Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454

Vol. 22 | No. 2 | pp. 1003-1007 | 2023

Original Research Paper

https://doi.org/10.46488/NEPT.2023.v22i02.046

Open Access Journal

Denaturing Gradient Gel Electrophoresis (DGGE) Analysis Indicating Increased Microbial Diversity in Landfill Area Near Conserved Wetland

M. B. Chakraborty†, S. R. Patgiri, A. S. Rahman, A. Dasgupta and G. Pegu
Department of Botany, Cotton University, Guwahati-781001, Assam, India
†Corresponding author: M. B. Chakraborty; moitrayeebchakraborty@cottonuniversity.ac.in

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 17-06-2022
Revised: 06-10-2022
Accepted: 15-10-2022

Key Words:
Anthropogenic disturbance
Landfill
Leachate
Microbial diversity
DGGE

ABSTRACT

The ecological balance of an ecosystem has a relation to its biodiversity. Although it has been established that biodiversity and ecological stability are related, generalization about the exact nature of this relation remains elusive and more so in microbial diversity. A growing volume of studies has indicated that anthropogenic activities impact biodiversity, but it is difficult to generalize the impact of anthropogenic activities on microbial diversity. Landfilling by municipal solid waste is one such activity where microbes play a major role, and leachates are released from the landfill, altering the soil's physical and chemical nature. Change in factors like carbon source, pH, and toxicity of the soil is most likely to affect the indigenous microflora of the soil. The present study was undertaken to compare the microbial diversity of soil receiving landfill leachate with that of the soil not receiving any landfill leachate to assess the impact of the landfilling activity on microbial diversity. The landfill site selected for the study was that of Kamrup Metro District of Assam, located at Boragaon, near the Ramsar wetland called Deeporbeel. By using the Denaturing Gradient Gel Electrophoresis (DGGE) method, it has been found that the microbial diversity of the soil receiving leachate was higher than that of the soil not receiving any leachate from the landfill.

INTRODUCTION

Most biodiversity studies have focused mostly on plants and animals. Ecological theories have been developed by studying aboveground ecosystems but have neglected the below-ground systems, despite the latter's importance to global nutrient cycling and life on the Earth (Lynch et al. 2004). The study of microbial diversity by culture-dependent methods had been limited earlier because only 1% of the total microflora can be cultured under lab conditions. Recent use of culture-independent methods like DGGE and metagenomics has brought newer insights into microbial ecology. Despite these advances, the link between microbial diversity and soil functions is still a major challenge. Several field studies have examined how disturbance affects microbial diversity, but the findings of different studies contradict each other. The lack of consensus on how disturbance affects microbial diversity highlights the need for more studies in this field.

In general ecology, Wilhm (1967) observed that benthic diversity was depressed in the Oklahoma stream, receiving inadequately treated municipal wastes. Increased exposure to mercury in the field decreased the sequence diversity of bacteria (Muller et al. 2001). No difference in bacterial diversity between operational and non-operational landfill was observed (Jayanthi et al. 2016). Song et al. (2015) investigated the bacterial communities of ten landfill leachate samples from five landfill sites in China and found that the bacterial community function (e.g., cellulolytic bacteria, sulfate-reducing bacteria (SRB), sulfate-oxidizing bacteria, and xenobiotic organic compound (XOC)-degrading bacteria) was diverse, but the pattern is unclear. The stored waste's conductivity, organic matter, and moisture content strongly correlate with microbial diversity (Wang et al. 2016). Pérez-Leblic et al. (2012) observed low diversity of microorganisms and decreased enzymatic activity with an increase in the concentration of hydrocarbons. Contradictory findings make generalizing the relationship between microbial diversity and ecological disturbance difficult. The dynamics of the biotic and abiotic components of the landfills remain far from being fully understood. According to Themelis and Ulloa (2007), it is inevitable to gain further knowledge about the microbially mediated processes in landfills since landfilling is still the major way of depositing waste at a global scale.

MATERIALS AND METHODS

Collection of Soil Samples

For this study, soil samples were collected from two different

sites, one adjacent to the landfill site and receiving leachate from it and the other situated away from the landfill site towards wetland Deepor Beel and not receiving any leachate from the landfill. A composite sample of the soil receiving leachate from the landfill was collected from five different sites within the inactive landfill site of Paschim Boragaon area (26°06.872′′ N and 91°40.896′′E Site Elevation: 46.9m above sea level), and another composite soil sample that did not receive any landfill leachate was collected from five different sites near the Ramsar wetland of Deeporbeel (located between latitude 26°03′26′′-26°09′26′′N and longitude 90°36′39′′-90°41′25′′E) were transported to the laboratory in ice-pack at 4°C. Soil samples were collected from a depth of 7.5 cm using a sterile spatula and transferred to a sterile container. Soil samples were labeled broadly as polluted (P_1 P_2) receiving leachate and non-polluted (N_1, N_2) not receiving any leachate. Each sample was a composite sample of 5 subsamples collected from 5 different sites.

Extraction of DNA from Soil

0.25 g of each sample was used to isolate the DNA. DNA was directly extracted from the soil samples using *PowerSoil© DNA Isolation Kit* (Mo Bio Laboratories Inc.), and nano reading was noted.

Gel Electrophoresis

0.8% Agarose gel is prepared in TAE buffer. The gel was poured into the gel cast, and a 1mm thick comb was put in to make wells. After about 30 minutes at room temperature, the comb was removed carefully, and DNA samples of each soil sample were loaded. The gel was then run in the electrophoretic unit to separate the DNA.

PCR Amplification

This study used the PCR technique to amplify DNA for 16S rRNA. Primers for PCR were designed to be specific for 16S rRNA. A forward primer designated as F-968 and a reverse primer situated at position 1401 were used to amplify bacterial 16S rRNA gene fragments. The PCR amplified the V6-V8 region of the DNA.

Denaturing Gradient Gel Electrophoresis (DGGE)

A 30% polyacrylamide gel with a 35-55% denaturant gradient was prepared. The comb of 1mm thickness was inserted to produce wells. The gel was allowed to cool for an hour. After the gel had cooled down, the wells were loaded roughly with an equal amount of DNA sample (50µl) with 5µl loading dye. Electrophoresis was carried out 1x TAE buffer at 100V for 16-17 hrs at 60°C. The gel was stained in 0.5µg/ml ethidium bromide for 15-20 mins. The gel was then destained in water, observed under a UV transilluminator at 256 nm, and photographed.

RESULTS AND DISCUSSION

DNA Concentration from Soil Sample

The DNA concentration in the samples N1, N2, P1, and P2 were studied using Nanodrop software (Table 1).

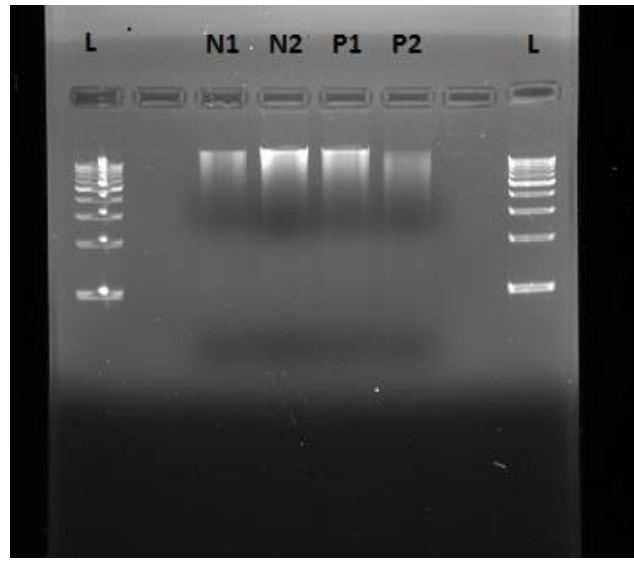

Fig. 1: Result of Gel Electrophoresis. L = Ladder DNA, N1 = DNA Sample of N1 Site, N2 = DNA Sample of N2 Site, P1 = DNA Sample of P1 Site, P2 = DNA Sample of P2 Site.

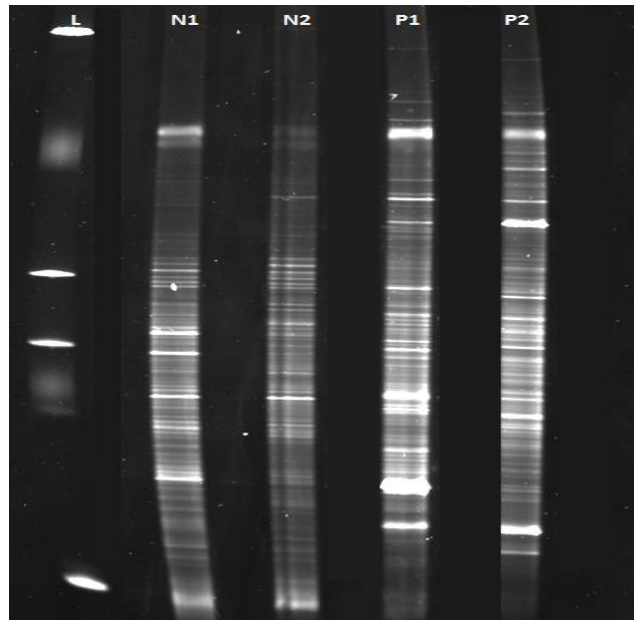

Fig. 2: Result of DGGE (Density Gradient Gel Electrophoresis) analysis. L = Ladder DNA, N1 = Bacterial Diversity of N1 Site, N2 = Bacterial Diversity of N2 Site, P1 = Bacterial Diversity of P1 Site, P2 = Bacterial Diversity of P2 Site.

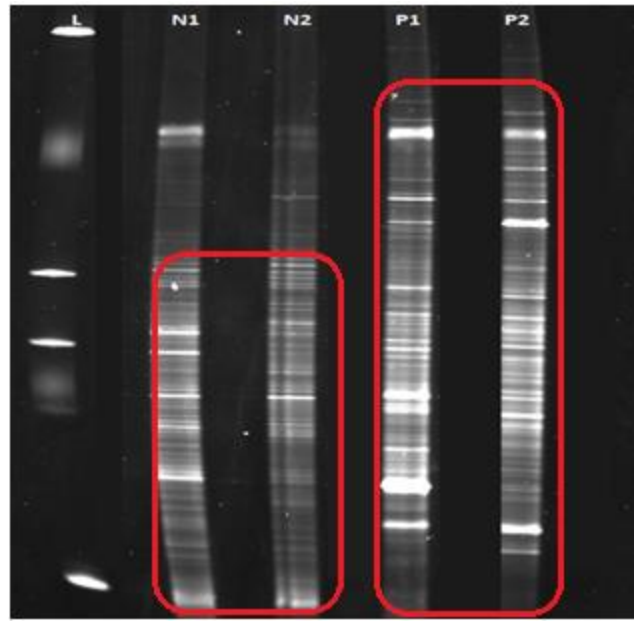

Fig. 3: Comparison of DNA bands separated by Density Gradient Gel Electrophoresis (DGGE). Polluted soil samples show more bands corresponding to higher bacterial diversity.

The nucleic acid concentration given by nanodrop reading gives an idea about the purity of the genomic DNA content extracted from the soil samples. The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA extracted from the sample (Fig. 1). A ratio of ~ 1.8 is accepted as pure for DNA, and a ratio of ~2.0 for RNA (Page 2010). In our samples, though the nucleic acid concentration in N1 is quite high, i.e., 50.4 ng.μl^{-1}, the A260/A280 ratio is appreciably less, which may indicate the presence of protein or other contaminants that also absorbs strongly at 280 nm. The 260/280 ratio of the N2, P1, and P2 samples is ~2.0, which may indicate RNA contamination. Sometimes

Table 1: The DNA concentration in the samples N1, N2, P1, and P2 using Nanodrop software.

SL.	Sample	Nucliec Acid Conc.(μg.ml^{-1})	A260	A280	260/280	260/230	Sample Type
1.	BLANK	0.0	0.001	-0.043	-0.02	0.01	DNA
2.	N1	50.4	1.007	2.066	0.49	0.48	DNA
3.	N2	26.1	0.522	0.266	1.96	23.51	DNA
4.	P1	22.6	0.453	0.277	2.00	-55.31	DNA
5.	P2	12.7	0.254	0.127	2.00	-3.89	DNA

variation in the ratio might also occur due to changes in sample pH (Wilfinger et al. 1997). The A260/A230 ratio is a secondary measure of nucleic acid purity. The A260/A230 ratio values for pure samples should be more than the respective A260/A280 values. In our samples, we got an appreciably low value indicating greater salt in the extract (Smith et al. 2010). Urea, carbohydrates, and phenolate ions also have absorbances near 230 nm, which might hinder the absorbance of nucleic acid.

Agarose gel electrophoresis of the purified DNA compliments the data obtained from absorbance readings. The concentration of DNA is determined after the gel electrophoresis is completed. The DNA in the agarose gel is visualized by staining with intercalating agents like ethidium bromide. This method is useful in cases where the contaminants absorbing at 260nm make accurate quantification impossible.

DGGE (Denaturing Gradient Gel Electrophoresis) of the amplified 16S rDNA is a culture-independent technique frequently used for bacterial community analysis in microbial ecosystems. DGGE separates the bacterial DNA of different species depending on their GC content. The different bands obtained after the DGGE indicate the diversity of bacterial species in the sample. The separated DNA from DGGE is visualized after staining with ethidium bromide. Each prominent band indicates a bacterial species. We can see a difference between the bacterial diversity of the non-polluted soil sample (N1 & N2) and the polluted soil sample (P1 & P2). A few species are also common to both sites. The polluted site is seen to be more diversified than the non-polluted site, as indicated by the DGGE bands.

Findings from DGGE analysis (Fig. 2) show that the number bands indicating different bacteria are more in the soil receiving leachate. In Fig. 3, it can be seen that some bacteria are common to both the polluted soil samples (box a, b, and e), while some bacteria are common to non-polluted soil samples (box c). Some bacteria have been found in all the soil samples (box d). Many ecological studies have indicated that high species diversity positively correlates with a system's ecological stability and that anthropogenic disturbances reduce species diversity. But findings from the present study indicate higher bacterial diversity in the disturbed soil receiving leachate from landfill. The findings may be interpreted in the light of the intermediate disturbance hypothesis, which suggests that at low disturbance frequency, species diversity is low because competitively dominant species exclude competitively inferior species, while at high disturbance frequency, species diversity is low because only species that quickly colonize and reach maturity can survive. Only at intermediate levels of disturbance do a mix of colonizers and competitors co-exist (Hughes 2010).

CONCLUSION

The study shows that the microbial diversity of the soil receiving landfill leachate differs from that of the indigenous microflora of the soil not receiving any leachate. Contradictory to the expectation of lower microbial diversity in the polluted soil, it has been found that the microbial diversity of the soil receiving landfill leachate was higher than that of the soil not receiving any leachate. The increase in diversity can be interpreted in the light of Such a shift in microbial community structure can have some serious implications in the long run and affect other ecosystems. The ecological balance of the adjacent Ramsar wetland of Deepor beel seems to be under threat due to the landfilling activity.

ACKNOWLEDGEMENT

We express gratitude to Dr. Madhusmita Dehingia and Dr. Manasi Das of the Institute of Advanced Study in Science & Technology (IASST), Gorchuk, Paschim Boragaon, Guwahati-35, Assam, for extending technical support.

REFERENCES

Hughes, A. 2010. Disturbance and diversity: An ecological chicken and egg problem. Nature Educ. Knowl., 3(10): 48.

Jayanthi, B., Emenike, C.U., Agamuthu, P., Simarani, K., Mohamad, S. and Fauziah, S.H. 2016. Selected microbial diversity of contaminated landfill soil of Peninsular Malaysia and the behavior towards heavy metal exposure. J. Catena, 147: 25-31.

Lynch, J.M., Benedetti, A., Insam, H., Nuti, M.P., Torsvik, K.V. and Nannipieri, P. 2004. Microbial diversity in soil: Ecological theories, the contribution of molecular techniques and the impact of transgenic plants and transgenic microorganisms. Biol. Fertil. Soils, 363e: 385.

Muller, A.K., Rasmussen, L.D. and Sorensen, S.J. 2001. Adaptation of bacterial community to mercury contamination. FEMS Microbiol. Lett., 204: 49-53. Doi. 10.1016/S0378-1097(01)00376-7.

Page, A.F. 2010. The MIQE Guidelines and Assessment of Nucleic Acids Prior to qPCR and RT-qPCR. Thermo Fischer Scientific, Wilmington, Delaware, USA, pp. 1-3.

Pérez-Leblic, M.I., Turmero, A., Hernández, M., Hernández, A.J., Pastor, J., Ball, A.S., Rodríguez, J. and Arias, M.E. 2012. Influence of xenobiotic contaminants on landfill soil microbial activity and diversity. J. Environ. Manag., 95: S285-S290. https://doi.org/10.1016/j.jenvman.2010.07.017

Smith, H.L., Hickey, J., Jablin, M.S., Trujillo, A., Freyer, J.P. and Majewski, J. 2010. Mouse fibroblast cell adhesion was studied by neutron reflectometry. Biophys. J., 98(5): 793-799. https://doi.org/10.1016/j.bpj.2009.11.019

Song, L., Wang, Y. and Tang, W. 2015. Bacterial community diversity in municipal waste landfill sites. Appl. Microbiol. Biotechnol., 99: 7745. https://doi.org/10.1007/s00253-015-6633

Themelis, N.J. and Ulloa, P.A. 2007. Methane generation in landfills. Renew. Energy, 32: 1243-1257

Wang, X., Cao, A., Zhao, G., Zhou, C. and Xu, R. 2016. Microbial community structure and diversity in a municipal solid waste landfill. Waste Manag., 66: 79-87. DOI: 10.1016/j.wasman.2017.04.023.

Wilfinger, W.W., Mackey, K. and Chomczynski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques, 22(3): 474-481. Doi: 10.2144/97223st01.

Wilhm, J.L. 1967. Comparison of some diversity indices applied to populations of benthic macroinvertebrates in a stream receiving organic wastes. J. Water Pollut. Control Fed., 39: 1673.