in JEOL GCMATE II in SAIF IIT-Madras. The following chromatographic conditions were followed for analysis. Front inlet temperature: 220°C, column: HP 5 Ms, carrier gas: high pure helium, flow rate: 1 ml/min, oven temperature: 50–250 at 10°/min, ion chamber temperature: 250°C, GC interface temperature: 250°C, mass analyzer: quadruple double focusing mass analyzer, detector: photon multiplier tube, scan range: 50–600 amu, and electron impact ionization: 70 eV.

Heavy metal analysis

The plants were air-dried followed by oven drying to get a constant weight. The samples were ground in a motor and pestle and sieved through 2 mm mesh. Five grams of the sample was accurately weighed and taken in a conical flask to which 30 mL of triacid mixture (Nitric acid:Sulfuric acid:Perchloric acid) was added and digested on hotplate at 200°C. The digested sample was filtered and made up to 100 mL with deionized water in a standard flask. The clear solution was analyzed for heavy metals such as cadmium, copper, chromium, nickel, lead, zinc, and iron in ICP-OES Perkin Elmer Optima 5300 DV in SAIF IIT-Madras. WINLAB 32 operating software was used for the determination of heavy metal concentration.

RESULTS AND DISCUSSION

Phytocomponents in the root exudates of control plant

Root exudates mainly consist of carbon-containing compounds. The quantity and composition of root exudates respond rapidly to very subtle environmental changes.[16] The phytocomponents in the root exudates of control plant identified by GC-MS technique are presented in Figure 1. Nine compounds were identified in the control plant which is devoid of heavy metal stress. The retention time, peak area %, International Union of Pure and Applied Chemistry (IUPAC) name, and the formula of the compounds are tabulated in Table 1. About 10% of the root exudate constituted hexadecanoic acid methyl ester whose biological properties are antioxidant, flavor, antifibrinolytic, hypocholesterolemic, antiandrogenic, lubricant, hemolytic, 5-alpha reductase inhibitor, and nematicide.[17] Hexadecanoic acid methyl ester is found in the leaves of *Cissus vitiginea*.[17] and exhibits acaricidal activity.[18] Methyl tetradecanoate constitutes about 8.84% of the peak area and is a constituent of fatty oil with versatile use such as flavor, emollient, and skin conditioner. It is present in the fruits of *Celtis australis*.[19] The compound 9-hexadecenoic acid methyl ester (9.92%) was previously identified in the leaf extract of *Jatropha curcas*[20] and is found to possess antimicrobial activity. No biological activity was reported for heptadecanoic acid methyl ester (10.65%) but is present in the seeds of *Satureja thymbra* and *Satureja cuneifolia*.[21] 9-octadecenoic acid methyl ester (11.07%) was identified in seeds of *S. thymbra* and *S. cuneifolia*[21] and in the roots of *Elephantorrhiza elephantina*.[22] Eicosanoic acid (12.13%) also called as arachidic acid is a saturated fatty acid and is abundantly found in *Citrullus vulgaris,*[23] but no biological activity is reported so far. The compound 3-methyl-2-undecyl-1H-quinolin4-one (13%) was released from the plant extract *Ruta graveolens*.[24] 2, 3 Dihydroxypropyl elaidate (13.12%) was already reported to be present in the essential oil of all parts of the plant *Etlingera elatior* except the leaves.[25]

Phytocomponents in the root exudates of plants exposed to 5 ppm heavy metal mixture

The phytocomponents in the root exudates of plant spiked with 5 ppm concentration of heavy metal mixture are presented in Figure 2. Eight components were identified. The retention time, peak area %, IUPAC name, and the formula of the compounds are tabulated in Table 2. The presence of compound 1 propanamine N-[phenylmethylene] (8.5%) and its biological property was not reported earlier. The compound 3,7,11-trimethyldodeca-2, 4, 6,10-tetraenal (9.9%) is also present in the essential oil obtained from *Citrus sinensis*.[26] Compound 2[[benzo (1,3) dioxole 4 carbonyl] amino] 3 hydroxy propionic acid (12.03%) was previously identified in the essential oil extracted from the leaves of *Perilla frutescens*.[27] Pentadecanoic acid, 13 methyl methyl ester (12.46%), 10 octadecenoic acid, 15 methy, ester (13.74%) possess the biological properties of antihelminthic, anti-inflammatory, antimicrobial, and anticancerous activity. It was previously identified

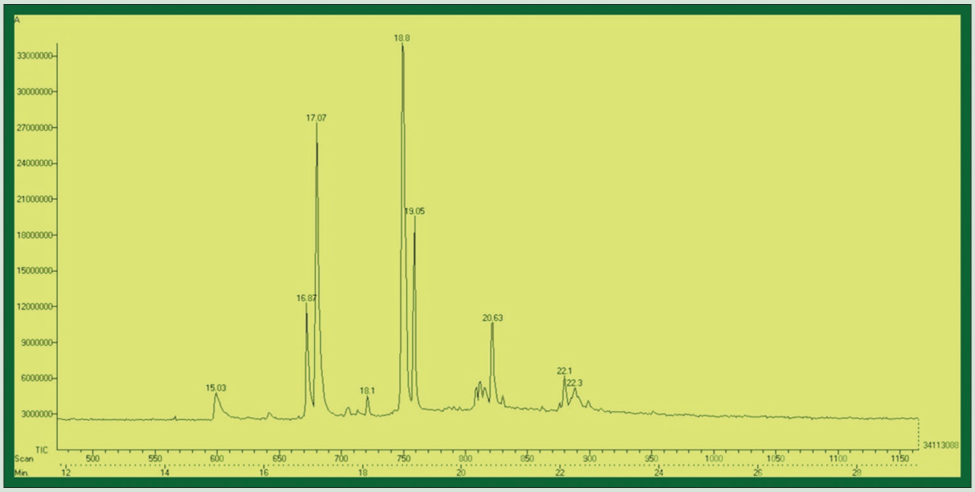
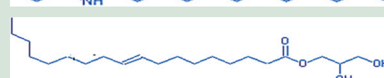


Figure 1: Phytocomponents in the root exudate of control plant

Table 1: Phytocomponents identified in the control plants

Peak	Retention time	Peak area percentage	IUPAC name	Chemical formula
1	15.03	8.84	Methyl tetradecanoate	
2	16.87	9.92	9 hexadecenoic acid, methyl ester	
3	17.07	10.04	Hexadecenoic acid, methyl ester	
4	18.1	10.65	Heptadecanoic acid, methyl ester	
5	18.8	11.07	9-octadecanoic acid, methyl ester	
6	19.05	11.20	Heptadecanoic acid, 15 methyl, methyl ester	
7	20.63	12.13	Eicosanoic acid	
8	22.1	13	3-methyl- 2-undecyl-1H-quinolin-4-one	
9	22.3	13.12	2,3-dihydroxypropyl elaidate	

IUPAC: International Union of Pure and Applied Chemistry

in the leaf extract of *Gmelina asiatica*[28] and stem extract of *Clausena anisata*.[29] Pentadecanoic acid is found in large quantities in blood orange varieties[30] and in essential oil obtained from *Malabaila suaveolens* fruits.[31] The compounds 2,4 oxymethano 1, 2 3 4 4a 4b, 5, 6, 7, 8, 8a, 9 dodecahydro phenanthren 9 one (15.19%) and 17 [1,5 Dimethylethyl] 3, 5 Dihydroxy, 10,13 Dimethyl hexadecahydrocyclopenta(a)phenanthren 6 one (16.59%) are not reported in previous literature. Dodecanamide, N (2 hydroxy ethyl) (11.33%) is found to be present in the roots of *E. elephantina*.[22]

Phytocomponents in the root exudates of plants exposed to 10 ppm heavy metal mixture

The phytocomponents in the root exudates of plants spiked with 10 ppm of heavy metal mixture are presented in Figure 3. Five compounds were identified. The retention time, peak area %, IUPAC name, and the formula of the compounds are tabulated in Table 3. The identified compounds 1-Cyclohexene-1-acrylic acid, 2,6,6-trimethyl-3-oxo-, methyl ester (17.37%), 1,3, Benzodioxole 5 (4-Keto butyric acid) (18.13%),1,2-benzenedicarboxylic acid, mono (2 ethyl ether) ester (25.19%) were not reported in literature. Pentadecanoic acid, 14 methyl, methyl ester (18.73%) is reported to possess antioxidant capacity and was found in the leaves of *Indigofera suffruticosa*.[32] 10-octadecenoic acid, methyl ester (20.56%) is previously reported in the leaf extract of *J. curcas*.[20]

Phytocomponents in the root exudates of plants exposed to 20 ppm heavy metal mixture

The phytocomponents in the root exudates of plants spiked with 20 ppm of heavy metal mixture are presented in Figure 4. Three compounds were identified. The retention time, peak area %, IUPAC name, and the formula of the compounds are tabulated in Table 4. Pentadecanoic acid, 14-methyl, methyl ester (29.76) possess antioxidant properties

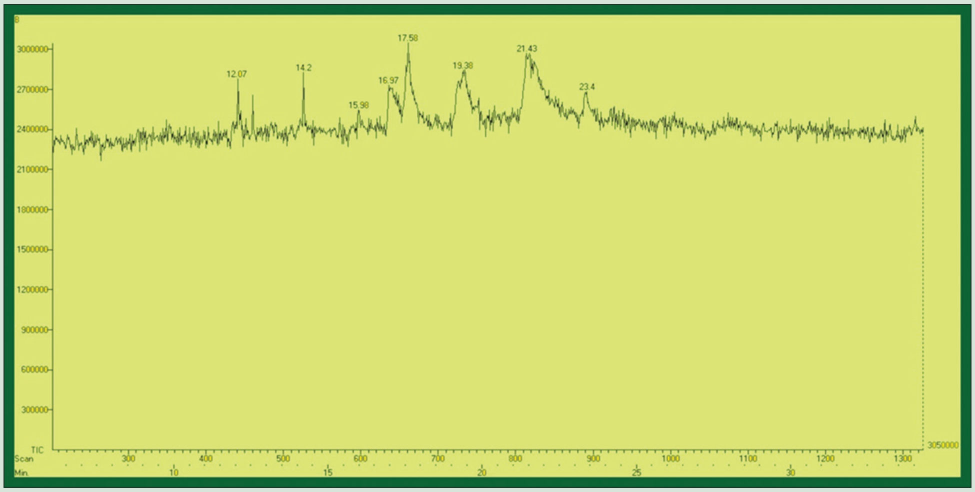


Figure 2: Phytocomponents in the root exudate of plant exposed to 5 ppm heavy metal mixture

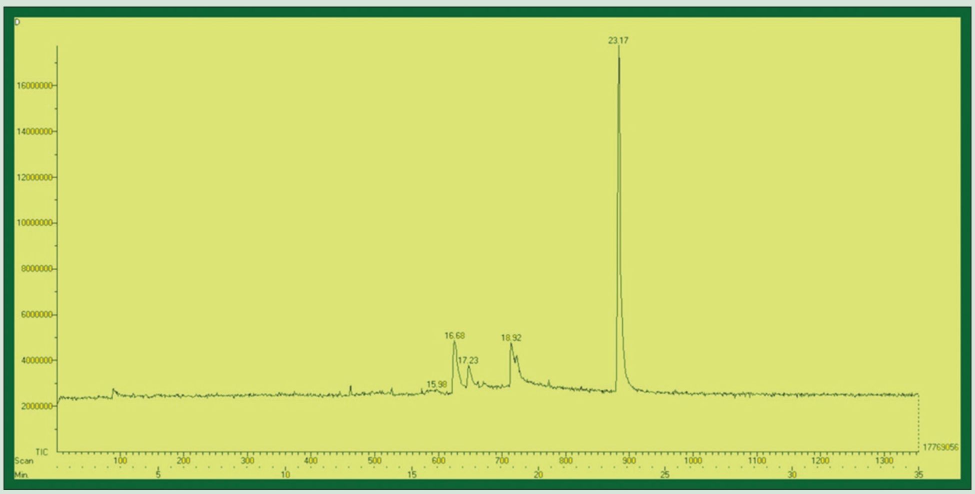


Figure 3: Phytocomponents in the root exudate of plant exposed to 10 ppm heavy metal mixture

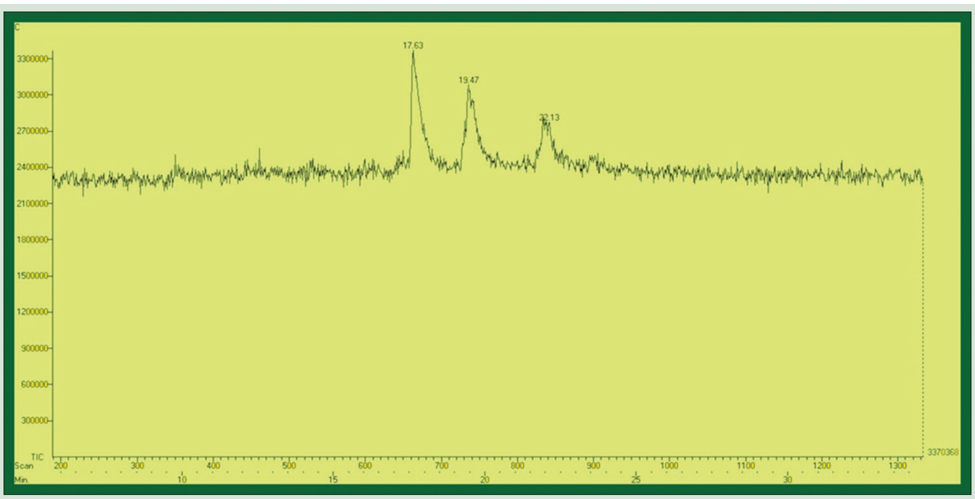


Figure 4: Phytocomponents in the root exudate of plant exposed to 20 ppm heavy metal mixture

and is found in the leaves of *I. suffruticosa*.[32] 16-octadecenoic acid methyl ester (32.87%) selectively inhibits eukaryotic DNA polymerase activities *in vitro*[33] and is also found in the whole plant extracts of *Iris germanica*.[34]

Table 2: Phytocomponents identified in the plants spiked with 5 ppm of metal mixture

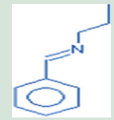
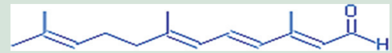
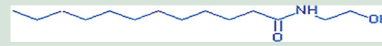
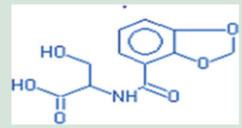
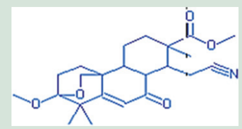
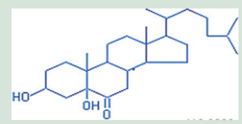
Peak	Retention time	Peak area percentage	IUPAC name	Chemical formula
1	12.07	8.5	1 propanamine N-[phenylmethylene]-	
2	14.2	9.9	3,7,11-trimethyl-dodeca- 2, 4, 6,10- tetraenal	
3	15.98	11.33	Dodecanamide , N [2 hydroxy ethyl]	
4	16.97	12.03	2[[benzo (1,3) dioxole 4 carbonyl] amino] 3 hydroxy propionic acid	
5	17.58	12.46	Pentadecanoic acid, 13-methyl-, methyl ester	
6	19.38	13.74	10-octadecenoic acid, 15-methyl ester	
7	21.43	15.19	2,4 oxymethano 1,2 3 4 4a 4b, 5,6,7,8,8a, 9 dodecahydro phenanthren 9 one	
8	23.4	16.59	17 [1,5 dimethylethyl] 3, 5 dihydroxy, 10,13 dimethyl hexadecahydrocyclopenta(a)phenanthren 6 one	

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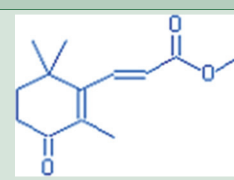
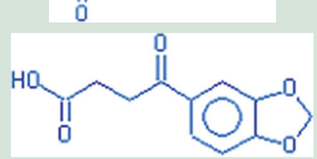
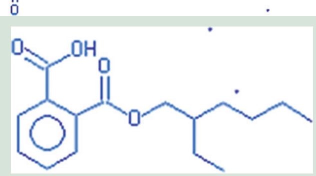
Table 3: Phytocomponents identified in the plants spiked with 10 ppm of metal mixture

Peak	Retention time	Peak area percentage	IUPAC name	Chemical formula
1	15.98	17.37	1-cyclohexene-1-acrylic acid, 2,6,6-trimethyl-3 oxo-, methyl ester	
2	16.68	18.13	1,3-benzodioxole-5-(4- Keto-butyric acid)	
3	17.23	18.73	Pentadecanoic acid, 14-methyl, methyl ester	
4	18.92	20.56	10-octadecenoic acid, methyl ester	
5	23.17	25.19	1,2 benzenedicarboxylic acid, mono (2 ethyl ether) ester	

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Heavy metal concentration in plant

Hydroponics is one of the excellent methods to remove heavy metals from wastewater. Heavy metal concentration in the plants is presented in Table 5. All the heavy metals are accumulated in different parts of plant tissues. The order of heavy metal accumulation in plant is Fe > Zn > Cu > Pb > Ni > Cd > Cr. *C. alternifolius* is capable of 100% removal of heavy metals Al, Cd, Cu, Fe, Pb, and Zn and 42.2% removal of Mn from the ecosystem.[35] About 85% of arsenic was taken up by *Brassica juncea* at different concentrations, namely 5, 10, 20, and 50 ppm under hydroponic conditions.[36] Ar, B, Cd, Cu, Pb, Ni, and Zn were removed in hydroponic condition by lettuce plants grown under glasshouse conditions.[37]

Table 4: Phytocomponents identified in the plants spiked with 20 ppm of metal mixture

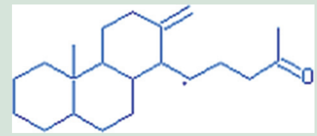
Peak	Retention time	Peak area percentage	IUPAC name	Chemical formula
1	17.63	29.76	Pentadecanoic acid, 14-methyl-, methyl ester	
2	19.47	32.87	16-octadecenoic acid, methyl ester	
3	22.13	37.36	13, 15 seco 5 a pregn 13(18) en 20 one	

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Table 5: Concentration of heavy metals uptake in *Cyperus alternifolius*

Heavy metal (mg/L)	5 ppm	10 ppm	20 ppm
Cd	2.4±0.2	4.6±0.4	7.8±0.3
Cr	2.0±0.3	4.2±0.5	7.4±0.2
Cu	3.0±0.3	5.6±0.4	9.5±0.2
Zn	3.2±0.2	6.5±0.3	10.8±0.4
Fe	4.4±0.4	7.3±0.2	12.6±0.3
Ni	2.2±0.3	5.2±0.4	8.2±0.2
Pb	2.3±0.2	5.5±0.3	8.9±0.4

CONCLUSION

This paper explains that the partial hydroponic condition not only helps in the removal of heavy metals but on the flip side but also it releases a diverse of organic acids and secondary metabolites. *C. alternifolius* is a promising plant in the removal of heavy metals. Apart from that, a dual role in the production of secondary metabolites with imperative biological properties is the key factor of this research. In the future, research can be enhanced in the studies pertaining to the production of secondary metabolites considering the importance of its biological properties.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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