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Characterization of induced metal responses of bacteria isolates from active non-sanitary landfill in Malaysia

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ABSTRACT

Microorganisms have specific genetic mechanisms towards their resistance to heavy metals, and may exhibit tolerance by immobilizing metal on cell surfaces or transforming them into less toxic forms. Unfortunately, selecting microbes that are metal-specific is a problem in remediation processes, and require identification of a process/strategy for evaluating metal interaction and bioreduction potential of microbial species before utilization for biodegradation/reduction in polluted soils. The study aimed to express the metal tolerance and interaction within the microbial abundance in an active non-sanitary landfill soil in Malaysia, as a developmental strategy for selecting bacterial species important for future remediation of metal-polluted soil. The characterized soil exposed the contamination level from heavy metals at the selected active non-sanitary landfill. Further assessment on the microbial community identified and typed the bacterial diversity in the contaminated area. Exposure to varying metal solutions showed the sensitivity of the bacteria species. Microbiological media infused with Pb, Al and Mn solutions demonstrated absolute heavy growth for all the six microbes studied at metal concentrations of 5–20 ppm. Comparison between the microbes indicates that *Burkholderia vietnaminesis* expressed higher metal resistance. In general all the isolated microbes demonstrated the ability to tolerate and resist metals at different concentrations. Bacterial isolates, mainly the gram-positives are metal-specific and may act as potential agent for remediation of heavy metal in contaminated sites.

1. Introduction

Whereas many sources of heavy metal contamination of soil abound, leachate pollution is one of such which is gradual but persistent, and harbours many environmental pollutants including heavy metals. Landfilling is one of the sources of heavy metals pollution due to leachate production (Agamuthu et al., 2014). The anthropogenic activities have led to their wide distribution in the environment and negatively impact human health in particular, and the ecosystem in general. The inevitable waste generation pattern, especially in the developing countries, often leads to the generation of high volume of leachate. Characterization of leachate, especially from municipal solid waste (MSW) landfills, has shown that it contains different groups of pollutants such as organics: alkenes, aromatic hydrocarbons, acids, esters, alcohols, hydroxybenzene, amides, and others, as well as ammonia nitrogen and high load of heavy metals (Emenike et al., 2013; Fauziah et al., 2013; Kjeldsen et al., 2002). Without proper collection system, raw leachate from landfills will laterally seep into soil compartments to cause soil contamination (Emenike et al., 2016). Areas near landfills have a greater possibility of groundwater contamination because of the leachate originating from the nearby site. Such contamination of groundwater resource poses a substantial risk to local resource users and to the natural environment. Heavy metal poisonousness depends on several conditions which includes level of pollutants, route of exposure, and chemical species. Health risks of leachate contamination due to groundwater contamination includes skin irritation, nausea, vomiting, and headache, while chronic exposure can lead to anemia, kidney damage, prostate cancer, lung cancer, memory loss, coma, headaches and depression. Arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. They are also classified as human carcinogens (known or probable) according to

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the U.S. Environmental Protection Agency (USEPA), and the International Agency for Research on Cancer. Therefore, the inevitable task faced by the society is identifying ways to prevent metal pollution in order to conserve the environment. Similarly, a more significant interest is to recover already polluted sites for the associated socio-economic benefits. Usually, the dynamic shift towards global sustainability is steering remedial or recovery activities towards the use of microbes. Hence, the use of microorganisms for cost-effective restoration of environment cannot be over emphasized. Microbes play an important role in the environmental fate of toxic metals (Polciniczak et al., 2013; Babu et al., 2013) by range of mechanisms for conversion between soluble and insoluble forms. Since heavy metals are increasingly found in microbial habitats due to natural and environmental processes, microbes have evolved several mechanisms to tolerate the presence of heavy metals (Lucious et al., 2013; Tiwary and Dubey, 2016). Microbes are capable of removing, concentrating and recovering metals through bioaccumulation or adsorption. Bacterial species found in leachate impacted environment or bioreactor influence degradation and removal of pollutants (Zhang et al., 2016), however, most studies on microbial interaction with leachate are limited to organic matters, xenobiotic organic chemicals, ammonia and few others (Gojski et al., 2012; Brkanac et al., 2014; Tigini et al., 2014) with less on heavy metals. The accumulation of heavy metals by microbes basically depends on the concentration and availability of heavy metals and is a complex process which is controlled by multiple factors, such as type of metal, the nature of the medium and microbial species. This research evaluated the difference in microbial species resident in non-sanitary landfill soil in relation to the heavy metals tolerance of the bacterial species. Bioremediation of metal pollution have received significant improvement with the use of biosorbents, especially when dead and living microorganisms are used (Wang and Chen, 2006; Gupta et al., 2010), but adsorption is often limited to metal ions from solution (Fosso-Kankeu et al., 2014) and there is need to explore microbial community for more bacterial species with the ability to concentrate metal ions. Therefore, the study aimed to express the metal tolerance and interaction within the microbial abundance in an active non-sanitary landfill soil of Peninsular Malaysia, as a developmental strategy for selecting bacterial species important for the future remediation of metal-polluted soil. Hence, its discrete objectives included diversity identification for polluted soil of active landfill and growth response to metal exposure.

2. Materials and method

2.1. Soil and leachate samplings and characterizations

Bukit Beruntung landfill site was selected for this study based on status and grade. Hence, soil samples were excavated at 30 cm depth from Bukit Beruntung landfill (BBL) (3° 32.14′N; 101° 25.80′E) in accordance to 2014 ASTME − 1197 standard guidelines for conducting terrestrial soil-core microcosm test (Emenike et al., 2012). The samples collected were analyzed for pH using multiprobe meter (YSI Professional Plus, USA), while the elemental concentrations of metals in the soil were evaluated based on the USEPA 3050B and USEPA 3052B protocol. Similarly, the raw leachate samples were collected from the environment and analyzed for similar parameters as with the soil samples. All assessments were duly replicated.

2.2. Bacteria isolation and identification

Bacteria species were isolated by mixing 1 g of soil sample with 10 ml of normal saline water (0.9% NaCl) as stock. The mixture was shaken vigorously (2 h at 180 rpm) using Lab-line 3521 orbit shaker and subjected to 20 times serial dilution. Dilutions (0.1 ml) were dispensed on freshly prepared nutrient agar under aseptic condition (Emenike et al., 2016). The plates (replicated) were incubated at 37 °C for 24 h. Developed colonies were further subcultured to ensure purity before identification. Subsequently, Biolog GEN III Microplate protocol was used to test the isolated microbes according to Bochner (1989a), (1989b). This involved the use of specific inoculation fluid (IF-A catalog no. 72401) to prepare suspension of the target cells. A multi-channel automated pipette was used to dispense 100 μL of the suspension prepared from inoculation fluid into each of the wells in a microplate (Catalog no.1030). The wells contain 71 carbon source utilization assays (columns 1–9) and 23 chemical sensitivity assays (columns 10–12), hence the isolates were identified at the species levels based on the "phenotypic fingerprint" of the microorganisms provided by the test panel. OmniLog reader was used to identify the bacteria species contained in the microbial identification systems software.

2.3. Heavy metal resistivity test

Bacteria isolated from the method discussed above were aseptically re-grown by inoculating each into discrete test tubes containing 5 ml of nutrient broth each at 37 °C for 18–24 h. Each inoculum was then transferred to the test tubes containing 4.5 ml of normal saline for standardization to obtain 0.1 ABS (absorbance)/0.5 McFarland at 860 nm. Final inocula required for the heavy metal sensitivity assessment were obtained by dispensing 0.1 ml of the resultant standard into corresponding test tubes containing 9.9 ml of normal saline for each test organism; hence approximate cell density of 5×10^5 CFU/ml was achieved. Therefore, the metal tolerance for each bacterial isolate was determined by agar-well diffusion method. The standard suspension of each organism (5×10^5 CFU/ml) was used to seed each sterile plate that contains 20 ml of nutrient agar. Pre-diffusion was allowed before cork borer was used to make 6 mm diameter well (Aweng et al., 2011) on the seeded plates. Four concentrations (5, 10, 15 and 20 ppm) of each metal were prepared. Table 1 shows list of metals used in this study. 70 μl of each concentration of the metals was dispensed into corresponding wells. Hence, the each plate accommodated four concentrations of designated heavy metal, and was allowed to stand 1 h for pre-diffusion. Plates were then incubated at 37 °C for 24 h. The minimum inhibitory concentrations (MIC) for the organisms were determined which is the lowest concentration at which no visible growth was observed. Diameters of the corresponding clear zones that characterized the concentrations of the heavy metals that showed no visible growth were measured to determine the inhibition zone diameter (IZD) (Jayanthi et al., 2016).

Table 1
Sources of utilized metal ions.

No	Heavy metals	Salts	Product
1	Lead (Pb)	$PbCl_2$	Merck
2	Manganese (Mn)	$MnSO_4$	Friendemann Schmidt
3	Iron (Fe)	$FeSO_4.7H_2O$	HumbG Chemicals
4	Mercury (Hg)	$HgSO_4$	Bendosen
5	Zinc (Zn)	$ZnSO_4.7H_2O$	AnalaR
6	Copper (Cu)	$CuSO_4$	Bendosen
7	Cadmium (Cd)	$CdCl_2$	Friendemann Schmidt
8	Nickel (Ni)	$NiCl_2.6H_2O$	Bendosen
9	Chromium (Cr)	$K_2Cr_2O_7$	HumbG Chemicals
10	Aluminium (Al)	$Al_2(SO_4).16H_2O$	Systerm

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3. Result and discussion

Bukit Beruntung landfill is a non-sanitary landfill without any proper collection system with Table 2 indicating the presence of heavy metals in the leachate. Comparison with local standards from Department of Environment, Malaysia under Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009 and International Standards, showed that almost all the metals content in Bukit Beruntung landfill leachate exceeded prescribed limits (Department of Environment (DOE), 2009; Aweng et al., 2011). Bukit Beruntung is an active landfill therefore waste is constantly deposited, and this may contribute to leaching of high load of heavy metals. Zinc (236 ± 11.8 mg/L) was found to be highest in the landfill leachate compared to other metals; Iron (60 ± 18.2 mg/L) and Chromium (17.3 ± 1.9 mg/L). Abdul Aziz et al. (2004) also reported higher concentration of Fe in the study conducted in non-sanitary landfill. Besides, another study by Sumaiya et al. (2014) also reported high Fe content in the leachate produced at Sultanate of Oman landfill (39.85 mg/L). The steel material dumped at the site may be the cause for the high concentration of Fe. The acceptable standard value for Zn is only 2.0 mg/L, however the leachate in Bukit Beruntung landfill showed much higher Fe concentration above allowable standard (5.0 mg/L). According to Emenike et al. (2013), the level of soluble metals tends to be higher in active landfills and supported by Yusof et al. (2009) and Lagerkvist (2003). Similarly the landfill soil was also analyzed for heavy metal concentration to identify the degree of contamination and it has been reported that Bukit Beruntung landfill contains higher concentration of heavy metals (Table 3). The concentration of heavy metal in the Bukit Beruntung landfill soil follow the order; Al (12,261 mg/L) > Fe (5367 mg/L) > Mn (174.8 mg/L > Zn (602 mg/L) > Cu (392 mg/L) > Pb (90.4 mg/L) > Cr (17.4 mg/L) > Ni (14.01 mg/L) > Cd (3.36 mg/L) > As (0.38 mg/L). Contamination from heavy metals in our environment is a major concern because of the toxicity and threat to human and the environment. The distribution of metal among specific forms differs widely based on the metal's chemical properties and soil characteristics (Soon and Bates, 1981; Olajire and Ayodele, 1998). Both leachate and soil from Bukit Beruntung recorded pH values between 7.09 ± 0.63 and 7.09–7.20 respectively. According to Kanmani and Gandhimathi (2013), there will be low metal solubility when the pH increases due to precipitation of metal ions as in soluble hydroxide at high pH value.

Table 3
Components of contaminated soil from Bukit Beruntung landfill.

Test Parameters	Methods	Range of values (mg/kg)
pH	Probe insertion	7.09–7.20
Total N	ASTM E778-87	0.46–0.49
Total K	ASTM E926-94	55.2–980
Total P	ASTM D5198-92	10.5–2170
As	USEPA 3050 B	0.1–0.38
Ca	USEPA 3050 B	63.4–9281
Fe	USEPA 3050 B	43.2–5367
Mn	USEPA 3050 B	2.42–928
Mg	USEPA 3050 B	35.2–723.8
Na	USEPA 3050 B	100.7–315
Cu	USEPA 3050 B	1.88–392
Zn	USEPA 3050 B	3.57–602
Pb	USEPA 3050 B	0.49–90.4
Cd	USEPA 3050 B	0.19–3.36
Hg	USEPA 3052	<0.002
Cr	USEPA 3050 B	0.57–17.4
Ni	USEPA 3050 B	0.67–14.01
Al	USEPA 3050 B	0.47–12,261

Range value (n = 5) *All parameters are in mg/Kg except pH and Total N (%).

Microbial isolation from Bukit Beruntung landfill soil indicated a significant bacterial distribution in the leachate contaminated soil. Six species of bacteria were isolated as shown in Table 4. *Burkholderia vietnamiensis*, *Rhodococcus rubber* and *Bacillus aryabhattai* are identified as gram positive microbes whereas *Cloacibacterium* sp. *Acidovorax ebreus* and *Brevundimonas diminuta* are gram negative bacteria. Basically, it is assumed that the morphology or some other characteristics of bacteria tend to influence the activities (Table 5).

Heavy metal resistance test as presented in Table 5 indicated that the exposure of microbes to the heavy metals at different concentrations showed that the overall growth of the bacteria species was significantly affected in a negative dimension (when compared with control experiment maintained at 0.0 ppm) as the metal concentrations increases. Hence it can be deduced that increased concentration reduces microbial growth. At 5 ppm of each metals solution except for Cu, strong positive growth was observed for all the six microbes except for *B. aryabhattai*. It may indicate strong metal-resistance potentials of the isolates because such concentration

Table 2
Characteristics of leachate from Bukit Beruntung landfill.

Test Parameters	Method	Range values (mg/L)	Standard (Environmental Quality Regulations, 2009; Malaysia)
pH	Probe insertion	7.09 ± 0.63*	6.0–9.0
BOD	APHA 5210 B	259 ± 37	20
COD	APHA 5220	985 ± 185	400
Total N	ASTM E778-87	0.32 ± 0.05*	5
Total K	ASTM E926-94	40.4 ± 6.04	N.A
Total P	ASTM D5198-92	24.3 ± 0.7	N.A
As	USEPA 3050 B	0.21	0.05
Ca	USEPA 3050 B	91.2 ± 11.6	N.A
Fe	USEPA 3050 B	60 ± 18.2	5.0
Mn	USEPA 3050 B	5.1 ± 0.5	0.2
Mg	USEPA 3050 B	96.6 ± 16	N.A
Na	USEPA 3050 B	242.1 ± 22.8	N.A
Cu	USEPA 3050 B	2.62 ± 0.8	0.2
Zn	USEPA 3050 B	236 ± 11.8	2.0
Pb	USEPA 3050 B	1.12 ± 0.04	0.10
Cd	USEPA 3050 B	0.4 ± 0.1	0.01
Hg	USEPA 3052	0.04	0.005
Cr	USEPA 3050 B	17.3 ± 1.9	0.20
Ni	USEPA 3050 B	12 ± 4.4	0.20
Al	USEPA 3050 B	13.1 ± 3.2	N.A

(Mean values n = 3) *All parameters are in mg/L except pH and Total N (%).

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Table 4
Microbes isolated from Bukit Beruntung landfill.

Gram Positive Bacteria	Gram Negative Bacteria
Burkholderia vietnamiensis	*Cloacibacterium* sp.
Rhodococcus rubber	*Acidovorax ebreus*
Bacillus aryabhattai	*Brevundimonas diminuta*

was enough to induce growth inhibition on bacterial species in the study by Amor et al. (2001), though species type and presence of co-contaminant may influence the toxic effect. Exposure to Pb, Al, and Mn (5–20 ppm) shows an absolute heavy growth with no inhibition pattern across all the six microbes from Bukit Beruntung landfill. For Fe, *B. diminuta* only showed inhibited growth pattern at the highest tested concentration (20 ppm), while all the other five microbes from the same landfill recorded absolute positive growth. The microbes exposed to Zn, revealed an inhibition for *B. aryabhattai* (at above 10 ppm) and *B. diminuta* (at above 5 ppm). No growth or a complete inhibition was observed for *B. aryabhattai* when exposed to Cu, and *B. diminuta* shows inhibition at 5 ppm while all the other microbes were resistant at 20 ppm and above. Exposure to Cd at different ppm (Aziz et al., 2004; Babu et al., 2013; Basu et al., 1997; Bochner, 1989a, 1989b; Brkanac et al., 2014; Department of Environment (DOE), 2009; Emenike et al., 2016, 2013, 2012; Fauziah et al., 2013; Fosso-Kankeu et al., 2014; Gabr et al., 2008; Gojski et al., 2012; Gupta et al., 2010; Jayanthi et al., 2016) shows that *B. vietnamiensis* has higher resistance above 20 ppm and *A. ebreus* at 10 ppm while all other isolates show inhibition at lower metal concentration (5 ppm). For Ni, *B. vietnamiensis*, *R. rubber* and *A. ebreus* showed absolute strong growth at 20 ppm of the tested metals while other three isolates demonstrated inhibition. Lastly, exposure to Hg shows heavy growth for all the microbes except for *B. diminuta*. All the six isolates exhibited variance in their growth and resistance towards different types of metals and showed significant

Table 5
Heavy metals resistance test.

Metal Conc (ppm)	*Burkholderia vietnamiensis*	*Rhodococcus rubber*	*Bacillus aryabhattai*	*Cloacibacterium* sp.	*Acidovorax ebreus*	*Brevundimonas diminuta*
Pb						
5	++	++	++	++	++	++
10	++	++	++	++	++	++
15	++	++	++	++	++	++
20	++	++	++	++	++	++
Fe						
5	++	++	++	++	++	++
10	++	++	++	++	++	++
15	++	++	++	++	++	++
20	++	++	++	++	++	+-
Zn						
5	++	++	++	++	++	+-
10	++	++	+-	++	++	+-
15	++	++	+-	++	++	+-
20	++	++	+-	++	++	+-
Cu						
5	++	++	–	++	++	+-
10	++	++	–	++	++	+-
15	++	++	–	++	++	+-
20	++	++	–	+-	++	+-
Cd						
5	++	+-	+-	+-	++	+-
10	++	+-	+-	+-	+-	+-
15	++	+-	+-	+-	+-	+-
20	++	+-	+-	+-	+-	+-
Ni						
5	++	++	++	+-	++	++
10	++	++	++	+-	++	+-
15	++	++	+-	+-	++	+-
20	++	++	+-	+-	++	+-
Cr						
5	++	++	++	+-	++	++
10	++	+-	+-	+-	++	+-
15	++	+-	+-	+-	+-	+-
20	+-	+-	+-	+-	+-	+-
Mn						
5	++	++	++	++	++	++
10	++	++	++	++	++	++
15	++	++	++	++	++	++
20	++	++	++	++	++	++
Hg						
5	++	++	++	++	++	+-
10	++	++	++	++	++	+-
15	++	++	++	++	++	+-
20	++	++	++	++	++	+-
Al						
5	++	++	++	++	++	++
10	++	++	++	++	++	++
15	++	++	++	++	++	++
20	++	++	++	++	++	++

–: no growth; +-: mild growth with some inhibition; ++ absolute growth.

difference at p > 0.05. Overall comparison between the isolates showed that *B. vietnamiensis* exhibited highest metal resistance towards all types of metals tested in this study except for Cr at 20 ppm. The nature of the microbes as gram positive and strictly aerobic influenced the interaction that existed when exposed to heavy metals. According to Mergeay et al., 2009, *B. vietnamiensis* have plasmid pMOL 30 which contains two large putative genomic islands compromising most of the gene involved in the response or resistance to heavy metals. Order of bacteria resistance is *B. vietnamiensis* > *A. ebreus* > *R. rubber* > *Cloacibacterium* sp. > *B. aryabhattai* > *B. diminuta*. The overall growth pattern was similar to study by Mgbemena et al. (2012), and can indicate that extreme exposure to metal concentrations will negatively affect microbial resistance to pollution. In general such information could serve as a comparative data to other tested species (Lucious et al., 2013). The maximum response similarity for the species was observed when all were exposed to Pb, Mn, Al (>20 ppm). However, *B. vietnamiensis* demonstrated the highest resistance across the species though the tolerance from *R. rubber and A. ebreus* also showed very strong resistance as well. Study by Maria et al. (2014) using *Rhodococcus* sp. showed that the microorganism also increased resistance to different heavy metals such as Cd and Cr, suggesting that *Rhodococcus* sp. may be useful for the bioremediation of sites contaminated with high concentrations of the metals (Van and Dijkhuizen, 2004). Changes in response may be due to some response peculiarity to selected metals rather than nature (morphology) in terms of being gram positive or gram negative organism. Yet the result concurs with Lucious et al. (2013) where it was established that both class of bacteria species exhibited metal resistance.

Minimum inhibitory concentration (MIC) was evaluated to determine the lowest concentration of heavy metal that will inhibit the growth of microorganisms. This is to compare the inhibitory zone between the microorganisms. Table 6 exhibit the MIC of various heavy metals towards the microbes isolated. The metal concentration tested in this study is between 5 and 20 ppm. The metal concentration is set to these concentrations because such is typical range in the most of contaminated sites and considered to be above the prescribed level. *B. vietnamiensis* revealed highest tolerance among all the microbes followed by *A. ebreus* and *R. rubber*. According to Ahmed et al. (2005) bacteria exposed to high levels of heavy metal in the environment have adapted to metal stress and develops various resistance mechanisms. Both *B. vietnamiensis* and *R. rubber* are gram positive bacteria which is a property that may contribute to higher tolerance towards metals. Basu et al. (1997) also reported higher metal resistance by gram positive bacteria. These resistance mechanisms also could be utilized for detoxification and removal of heavy metals in polluted environment.

Inhibition zone diameter (IZD) was measured against the heavy metal for all the isolates from Bukit Beruntung landfill as reflected in Figs. 1–6. IZD measurement shows that the size of the zone of inhibition denotes the degree of sensitivity of bacteria to a particular metal concentration. The highest inhibition zone diameter was 4.5 cm from *B. aryabhattai* and *A. ebreus* for 10, 15 and 20 ppm of Cu and Cd respectively. Among the six isolates studied, three of the isolated bacteria showed highest IZD on exposure to Cr concentrations. *R. rubber* (3.6 cm), *Cloacibacterium* sp. (3.4 cm) and *B. vietnamiensis* (1.1 cm) were reported to have highest IZD value for Cr among the different metals tested. However the IZD is observed to be different between the microbes. For *B. diminuta* highest IZD value is from Cd at 2.8 cm. It seems to be logical that the size of inhibition zone, increase as the concentration of metal increases. Similar trend is observed in this study. Based on the results most of the low or zero value for IZD shows that the isolates were highly resistant to the metal concentrations. Therefore the potential isolates can be highly recommended for bioremediation of heavy metal contaminated sites. According to Ruban and Gunaseelan (2011), the tolerance of isolates towards heavy metal and inhibition may depend on the binding ability to the metal, precipitation ability of the isolate and the ionic interaction or complex formation.

Microbial metal response in the study varied in a pattern that may imply the presence of specific characteristics of bacterial species that influence metal binding, biosorption or bioimmobilization of metal ions in aggregate state. The calculated variance (F) significantly supports the differences in the behavior of the studied bacteria species in metal-induced environment. Low F-value found on *B. vietnamiensis* (4.42) is an indication of low variability in resistance pattern to the different metal solutions utilized in the study. The variance (F) across the bacteria species reflected in the order of resistance; *B. vietnaminesis* [F ($p < 0.05$) = 4.42] > *A. ebreus* [F ($p < 0.05$) = 27.22] > *R. rubber* [F ($p < 0.05$) = 48.94] > *Cloacibacterium* sp. [F ($p < 0.05$) = 82.31] > *B. aryabhattai* [F ($p < 0.05$) = 94.95] > *B. diminuta* [F ($p < 0.05$) = 123.89]. Furthermore, a correlation exists between metal resistance of the bacterial species and the measured IZD. Observed IZD was inversely proportional to resistivity. Even *B. aryabhattai* showed pronounced correlation from a plot, $y = -0.2424x + 3.8243$ with an R^2 value of 0.94, despite showing an absolute susceptibility to Cu exposure. Hence, the distribution nullified the hypothesis that all the bacterial species will exhibit the same response to metal contamination. Differences in metabolic rates and associated genetic alterations within each microbial species may cause varied responses. Yet, the overall distribution revealed that all the bacterial species were resistant to the metal exposure. This result is in concord with Lin et al. (2007) and Amor et al. (2001), who demonstrated that microbes exhibited various growth and degradation rates reductions in the presence of different types and concentrations of heavy metals. However, all the microbes from the present study demonstrated high resistance. Differences in response to inhibition may be a reflection of variation in metal uptake mechanism of the individual isolates. This is supported by Fosso-Kankeu et al. (2014) that pointed out that accumulation of some metals in the cells could

Table 6
Minimum inhibitory concentrations of heavy metals on the isolates.

Metal	*Burkholderia vietnamiensis*	*Rhodococcus rubber*	*Bacillus aryabhattai*	*Cloacibacterium* sp.	*Acidovorax ebreus*	*Brevundimonas diminuta*
Pb	>20	>20	>20	>20	>20	>20
Fe	>20	>20	>20	>20	>20	20
Zn	>20	>20	10	>20	>20	5
Cu	>20	>20	<5	20	>20	5
Cd	>20	5	5	5	10	5
Ni	>20	>20	15	5	>20	10
Cr	20	10	10	5	15	10
Mn	>20	>20	>20	>20	>20	>20
Hg	>20	>20	>20	>20	>20	5
Al	>20	>20	>20	>20	>20	>20

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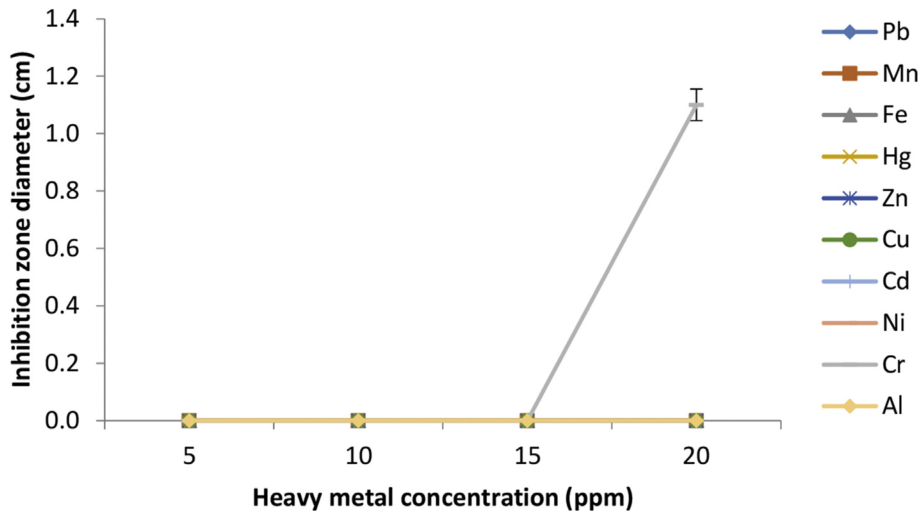

Fig. 1. Inhibition zone diameter (IZD) of *Burkholderia vietnamiensis* exposed to metals.

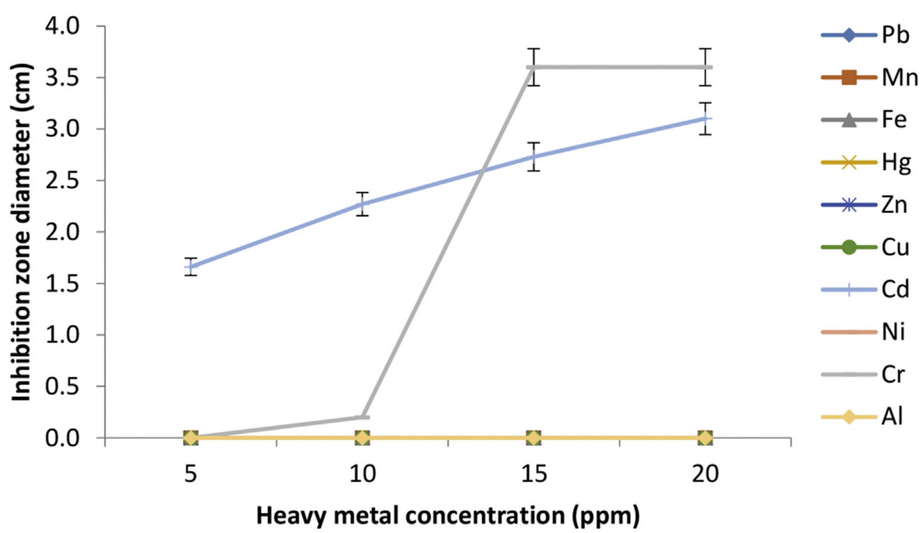

Fig. 2. Inhibition zone diameter (IZD) of *Rhodococcus rubber* exposed to metals.

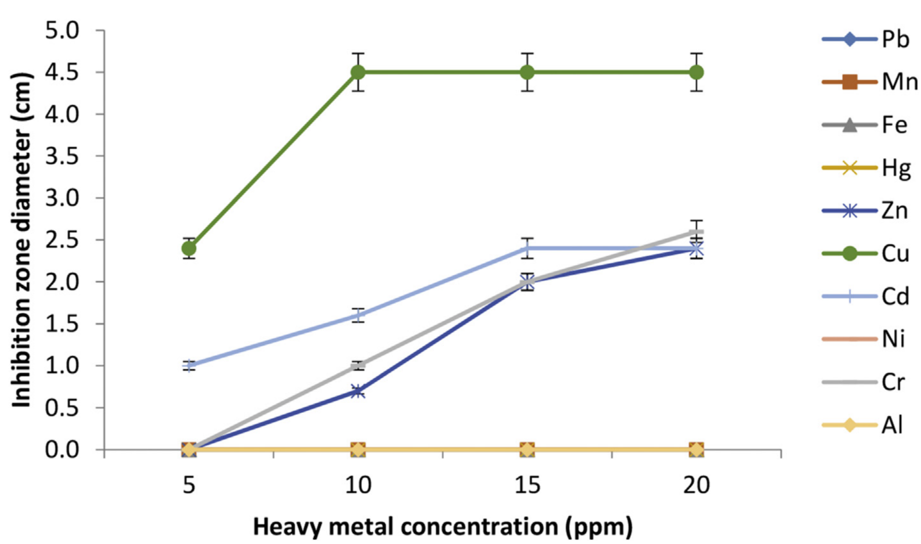

Fig. 3. Inhibition zone diameter (IZD) of *Bacillus aryabhattai* exposed to metals.

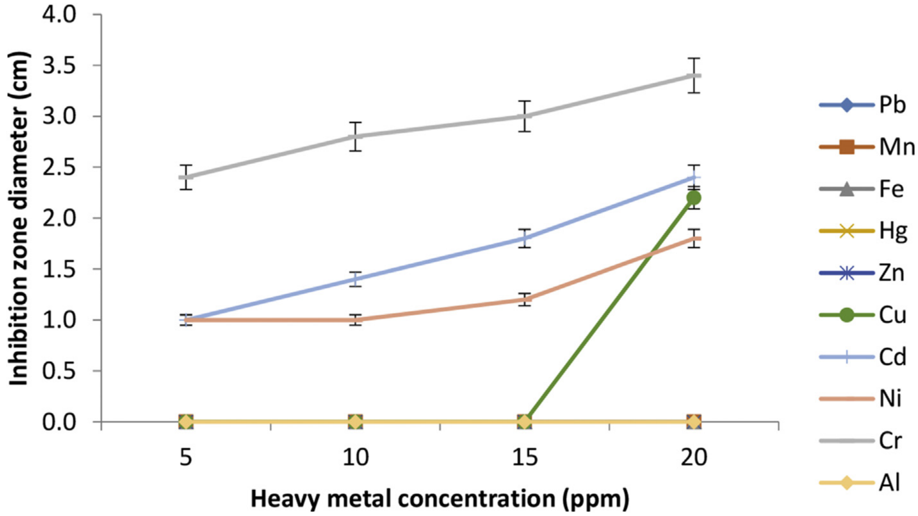

Fig. 4. Inhibition zone diameter (IZD) of *Cloacibacterium* sp exposed to metals.

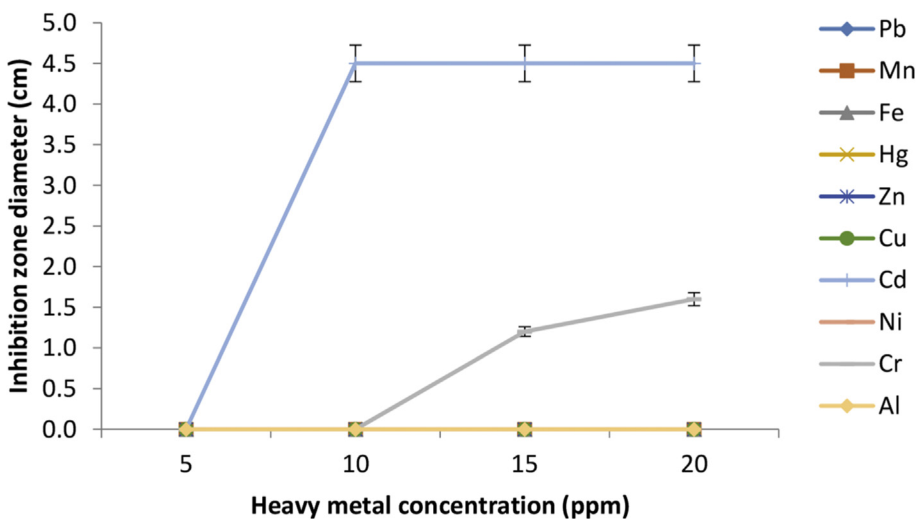

Fig. 5. Inhibition zone diameter (IZD) of *Acidovorax ebreus* exposed to metals.

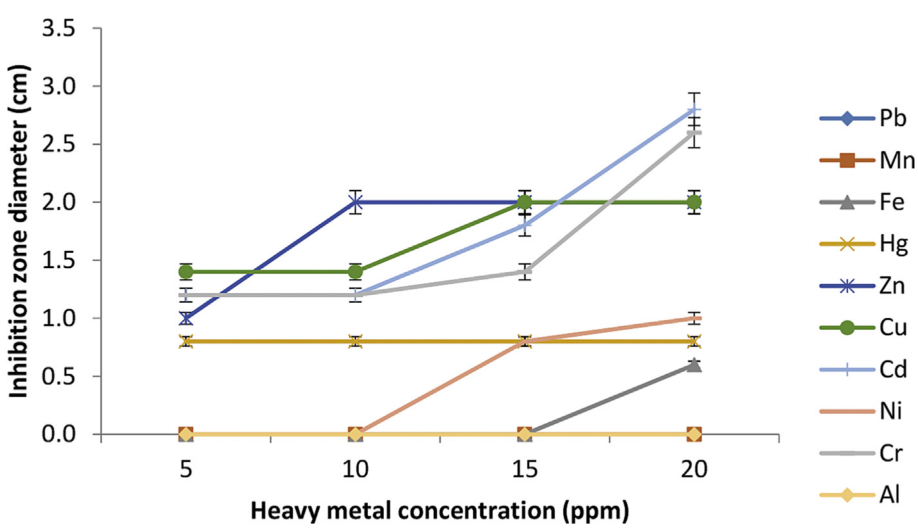

Fig. 6. Inhibition zone diameter (IZD) of *Brevundimonas diminuta* exposed to metals.

be facilitated through the existence of a pathway with specific protein transporters in the cell membrane. Hence, the interaction between bacterial species and the metal-induced media may help to predict the overall behavourial pattern of co-habiting microbes in metal polluted system because there could be a change in community structure due to the ions present.

B. vietnamiensis may be metabolizing significantly in the presence of metals just as it is common to discover that substrate-degraders easily adapt to the discrete substrate environment and become dominant microbe. These microbes could adopt metabolically mediated or physicochemical pathways of uptake when introduced into a contaminated system. Resistivity may indicate inherent potentials to bind and concentrate heavy metals even from very dilute aqueous solution by the non-living microbial biomass (Joo et al., 2010). The functional groups present in the microbial cell, especially carboxylic, hydroxyl and amino groups, enhance biosorption of heavy metals. Joo et al. (2010) further confirmed this after the band characterization (not done in the present study) through Fourier transform infrared (FTIR) spectroscopy analysis showed presence of C═O groups at the range of 1650–1660 cm^{-1}, indicating interaction between Zn(II) and C═O groups at the surface of the biomass. Associated broadenings depicted the involvement of H– bonding (Sar et al., 1999). Furthermore, the metal–microbe interaction could occur through passive mechanism of metal-to-cell surface physical/chemical binding (usually electrostatic attraction, cellular affinity, and ion exchange), or by squarely accumulating the metals in the cell. However, considering the short exposure time of the experiment (agar-well diffusion), it is possible that passive mechanism took place since it is a quick process (Ye et al., 2013).

The study views microbes as essential requirement in any bioprocess for the removal/reduction of heavy metals in polluted soil environment. Hence, knowledge derived from the assessment of the microbial interaction is important to biologists and engineers in order to execute effective bioremediation programme on metal-impacted soil. Understanding behavioural response to metal induced media (water and soil) will provide adequate technical information pivotal to implementation of a bioremediation technique. The isolates used in the study have little or no known prior evidence of involvement in bioremediation especially heavy metals situation. Therefore, once proven to be resistant to metal pollution, such bacteria species can be of significant benefit in tropical climes of Southeast Asia and Africa, and other developing countries with similar climatic condition where metal pollution from landfill/waste dumps is prevalent. The bacteria species are tolerant to tropical conditions, hence the isolation from landfill in Peninsular Malaysia. Such development will encourage cost-effective execution of bioremediation exercise since resident/indigenous microbes can be manipulated. This development will minimize the impact of alien microbes common to most bioaugmentation processes. In some cases, use of alien microbes caused displacement of species, change in population structure, possible loss of certain functions, production of toxic metabolites which might lead to disturbance of key ecological processes (Viebahn et al., 2009). However, when the known degraders or metabolizing microbe is isolated from source similar to the target site for bioremediation, its use in bioaugmentation is often positive (Pimmata et al., 2013).

4. Conclusion

Soil contaminated with leachate from landfill is a significant source of heavy metal contamination. However, the leachate polluted soil allows for the co-existence of diverse genera of bacteria species. It implied that bacteria species, especially those with gram-positive morphology are culturable under stress induced by metal pollution; hence a niche for microbial abundance is sustained. Growth of the bacteria species in the study indicate that the isolates are potential chemolithotrophs, hence the induced metal condition was partly source of energy needed for metabolic activities. Isolated microbes exhibit metal tolerance but differ across species. About 85% susceptibility to chromium ion recorded in the study prioritized the metal in the toxicity order above more established toxic ions of lead and mercury. An overall assessment of metal toxicity response from the study concludes that *B. aryabhattai* is highly (100%) susceptible to the presence of copper ions, and could indicate a specific cell membrane modification from other gram-positive bacteria species in the study. The study further deduced from the IZD assessment that increase in dimension is a reflection of reduced bacteria tolerance. Resistance shown by *B. vietnamiensis* and *R. rubber* is a significant property that may influence the potential of such bacterial species to assist in remediation of heavy metal polluted soil. Bacteria resistance to heavy metals is a vital factor to be considered in the development of any plan for remediation because it is directly related to the survival and growth of the bacteria species being used in the recovery of contaminated site.

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