

DEDICATION

Covered in the Special Blood of Jesus Christ, my personal Lord and Saviour, I herein dedicate this work to His Grace, The Most Rev. Prof. Daddy Hezekiah, MFR, NFNY, JP Odum Ebo Igbo (Lion of the Tribe of Igbo), Founder and Leader of Living Christ Mission Inc, and his anointed daughter, Princess Chukwunazaekpere Uremma Hezekiah.

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ORIGINAL LITERARY WORK DECLARATION

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Field of Study: **ENVIRONMENTAL TOXICOLOGY AND BIOREMEDIATION**

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ABSTRACT

Chemical evaluations and characterization had often served as the commonly adopted options for assessing the potential impact of pollutants, which at the same time provide insight into the possible remediation technologies. However, heterogeneous substances may not be best studied in aforementioned forms because of the varied characteristics and concentrations of the discrete components. Leachate which is the liquid by-product of waste decomposition in landfills or dump sites is one such heterogeneous fluid that can easily pollute the environment (terrestrial and aquatic). With increase in waste generation in Malaysia, leachate production becomes inevitable especially as landfilling is the ultimate predominant waste disposal option. An average of 300,000 L of leachate is produced daily from each landfill and there are over 200 landfills in operation. Considering the high distribution of waste disposal sites in Malaysia, the study was designed to characterize leachate from the different landfill types in relation to their operational status. It aimed to assess the mortality effects of the leachate on aquatic life such that leachate toxicity index can be proposed. Similarly, the study was undertaken to investigate a possible accumulation of heavy metals in fish species due to leachate pollution and at the same time adopting a bioremediation option that will help remove the heavy metal that percolate in the soil due to leachate leaks, as a way of preventing heavy metals from getting to nearby surface water. Various methods adopted in the study ranged from the physico-chemical characterization of leachate, acute toxicity exposure, histopathology, to microbial characterization and bioaugmentation of leachate contaminated soil. Microsoft Excel, SPSS, EPA Finney's Probit and Datafit were statistical tools used in the study. Some similarities were found among the characterized leachate samples. At 482 – 51,200 mg/L COD and 127 – 27,000 mg/L BOD₅, the organic concentrations of the landfills were above the landfills' discharge limits in Malaysia regardless of the landfill type. Ammonical-nitrogen was present in all the

landfills and the highest value (880 mg/L) was obtained in the non-active sanitary landfill, whereas concentrations of heavy metals were highest (540 mg/L) in the active sanitary landfill leachate. The acute toxicity test revealed that LC₅₀ as low as 1% v/v of the raw leachate was possible and showed that mortality and the corresponding Fish Leachate Toxicity index (FLLTI) decreased in the order of non-active sanitary (5.46), active sanitary (3.87), non-active non-sanitary (1.76) and the active non-sanitary (1.28) landfills, respectively. Bioaccumulation study revealed that about 3.2 µg/g and 2.1 µg/g of Zn and Fe, respectively were found in the fish after exposure to leachate pollution for 96 hours, as against 0.21 µg/g and 0.8 µg/g of the same heavy metals obtained in the control experiment (non-exposed fish), respectively. Similarly, tissue analysis of fish showed pale stains on the cellular compartments of the gills and liver. Microbial characterization of the leachate polluted soil indicated that regardless of the toxic effect on the environment, some bacteria species can survive namely *Bacillus sp.*, *Pseudomonas sp.*, *Lysinibacillus sp.*, *Rhodococcus sp.*, *Acinetobacter sp.*, *Microbacterium sp.*, *Brevundomonas sp.*, *Stenotrophomonas sp.*, and *Flavomonas sp.* Bioaugmenting the polluted soil with different treatments (A,B,C) formed from the combination of the microbes showed that the highest heavy metal removal was achieved when *Bacillus sp.*, *Lysinibacillus sp.* and *Rhodococcus sp.* were combined as compared to other treatments ($P < 0.05$). Cu (86%), Zn (73%), Pb (70%), Fe (67%) and Mn (64%) were removed after 100 days of biomonitoring. The least heavy metal removal rate constant was observed for Cd ($K = 0.0053 \text{ day}^{-1}$). Therefore, the study concludes that raw landfill leachate in Malaysia is toxic to these fish species, yet the varied impact of leachate is a reflection of the variation in the heterogeneous nature of leachate across different landfills rather than differences in exposed fish species/types. Also, the use of isolated microbes from leachate contaminated soil to bioaugment the polluted soil is a potential approach for optimal removal of heavy metals from leachate polluted soil.

ABSTRAK

Penilaian dan pencirian kimia telah diambil kira sebagai pilihan yang sering digunakan untuk menilai potensi kesan pencemaran dan yang pada masa yang sama memberi gambaran tentang teknologi “Bioremediation”. Walau bagaimanapun, bahan-bahan yang berbeza mungkin tidak dapat dikaji dalam bentuk yang disebutkan di atas kerana ciri-ciri kepekatan komponen diskret dan kepelbagaiannya. “Leachate” adalah sejenis cecair yang dihasilkan daripada proses penguraian sisa di tapak pelupusan atau tapak pembuangan and ia adalah cecair heterogen yang boleh mencemarkan alam sekitar sama ada di daratan atau akuatik. Dengan kadar peningkatan dalam penjanaan sisa di Malaysia, penghasilan “leachate” tidak dapat dielakkan terutama daripada tapak penimbunan sampah kerana ia adalah kaedah pelupusan sisa yang paling digemari. Purata 300,000 L “leachate” dihasilkan setiap hari dari setiap tapak pelupusan sampah dan terdapat lebih 200 tapak pelupusan yang beroperasi di Malaysia. Memandangkan kadar pengagihan tapak pelupusan sisa yang tinggi di Malaysia, kajian atau penyelidikan ini bertujuan untuk mencirikan “leachate” dari pelbagai jenis tapak pelupusan sisa yang juga berhubung dengan status operasi mereka. Ia bertujuan untuk menilai kesan “mortality leachate” pada kehidupan akuatik dengan menggunakan indeks ketoksikan “leachate” seperti yang dicadangkan dalam kajian ini. Selain itu, kajian ini juga dijalankan untuk menyiasat kemungkinan pengumpulan logam berat dalam spesis ikan akibat pencemaran dari “leachate” dan pada masa yang sama menggunakan teknologi biopemulihan yang akan membantu penyingkiran logam berat yang meresap di dalam tanah kerana kebocoran “leachate”, dan merupakan satu cara yang mengelakan logam berat dari memasuki ke permukaan air yang berhampiran. Pelbagai kaedah telah digunakan dalam kajian ini yang terdiri daripada pencirian fiziko-kimia “leachate”, pendedahan ujian “acute toxicity”, histopatologi, pencirian mikrob dan “bioaugmentation” tanah yang tercemar dengan “leachate”. Penggunaan kiraan

statistik Microsoft Excel, SPSS, PROBIT EPA Finney dan Datafit telah digunakan dalam kajian ini. Beberapa ciri ciri persamaan di kalangan sampel leachate telah ditemui dalam kajian ini. Pada 482 - 51,200 mg / L COD dan 127 - 27,000 mg / L BOD₅ , kepekatan organik daripada tapak pelupusan adalah melebihi had pelepasan tapak pelupusan di Malaysia tanpa mengira jenis tapak pelupusan sisa. Ammonia -nitrogen telah dikenalpasti hadir dalam semua tapak pelupusan sisa yang dikaji dalam penyelidikan ini dan nilai yang paling tinggi (880 mg / L) telah diperolehi dari tapak pelupusan sanitari yang tidak aktif , manakala kepekatan logam berat yang paling tinggi (540 mg / L) ditemui dalam “leachate” tapak pelupusan sanitari yang aktif. Ujian “acute toxicity” mendedahkan bahawa LC₅₀ serendah 1% v / v dari “leachate” mentah adalah kemungkinan dan menunjukkan bahawa kadar kematian dan Fish Leachate Toxicity index (FLLTI) (FLLTI) menunjukkan kadar menurun dalam perintah tapak pelupusan sanitari yang tidak aktif(5.46) , tapak pelupusan sanitari yang aktif (3.87) , tapak pelupusan yang bukan aktif dan bukan sanitari (1.76) dan tapak pelupusan aktif tapi bukan sanitari (1.28) , masing-masing . Kajian “Bioaccumulation” mendedahkan bahawa kira-kira 3.2 µg / g dan 2.1 µg / g Zn dan Fe, masing-masing telah ditemui dalam ikan selepas terdedah kepada pencemaran “leachate” untuk tempoh 96 jam , berbanding dengan 0.21 µg / g dan 0.8 µg / g logam berat yang sama diperolehi dalam eksperimen kawalan (ikan yang tidak terdedah). Begitu juga , analisis tisu ikan menunjukkan kesan pucat pada dalam sel insang dan hati. Pencirian mikrob tanah “leachate” tercemar juga menunjukkan bahawa tanpa mengira kesan toksik kepada alam sekitar, beberapa spesies bakteria ditemui hadir yang terdiri daripada *Bacillus sp.* , *Pseudomonas sp.* , *Lysinibacillus sp.* , *Rodococcus sp.* , *Acinetobacter sp.* , *Microbacterium sp.* , *Brevundomonas sp.* , *Stenotrophomonas sp.* , dan *Flavomonas sp.* Kajian “Bioaugmentation “ dengan tanah yang tercemar dengan rawatan yang berbeza (A, B , C) yang dibentuk daripada gabungan mikrob menunjukkan penyingkiran logam

berat yang tertinggi telah dicapai apabila spesis mikrob *Bacillus sp.* , *Lysinibacillus sp.* dan *Rodococcus sp.* telah digabungkan berbanding dengan rawatan lain ($P < 0.05$). Kajian “Biomonitor” untuk tempoh 100 hari menunjukkan penyingkiran Cu (86%) , Zn (73%) , Pb (70%) , Fe (67%) dan Mn (64%). Sekurang-kurangnya berat pemalar kadar penyingkiran logam diperhatikan untuk Cd ($K = 0.0053 \text{ hari}^{-1}$). Oleh itu , kajian ini menyimpulkan bahawa tapak pelupusan sampah “leachate” mentah di Malaysia adalah toksik kepada spesies ikan , namun pelbagai kesan daripada “leachate” mencerminkan perubahan dalam sifat heterogen “leachate” di tapak pelupusan yang berbeza dan bukannya perbezaan dalam pendedahan kepada spesies atau jenis ikan. Selain itu, penggunaan mikrob yang diasingkan dari tanah yang tercemar dengan “leachate” untuk kajian “Bioaugmentation” tanah yang tercemar adalah satu pendekatan yang berpotensi untuk pembuangan optimum logam berat daripada tanah yang tercemar dengan” leachate”.

ABBREVIATIONS

3R	Reduce, Reuse and Recycle
ABS	Absorbance
AHL	Air Hitam Sanitary Landfill
ANOVA	Analysis of Variance
ANSL	Active Non-Sanitary Landfill
AOAC	Association of Official Analytical Chemists
APHA	American Public Health Association
ASL	Active Sanitary Landfill
ASTM	American Society for Testing and Materials
BBL	Bukit Beruntung Landfill
BDAT	Best Demonstrated Available Technologies
BGC	Biogeochemical Cycles
BOD	Biochemical Oxygen Demand
C/N	Carbon/Nitrogen
CEN	Commission of European Nations
CFU	Colony Forming Unit
CNS	Central Nervous System
COD	Chemical Oxygen Demand
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethylene
DO	Dissolved Oxygen
DOE	Department of Environment
EC	European Commission
EPA	Environmental Protection Agency
EQA	Environmental Quality Act

EU	European Union
FLLTI	Fish Lethal Leachate Toxicity Index
GC – MS	Gas Chromatography Mass Spectrometry
GDP	Gross Domestic Product
H & E	Haematoxylin & Eosin
HDPE	High Density Polyethylene
ICP – MS	Inductively –coupled Plasma Mass Spectrometry
IQ	Intelligence Quotient
ISWA	International Solid Waste Association
JSL	Jeram Sanitary Landfill
LC	Lethal Concentration
MHLG	Ministry of Housing and Local Government
MSW	Municipal Solid Waste
MSWG	Municipal Solid Waste Generation
NA	Nutrient Agar
NANSL	Non-Active Non-Sanitary Landfill
NASL	Non-Active Sanitary Landfill
OECD	Organisation of Economic Cooperation and Development
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PCE	Perchloroethylene
POP	Persistent Organic Pollutants
PTI	Pesticide Toxicity Index
PVC	Polyvinyl Chloride
RCRA	Resource Conservation and Recovery Act
RSD	Relative Standard Deviation

Std. dev	Standard Deviation
T	Transmittance
TBL	Taman Beringin Landfill
TCE	Trichloroethylene
TDS	Total Dissolved Solid
TNT	Trinitrotoluene
TOC	Total Organic Carbon
UK	United Kingdom
USA	United States of America
USEPA	United States Environmental Protection Agency
v/v	Volume/Volume
VEB	Vertical Engineered Barriers
VFAs	Volatile Fatty Acids

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