Uncovering the Phytochemicals of Root Exudates and Extracts of Lead (Pb) Hyperaccumulator Vetiveria Zizanioides (L.) in Response to Lead Contamination and their Effect on the Chemotactic Behaviour of Rhizospheric Bacteria

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Research Article

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Abstract

Chemical composition of root exudates and root extracts from *V. zizanioides* cv KS-1 was determined, in the presence of lead [Pb(II)]. Hitherto, no information is available in the literature concerning the phytochemical components of root exudates of *Vetiver zizanioides*. Significantly higher concentrations of total carbohydrates (26.75 and 42.62% in root exudates and root extract, respectively), reducing sugars (21.46 and 56.11% in root exudates and root extract, respectively), total proteins (9.22 and 23.70% in root exudates and root extract, respectively), total phenolic acids (14.69 and 8.33% in root exudates and root extract, respectively), total flavonoids (14.30 and 12.28% in root exudates and root extract, respectively) and total alkaloids (12.48 and 7.96% in root exudates and root extract, respectively) were observed in samples from plants growing under Pb(II) stress in comparison to the respective controls. GC-MS profiling showed the presence of diverse group of compounds in root exudates and extracts including terpenes, alkaloids, flavonoids, carotenoids, plant hormones, carboxylic/organic acids and fatty acids. Among the detected compounds, many have important role in plant development, regulating rhizosphere microbiota, and allelopathy. Furthermore, the results indicated that *V. zizanioides* exudates possess a chemotactic response for rhizospheric bacterial strains *Bacillus licheniformis*, *Bacillus subtilis* and *Acinetobacter junii* Pb1.

1. Introduction

Rapid global industrialization and urbanization has caused severe soil contamination problems. Among them, pollution due to heavy metals (HM) has been a serious concern due to its toxic effects on human health and the ecosystem, including the flora and fauna (Arul et al. 2016; Bind et al. 2019). Among all HM contaminants, Pb is considered as one of the most toxic pollutant because it tends to (bio)accumulate at high rates in soil environments (Kushwaha et al. 2017, Singh et al. 2019; Sathe et al. 2020). The common anthropogenic sources of Pb contamination in the environment include smelting of ores, burning of coal, mining, effluents from battery industries, automobile exhausts, metal plating, leather tanning, finishing operations, and the overuse of fertilizers and pesticides (Goswami et al. 2017, Kushwaha et al. 2020). The bioavailability of HM in soil is a serious aspect which has to be monitored in order to restore a contaminated site. Thus, environment researchers have given it a serious concern, thereby, ensuring human health (Bind et al. 2018; Goswami et al. 2020).

In this line of progressive research, phytoremediation is a novel, cost-effective, efficient, environment friendly and solar-driven remediation technology, which has proven to improve the soil quality (Kushwaha et al. 2015). *Vetiveria zizanioides* cv KS-1 (VZ) was selected in this study due to its ability to grow in extreme environmental conditions and demonstrate high biomass yield within short time durations. A native of India, it is also known as the 'wonder grass' and it is extensively used in majority of the tropical countries for the phytoremediation of various contaminants present in water bodies. Since, it grows rapidly, it is more effective for the restoration of contaminated sites in only 4–5 months as compared with trees and shrubs which usually require ~ 2–3 years (Sinha et al. 2009). *Vetiveria zizanioides* is known to tolerate high alkalinity and acidity (pH 3.0 to 10.5), salanity (EC 8 dSm^{-1}), high concentration of heavy metals (lead, mercury, copper, zinc, arsenic, cadmium, nickel, magnesium, manganese, aluminium and selenium), pesticides and herbicides. It has been reported that vetiver grass stabilized Pb(II) in root zone with moderate translocation) to above ground biomass (shoots). Thus, its shoots can be safely used as fodder or grazed by animals. Earlier literatures have reported the mechanisms involved in the accumulation and transportation of inorganic pollutants in *V. zizanioides*, but still some mechanisms remain rather unclear, especially the role of root exudates (REs).

REs plays a significant role in phytoremediation. The high density and diversity of rhizopsheric microorganisms is due to the effect of various RE components. The secondary metabolites released from plant roots during stressful environmental conditions could improve the nutrient uptake and help the plant to cope with those stresses (Luo et al. 2015). The chemical constituent of REs, root extracts (Ret) and their concentration depends upon various factors such as the plant type, plant species, soil, environmental and geographical conditions, biotic and abiotic stress and collection method (Rani and Juwarkar 2016; Dutta et al. 2019). REs is mainly classified into two types, i.e. low molecular weight (LMW) and high molecular weight (HMW) compounds. LMW REs is typically composed of organic acids, sugars, phenols and various amino acid and non-proteinaceous amino acids (phytosiderophore), while HMW is majorly composed of mucilage and proteins. Root secretions are known to stimulate the solubility and mobility of HM ions in soil thereby enhancing the phytoextraction efficiency of the plant (Rajkumar et al. 2012). Organic acids (citric and oxalic acid) released by the roots of *Echinochloa crusgalli* have been reported to increase the Cu(II), Cd(II) and Pb(II) translocation from below ground biomass to above ground biomass (Kim et al. 2010).

To the best of the authors knowledge, this is the first report that describes the exudation behaviour of VZ in presence of Pb(II) and its role as chemoattractant. Besides, very little data are available in the literatures concerning the effect of different level Pb(II) on the phytocomponents of vetiver grass and its chemotactic effects. Therefore, the present study will help to envisage the role of various phytocomponents of vetiver grass on the Pb(II) tolerance capacity. Thus, the specific objectives of this work can be stated as follows: (i) to perform chemical characterization of the REs and REt from the Pb hyperaccumulator VZ, and (ii) to determine the chemotaxis response of rhizospheric bacteria *(Bacillus licheniformis, Bacillus subtilis and A. junii* Pb1) towards VZ exudates.

2. Materials And Methods

2.1 Materials

Analytical grade chemicals were used in this study. Lead nitrate [$Pb(NO_3)_2$] (Merck, India) was used for preparing the stock solution of Pb(II). The respective concentrations of the working solution were made from the stock solution using deionised water (18Ω). The plant slips of VZ were purchased from the Central Institute of Medicinal and Aromatic Plants, Lucknow (Uttar Pradesh, India). All experiments were performed in triplicates.

2.2 Collection of root exudates and root extracts

REs from VZ were obtained by the sand culture method described by Gaidamak (1971) and REt were collected by extracting the roots in calcium chloride solution described by Liebersbach et al. (2004). Briefly stating, for REs, the roots of VZ was washed with tap water followed by distilled water. Hoagland solution was sprinkled periodically in order to maintain 30–40% of the water holding capacity of sand and for the growth of plant. To study the variance in the

exudation behaviour of plant in the presence of Pb(II), one set of experiment was spiked with Pb(II) (100 mg kg^{-1}), while the other was used as the control (non-spiked). For the collection of REs, the top excess portion of Hoagland solution was withdrawn so that the Hoagland solution along with root washings overflows and gets collected in the beaker at the bottom of the experimental setup. In this manner, REs was collected on the 20th and 60th day.

REt were collected according to the $CaCl_2$ extraction method described by Liebersbach et al. (2004). Experiments for two treatments were performed, i.e. one spiked with Pb(II) (experimental), while the other served as the control (non-spiked). For the collection of REt, the plants were uprooted and placed in freshly prepared 0.5 mM $CaCl_2$ solution for 2 h. Thus, the REt were collected on the 20th and 60th day. After collecting the samples, the REs and REt were sterilized by passing through 0.22 µm membrane filter, purified by dialysis, concentrated 15-fold by lyophilisation, and stored at -4°C until further analysis.

2.3 Chemical characterization of root exudates and root extracts

REs and REt were analysed for total carbohydrate content using the Anthrone's method (Hedge and Hofreiter, 1962), reducing sugars concentration by the Dintrosalicylic Acid (DNS) (Miller 1959), proteins by performing the Bradford's test (Bradford, 1976), total phenolic acid content by performing Folin-Ciocalteu assay tests (Kaur et al. 2002), total flavonoid content using aluminium chloride (Chang et al. 2002) and total alkaloids by the acid dye method (Tambe and Bhambar 2014).

2.4 GC-MS detection of root exudates

REs and REt of VZ was further analysed by gas chromatography-mass spectrometry GS-MS (Thermo Scientific TSQ 8000). The MS part consisted of Triple Quadrupole and it was paired with the TRACE 1300 GC, fitted with an auto-sampler. REs (control and 100 mg kg^{-1}) and REt (control and 100 mg kg^{-1}) were lyophilized 20 times and suspended in acetone and subsequently subjected to GC-MS analysis. Chromatographic separation was achieved under the following conditions: injection temperature 240°C, 3 min @ 80°C isothermal followed by a ramp of 5°C min^{-1} to 320°C for 5 min and gas flow rate 2 mL min^{-1}. Mass spectra were recorded at 10 spectra sec^{-1} with an m/z scanning range of 50 to 700. The compounds were identified by comparing the mass spectra from the NIST library database.

2.5 Chemotactic effect of root exudates on rhizospheric microorganisms

The chemotactic effect of REs on rhizospheric microorganisms was assessed by the agar diffusion method. The minimal media was prepared with 0.75% agar powder in petri plates. The 14 h old culture having an optical density (OD) of 1.0 contained different bacterial strains, *Bacillus subtilis*, *Bacillus licheniformis* and the lead resistant bacterium *Acinetobacter junii* Pb1 were used for performing chemotactic studies. Two wells were created in petri plates and 80 µl of root exudates was added in one, while distilled water was added to the other well that acted as the control. At the centre of the petridish, equidistant from both the wells, 5 µl of culture (OD: 1.0) was inoculated and incubated for 12 h at 30°C. The chemotactic effect of REs towards the different bacterial strains was observed after 12 h of incubation.

2.6 Statistical analysis

All the experiments were carried out in triplicates. The experimental data were statistically analysed and presented with the appropriate standard deviation values. The data was also subjected to Student's t-test using the RAUSTAT software (Dr. R.C. Bharti, PUSA). $P < 0.05$ were considered to be statistically significant. The data analysis and preparation of graphs were done using the GraphPad Prism 5software.

3. Results And Discussion

3.1 Chemical characterisation of root exudates and extract of VZ

3.1.1 Total carbohydrates content

The concentration of total carbohydrates in REs and REt on the 20 and 60th day of plant growth are given in Table 1. It can be seen that, there was insignificant change ($P > 0.05$) in the carbohydrate content of both REs and REt on the 20th day between the control and test samples (Fig. 1). However, a significant difference ($P < 0.05$) was observed in the carbohydrate content in both REs and REt on the 60th day (Fig. 1). On the 60th day, the total carbohydrate content increased by 26.75 and 42.67% in the REs and REt, respectively, in the presence of Pb(II) when compared to control (Table 2). The carbohydrate content varied significantly in REs and REt on the 60th day, wherein the highest carbohydrate concentration was observed in REt as compared to REs (Table 2). The difference in carbohydrate content suggest that the concentration of the same component differ depending upon the technique used for its collection. Rani and Juwarkar (2015; 2016) reported that the chemical composition of both REs and REt depends not only on the plant species, soil, environmental and geographical surroundings, but also on the technique used for its collection. In a previous work, Krishnaraj et al. (2012) studied the effect of biologically synthesized silver nanoparticles (AgNPs) on *Bacopa monnieri* (Linn.) Wettst. and reported an increase in the carbohydrate content in AgNPs treated plant when compared to the control. The carbohydrate content of REs mainly includes mucilaginous substances (HMW) secreted by the root tissues; however, it also includes a minor portion of sugars (LMW). These carbohydrates act as the carbon source for microorganisms and alter the activity and number of microorganisms, thereby affecting the HM bioavailability in soil.

Table 1
Characterization of root exudates and root extracts in the presence and absence of Pb(II)

Component		Concentration of root exudates		Concentration of root extract	
		20th day	60th day	20th day	60th day
Total carbohydrate ($\mu g\ ml^{-1}$)	Control	0.404 ± 0.021	0.459 ± 0.0407	0.413 ± 0.0282	0.456 ± 0.0497
	Test	0.438 ± 0.049	0.626 ± 0.044	0.422 ± 0.0502	0.651 ± 0.0498
Reducing sugar ($\mu g\ ml^{-1}$)	Control	0.366 ± 0.012	0.419 ± 0.004	0.392 ± 0.017	0.377 ± 0.017
	Test	0.399 ± 0.008	0.509 ± 0.009	0.451 ± 0.013	0.530 ± 0.031
Total Protein ($\mu g\ ml^{-1}$)	Control	1.683 ± 0.125	1.982 ± 0.074	1.806 ± 0.099	1.992 ± 0.067
	Test	1.928 ± 0.104	2.165 ± 0.149	2.130 ± 0.200	2.464 ± 0.253
Total Phenolic ($\mu g\ ml^{-1}$)	Control	54.92 ± 2.5	56.27 ± 2.57	57.74 ± 1.96	73.74 ± 2.1
	Test	60.385 ± 2.965	64.535 ± 2.965	54.999 ± 1.929	79.888 ± 2.468
Total Flavonoids ($\mu g\ ml^{-1}$)	Control	155.727 ± 5.364	162.090 ± 5.818	169.636 ± 4.000	173.999 ± 3.454
	Test	161.136 ± 7.409	185.272 ± 4.818	177.272 ± 4.727	195.363 ± 10.454
Total Alkaloids ($\mu g\ ml^{-1}$)	Control	7.579 ± 0.301	8.05 ± 0.25	7.939 ± 0.301	9.638 ± 0.302
	Test	8.237 ± 0.335	9.055 ± 0.445	8.584 ± 0.296	10.405 ± 0.495

Table 2
Change in the root exudate and extract profiles (in %) on the 60th day

Component	Percent change in root exudates on lead exposure	Percent change in root extract on lead exposure
Total carbohydrate ($\mu g\ ml^{-1}$)	+ 26.75*	+ 42.62*
Reducing sugar ($\mu g\ ml^{-1}$)	+ 21.46*	+ 56.11*
Total Protein ($\mu g\ ml^{-1}$)	+ 9.22*	+ 23.70*
Total Phenolic ($\mu g\ ml^{-1}$)	+ 14.69*	+ 8.33*
Total Flavonoids ($\mu g\ ml^{-1}$)	+ 14.30*	+ 12.28*
Total Alkaloids ($\mu g\ ml^{-1}$)	+ 12.48*	+ 7.96*

3.1.2 Reducing sugars content

Reducing sugars in REs and REt on the 20 and 60th day of plant age is given in Table 1. On the 20th day, insignificant difference ($P > 0.05$) was observed in the total reducing sugar concentrations of REs and REt, between the control and test samples (Fig. 2). However, on the 60th day, the total reducing sugar concentration increased by 21.46% and 56.11% in REs and REt, respectively ($P < 0.05$) in the presence of Pb(II) (Table 2). Increase in the reducing sugar content in plants, upon expose to HM has been reported recently (Shah et al. (2017). The authors observed an increase in the reducing sugar content by 47.95% in *Tagetes erecta* L. when grown in cadmium (18 mg kg^{-1}) treated soil, while a further increase in the cadmium levels decreased the reducing sugar content significantly.

3.1.3 Total protein content

Total protein in REs and REt on the 20th and 60th day of plant age is given in Table 1. As observed previously for the carbohydrates and reducing sugar contents, on the 20th day, insignificant change ($P > 0.05$) was observed in the protein content for REs and REt between the control and test samples (Fig. 3). However, in the case of REt, a significant difference ($P < 0.05$) was observed in the protein content on the 60th day (Fig. 3). The protein concentration was found to increase by 9.22 and 23.70% in REs and REt, respectively ($P < 0.05$) in presence of Pb(II) (Table 2). Thus, the protein content in both REs and REt was found to increase in presence of lead when compared to the control experiments. Gohari et al. (2012) reported an increase in the protein content in roots of two varieties (Hyola308 and RGS003) of *Brassica napus L*. with an increase in Pb(II) concentration (0 to 400 µM). Increase in protein secretion from plant roots in the presence of Pb(II) implies significant biological functions, enzymatic changes, and alteration in the defence mechanisms related to the uptake and transformation of lead compounds.

3.1.4 Total phenolic acids content

The total phenolic acids concentration in REs and REt are presented in Table 1. As shown in Fig. 4, no significant difference ($P > 0.05$) in the phenolic acids content was observed in REs and REt between the control and test samples on the 20th day. However, on the 60th day, a significant increase ($P < 0.05$), in REs

and REt, i.e. 14.69% and 8.33%, respectively, in the phenolic acids content was observed in the presence of Pb(II). It is noteworthy to mention that, increased secretion of phenolic acids from roots under heavy metal stress have been reported in several previous studies. For example, Irtelli and Navari-Izzo (2006) reported that in the presence of Cd(II) (150 mg kg^{-1}) an increase in phenolic acids content was noticed in *Brassica juncea*. Jung et al. (2003) also reported an increase in the phenolic acids content (particularly genistein and genistein-(malonyl)-glucoside) in the roots of *Lupinus albus* L. when exposed to 20 µM copper solution. Márquez-García et al. (2012) reported an increase in the total phenolic content in *Erica andevalensis* when treated with Cd(II) (5 µg g^{-1} soil).

Phenolic acids present in REs and REt are usually linked with numerous functions. They have redox properties, which act as antioxidants. The presence of hydroxyl groups is known to be responsible for scavenging the free radicals generated during periods of unexpected environmental stress. Thus, the total phenolic acids concentration could be used to access the antioxidant activity (Baba and Malik 2015). The antioxidant activity of phenolic compounds is mainly due to their reduced properties which allow them to act as metal chelators, absorb and neutralize the free radicals. They also act as growth promoters and chemoattractant for microorganisms present in soil. Many phenolic compounds act as defence mechanism in plants against pathogens, have allelophatic activity and act as phytoalexins (Rani and Juwarkar 2016).

3.1.5 Total flavonoid content

The results of total flavonoid content in REs and REt are presented in Table 1. On the 60th day, an increase by 14.30% and 12.28% in the flavonoid content of REs and REt, respectively, with $P < 0.05$, was observed in the presence of Pb(II) (Fig. 5). In a previous study, Parry et al. (1994) reported the increase in isoflavonoid content in roots of Alfalfa when treated with 1 mM $CuCl_2$. Tumova and Ruskova (1998) reported an increase in the flavonoid content in callus culture of *Ononis arvensis* when $CuSO_4$ and $CdCl_2$ concentrations were increased. Flavonoids, flavones, condensed tannins and flavanols are secondary metabolites present in plant. Flavonoids are known to act as the scavengers of free radicals generated under oxidative stress. Due to the presence of the free hydroxyl groups, especially 3-OH, flavonoids exhibits antioxidant activity. Besides radical scavengers, flavonoids can also act as metal chelators depending on the molecular structure (Kumar and Pandey 2013). In addition, anthocyanins which are synthesized through the same pathway as that of flavonoids, are known to be tolerant towards metals.

3.1.6 Total alkaloids content

The total alkaloid content of REs and REt are given in Table 1. Similar to prior observations, on the 20th day, insignificant change ($P > 0.05$) was observed in the content of alkaloids in REs and REt between control and test samples (Fig. 6). Conversely, in the presence of Pb(II), on the 60th day, the alkaloid concentrations increased by 12.48% and 7.96% in REs and REt, respectively ($P < 0.05$) (Table 2). Srivastava and Srivastava (2010) reported that when *Catharanthus roseus* L. was exposed to 5 mM of Mn, Ni, and Pb, the alkaloid contents increased significantly in the roots. ArefiFard (2017) also reported an increase in the alkaloid content in shoots and roots of *Catharanthus roseus* when exposed to $NiCl_2$. It is well-known that, under harsh environmental conditions or environmental stress, an alteration in metabolic activity towards protective secondary metabolites can be expected to occur as a mode of strengthening the defence mechanism. The increase in alkaloid content is presumably due to the increase in endogenous methyl jasmonate which is involved in catalysing the enzymes responsible for alkaloid biosynthesis (Turner et al. 2002). Thus, the accumulated alkaloids are known to be responsible for inducing the defence mechanism in plants. The results from this study showed that, REs and REt of *V. zizanioides* comprises of varied chemical components including carbohydrates, proteins, alkaloids, flavonoids and phenolic.

3.2 GC-MS analysis of root exudates

The results from GC-MS analysis led to the identification of a number of compounds from the GC fractions of REs and REt. The compounds identified in REs and REt in absence and presence of Pb(II) through mass spectrometry are shown in Figs S1 and S2, respectively, and Tables 3 to 6 with their retention time (RT), molecular formula and their functions. Figure 7 and Tables 5 and 6 provides the detailed list of compounds identified in the extract, i.e. in the absence and presence of Pb(II). In presence of Pb(II) various phyto-compounds such as 1-Phenanthrenecarboxylic acid; tetradecahydro-7-(2-methoxy-2-oxoethylidene)-1,4a,8-trimethy – 9-oxo,methylester; hexatbutylselenatrisiletane; 1,3-cyclohexadiene,1-methyl-4-(1-methyl ethyl); cyclohexene-1-methyl-4-(1-methylethylidene); 2,4,6-octatriene, 2,6dimethyl-; (+)-4-carene; benzene-1-methyl-3-(1-methylethyl); ç-terpinene; 9,12,15-octadecatrienoicacid, 2,3-bis[(trimethylsilyl)oxy]polylester are known to have antioxidant properties and act as a defence response against insect attack and fungal pathogen infection. Among the identified compounds some are reported to be potential therapeutic agents. They inherently possess antimicrobial, antioxidant, anti-inflammatory, insecticide, and antifungal characteristics. Huang et al. (2010) also reported the presence of 9,12,15-octadecatrienoicacid,2,3-bis [(trimethylsilyl)oxy]polylester and 1-Phenanthrenecarboxylic acid in REs of cowpea. The presence of 9,12,15-octadecatrienoicacid,2,3-bis[(trimethylsilyl)oxy]polylester has been reported in *Cyperus alternifolius* REs when exposed to a mixture of HM (Cd, Cu, Cr, Ni, Zn, Pb, and Fe) (Usharani and Vasudevan 2017).

Table 3
Chemical composition of the root exudates (control)

Sl. No.	RT (min.)	Area %	Compound name and Molecular formula	Function and Class	References
1	6.58	29.19	2,4,6 Octatriene, 2,6 dimethyl ($C_{10}H_{16}$)	Organic compounds, pleasant odor	Kishimoto et al. 2005
2	6.58	29.19	(+)4Carene ($C_{10}H_{16}$)	Antimicrobial activity, monoterpenes, hydrocarbons	Omoruyi and Muchenje (2017)
3	6.58	29.19	1,3Cyclohexadiene,1methyl4(1methylethyl) ($C_{10}H_{16}$)	Alpha terpinene, isomeric hydrocarbons	Najafian and Zahedifar (2015)
4	6.73	16.90	Benzene, 1ethyl2,4dimethyl ($C_{10}H_{14}$)	Organic compound, insecticide	Runde et al. (2015)
5	6.73	16.90	Benzene, 1methyl3(1methylethyl) ($C_{10}H_{14}$)	Aromatic organic compound, alkyl benzene	Tang et al. (2003)
6	6.73	16.90	Benzene, 1,2,4,5tetramethyl ($C_{10}H_{14}$)	Organic compound, antioxidant activity under Al stress	Wang et al. (2006)
7	6.82	4.59	Cyclohexene,1methyl5(1methylethenyl),(R) ($C_{10}H_{16}$)	Antimicrobial activity, hydrocarbon compound	Mohammed et al. (2016)
8	6.82	4.59	Limonene ($C_{10}H_{16}$)	Monoterpene, found in citrus fruits, induces shifts in bacterial community composition	Musilova et al. (2016); Jha et al. (2015)
9	6.89	3.92	Eucalyptol ($C_{10}H_{18}O$)	Organic compound, antifungal, insect repellent and insecticide	Tripathi et al. (2016), Tayade et al. (2013)
10	6.89	3.92	Trifluoroacetylàterpineol ($C_{12}H_{17}F_3O_2$)	Monoterpene alcohol, antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive, anti-nociceptive	Khaleel et al. (2018)
11	7.44	40.59	çTerpinene ($C_{10}H_{16}$)	Monoterpene, antimicrobial activity	Lim (2016)
13	8.10	3.94	2,4,6 Octatriene,2,6-dimethyl ($C_{10}H_{16}$)	Volatile organic compound, resistant against the fungal disease, ethylene and jasmonic acid signalling pathways	Kishimoto et al. (2005); Kishimoto et al. (2006); Silva et al. (2013)
14	50.20	0.87	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl) ($C_{27}H_{42}O_4$)	Carboxylic acid, antimicrobial, antitumor, insecticidal	Kadhim et al. (2016)
15	50.20	0.87	9,12,15Octadecatrienoicacid, 2,3bis[(trimethylsilyl)oxy]propylester, (Z,Z,Z) ($C_{27}H_{52}O_4Si_2$)	Flavonoids, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary activity	Rajendran et al. (2017)

Table 4

Chemical composition of the root exudates at 100 mg kg^{-1}

Sl. No.	RT (min.)	Area %	Compound name and Molecular formula	Function and Class	References
1	5.51	3.46	1-Phenanthrenecarboxylic acid, tetradecahydro-7-(2-methoxy-2-oxoethylidene)-1,4a, 8-trimethyl-9-oxo-, methyl ester, [1S(1à,4aà,4bá,8á,8aà,10aá)-($C_{22}H_{32}O_5$)	Antioxidant activity under stress conditions, it also has defense response against insect attack and fungal pathogen infection.	Ning-Nan et al. (2007)
2	6.02	7.10	9,12,15Octadecatrienoic acid, 2,3bis[trimethylsilyl) oxy]propyl ester, (Z,Z,Z) ($C_{27}H_{52}O_4Si_2$)	Flavonoids. antimicrobial, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary	Rajendran et al. (2017)
3	6.41	6.24	Hexa-t-butylselenatrisiletane ($C_{24}H_{54}SeSi_3$)	Antioxidant activity, Antimicrobial, antitumor, antiseptic, preservative, insecticidal	Abdul-Hafeez et al. (2015)
4	6.41	6.24	Heptadecane, 9-hexyl ($C_{23}H_{48}$)	Antifungal, pharmacological properties	Abubacker and Devi (2015); Tayade et al. (2013)
5	6.41	6.24	Octadecane, 3-ethyl-5-(2-ethylbutyl) ($C_{26}H_{54}$)	Antifungal activity, anti-inflammatory, antioxidant	Morah et al. (2017)
6	6.57	28.90	1,3-Cyclohexadiene,1-methyl-4-(1-methylethyl) ($C_{10}H_{16}$)	γ-Terpinene, Antioxidant, Antibacterial, allelopathic, herbivore deterrent activity	Radmanesh et al. (2015); Raj et al. (2014); Jiménez-Osornio et al. (1996)
7	6.57	28.90	Cyclohexene, 1-methyl-4-(1-methylethylidene) ($C_{10}H_{16}$)	Terpinolene, Antioxidant activity	Ruberto et al. (2000)
8	6.57	28.90	(+)4-Carene ($C_{10}H_{16}$)	Monoterpene, insecticide, antimicrobial activity	Mneimne et al. (2016); Bautista-Lozada et al. (2013) Smeriglio et al. (2017)
9	6.72	20.19	Benzene, 1-ethyl-2,4-dimethyl ($C_{10}H_{14}$)	Antimicrobial, Insecticide	Choi et al. (2008)
10	6.72	20.19	Benzene, 1,2,4,5-tetramethyl ($C_{10}H_{14}$)	Aromatic compound, resistance against Al^{+3}, provide protection against insects and other herbivores	Wang et al. (2006)
11	6.72	20.19	Benzene, 4-ethyl-1,2-dimethyl ($C_{10}H_{14}$)	Aromatic compounds	http://foodb.ca/compounds/FDB007614
12	6.81	6.12	Docosahexaenoic acid, 1,2,3-propanetriylester ($C_{69}H_{98}O_6$)	Phytochemical compound	Srivastava et al. (2015)
13	7.42	27.99	çTerpinene ($C_{10}H_{16}$)	Antimicrobial activity, Monoterpenoid	Lim (2016)
14	7.42	27.99	3-Carene ($C_{10}H_{16}$)	Terpenoids, resist attack from pathogens	Keeling and Bohlmann (2006); Arora (1991)

Table 5
Chemical composition of the root extract (control)

Sl. No.	RT (min.)	Area %	Compound name and Molecular formula	Function and Class	References
1	6.05	4.33	17-Pentatriacontene ($C_{35}H_{70}$)	Found in plant extracts	Tayade et al., 2013
2	6.05	4.33	Oleic acid, eicosyl ester ($C_{38}H_{74}O_2$)	Resistance against pathogen, antibacterial, anti-inflammatory, cancer preventive, dermatitigenic Hypocholesterolemic and anemiagenic Insectifuge	Upchurch (2008); Dilika et al. (20
5	15.11	17.52	Fluprednisolone ($C_{21}H_{27}FO_5$)	Anti-inflammatory agent	https://pubchem.ncbi.nlm.nih.go
6	15.11	17.52	3Methoxymethoxy3,7,16,20tetramethylheneicosa1,7,11,15,19pentaene ($C_{27}H_{46}O_2$)	Found in plant extracts	Jisha et al., 2016
7	16.94	14.32	9,12,15-Octadecatrienoicacid, 2,3-bis [(trimethylsilyl)oxy]propyl ester, (Z,Z,Z) ($C_{27}H_{52}O_4Si_2$)	Flavonoids. antimicrobial, anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary	Rajendran et al. (2017)
8	18.34	5.49	.psi.,.psi.Carotene,1,1',2,2'-tetrahydro-1,1'-dimethoxy ($C_{42}H_{64}O_2$)	Repellant, antibacterial, antifungal, anti-inflammatory	Mebude and Adeniyi (2017)
9	18.34	5.49	Lycoxanthin ($C_{40}H_{56}O$)	Alcohols, carotenoid	Prasad et al. (2017); Markham ar
11	36.13	44.82	6,9,12,15-Docosatetraenoicacid, methyl ester	Polyunsaturated fatty acid, anti-cholesterol, anti-carcinogenic and anti-atherosclerotic	Hussein et al. (2016)
12	36.13	44.82	1-Heptatriacotanol	Alcoholic compound, antimicrobial, anticancer, nematicide, anti-inflammatory	Anburaj et al. (2016); Jegadeesw

Table 6

Chemical composition of the root extract at 100 mg kg^{-1}

Sl. No.	RT (min.)	Area %	Compound name and Molecular formula	Function and Class	References
1	5.98	12.49	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'd]pyridazine ($C_{20}H_{13}N_5O_2$)	Alkaloid compound, antioxidant, anti-helminthic anti-inflammatory, anti-microbial, antimalarial, antiasthma and anti-cancerous	Azhagumurugan and Rajan (2014); Altameme et al. (2015)
2	5.98	12.49	Gibberellic acid ($C_{19}H_{22}O_6$)	Hormone found in plants, abolishing the detrimental effects of Cd and Pb	Sharaf et al. (2009)
3	6.02	3.71	3[3Bromophenyl]7chloro3,4dihydro10hydroxy1,9(2H,10H)acridinedione ($C_{19}H_{13}BrClNO_3$)	Alkaloid compound, Antioxidant, Hepatostimulant, hepatocarcinogenic, herbicide	Jisha et al. (2016)
4	6.02	3.71	3HCyclodeca[b]furan2one,4,9dihydroxy6methyl3,10dimethylene3a,4,7,8,9,10,11,11 Aoctahydro ($C_{15}H_{20}O_4$)	Ketone compound, herbicide, hirudicide, hormone	Jisha et al. (2016)
5	6.02	3.71	9Oximino2,7diethoxyfluorene ($C_{17}H_{17}NO_3$)	Aromatic hydrocarbon	Chaudhari and Mahajan (2015)
6	6.26	27.93	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl]cy ($C_{23}H_{32}O$)	Antimicrobials and anti-viral, found in plant extracts	Hussein et al. (2016); Dey et al. (2016)
7	6.26	27.93	9,12,15Octadecatrienoicacid, 2[(trimethylsilyl)oxy]1[[(Trimethyl silyl)oxy]methyl] ethyl ester, (Z,Z,Z) ($C_{27}H_{52}O_4Si_2$)	Flavonoids, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary activity	Rajendran et al. (2017)
8	6.57	6.87	.psi.,.psi.Carotene,1,1',2,2'tetrahydro1,1' Dimethoxy ($C_{42}H_{64}O_2$)	Repellant, antibacterial, antifungal, anti-inflammatory	Mebude and Adeniyi (2017)
11	7.67	17.97	Astaxanthin ($C_{40}H_{52}O_4$)	Keto-carotenoid, antioxidant activity	Cunningham and Gantt (2011); Ambati et al. (2014)
12	7.67	17.97	4Hexyl1(7methoxycarbonylheptyl)bicyclo [4.4.0]deca2,5,7triene ($C_{25}H_{40}O_2$)	Found in plant extracts	Karthikeyan et al. (2016)

3.3 Chemotactic effects of root exudates on rhizospheric microorganisms

The exudates secreted by plants act as a rich source of nutrients for the native microorganisms present in rhizosphere and they participate in early colonization, thereby inducing chemotactic responses of rhizospheric bacteria (Yuan et al. 2015; Sood 2003). The REs from different plant species could alter the physiological properties and type of soil microorganisms (Shi 2004). In this study, after 12 h of incubation, the microbial growth was found to be inclined towards the REs as compared to DW suggesting chemotactic response of *A. junii* Pb1, *Bacillus subtilis* and *Bacillus licheniformis*, respectively, towards REs and it was visualized by their growth on the petri-dishes (Fig. 7). The composition and concentration of sugars, phenolic acids and flavonoids present in REs from VZ act as an attractant for microorganisms. This result confirms the chemo-attractant property of REs from VZ and thus favours the colonization of microorganisms from the ecological niche. Lei et al. (2017) also reported the positive chemotactic response of Ginseng bacterial soft-rot towards ginseng REs. Bacilio-Jiménez et al. (2003) assessed the chemotactic response of endophytic bacteria towards REs from rice. The authors observed that rice exudates induced high chemotactic response for both *Corynebacterium flavescens* and *Bacillus pumilus*. Similarly, Yuan et al. (2015) reported that banana exudates induced chemotaxis and biofilm formation in plant growth promoting rhizobacteria strain *Bacillus amyloliquefaciens* NJN-6. Thus, evidently, the chemical compounds present in REs of VZ served as a chemoattractant for these bacterial cultures. The chemotaxis between REs and bacteria act as one of the several mode of interaction between bacteria and the plant. Anew, it was demonstrated that the root exudates play an important role for the survival of plants under abiotic stress and also in initiating and enticing PGPR colonization on the host roots. From a practical knowledge perspective, further research is required to analyse the components of REs and their contribution using sophisticated techniques such as UPLC-Q-TOF or NMR and ascertain their role in HM bioremediation by performing (bio)leaching experiments and pot soil culture experiments.

4. Conclusions

The results from photochemical tests show that VZ REs and REt contains various bioactive phyto-constituents such as carbohydrate, protein, phenolic, flavonoid and alkaloid. When treated with Pb(II), the presence of phyto-constituents in REs and REt improved the defence mechanism and enhanced heavy metal stress tolerance. The chemotactic response of rhizospheric bacterial strains towards VZ exudates demonstrated the crucial role in attracting and initiating colonization on the host roots. A dual role in the production of secondary metabolites with imperative biological properties appears to have good practical implications in the field of bioremediation.

Declarations

Ethics approval:

The article does not contain any studies performed on animals; Approval from "Institute Ethical Committee" not required.

Consent to participate:

Not Applicable

Consent for publication:

Not Applicable

Availability of data:

All the experimental data of the study is included in this article, some of it has been provided as supplementary files.

Competing interests:

The authors AK, NH, BSG, ER, RR declare no competing interests.

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Authors contribution:

AK, RR conceived and planned the study; AK, NH, RR performed the experiments; AK, RR, BSG, ER performed data interpretation; AK,RR,BSG,ER wrote and refined the manuscript

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Figures

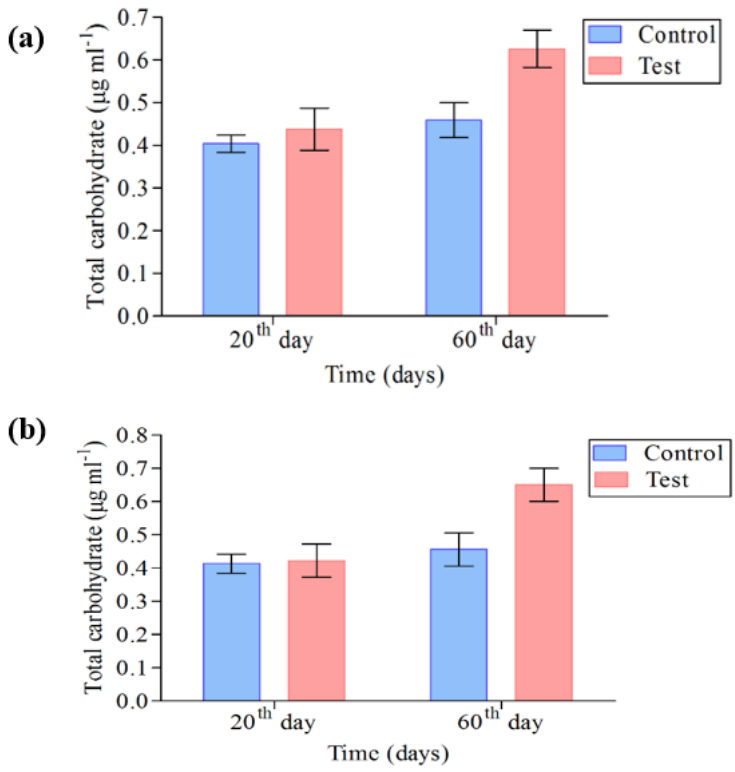

Figure 1

Total carbohydrate content: (a) root exudates, and (b) root extract

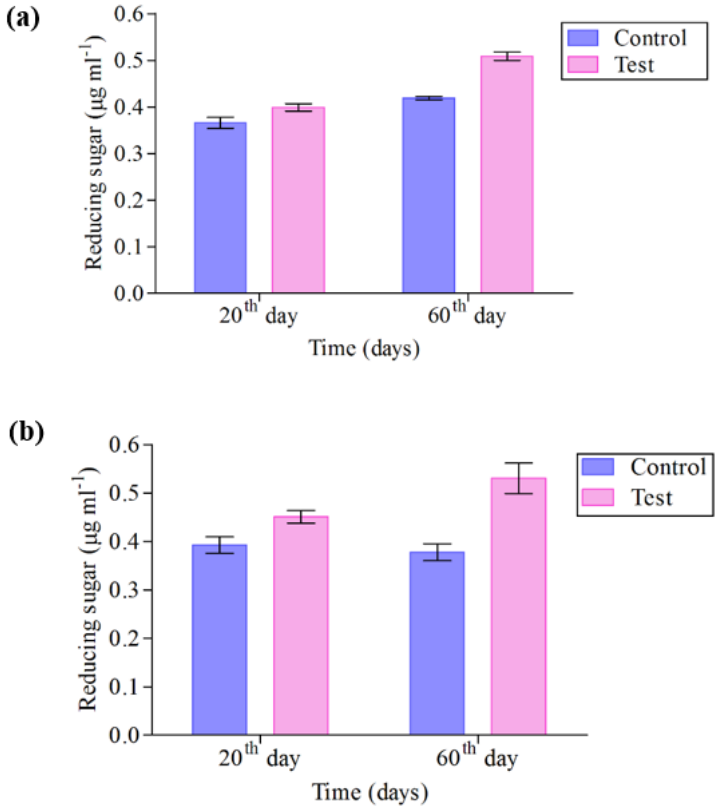

Figure 2

Reducing sugar content: (a) root exudates, and (b) root extract

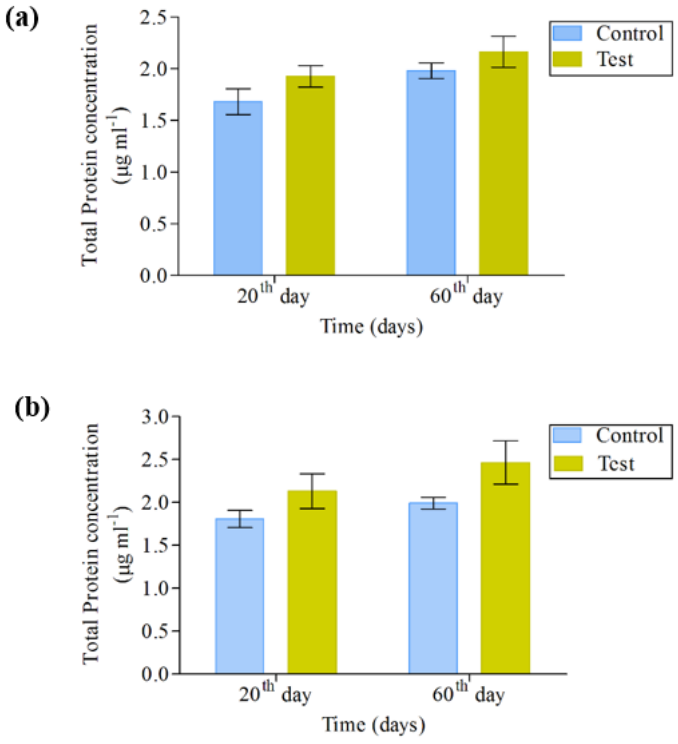

Figure 3

Total protein estimation: (a) root exudates, and (b) root extract

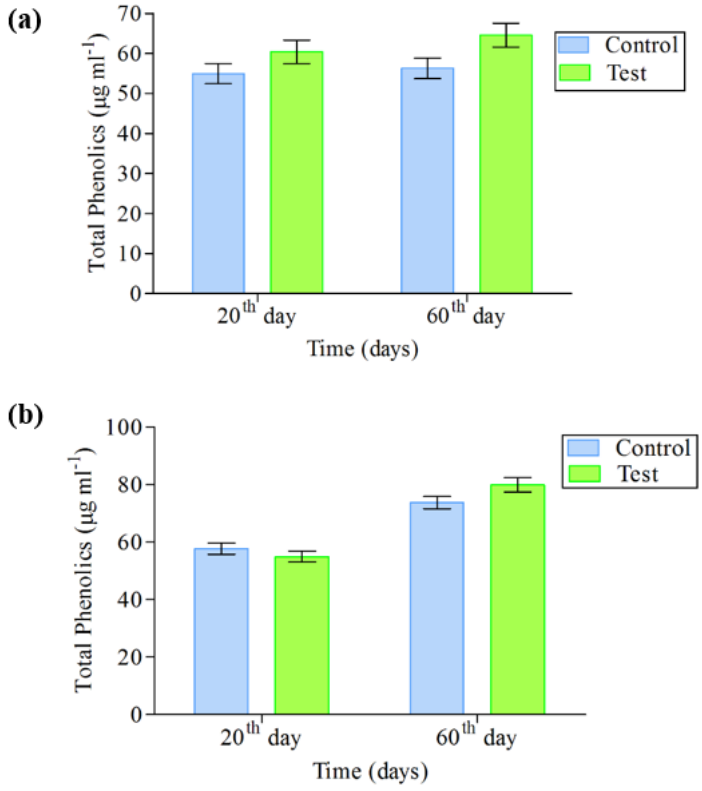

Figure 4

Total phenolic content: (a) root exudates, and (b) root extract

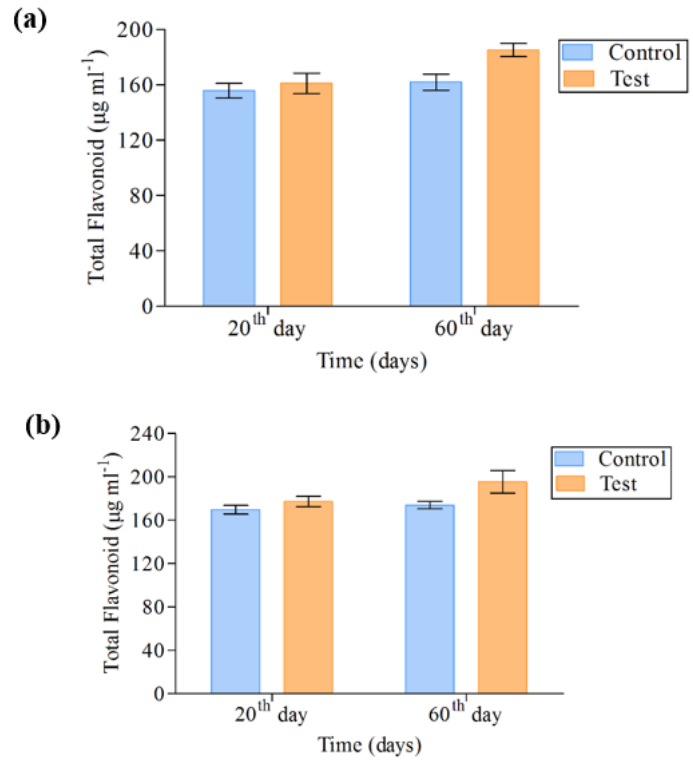

Figure 5

Total flavonoids content: (a) root exudates, and (b) root extract

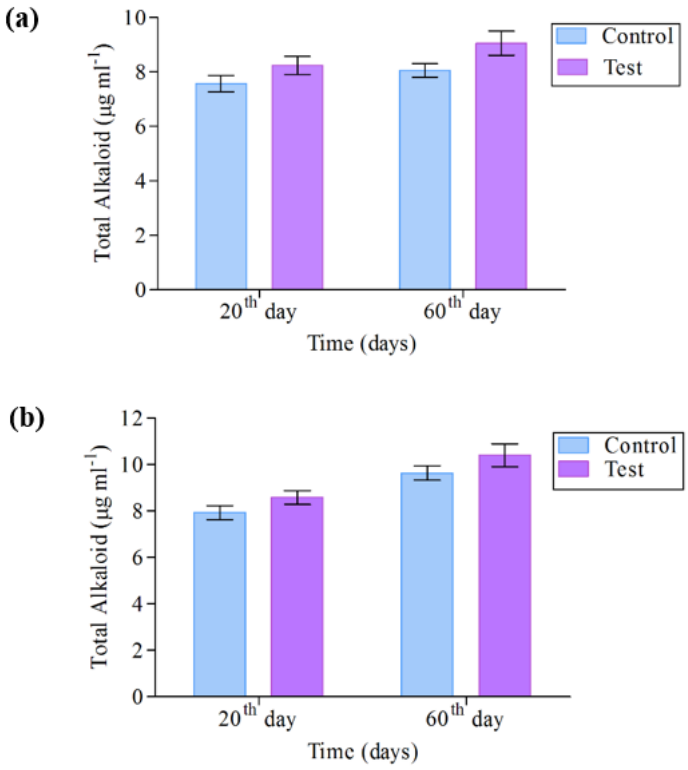

Figure 6

Total alkaloid content: (a) root exudates, and (b) root extract

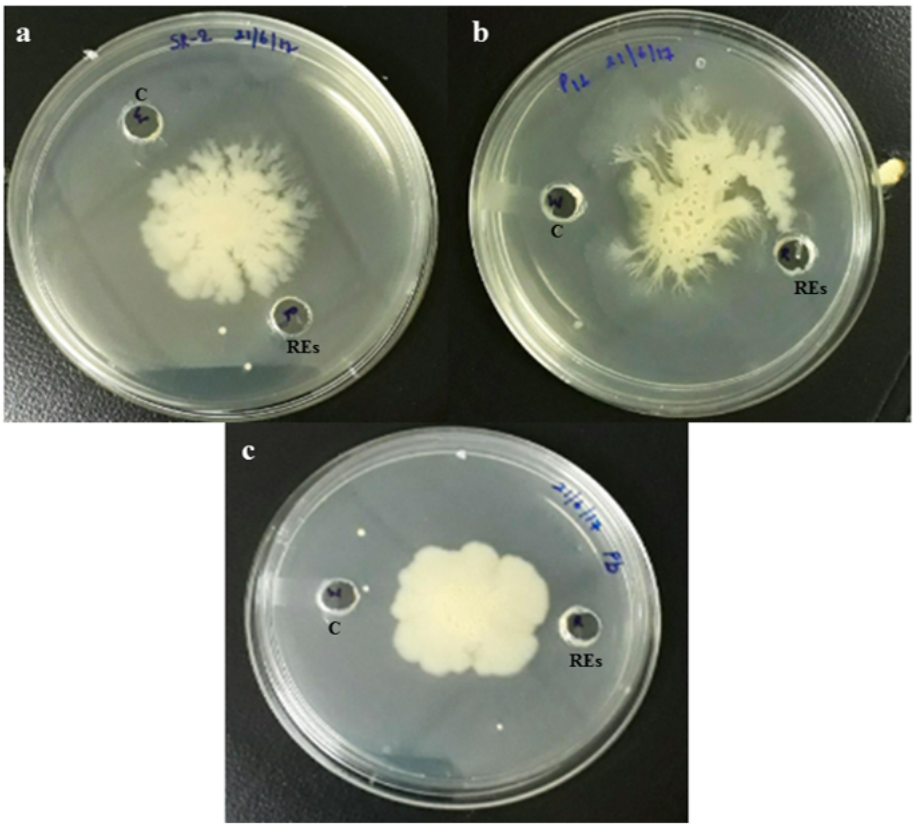

Figure 7

Chemotactic effect of REs on bacterial isolates: (a) Bacillus subtilis, (b) Bacillus licheniformis and (c) A. junii Pb1 (C: control and REs: root exudates)

Supplementary Files

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