Catena 147 (2016) 25–31

Contents lists available at ScienceDirect

Catena

journal homepage: www.elsevier.com/locate/catena

Selected microbial diversity of contaminated landfill soil of Peninsular Malaysia and the behavior towards heavy metal exposure

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ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form 19 May 2016
Accepted 23 June 2016
Available online 30 June 2016

Keywords:
Bioremediation
Heavy metals
Landfills
Leachate
Metals resistance

ABSTRACT

Microbes function normally and effectively when the site of action or immediate environment is intact and unpolluted. Given that microbes can significantly improve the bio-geochemical cycling of toxic heavy metals or the remediation of metal-contaminated environments, understanding the effect of microbe diversity on heavy metal pollution caused by leachate seepage is imperative. This study focused on the difference in the distribution of microbial species in non-sanitary landfill soil of Peninsular Malaysia (operational and non-operational status) regarding the heavy metal tolerance of the bacterial species. Soil and leachate characterization identified the level of pollutants in the landfill environment. Hence, microbial isolation and identification generated the microbial diversity of the contaminated landfill soils of Peninsular Malaysia. Exposure to Hg concentrations (5–20 ppm) showed that all the organisms survived with a heavy growth pattern. All strains showed varied resistance to the heavy metals. *Pseudomonas mendocina* demonstrated the highest resistance to metal exposure. *Bacillus pumilus* was absolutely resistant to the heavy metals used in the study, except Ni. A comparison of isolates from operational and non-operational landfill sites in Peninsular Malaysia suggests higher susceptibility to chromium ions than to other highly toxic metals, especially lead and mercuric ions. The behavior/response of the isolated microbes suggests suitability for enhanced bioremediation of heavy-metal-polluted environments.
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1. Introduction

Microorganisms are integral components of the ecosystem. The presence of microbes in atmospheric, terrestrial, and aquatic environments enhances different dimensions of metabolism and transformations. This phenomenon explains the importance of microbes in synthesis and degradation. Basically, microbes thrive more optimally when the site of action or the immediate environment is intact than when the environment is contaminated. However, totally intact or undisturbed environments are gradually disappearing because of human interference. Anthropogenic activities that are guided by the insatiable societal desires are now a global concern, as most green environments have been negatively affected by man-made pollutants. The list of pollutants is long and has been updated every now and then by professional bodies and organizations based on research findings.

However, the distribution of numerous pollutants in the environment does not imply equal prevalence and influence. Several pollutants are very toxic and dangerous to every form of life, whereas other pollutants are considered negligible. Heavy metals are pollutants that are classified as toxic to the environment, even at minute concentrations. Although different sources of heavy metals exist, metals also occur naturally in the environment as ores and few other complexes; hence, several metals play useful roles in the environment, particularly in plant growth (Aydinalp and Marinova, 2009; Sing et al., 2011; Galal and Shehata, 2015). However, when heavy metals exist in excessively high concentrations, pollution occurs and can be catastrophic because of its effect on microbes. Sometimes, metals distinctively perturb soil microbial biomass and activity and even reduce the composition and diversity of the microbial community of soil (Xu et al., 2015). Free radical nanoparticles of CuO exhibit toxicity towards bacteria (Gajjar et al., 2001). Similarly, nanoparticles of TiO_2 rupture cell wall/membrane of *Nitrosomonas europaea* to induce cell permeability and mortality (Fang et al., 2010). One source of heavy metal pollution is the leachate produced from landfill operation. The inevitable waste generation pattern, especially in developing countries, often leads to daily generation of high volumes of leachate. Leachate is the liquid/fluid that flows out from waste because of increased moisture levels from water penetration or degradation. The global characterization of leachate, especially from municipal solid waste (MSW) landfills, has shown that it is highly heterogeneous and often contains massive amounts of dissolved organic matter, pesticides, xenobiotics, and heavy metals (Emenike et al., 2013, 2012; Fauziah et al., 2013; Kjeldsen et al., 2002). When any

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http://dx.doi.org/10.1016/j.catena.2016.06.033
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Table 1
General conditions of the landfill sites.

Condition class	Taman Beringin landfill	Bukit Beruntung landfill
Landfill type	Non-sanitary (non-operational)	Non-sanitary (operational)
Period of landfilling	1991–2005	2001 - date
Age classification	Stabilized	Mature
Daily average of waste disposed (tonnage)	1800–2000	1500
Waste type	Household, commercial and industrial	Household, commercial and industrial
Form of leachate treatment	Physical and biological	Biological
Distance to river/stream (m)	5	NA
Fate of generated landfill gas	No facility	No facility
Coordinates of sampling spots	A 3° 13′ 40.17 N 101 39′43.48 E B 3 13′ 43.86 N 101 39′51.74 E C 3 13′37.91 N 101°39′51.74 E D 3°13′36.44 N 101°39′46.72 E	A 3°42′49.21 N 101 54′55.87 E B 3 42′49.81 N 101 54′53.35 E C 3 25′31.88 N 101°32′48.92 E

NA – not available.

landfill lacks proper liners and an adequate collection system for the leachate generated from waste degradation, raw leachate will laterally seep into soil compartments and contaminate the soil. Therefore, the interactions and responses of microbes in the presence of leachate as a soil contaminant are important. The responses of microbes to pollution may vary from one environment to another or may vary among species because of the nature of pollutants and their varying concentrations. For instance, microbial growth can be enhanced at low concentrations of copper (Cu), but will be repressed at high concentrations. Meanwhile, low concentrations of cadmium (Cd) can cause severe toxicity (Lucious et al., 2013; Wei et al., 2009; Karnachuk et al., 2003). Given that microbes can significantly improve the bio-geochemical cycling of toxic heavy metals or remediating metal-contaminated environments, understanding the diversity of microbes during heavy metal pollution caused by leachate seepage into soil is imperative. Similarly, increasing evidence on the metal resistance among naturally resident microbes found in the contaminated sites has become available (Lucious et al., 2013). However, studies on understanding similar resistance within landfill sites still remain limited. Considering the indiscriminate distribution of non-sanitary landfills in Southeast Asia, including Malaysia, contamination of soil with leachate is common, and the effect of contamination on microbial diversity has not been thoroughly investigated. Therefore, this research focuses on the difference in microbial species resident in non-sanitary landfill (operational and non-operational)

Table 2
Characteristics of heavy metals used.

S/N	Metal	Salt	Product	Mol.wt (g/mol)	Atomic wt (g)
1	Pb	$PbCl_2$	Merck	278.1	207.2
2	Mn	$MnSO_4$	Friendemann Schmidt	169.02	54.93
3	Fe	$FeSO_4 \cdot 7H_2O$	HumbG Chemicals	278.02	55.85
4	Hg	$HgSO_4$	Bendosen	296.65	200.59
5	Zn	$ZnSO_4 \cdot 7H_2O$	AnalaR	287.55	65.38
6	Cu	$CuSO_4$	Bendosen	159.60	159.60
7	Cd	$CdCl_2$	Friendemann Schmidt	228.85	112.41
8	Ni	$NiCl_2 \cdot 6H_2O$	Bendosen	237.73	58.69
9	Cr	$K_2Cr_2O_7$	HumbG Chemicals	294.19	103.8
10	Al	$Al_2(SO_4) \cdot 16H_2O$	Systerm	630.39	53.92

soils across Peninsular Malaysia in relation to the heavy metal tolerance of the bacterial species.

2. Materials and methods

2.1. Soil and leachate samplings and characterizations

Municipal solid waste (MSW) landfill sites were selected for the study based on their status and grade. The two selected landfills were graded non-sanitary because membrane liners were not installed. One of the landfills was "operational" because waste was dumped at the site. The other landfill had not received MSW for more than 10 years and was considered "non-operational". Table 1 reflects the general conditions of the landfill sites. Hence, soil samples were excavated at 30 cm depth from each of Taman Beringin (TBL) (3° 13.78′N; 101° 39.72′E; non-operational) and Bukit Beruntung (BBL) (3° 32.14′N;101° 25.80′E; operational) landfills in accordance with the 2014 ASTME–1197 standard guidelines for conducting terrestrial soil-core microcosm tests (Sprocati et al., 2011). The excavated samples were analyzed for pH using a multiprobe meter (YSI Professional Plus, USA), while the soil total nitrogen, total potassium, and total phosphorus were analyzed by adopting ASTM E778-87, ASTM E96-94, and ASTM D5198-92 methods, respectively. Elemental concentrations of metals in the soil were analyzed based on the USEPA 3050B guidelines except for mercury (Hg), which was analyzed based on the USEPA 3052 method. All assessments were duly replicated (including different trials). Similarly, the raw leachate samples were collected from the environment and analyzed for parameters similar to the soil samples. Part of the leachate assessment included on-the-spot analysis of raw leachate collection for several parameters, especially pH (HANNA HI 8424). Similarly, several other physico-chemical properties of the leachate samples determined in the laboratory were BOD_5, COD, total N, P, K, and the metal distribution. The assessment was conducted based on APHA (1998) standards. Preliminary investigation and assessment of the landfill site, which include soil testing, topographic outlay, and visual observation, determined the degree of heterogeneity and siting of the sampling spots.

2.2. Bacteria isolation and identification

Bacterial 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 (3 h at 180 rpm) with the aid of a Lab-line 3521 orbit shaker, and the resulting suspension was subjected to 20 times serial dilution. Dilutions (0.1 ml) were dispensed on freshly prepared nutrient agar under aseptic conditions (Kauppi et al., 2011). The inoculated media plates and associated replicates were incubated at 37 °C for 24 h. Developed colonies were further sub-cultured to ensure the purity of samples prior to identification. Subsequently, the Biolog GEN III Microplate protocol was used to test the isolated microbes according to Bochner (1989a,1989b). An omni log reader was used to identify the bacteria species contained in the microbial identification system software.

2.3. Heavy metal resistivity test

Isolated bacteria were aseptically re-grown by inoculating each species into individual test tubes containing 5 ml of nutrient broth at 37 °C for 18–24 h. Each inoculum was later introduced into test tubes containing 4.5 ml of normal saline water for standardization (NCCLS, 1993) to obtain 0.1 ABS (absorbance)/0.5 McFarland at 860 nm. However, the final inoculums 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 water for each test organism to provide an approximate cell density of 5×10^5 CFU/ml.

Furthermore, the chemical characteristics of heavy metals used for the resistivity test are in Table 2.

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