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Research Article

Bacterial community structure in soils contaminated with electronic waste pollutants from Delhi NCR, India

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ABSTRACT

Background: Microbial community analysis of electronic waste (e-waste)-polluted environments is of interest to understand the effect of toxic e-waste pollutants on the soil microbial community and to evaluate novel microorganisms resisting the toxic environment. The present study aims to investigate the bacterial community structure in soils contaminated with e-waste from various sites of Loni and Mandoli (National Capital Region (NCR), India) where e-waste dumping and recycling activities are being carried out for many years.

Results: Interferences to soil metagenomic DNA extraction and PCR amplification were observed because of the presence of inhibiting components derived from circuit boards. Whole-metagenome sequencing on the Illumina MiSeq platform showed that the most abundant phyla were *Proteobacteria* and *Firmicutes*. *Deltaproteobacteria* and *Betaproteobacteria* were the most common classes under *Proteobacteria*. Denaturing gradient gel electrophoresis (DGGE) analysis of the bacterial 16S rRNA gene showed that e-waste contamination altered the soil bacterial composition and diversity. There was a decrease in the number of predominant bacterial groups like *Proteobacteria* and *Firmicutes* but emergence of *Actinobacteria* in the contaminated soil samples.

Conclusions: This is the first report describing the bacterial community structure of composite soil samples of e-waste-contaminated sites of Loni and Mandoli, Delhi NCR, India. The findings indicate that novel bacteria with potential bioremediating properties may be present in the e-waste-contaminated sites and hence need to be evaluated further.

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1. Introduction

The top electronic waste (e-waste)-producing countries are China, USA, Japan, India, Germany, Brazil, Russia, France, Indonesia, and Italy. India is among the top five countries producing 2 million tonnes of e-waste annually [1]. Although many countries have come up with the e-waste management legislations, very few have started implementing in a proper way. In India, according to e-waste management guidelines (October 2016), manufacturers of electronic equipment should make sure of e-waste collection and their transport to authorized recycling centers. However, this process is not being implemented by the manufacturers. Despite the Basel Convention on the control of illegal transboundary movement of hazardous waste, actual enforcement remains a challenge. A recent study pointed out evidence of transport of e-waste across countries through location tracking of discarded computer monitors and other devices. Wireless GPS location trackers were planted inside discarded computer devices, and it was found that a huge amount of e-waste from California were transported to various ports in China, Malaysia, Pakistan, UAE, and African countries [2]. Illegal processes of recycling are still in continuation, which involve manual dismantling, separation, heating, acidic extraction of metals, and burning of e-waste. The major cities in India where illegal recycling processes of e-waste are conducted are Karnataka, Maharashtra, Uttar Pradesh, and West Bengal [3,4]. Burning of e-waste causes severe environmental issues and affects air, water, and soil adversely. Components of e-waste may cause leaching and bioaccumulation in the environment [5].

E-waste contains hazardous components such as heavy metals (Pb, Sb, As, Cd, Ni, Hg, and Cr), plastics, and polychlorinated biphenyls (PCB). When these components are present beyond threshold quantities, they are classified under hazardous waste [6]. Bromine has also been found as a substitute for brominated flame retardants in many of the

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electronic items [7]. Microorganisms play an important role in the soil ecological function, soil health, and bioremediation. A number of investigations have been made on the toxic effects of pollutants on soil microbial population and diversity; yet, more studies are required in this area to draw a final conclusion of the effects of toxic pollutants on soil microbial community. Co-occurrence of multiple pollutants may give rise to complicated interactions and affect the soil microbiological processes [8]. In a recent study, the effect of different levels of heavy metal contamination (Cu, Pb, and Zn) on soil bacterial community was studied at an e-waste site in Nigeria, and it was found that soil properties such as soil texture, pH, and soil carbon had more influence on the soil bacterial composition and diversity than heavy metals [8].

Soil samples heavily contaminated with e-waste recycling in China and Pakistan were also studied, and the indigenous microbial communities were analyzed [9]. It was revealed that the microbial community composition and diversity were affected significantly by polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDE), and heavy metals (e.g., Cu, Zn, and Pb). The constructed ecological network of soil microbial communities illustrated microbial co-occurrence, competition, and antagonism across soils, revealing the response of microbes to soil properties and pollutants. In addition, microorganisms were sensitive to nutrition (total organic carbon and total nitrogen) and pollutants [9].

In a recent study, using a culture-independent approach, identification of biphenyl and PCB degraders in e-waste-contaminated soil was performed. The study provided a deeper understanding of the mechanisms underlying PCB degradation, and 19 rare operational taxonomic units along with three genera were enriched with the DNA stable-isotope probing, hence confirming the presence of PCB degradation pathways [10].

The toxic compounds and heavy metals present in e-waste create an environment difficult for microorganisms to sustain, but there are certain microorganisms that can resist these harsh conditions. Novel microorganisms that can simultaneously remediate both heavy metals and persistent organic pollutants have been discovered [11]. However, presently, there are only few reports available and are yet to be investigated.

There are certain bacteria with bioleaching properties in the environment and can be used for recovery of metals from industrial wastes [12]. A sponge bacterium, *Bacillus* sp. Hyhel-1, was shown to exhibit bioleaching property of e-waste by producing a lipopeptide substance that can bind to the leached copper-solubilizing 17 g L^{-1} copper and has the potential for efficient recovery of copper [13]. Fungi also have immense potential to be used for the biorecovery of gold through biochemical leaching [14].

Studies on the combined effect of different pollutants of e-waste on the composition of soil microbial community showed that there is a significant difference between the contaminated and the reference soils in many of the reports [9,15].

In our previous study, it was found that the composite soil samples from the Mandoli area were found to be contaminated with the heavy metals arsenic, nickel, and chromium with values of 3.15 mg kg^{-1}, 89.4 mg kg^{-1}, and 35.5 mg kg^{-1}, respectively [16]. It was also observed that in all the samples collected from Mandoli, there was presence of BDE-7, BDE-28, and BDE-100, which are lower brominated congeners of PBDE and are considered harmful pollutants. Therefore, the present study was undertaken, to investigate the general impact of e-waste disposal and burning on soil bacterial community structure as compared to that in the adjacent noncontaminated soil.

2. Materials and methods

2.1. Samples

Soil samples were collected from two different e-waste dumping and recycling sites in Delhi (NCR) where most of the hazardous practices for recovery of materials have been occurring for a significant period of time. The sites chosen were (i) Loni village with geolocations 28°45′09.17″N and 77°18′16.14″E, (ii) Mandoli Tilla Shabazpur village with geolocations 28°42′14.90″N and 77°18′50″E [Location map provided in Fig. S1. Four different sampling areas were selected in each site as (i) disposal area, (ii) contaminated grassland area, (iii) area near the open burning site, and (iv) area at the open burning site. The soil samples were collected from a depth of 0–15 cm from the surface and were then mixed to obtain a composite mixture of the e-waste-contaminated sites. The composite soil mixtures of Loni and Mandoli e-waste disposal sites have been referred to as MD2 and MD3, respectively. Reference soil sample, referred to as MD1, was collected from an area located approximately 1 km away with no e-waste dumping or recycling activities.

2.2. Isolation of metagenomic DNA and PCR amplification

Metagenomic DNA was isolated from 500 mg of soil samples using the Favorgen soil DNA isolation kit (Favorgen Biotech Corp., Taiwan) following the manufacturer's instructions. The NanoDrop quantitative analysis of the DNA was performed to check the purity of the DNA. To recover humic acid-free metagenomic DNA, the metagenomic samples were electrophoresed in 0.8% (*w*/*v*) agarose TAE buffer [2 M Tris-acetate (pH 8), 100 mM Na_2EDTA] and the DNA bands were sliced from the gel after a long run to separate humic acid contamination. The DNA was then extracted from the agarose gel using the QIA Quick Gel Extraction Kit (Qiagen, Germany).

Purified DNA from all the soil samples were used as a template for PCR amplification. Bacterial 16S rRNA genes were amplified using the primer sets (i) GC-342F (5′-GC-clamp-CTACGGGGGCAGCAG-3′) and 806R (5′-GGACTACCGGGGTATCT-3′) [17], (ii) GC-338F (5′-GC-clamp-ACTCCTACGGGAGGCAGCAG-3′) and 518R (5′ATTACCGCGGC TGCTGG3′) [18]. A GC clamp (5′-CGCCCGCCGCGCGCGGCGGGCGGGGC GGGGGCACGGGGGG-3′) was attached to the 5′ ends of both the forward primers.

PCR was performed in a thermocycler (Bio-Rad) with the amplification mixture containing 0.25 μM of each primer, 3 ng of template DNA, and 1X Dream Taq PCR Master mix (Thermo Fisher Scientific, USA) in a 40 μl final reaction volume. Control PCR was set up using genomic DNA (gDNA) from *E. coli* BL-21 DE3, and a negative control was set up with nuclease-free water in place of template DNA. For the primer set GC-342F and 806R, PCR was performed at 94°C for 5 min as the initial denaturation step, followed by 35 cycles of 94°C for 30 s, 53°C for 45 s, and 72°C for 1 min, and a final extension of 72°C for 7 min. For the second set of primer with GC-338F and 518R, PCR was performed with an initial denaturation step of 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 60 s, and a final extension of 72°C for 10 min. The amplified PCR products were detected on a 1.5% (*w*/*v*) agarose gel stained with ethidium bromide (0.5 μg ml^{-1}).

2.3. DGGE analysis

Bio-Rad DCode system, USA, was used for DGGE. The PCR products were resolved using 8% (w/v) polyacrylamide gels in 0.5 × TAE [20 mM Tris-acetate (pH 7.4), 10 mM acetate, 0.5 mM Na_2EDTA]. Denaturing gradients ranged from 20% to 50% for samples MD1 and MD2 and 30–50% for sample MD3 (where a 100% denaturant contains 7 M urea and 40% [*v*/v] formamide). The gels were prepared by using an 8% acrylamide/bis-acrylamide (37.5:1) gel. It is advisable to have a high concentration of DNA as the starting sample to obtain properly visible bands in the DGGE gel after staining. Thus, 500 μl of a 30-cycle PCR product of both the primers was concentrated to a 50 μl sample using the UniPro GelEx and CleanUp Kit (KPC Life Sciences, India). Twenty-five microliters of each sample was mixed with 5 μl of 6× DNA loading Dye and loaded onto the gel. Electrophoresis was carried

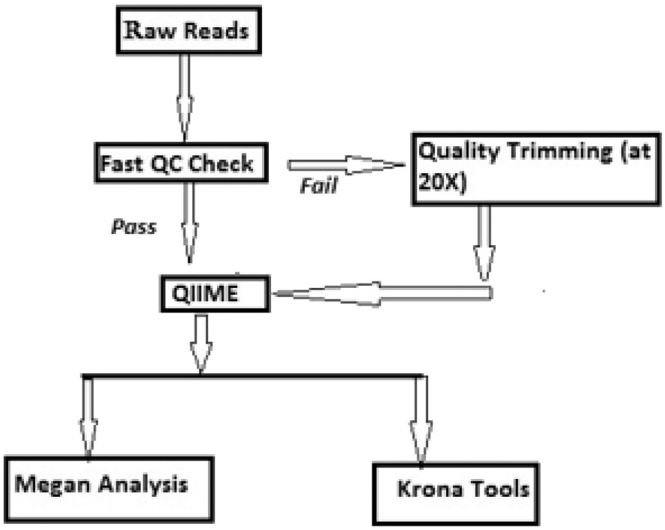

Fig. 1. Workflow for metagenomic DNA sequencing analysis.

out at 60 V for 12–16 h at a constant temperature of 60°C. The gels were stained for 20 min with ethidium bromide and washed twice for 5 min with Milli-Q water before UV transillumination.

Gel staining was performed by adding 40 μl of EtBr (10 mg ml^{-1}) to 100 ml of 0.5× TBE to prepare the staining solution. Gel staining was performed in a polypropylene box for 45 min^{-1} h under shaking condition and then visualized in gel imaging equipment.

2.4. Extraction of DNA from acrylamide gel

The DNA bands were cut off from the urea-acrylamide gel, and each band was collected in a 1.5 ml microfuge tube. The acrylamide gel was then properly crushed, and 200 μl of nuclease-free water was added to it. The tubes were incubated at 65°C overnight, and the contents were centrifuged at 13000 rpm for 5 min. The supernatant was carefully collected from the top keeping the acrylamide layer below, undisturbed. Five microliters of this supernatant was used as the template for the 25 μl re-PCR reaction with the respective primers. Sequencing of the DNA eluted from the excised DGGE bands was performed through a commercial sequencing facility (KPC Life Sciences, Kolkata, India).

2.5. Sequence analysis

The sequence data obtained for the DGGE bands were searched in the GenBank using the BLAST program to determine the closest known relatives of the partial 16S rDNA sequences.

2.6. Investigation of the bacterial phylogeny structure of the e-waste-contaminated site by using metagenomics approach

Bacterial phylogenetic structure investigation through the metagenomics approach was performed by preparing gDNA shotgun library and sequencing on Illumina HiSeq platform with 2 × 150 bp paired end sequencing. Analysis of genomic sequences was carried out by MEGAN analysis (6.11), Quantitative Insights Into Microbial Ecology (QIIME 1.1), and Krona tool (2.7). The process was carried out according to the workflow shown in Fig. 1.

3. Results

3.1. Isolation of metagenomic DNA and PCR amplification

Metagenomic DNA was obtained from the three different samples MD1, MD2, and MD3. It was found that the DNA samples recovered using the soil DNA isolation kit were of good quality (A260/280 approximately 1.7–2.0) (Fig. 2a). In the PCR amplification, the control sample with *E. coli* BL-21 DE3 genomic DNA showed amplified bands of expected sizes 200 bp using the primer pair GC 338F-518R and 500 bp with the primer pair GC 342F-806R. However, no amplification was seen in the PCR assays for samples MD1, MD2, and MD3 using the primer pairs GC 338F-518R and GC 342F-806R. This may be because soil samples have a plenty of PCR inhibitors such as humic and tannic acids, complex polysaccharides from plants, and many other organic substances. When we extract DNA from the soil, generally, humic substances are coextracted, as they bind to the reagents and to the DNA in the reaction and inhibit the PCR assay. Humic acid-free DNA samples were obtained after electrophoresing the DNA samples in 0.8% (*w*/*v*) agarose gel for a longer time so that the samples reach the bottom part of the gel and the humic acid contamination is removed. Twenty microliters of each of the genomic DNA samples was gel-purified using the gel extraction kit and eluted in 35 μl nuclease-free water. Two microliters of each of the gel-extracted DNA samples was electrophoresed, and the samples were found to be free from humic acid (Fig. 2b). The purified metagenomic DNA samples produced

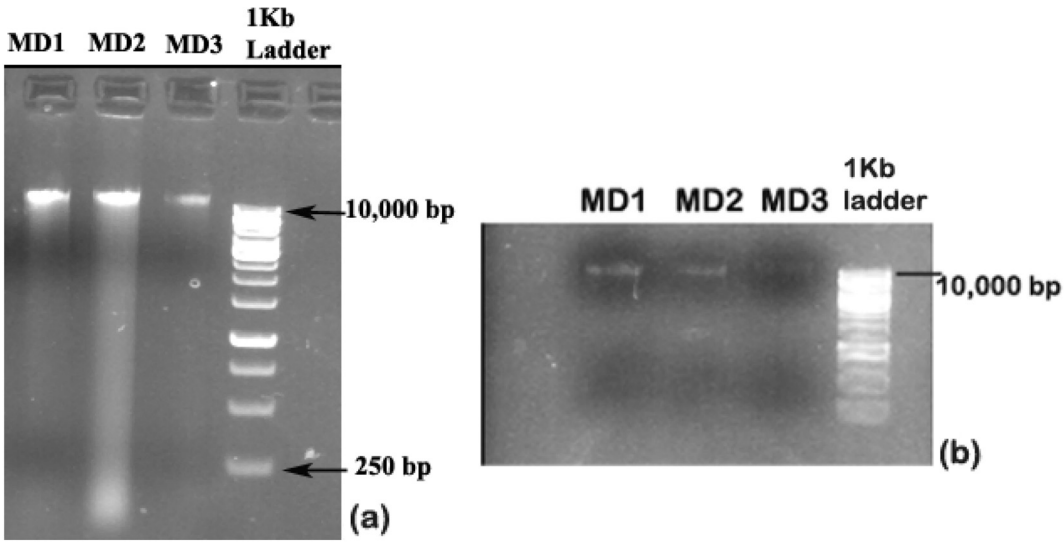

Fig. 2. Metagenomic DNA obtained for the three different samples MD1, MD2, and MD3 (a) before purification and (b) after purification.

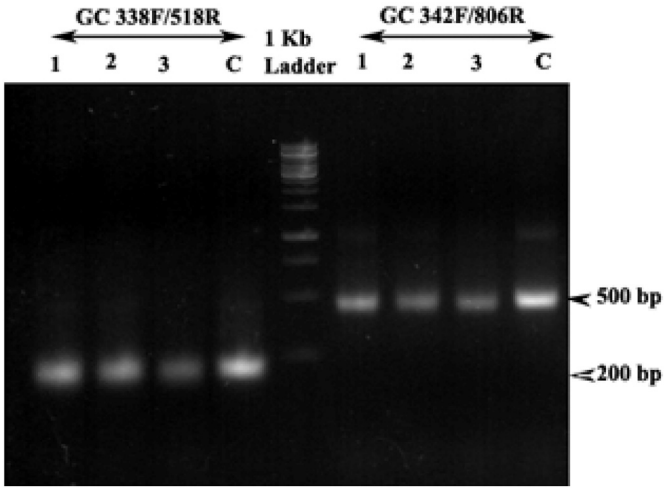

Fig. 3. PCR amplification of purified metagenomic DNA samples: Lane 1: MD1, Lane 2: MD2, Lane 3: MD3, Lane C: Positive control using gDNA from *E. coli* BL-21 DE3.

amplified bands of expected sizes 200 bp and 500 bp with the primer pairs GC 338 F/518 R and GC 342 F/806 R, respectively (Fig. 3).

3.2. DGGE analysis

DGGE band pattern was obtained after optimization with gradients ranging from 20% to 50% denaturing condition for samples MD1 and MD2 and 30–50% for sample MD3. DNA purified from the DGGE bands, when subjected to PCR with the same primer sets GC 338 F-518 R and GC 342 F-806 R, produced the expected amplicon sizes of 200 bp and 500 bp, respectively. Identification of the bacterial community members was done by sequencing of DNA eluted from excised DGGE bands, which revealed the predominant members of bacteria as listed in Table 1. The sequencing results showed the presence of bacteria with high sequence similarity (90–99%) to different bacterial groups (Table S1). Most of the dominant bands selected for sequencing showed sequence data of less than 200 bp, except five of them that provided significant sequence information for submission to GenBank. These are the bands corresponding to band numbers 12, 14, 15, 17, and 18. These sequences are available from GenBank under accession numbers MK318643, MK318644, MK318645, MK318646, and MK318647.

The DGGE community profile showed that the number of predominant bacterial groups is decreased in the samples contaminated with e-waste pollutants as shown by the decreasing number of bands in samples MD2 and MD3 as compared to that in the reference sample MD1 (Fig. 4). The results showed that *Proteobacteria* was the predominant group in all samples.

3.3. Investigation of the bacterial phylogeny structure of the e-waste-contaminated site by using metagenomics approach

3.3.1. Microbial community composition

gDNA shotgun library preparation and sequencing on the Illumina HiSeq platform with 2 × 150 bp paired end sequencing were carried out with a composite mixture of the e-waste-contaminated soil sample. Library preparation and analysis of sequencing results were performed. Quality check for the raw reads of the sequences was done through the FastQC tool [19], and the results of the quality check that show pass are shown in Fig. 5. The total raw reads were 6,326,240, 150 bp long each, and the %GC per sequence is 50. For microbiome analysis of the raw sequence data, QIIME (1.1) was performed at a sequence similarity threshold of 97%. Taxonomical annotation was done using the most recent Greengenes database taxonomy. Finally, the sequence alignment results were parsed with MEGAN (6.11) using the default parameter. Results of the analyses are as shown in Fig. 6 where the taxonomy profile at the genus level is shown as a bar chart representing the proportion of sequences in percentage. Hierarchical cluster analysis of the predominant genera and species richness of the analyzed sample are shown in Fig. 7a and b**,** respectively. Individual rarefaction curve follows the typical plateau formation but indicates that rare species may be present, as the curve shows some increase toward the end (Fig. 7b). Sequencing results showed that in the e-waste microbiome, there were a significantly higher number of sequences classified under the phyla a) *Firmicutes*, b) *Proteobacteria*, c) *Bacteroidetes*, d) *Chloroflexi*, e) *Euryarchaeota*, f) *Planktomycetes*, g) *Spirochaetes*, and h) Candidate division H-178 (unclassified division). Krona graph tool was used to display abundance and hierarchy simultaneously using a radial space-filling display. The Krona tool revealed that the most abundant phyla were *Firmicutes* and *Proteobacteria*. Bacilli and Clostridia were the most common forms under *Firmicutes,* and *Deltaproteobacteria* and *Betaproteobacteria* were the most common classes under *Proteobacteria* (Fig. 8).

4. Discussion

Soil samples from e-waste dumping and recycling sites were taken and different composite soil mixtures (MD2 and MD3) were used to study the effect of e-waste contaminants on microbial community structure. In our earlier study, the soil samples collected from various

Table 1
Predominant bacterial members in the different samples as obtained after DNA elution from excised DGGE bands and sequencing.[a]

S. No.	Samples					
	MD1		MD2		MD3	
	Microorganisms	Phyla	Microorganisms	Phyla	Microorganisms	Phyla
1.	*Aquimonas voraii* **(Band 5)**	*Proteobacteria*	*Pontibacter odishensis* **(Band 10)**	*Bacteroidetes*	*Pantoea theicola* **(Bands 13,16)**	*Proteobacteria*
2.	*Bacillus cucumis* **(Band 6)**	*Firmicutes*	*Aureimonas glaciistagni* **(Band 11)**	*Proteobacteria*	*Arthrobacter tumbae* **(Bands 14,17)**	*Actinobacteria*
3.	*Nitrosomonas communis* **(Band 7)**	*Proteobacteria*	*Arthrobacter globiformis, Arthrobacter parietis* **(Band 12)**	*Actinobacteria*	Uncultured Gamma proteobacterium **(Bands 15,18)**	*Proteobacteria*
4.	*Vibrio cidicii* **(Band 1)**	*Proteobacteria*	*Nitratifactor salsuginis* **(Band 8)**	*Proteobacteria*	–	–
5.	*Microbulbifer rhizosphaerae* **(Band 2)**	*Proteobacteria*	*Aeromicrobium choanae* **(Band 9)**	*Actinobacteria*	–	–
6.	*Rhizobacter profundii* **(Band 3)**	*Proteobacteria*	–	–	–	–
7.	*Streptomyces virens* **(Band 4)**	*Actinobacteria*	–	–	–	–

[a] The DGGE band numbers according to Fig. 4 are given in brackets for each bacterial member identified.

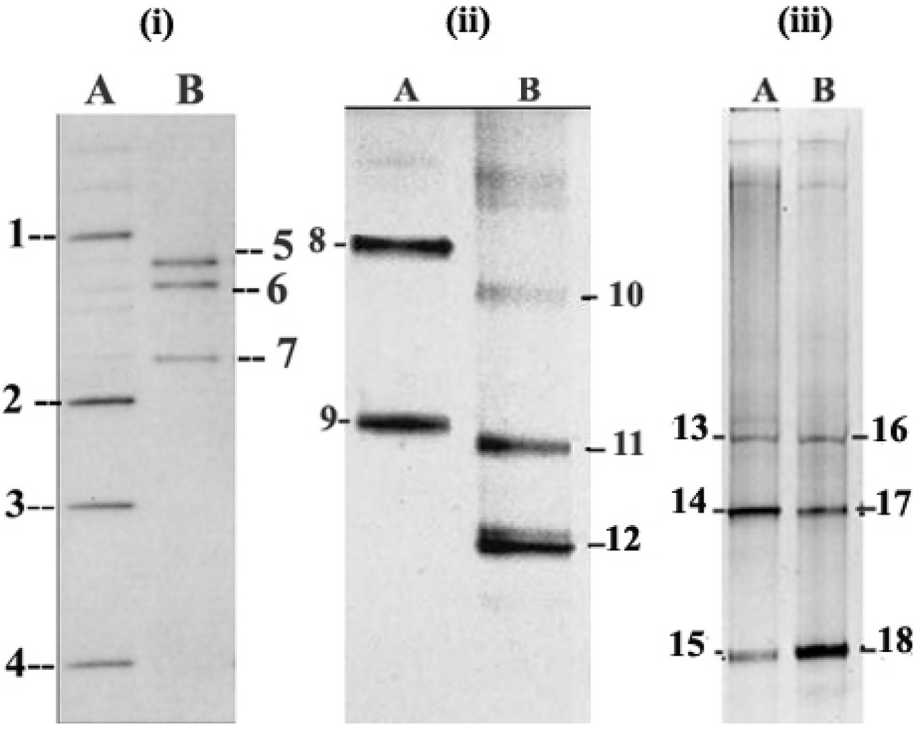

Fig. 4. DGGE of Samples (i) MD1, (ii) MD2, and (iii) MD3; (A) Results from the primer pair GC 338F-518R, (B) Results from the primer pair GC 342F-806R.

areas of the e-waste dumping and recycling sites of Loni and Mandoli villages have been reported to be contaminated with the heavy metals arsenic, nickel, chromium, and PBDEs. pH of the soil samples ranged between 5.5 and 6.5 [16]. Among the heavy metals, chromium concentration was found to be 7-fold higher than that of the control sample collected from sites with no e-waste activities. Arsenic was

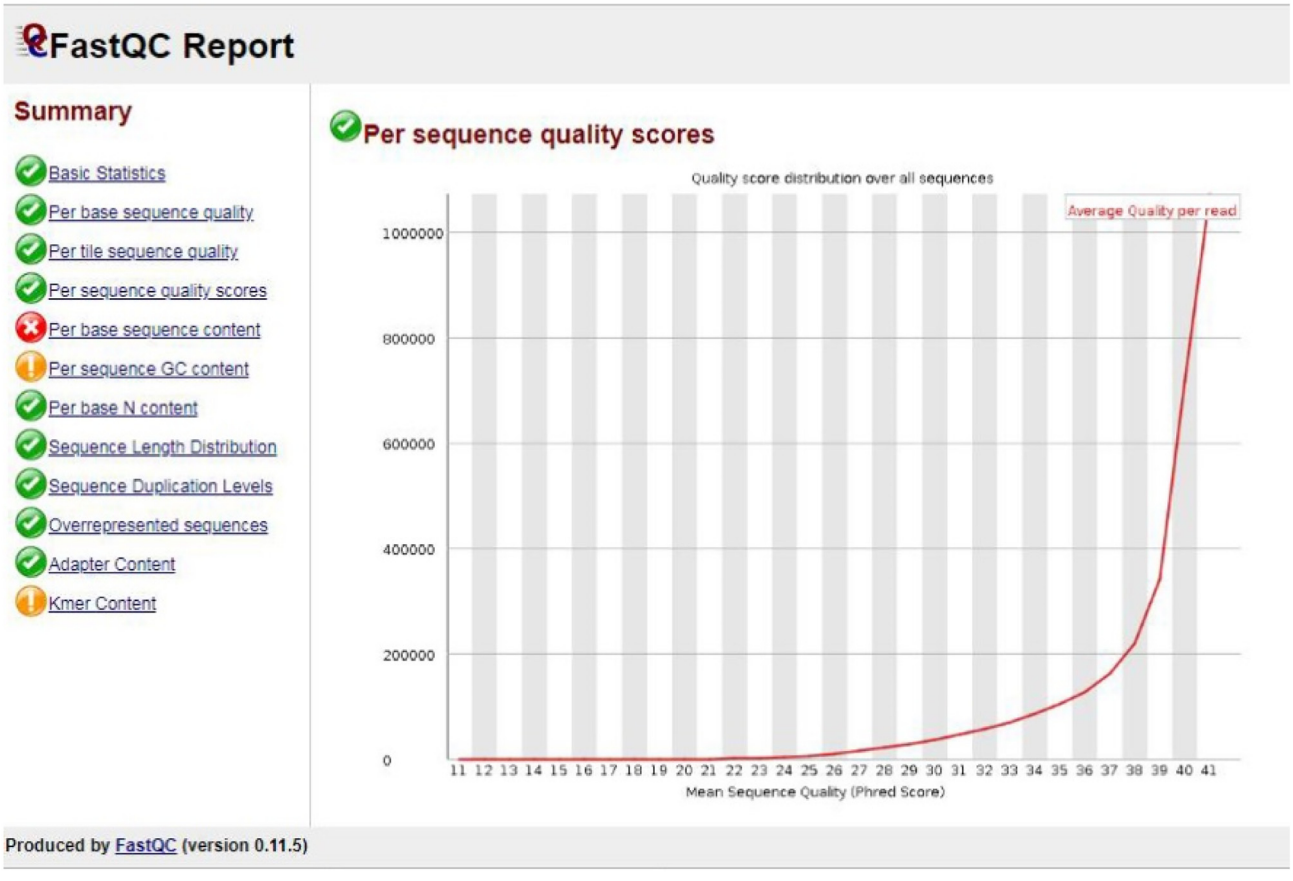

Fig. 5. Quality check of raw sequence data through the FastQC tool.

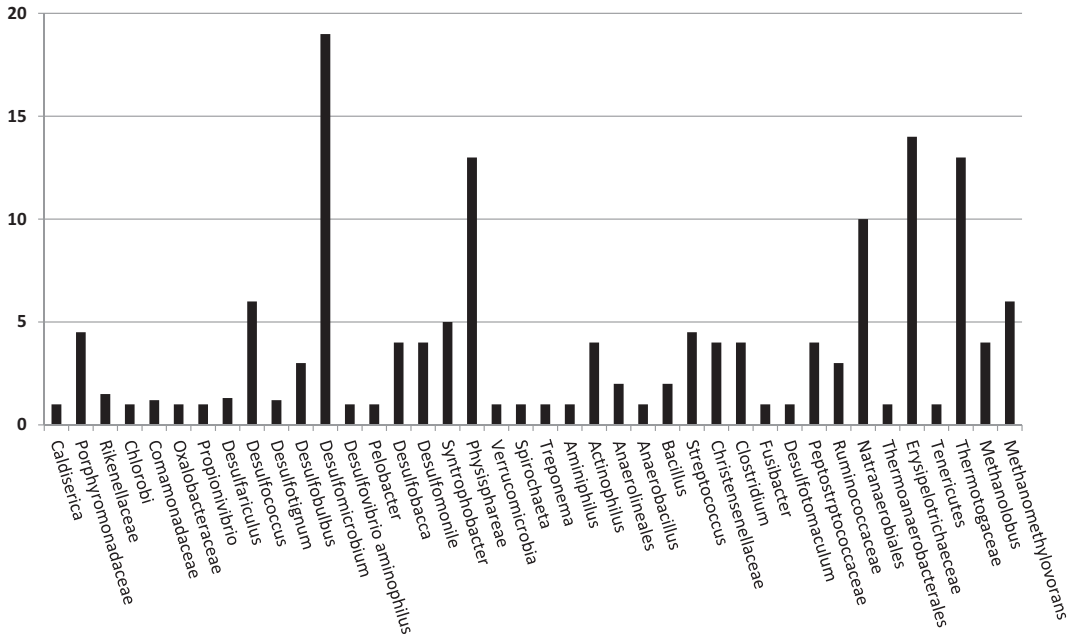

Fig. 6. Taxonomy profile showing the number of reads (%) at the genus level.

found to be nearly 8-fold higher than that in the control in two of the sampling sites of Mandoli, i.e., the disposal area and the area of open burning site. Nickel was also found to be significantly higher than that in the control, with approximately 10-fold higher concentration in one of the sampling sites than that in the control, i.e., the disposal site of Loni, and approximately 15-fold higher in the area of open burning site than that in the control, i.e., in Mandoli. PBDE was detected in all the soil samples collected from Mandoli in the range of 6.5 to 57.5 ng g^{-1} dry weight [16]. As an extension of our previous work [16], we, therefore, carried out the present study of soil bacterial composition with the identical samples used in our previous study and stored at −20°C. Soil composite mixtures were made for Loni and Mandoli sampling sites and referred to as MD2 and MD3, respectively. Sample MD1 was taken as the reference sample and was collected from a site where there was no e-waste disposal or recycling activity.

Metagenomic DNA isolation from the contaminated samples was a challenge because of the heavy contamination of e-waste components such as pieces of circuit board. Using 500 mg of soil sample, the DNA was extracted using the commercial soil DNA isolation kit. However, DNA samples had to be electrophoresed and then purified using the gel extraction kit to remove the co-extracted humic acid. Extraction of metagenomics DNA from soil is a challenging step, and if the quality of DNA is low, it may affect the subsequent steps such as PCR and library construction. Various methods of metagenomic DNA isolation from various samples have been developed thus far [20,21].

For PCR amplification of the extracted DNA, we used the primer pair GC-338 F and 518 R, which anneal to the V3-V5 region of the 16S rRNA gene. This pair of primers is commonly used for studying the composition of bacteria in samples, as it ideally targets most taxa of bacteria. However, there is a chance of co-amplification of eukaryotic

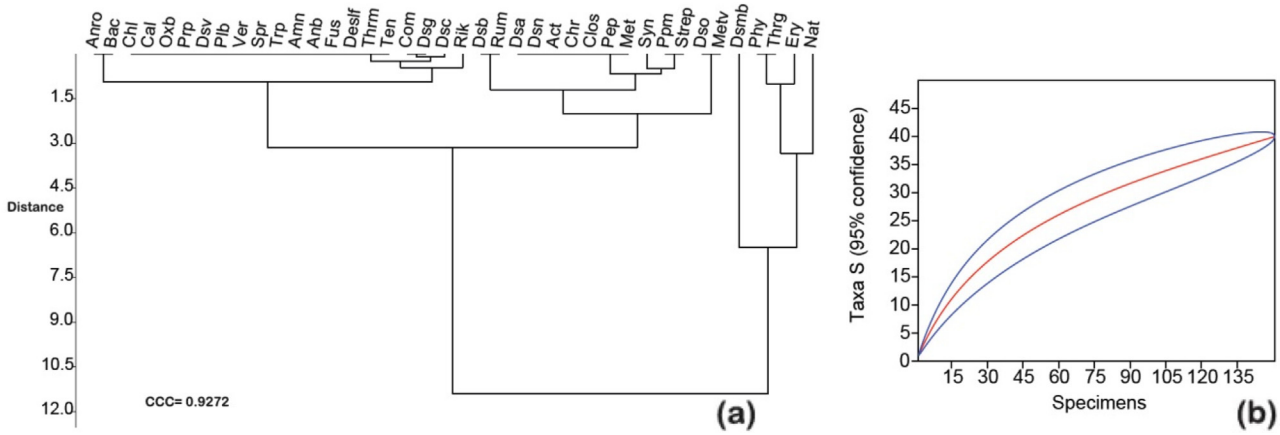

Fig. 7. (a) Cluster analysis (hierarchical clustering) of the different genera: Cal: *Caldiserica;* Ppm: *Porphyromonadaceae*; Rik: *Rikenellaceae*; Chl: *Chlorobi*; Com: *Comamonadaceae*; Oxb: *Oxalobacteraceae*; Prp: *Propionivibrio*; Dsc: *Desulfariculus*; Dso: *Desulfococcus*; Dsg: *Desulfotignum*; Dsb: *Desulfobulbus*; Dsmb: *Desulfomicrobium*; Dsv: *Desulfovibrio aminophilus*; Plb: *Pelobacter*; Dsa: *Desulfobacca*; Dsn: *Desulfomonile*; Syn: *Syntrophobacter*; Phy: *Physisphareae*; Ver: *Verrucomicrobia*; Spr: *Spirochaeta*; Trp: *Treponema*; Amn: *Aminiphilus*; Act: *Actinophilus*; Anro: *Anaerolineales*; Anb: *Anaerobacillus*; Bac: *Bacillus*; Strep: *Streptococcus*; Chr: *Christensenellaceae*; Clos: *Clostridium*; Fus: *Fusibacter*; Deslf: *Desulfotomaculum*; Pep: *Peptostreptococcaceae*; Rum: *Ruminococcaceae*; Nat: Natranaerobiales; Thrm: *Thermoanaerobacterales*; Ery: *Erysipelotrichaceae*; Ten: *Tenericutes*; Thrg: *Thermotogaceae*; Met: *Methanolobus*; Metv: *Methanomethylovorans.* (b) Individual rarefaction and 95% confidence interval of the studied sample.

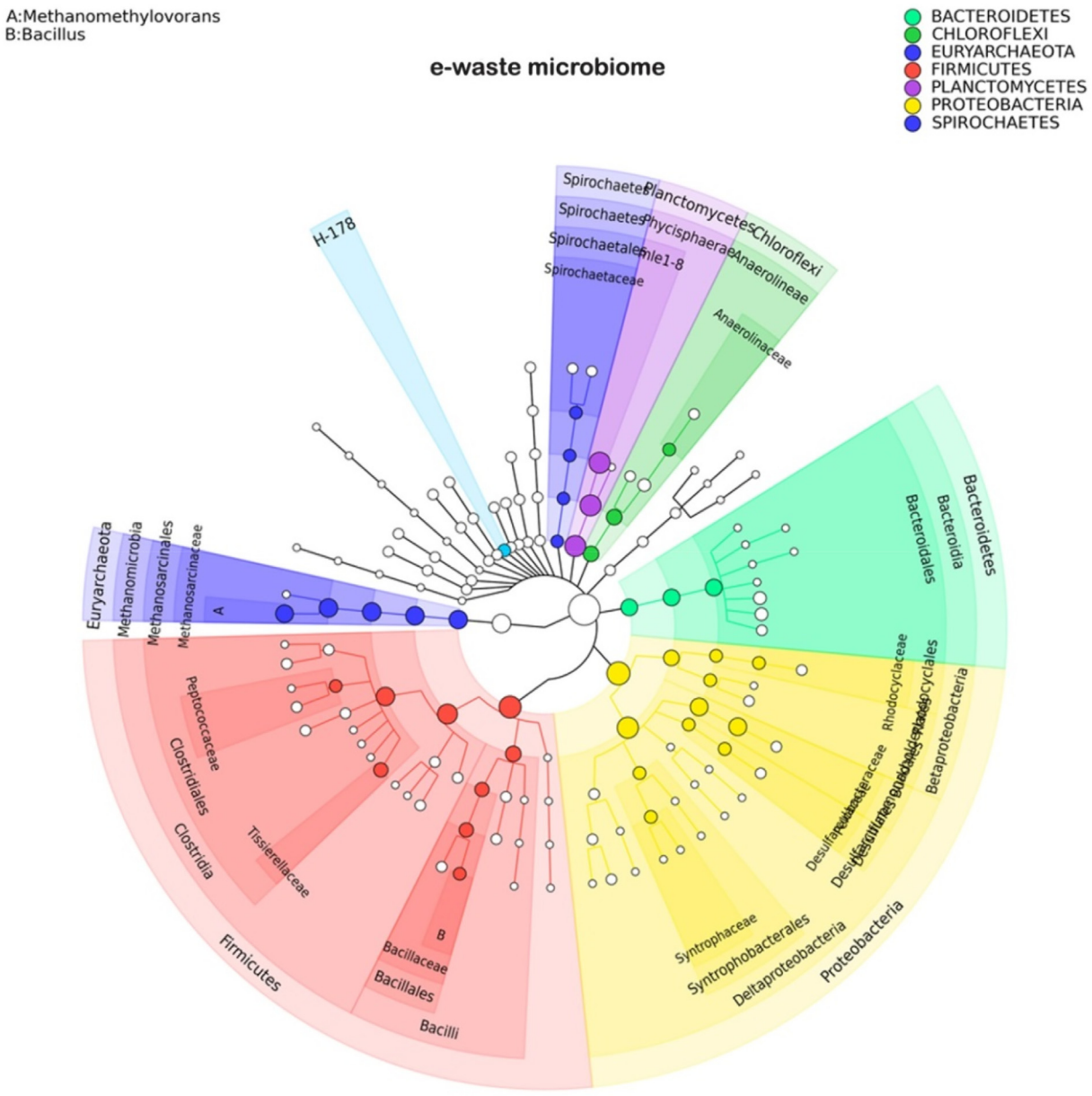

Fig. 8. Krona graph of the e-waste microbiome.

sequences, as these primer sequences have similarity to conserved regions in eukaryotic rRNA genes [22]. Therefore, in the present study, we used another primer set, i.e., GC 342 F-806 R, which amplifies only bacterial and archaeal 16S rRNA gene sequences and, therefore, can omit the amplification of eukaryotic rRNA genes [23]. Moreover, a comparison of the bacterial composition could be drawn out by using two different sets of primers. PCR amplification of the 16S rRNA gene with the primer sets GC 338 F-518 R and GC 342 F-806 R yielded amplicons of 200 bp and 500 bp, respectively.

DGGE analysis after optimization showed band patterns at gradients ranging from 20% to 50% denaturing condition for samples MD1 and MD2 and 30–50% for sample MD3. A denaturing gradient of 40–80% (100% denaturant with 7 M urea, 40% (v/v) formamide) was used in DGGE analysis of bacterial communities from e-waste-contaminated samples in one report [23]. In another report on bacterial community analysis of e-waste-contaminated soil using DGGE, the amplified DNA was separated on a 10% acrylamide gel with a denaturant gradient ranging from 35% to 60% [24].

The DGGE community profiling was carried out to compare the microbial composition of the samples; and the results showed that the number of predominant bacterial groups was decreased in the samples contaminated with e-waste pollutants as shown by the decreasing number of bands in samples MD2 and MD3 as compared to that in the reference sample MD1. The results showed that *Proteobacteria* was the predominant group in all samples. In our earlier study, the effect of e-waste pollutants on soil enzyme activities was studied, and soil dehydrogenase, β-glucosidase, and arylsulfatase activities were largely reduced in the e-waste-contaminated sites as compared to those of the control [25]. Reduced soil enzyme activities in relation to soil contamination with organic pollutants and heavy metals have been reported by many authors [26,27]. DGGE band pattern when compared with the control also shows that the number of predominant bacterial groups is decreased in the e-waste-contaminated soil. On the contrary, in one report, it was observed that e-waste pollution altered the bacterial composition by promoting species richness and diversity. It was observed that the diversity was

not decreased when compared with that in the noncontaminated site, and *Proteobacteria* and *Firmicutes* were the abundant phyla in the PAH [23].

In another study, the microbial analysis of an e-waste recycling site of the Taizhou region of China revealed that there was no apparent correlation between the microbial community and the pollutants. However, through DGGE analysis, it was found that contamination with heavy metals and PCBs had a slight influence on soil microbial community [28].

Sequencing of the excised DGGE gel bands from sample MD2 revealed the presence of bacteria having a high sequence similarity (90–99%) to *Pontibacter odishensis* strain JC130, *Aureimonas glaciistagni* strain PAMC 27157, *Arthrobacter globiformis* strain DSM 20124, *Arthrobacter parietis* strain LMG 22281, *Nitratifactor salsuginis* strain DSM 16511, *Aeromicrobium choanae* strain 9H-4, while sequences from sample MD3 showed the presence of bacteria similar to *Pantoea theicola* strain QC88-366, *Arthrobacter tumbae* strain LMG 19501 [29] and uncultured *GammaProteobacterium* strain CWEG_LIB158 (Table S1). Apart from the above data, the general outline of the e-waste microbiome determined through whole metagenome sequencing showed that there were a significantly higher number of sequences classified under the phyla *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Euryarchaeota*, *Planktomycetes*, *Spirochaetes,* and Candidate division H-178 (unclassified division). Most abundant phyla were *Firmicutes* and *Proteobacteria*. Bacilli and Clostridia were the most common forms under *Firmicutes,* and *Deltaproteobacteria* and *Betaproteobacteria* were the most common classes under *Proteobacteria*. This finding is similar to that determined for the analysis of e-waste-contaminated soils collected from Guiyu, China, where *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, and *Firmicutes* were the dominant phyla of the e-waste-affected communities. The most commonly found bacteria were *Acinetobacter*, *Pseudomonas*, and *Alcanivorax* which are known to be organic pollutant-degrading bacteria [15].

Among the bacteria identified by sequencing of the DGGE bands, band 10 having 92% similarity with that of *Pontibacter odishensis* may have bioremediation properties. *Pontibacter* spp. have been reported from hexachlorocyclohexane and lindane-contaminated soils and dumpsites and may have the ability to degrade organic pollutants [25,30,31,32]. Band 12 shows 99% similarity with *Arthrobacter globiformis,* and it has been reported that this bacterium has been used to study at field-scale for the bioremediation of a farmland contaminated with dichlorodiphenyl trichloroethanes (DDT) and PAH in Shenyang North New Area of China [33]. It was observed that removal of DDT and PAH after addition of surfactants at an optimized concentration was 64.3% and 35.6%, respectively, at 150 days. *Arthrobacter globiformis* has also been reported to be able to detoxify Hg and hexavalent Cr with high efficiency showing its bioremediation potential of heavy metal-contaminated sites [24]. Bands 14 and 17 showed 99% similarity to that of *Arthrobacter tumbae,* which has been reported as a Cu-tolerant root nodule endophytic bacterium. *Arthrobacter tumbae* MYR1 has been shown to exhibit maximum copper removal of 84% in a study on bioremediation of copper-contaminated soil using rhizospheric bacteria [34]. Bands 15 and 18 showing 86% similarity to that of uncultured γ-proteobacterium may also contain various bacterial species able to degrade PAH and PBDE [35].

5. Conclusion

The present study provides a general account of the bacterial composition of soil samples collected from e-waste-contaminated sites of Mandoli and Loni regions of Delhi, NCR. Thus far, no studies have been conducted to describe the bacterial community structure of these e-waste-contaminated sites where illegal dumping and burning of e-waste have been continued for many years. The study showed that, although there is a decrease in the number of predominant bacterial groups in the contaminated soils as compared to that in the reference soil, the emergence of bacterial groups tolerant to the e-waste pollutants is predicted. Furthermore, a thorough investigation is required to assess novel bacteria able to remediate the combined toxic effects of heavy metals and PCBs.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

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