Folia Microbiologica (2020) 65:995–1007
https://doi.org/10.1007/s12223-020-00808-2

ORIGINAL ARTICLE

16S rRNA molecular profiling of heavy metal tolerant bacterial communities isolated from soil contaminated by electronic waste

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Received: 19 February 2020 / Accepted: 29 June 2020 / Published online: 21 July 2020

Abstract

Electronic waste is an evolving source of harmful pollutants in our surrounding environments and considered to be perilous as it contains toxic metals such as chromium, cadmium, lead, mercury, zinc, and nickel in huge quantities. Heavy metals are harmful contaminants and accumulated in the environment due to various anthropogenic activities. The present study was conducted to isolate and characterize different heavy metal tolerant bacterial species, based on molecular techniques from soil contaminated by electronic waste. The contaminated soil samples were analyzed for various physicochemical properties such as pH, electrical conductivity, soil moisture, water holding capacity, organic carbon, organic matter, available phosphorus, total nitrogen, and potassium using standard procedures. The soil samples were found to contain a higher amount of different heavy metals such as copper, chromium, lead, iron, cadmium, and nickel. Serial dilution and spread plate techniques have been used for bacterial isolation. The identification and molecular characterization of isolated bacterial species were done by biochemical tests and 16S rRNA gene sequencing technique. The 16S rRNA sequencing analysis confirmed the presence of different bacterial species as, *Micrococcus aloeverae*, *Kocuria turfanensis*, *Bacillus licheniformis*, *Bacillus jeotgali*, *Bacillus velezensis*, and *Bacillus haikouensis*. The findings indicated that the e-waste dumping sites are the storehouse of elite bacterial species. The present research study offers a platform for systematic analysis of e-waste sites by microbial profiling that may help in the innovation of novel microorganisms of scientific importance and better biotechnological potential.

Keywords Electronic waste · Heavy metals · Microbial diversity · 16S rRNA gene sequencing · Phylogenetic tree analysis

Introduction

Electronic industries are releasing a huge amount of electronic waste (e-waste) in the environment at a rate of 20–50 million tons per year (Duan et al. 2009; Wang et al. 2013). According to the assessment of the United States Environmental Protection Agency (USEPA), only 15–20% of e-waste can be recycled, whereas the remaining parts are often disposed of in landfills (USEPA 2012). E-waste is supposed to be very diverse and composite that significantly involves disposed electrical and electronic parts (Kiddee et al. 2013; Heacock et al. 2016). E-waste usually comprises a high amount of toxic substances including organic pollutants (e.g., polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs)) and heavy metals (Pb, Zn, Cu, and Cd) (Kumar 2018). Therefore, if these e-wastes are disposed of improperly, these harmful substances pose a severe risk to the environment as well as human health. The constituents of e-waste are chemically and physically distinct from other municipal or industrial wastes which pose a different type of health risk. The chemical compositions of e-waste depending on the age and type of the discarded items mostly composed of a mixture of heavy metals attached to, covered with, or mixed with various types of plastics and ceramics (Hoffmann 1992; Robinson 2009).

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12223-020-00808-2) contains supplementary material, which is available to authorized users.

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Eventually, extensive contamination of heavy metals created from different modern and rural activities is responsible for human health hazards as well as biological communities (Nriagu and Pacyna 1988; Kumar and Fulekar 2017; Kumar and Fulekar 2018; Singh et al. 2019). The e-waste has been transported to developing countries such as China, India, and Pakistan to handle the recycling and disposal due to the less stringent environmental regulations (Wu et al. 2019). E-waste causes serious environmental pollution in developing countries due to uncontrolled recycling and unregulated disposal methods (Leung et al. 2006). Most of the e-waste in the developing countries are treated illegally with primary techniques, including open burning and acid digestion, to recover the precious metals. These activities could be eradicated by regulations and enforcement (Lu et al. 2015).

Even in developed countries, e-waste processing may include serious hazards to workers and societies. After the e-waste recycling process, recycling sites can further threaten public health due to their persistence in the environment (Wu et al. 2015). Therefore, it is needed to have a safe transfer of e-waste turning into a significant environmental concern due to the presence of different harmful organic and inorganic compounds (Bhattacharya and Khare 2016). Many research studies have been performed to explore the considerable effects of heavy metals on the microorganisms isolated from various habitats (Duxbury and Bicknell 1983; Jonas 1989; Hiroki 1992; Jansen et al. 1994).

Several studies have indicated that environmental pollution by heavy metals may cause drastic changes in the composition and activity of the microbial community which have a significant correlation between microbial diversity and contamination gradients (Li et al. 2006; Shentu et al. 2008). Some toxic metals (As, Cd, Hg, and Pb) are dangerous to humans as well as nature and may cause severe deterioration (Gall et al. 2015). Apart from these heavy metals, Cu, Ni, Fe, Zn, and Mn are essential for humans (Kumar et al. 2017; Rai et al. 2019). The structure and variety of microbial networks are an ample pointer of the impact of anthropogenic activities on soil nature.

Since just less than 1% of soil microorganism is cultivable by existing tools, the configuration of microbial populations by incubation-independent tools was frequently studied to explore the impact of anthropogenic activities on the soil quality (Renella et al. 2005; Wang et al. 2007). Researches have confirmed that the presence of toxic metals and organic substances might influence the biological network in the soil (McGrath et al. 2001; Baker et al. 2001; Lasat 2002; Suhadolc et al. 2004; Wang et al. 2007; Lee et al. 2010).

The present research study deals with the physicochemical properties of soil collected from e-waste dumping sites and also includes the isolation of heavy metal tolerant indigenous microorganism and their biochemical and molecular characterization using 16S rRNA techniques. The study delivers an inclusive phylogenetic look of the microbial assemblies in heavy metal polluted soils. This reveals that the contamination due to inappropriate e-waste dumping may fundamentally cause changes in soil microbiota. The present study recommends that the pollution concern of heavy metals may affect microbial activity.

Materials and methods

Description of the study area, sampling procedure, and analytical methodology

The present study has been conducted at different e-waste dumping sites close to Ahmedabad railway station. Ahmedabad, the modern capital of Gujarat, lies at 23.03°N latitudes 72.58°E longitudes on the bank of River Sabarmati, with a height of 49 m above mean sea level. The soil samples were collected from the depths of (15–30 cm) at three different sampling locations using stainless steel scoop and transferred into airtight bags and transported to the laboratory for physicochemical and microbial study. Soil samples were preserved at 4 °C to avoid any deviation and contamination for microbial characterization. The sample's pH was estimated (1:2.5 w/v) by digital pH meter (Woermann 1973); electrical conductivity was determined (1:2.5 w/v) by conductivity meter. Organic carbon and organic matter were calculated as per the methodology described by Osuji and Adesiyan (2005), while available phosphorus was determined by Brays No. 1 method (Kovar and Pierzynski 2009). Soil moisture, water holding capacity, nitrate, and potassium concentrations were also analyzed as per the standard methods.

Heavy metal analysis in e-waste contaminated soil samples

Soil samples were prepared for heavy metal analysis by taking 1 g of a well-normalized sample and 10 mL of freshly prepared aqua regia (HNO_3 + HCl, i.e., ratio 1:3). The mixture was heated for 4 h at a temperature of 120 °C on a hot plate. The solution was cooled and filtered using Whatman filter paper No. 42. The filtered solution was then diluted to 20 mL with distilled water. The concentrations of Cr, Pb, Cd, Fe, Cu, and Ni were calculated using Perkin Elmer Optima 7300 DV ICP (OES). The samples were analyzed in triplicate and the concentrations of the metals were reported in mg/kg.

Isolation and enumeration of bacteria

Isolation and enumeration of microorganisms were achieved by serial dilution procedures using agar media. Soil sample of 1 g was taken and serially diluted in 5 test tubes each

containing 9 mL of sterile distilled water in the order of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. For this, 1 mL of the solution was transported to 10^{-2} from 10^{-1} dilution. Then 1 mL was transferred to 10^{-3} from 10^{-2} dilution, then to 10^{-4} from 10^{-3} dilution and then to 10^{-5} from 10^{-4} dilution. After that, 1 mL of the solution was taken from all dilutions and transported onto agar plates and kept at 37 °C for 24 h. The isolated bacterial colonies were refined by frequent streaking and well-preserved in slants at 4 °C.

Morphological and biochemical characterization of bacterial isolates

The isolated bacterial strains were grown on nutrient agar media and gram stained according to the procedure (Cappuccino and Sherman 2002). The bacterial isolates were smeared in the slide and heat-fixed. The crystal violet dye was added, kept for a minute, and washed out in running water. After that, Gram's iodine was added and washed in running water after a minute then the smear was decolorized with ethanol, and finally, the counterstain safranin was added after a minute and washed in running water. The color and shape of the bacterial strains were observed under the binocular microscope. The different biochemical characteristics such as xylose, maltose, fructose, dextrose, trehalose, sucrose, L-arabinose, glycerol, inositol, melezitose, citrate utilization, lactose, esculin hydrolysis, amylase, gelatinase, urease, oxidase, catalase, indole production, methyl-red (MR) test, Voges-Proskauer (VP) test, and nitrate reduction test were performed by KB001 Biochemical Test Kit (HI Media, India).

Molecular classification of isolated bacterial species

The 16S rRNA sequences were achieved by NCBI BLAST tools and the accession numbers were assigned by Gujarat State Biotechnology Mission, Gandhinagar, Gujarat, India. The isolated bacterial strains have been deposited in the National Center for Biotechnology Information, US National Library of Medicine.

Extraction of genomic DNA

For the extraction of Genomic DNA, 1 mL of broth culture was centrifuged at 5000 rpm for 10 min. The pellet was again suspended in 200 μL of distilled water and 200 μL of buffer-saturated phenol was added and incubated at 60 °C for 1 h and centrifuged at 8000 rpm for 5 min. Again 400 μL of chilled ethanol was added to the aqueous stage for DNA precipitation. The pellet of precipitated DNA was washed with 70% ethanol and resuspended in nuclease-free water (Das et al. 2019).

Amplification of 16S rRNA gene using PCR

The extracted DNA was used for the amplification of 16S rRNA gene fragment with primers A109 (F) AC(G/T) GCTCAGTAACACGT and 1510 (R) GGTTACCT TGTTACGACTT (Birbir et al. 2007; Mani et al. 2012). PCR reaction combination contained 10 × Taq buffer, 10 mM of dNTPs, 2-mM $MgCl_2$, 10 μM of each primer, 1 μL of DNA, and 2 U Taq Polymerase. The reaction was initiated by denaturation at 94 °C for 5 min followed by 35 cycles of denaturation, annealing, and elongation. The reaction was terminated after a final elongation. The amplified product was exposed to electrophoresis on 1.5% agarose gel. The refined product was sequenced bidirectionally, using an automated DNA sequencer.

Construction of phylogenetic tree analysis

The phylogenetic tree was constructed using 16S rRNA sequences obtained from isolated bacteria in FASTA format. The sequences closely related to the bacteria of the current study were obtained from the NCBI and aligned using Clustal W. The phylogenetic tree was prepared using MEGA software version 7.0 and the maximum likelihood method (Tamura and Nei 1993; Kumar et al. 2016).

Statistical analysis

The physicochemical properties and heavy metals' characterization were analyzed in triplicates and the results were described as mean with standard deviations (±SD). Pearson's correlation coefficient was determined to establish the affiliation between the physicochemical properties of the soil and among the heavy metals present in the soil. Pearson's correlation coefficient was executed by Origin software.

Accession numbers of the nucleotide sequence

The nucleotide 16S rRNA gene sequences of six bacterial strains were submitted to the National Center for Biotechnology Information database of GenBank under the accession numbers MH665549, MH665548, MH714558, MH665547, MH714559, and MH714557.

Results

Physicochemical analysis of e-waste contaminated soil samples

The characterization of different bacterial species isolated from e-waste contaminated soil was attempted in the present study. The growth of microorganisms relies on the different

physicochemical properties of soil such as pH and temperature. Therefore, various conditions of any particular habitats from where the microorganisms are being isolated must be considered. The physicochemical properties of the contaminated soil samples were highly varied (Table 1). The pH of the contaminated sites was observed slightly basic with an average of 7.46. Electrical conductivity was observed highest at site 2 tailed by site 1 and site 3 with an average of 2.46 mS/cm. The water holding capacity was found to be higher at site 1 as compared with site 2 and site 3 with an average of 39.50%. Organic carbon and organic matter were perceived with an average of 1.45% and 2.49%, respectively. The total nitrogen in the soil was found with an average of 0.12%, whereas the available phosphorus and potassium were found 1.74 mg/kg and 60.86 mg/kg respectively.

Two-tailed test of the significance level of $P < 0.05$ was used to analyze the correlation coefficient among the various physicochemical characteristics of the soil samples (Table 2). A significant positive relationship was found between the pairs of some physicochemical properties of soil as, soil pH with soil moisture ($r = 0.99$), electrical conductivity with potassium ($r = 0.99$). Water holding capacity is showing highly positive correlation with organic carbon ($r = 0.99$), organic matter ($r = 0.99$), and total nitrogen ($r = 0.99$). Some other properties are showing a negative correlation with each other.

Distribution of heavy metals

Heavy metal concentrations were found higher than the USEPA screening standards. The findings specify that the collected soil samples were enormously polluted by the higher concentration of different heavy metals. The predominant toxic metals in the contaminated soil were copper, cadmium, chromium lead, iron, and nickel. The average concentrations that were perceived for these metals are presented in Fig. 1. The values for Cu, Cr, Pb, Fe, Cd, and Ni were (152.74 ± 0.44 mg/kg), (151.59 ± 0.47 mg/kg), (65.81 ± 0.68 mg/kg), (15.58 ± 0.33 mg/kg), (14.86 ± 0.54 mg/kg), and (14.54 ± 0.46 mg/kg), respectively.

Similarly, a two-tailed test of the significance level of ($P < 0.05$) was used to analyze the correlation coefficient among the various heavy metals (Table 3). Significant positive correlations were observed between the pairs of some heavy metals in the contaminated soil samples. The correlation analysis revealed that copper showed a highly positive correlation with chromium ($r = 0.99$) and nickel ($r = 0.98$). A positive correlation was also observed between iron and lead with a correlation coefficient of 0.87. Also, chromium has shown a very high positive correlation with nickel having a correlation coefficient of 0.99.

Table 1 Characterization of soil samples at three different sites with their mean values

Parameters	Site 1	Site 2	Site 3	Mean ± SD
pH	7.22	7.44	7.72	7.46 ± 0.25
EC (mS/cm)	2.30	4.16	0.91	2.46 ± 1.63
Soil moisture (%)	3.58	5.88	8.04	5.83 ± 2.23
Water holding capacity (%)	41.48	39.99	37.02	39.50 ± 2.27
Organic carbon (%)	1.55	1.47	1.32	1.45 ± 0.12
Organic matter (%)	2.67	2.52	2.27	2.49 ± 0.20
Total nitrogen (%)	0.133	0.126	0.113	0.12 ± 0.01
Available phosphorus (mg/kg)	1.82	1.51	1.89	1.74 ± 0.20
Potassium (mg/kg)	58.65	79.91	44.03	60.86 ± 18.04

Morphological characteristics of bacterial isolates

The bacterial strains were isolated from the e-waste contaminated soil samples and several morphological properties such as size, shape, pigmentation, margin, surface, consistency, and optical characteristics were observed (Table 4). The identified isolates were *Micrococcus aloeverae*, *Kocuria turfanensis*, *Bacillus licheniformis*, *Bacillus jeotgali*, *Bacillus velezensis*, and *Bacillus haikouensis*.

Small subunit rRNA gene sequencing and phylogenetic analysis indicated that isolated bacteria belonged to three different genera including *Micrococcus*, *Kocuria*, and *Bacillus*. *Micrococcus aloeverae* was observed as a yellow gram-positive, non-motile, non-endospore forming, and spherical endophytic actinobacterium that can be found in many places such as the human skin, water, dust, and soil and mostly considered a harmless microorganism (Fig. 2 a). *Kocuria turfanensis* was found to be a gram-positive, non-motile, coccoid cell organized in pairs, short chains, tetrads, and irregular clusters that can be found in several environmental and ecological niches and usually considered as a non-pathogenic bacteria (Fig. 2 b). *Bacillus licheniformis* was observed as saprophytic gram-positive, rods with rounded ends, motile bacterium generally found in soil with finely wrinkled, dull, opaque, adherent colonies (Fig. 2 c). *Bacillus jeotgali* was perceived as a gram-variable, rod-shaped, endospore-forming bacterial strain, which is motile with peritrichous flagella (Fig. 2 d). The colonies of the strain were smooth, irregular, flat, and cream-yellow colored. *Bacillus velezensis* was found to be a gram-positive, rod-shaped, endospore-forming bacterium (Fig. 2 e). *Bacillus haikouensis* was observed to be a gram-stain positive, rod-shaped, endospore-forming, motile bacterium with peritrichous flagella (Fig. 2 f). The colonies of *Bacillus haikouensis* grown on nutrient agar medium at 37 °C for 48 h are circular, convex, and orange-red.

Table 2 Pearson's correlation coefficient (*r*) among physicochemical factors

	pH	EC	SM	WHC	OC	OM	TN	AP	K
pH	1								
EC	− 0.48	1							
SM	0.99*	− 0.40	1						
WHC	− 0.99	0.58	− 0.97	1					
OC	− 0.99	0.57	− 0.98	0.99*	1				
OM	− 0.99	0.55	− 0.98	0.99*	0.99*	1			
TN	− 0.99	0.57	− 0.98	0.99*	0.99*	0.99*	1		
AP	0.24	− 0.96	0.15	− 0.35	− 0.34	− 0.31	− 0.33	1	
K	− 0.46	0.99*	0.38	0.57	0.55	0.53	0.55	− 0.97	1

Biochemical characterization of isolated bacterial colonies

Several biochemical analyses were carried out to describe the isolated bacterial strains. All the bacterial isolates were found to be gram-positive. The biochemical studies indicated that isolates PK-1 (*Micrococcus aloeverae*) and PK-4 (*Bacillus jeotgali*) were negative for citrate utilization test, while PK-3 (*Bacillus licheniformis*) and PK-5 (*Bacillus velezensis*) were showing variability to citrate test. Isolate PK-2 (*Kocuria turfanensis*) was positive for the citrate test (Table 5). All the isolates were negative for indole production test. Isolates PK-1 (*Micrococcus aloeverae*), PK-3 (*Bacillus licheniformis*), and PK-5 (*Bacillus velezensis*) were negative for the Voges-Proskauer test, while PK-2 (*Kocuria turfanensis*) was positive for the Voges-Proskauer test. Strain PK-1 (*Micrococcus aloeverae*), PK-2 (*Kocuria turfanensis*), PK-3 (*Bacillus licheniformis*), and PK-5 (*Bacillus velezensis*) were positive for nitrate reduction ability. Table 5 shows the utilization of different substrates by bacterial isolates and the utilization of lactose and L-arabinose. Isolates PK-1 (*Micrococcus aloeverae*), PK-2 (*Kocuria turfanensis*), PK-3 (*Bacillus licheniformis*), PK-4 (*Bacillus jeotgali*), and PK-6 (*Bacillus haikouensis*) were positive for trehalose, while PK-5 (*Bacillus velezensis*) showed variability.

Molecular (16 S rRNA) profiling of isolated bacterial strains

The gene sequences of *Micrococcus aloeverae, Kocuria turfanensis, Bacillus licheniformis, Bacillus jeotgali, Bacillus velezensis,* and *Bacillus haikouensis* were submitted to the National Center for Biotechnology Information (NCBI) under accession number MH665549, MH665548, MH714558, MH665547, MH714559, and MH714557, respectively. The molecular characterization of bacterial strains based on the 16S rRNA gene sequencing was presented in Table S1.

Phylogenetic tree

The sequences have been studied to define the relationships between the development and nomenclature using the phylogenetic tree. The partially enlarged sequences of the 16S rRNA gene from isolates (PK-1 to PK-6) were associated to find the closest match using the Basic Local Alignment Search

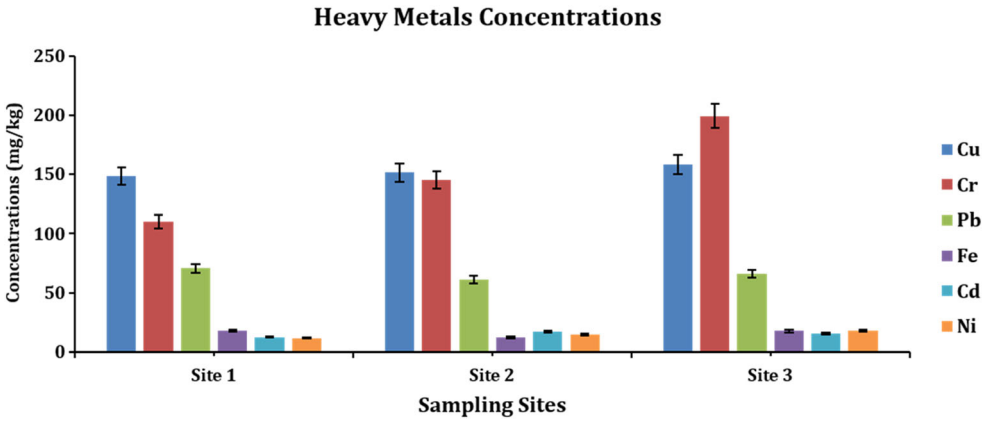


Fig. 1 Mean concentrations (mg/kg) of different heavy metals

Table 3 Pearson's correlation coefficient (*r*) among heavy metals

	Fe	Cu	Cd	Cr	Pb	Ni
Fe	1					
Cu	0.22	1				
Cd	− 0.84	0.34	1			
Cr	0.10	0.99*	0.45	1		
Pb	0.87	− 0.28	− 1.00	− 0.39	1	
Ni	0.04	0.98*	0.50	0.99*	− 0.45	1

Tool (BLAST). The BLAST analysis revealed that the genes were 98.74% similar to *Micrococcus aloeverae* (MH665549; PK-1), 100% similar to *Kocuria turfanensis* (MH665548; PK-2), 100% similar to *Bacillus licheniformis* (MH714558; PK-3), 100% similar to *Bacillus jeotgali* (MH665547; PK-4), 99.86% similar to *Bacillus velezensis* (MH714559; PK-5), and 99.22% homologous to *Bacillus haikouensis* (MH714557; PK-6). The construction of a phylogenetic tree was done by aligning the 16S rRNA sequences of different bacterial colonies (Fig. 3).

Discussion

In the present study, six different bacterial species, *Micrococcus aloeverae, Kocuria turfanensis, Bacillus licheniformis, Bacillus jeotgali, Bacillus velezensis,* and *Bacillus haikouensis*, were isolated from heavy metal contaminated soil. The isolated bacterial species were further characterized by different biochemical tests and molecular techniques.

Heavy metal contamination posed a significant influence on microbial diversity. The assessment of microbial diversity is an imperative indicator of soil quality. The physicochemical properties and soil microbial community have been related to the soil enzyme activities (Waldrop et al. 2000; Caldwell 2005). The occurrence of microbial population specifies the deprivation of the discarded ingredients. The soil contamination due to the e-waste recycling activities linked to the higher concentrations of heavy metals and other harmful toxins such as PCBs and PBDEs. The mixture of harmful toxic substances in the soil was altogether higher than the other polluted soils (Liu et al. 2015). Modern studies are dedicated to both single and consolidated impacts of individual metals or individual congeners of natural contamination on microbial communities of the soil (Muhammad et al. 2005; Zhu et al. 2010; Zhang et al. 2012).

Soil pH and nutrient properties

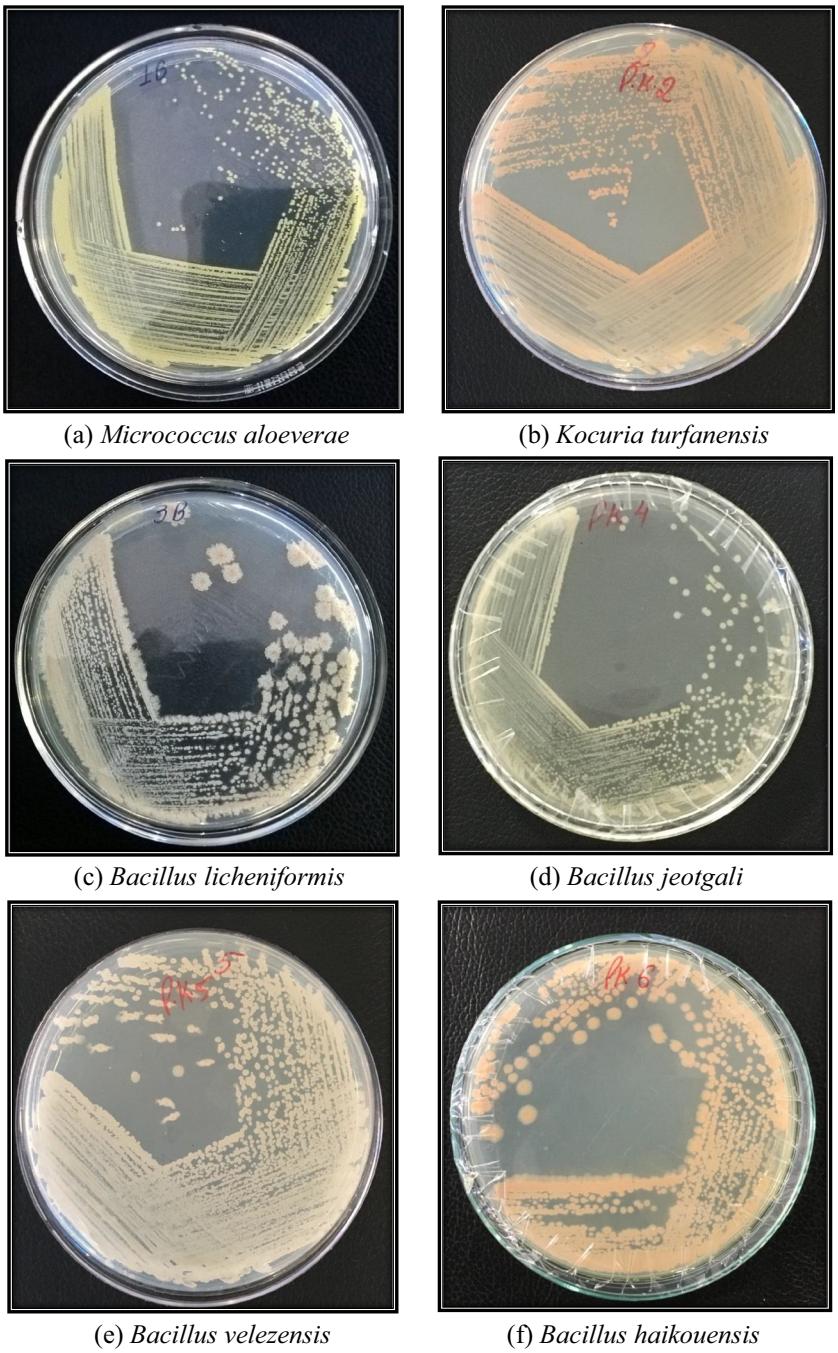
The soil properties which were characterized by analyzing soil organic carbon, organic matter, total nitrogen, available phosphorus, potassium, and pH were found to be quite different. The soil was found marginally alkaline due to immense humus accumulation. The soil pH is mainly influenced by the conversion of soluble phosphate, nutrients discharge, and bacterial activities in the soil (Mahajan and Billore 2014). However, the pH value specifies the suitability of the soil as a landfill for wastes rich in heavy metals. The higher pH in the soil may affect the concentration of heavy metals in the soil. The greater pH favors the heavy metal retention in the soil (Nath 2013). The electrical conductivity varied from 0.91 to 4.16 mS/cm with an average value of 2.46 mS/cm. The moderate electrical conductivity might be attributed to the discharge of salts to the inferior horizon.

The soil organic carbon was found low due to the high temperature and more aeration that occurred in the soil, and this raises the oxidation rate of organic matter (Singh and Mishra 2012). Total nitrogen in the soil samples varied from 0.113 to 0.133% with a mean of 0.12%. The little value of nitrogen was found due to the low organic carbon content in the soil. A highly positive correlation ($r = 0.99$) was found between nitrogen content and organic carbon of the soil (Table 2). Subsequently, maximum soil nitrogen was found in the organic form; thus, a positive correlation trend was observed.

Table 4 Morphology of isolated bacterial strains found in the soil samples

Strain	Bacteria name	Morphology	Accession number
PK 1	*Micrococcus aloeverae*	Gram-stain-positive, yellow, round convex, small spherical, non-endospore forming	MH665549
PK 2	*Kocuria turfanensis*	Gram-positive, non-motile, coccoid cells	MH665548
PK 3	*Bacillus licheniformis*	Gram-positive, round, irregular, whitish, and medium-sized colonies	MH714558
PK 4	*Bacillus jeotgali*	Gram-variable, cream yellow or light orange-yellow, smooth, flat with irregular margins	MH665547
PK 5	*Bacillus velezensis*	Gram-positive, rod-shaped, whitish, endospore-forming	MH714559
PK 6	*Bacillus haikouensis*	Gram stain–positive, rod-shaped, light orange, endospore-forming	MH714557

The bacterial species *Micrococcus aloeverae*, *Kocuria turfanensis*, *Bacillus licheniformis*, *Bacillus jeotgali*, *Bacillus velezensis*, and *Bacillus haikouensis* have been determined by 16S rRNA gene sequencing analysis



(a) *Micrococcus aloeverae* (b) *Kocuria turfanensis*

(c) *Bacillus licheniformis* (d) *Bacillus jeotgali*

(e) *Bacillus velezensis* (f) *Bacillus haikouensis*

Fig. 2 **a** *Micrococcus aloeverae.* **b** *Kocuria turfanensis.* **c** *Bacillus licheniformis.* **d** *Bacillus jeotgali.* **e** *Bacillus velezensis*. **f** *Bacillus haikouensis*

Available phosphorus was found in the range of 1.51 to 1.89 mg/kg with a mean of 1.74 mg/kg (Table 1). The availability of phosphorus in the soil might have increased due to the presence of organic matter. Approximately 50% of the phosphorus content was found in organic form and the breakdown of organic matter creates humus and forms a network with aluminum and iron and defends the phosphorus fixation (Havlin et al. 2004). The potassium varied from 44.03 to 79.91 mg/kg with an average of 60.86 mg/kg (Table 1). The availability of potassium in the soil depends on a favorable environment of the soil and the existence of organic substances (Nagaraja et al. 2014).

Microbial community profile

Six bacterial species were isolated from heavy metal contaminated soil and further characterized by their molecular profiling for identification. Many novel bacterial species were categorized into rare phyla with insignificant abundance. Among the core operational taxonomic units, which indicate the main

Table 5 Biochemical characteristics of isolated bacterial strains

Biochemical test	*Micrococcus aloeverae* PK1	*Kocuria turfanensis* PK2	*Bacillus licheniformis* PK3	*Bacillus jeotgali* PK4	*Bacillus velezensis* PK5	*Bacillus haikouensis* PK6
Gram reaction	+	+	+	+	+	+
Endospore formation	–	–	–	–	+	+
Xylose	–	+	–	–	+	–
Maltose	+	+	+	+	+	+
Fructose	–	+	+	+	+	+
Dextrose		+	–	+	–	+
Trehalose	+	+	+	+	Variable	+
Sucrose	–	+	+	Variable	+	–
L-arabinose	–	+	+	+	Variable	+
Glycerol	–	+	+	–	+	–
Inositol	–	+	–	+	–	+
Melezitose	–	+	+	+	+	+
Citrate	–	+	±	–	Variable	+
Lactose	+	–	±	–	+	–
Esculin hydrolysis	–	+	+	+	–	+
Amylase	+	–	+	+	–	+
Gelatinase	+	–	+	+	+	+
Urease	+	–	–	+	–	+
Oxidase	+	–	+	–	+	+
Catalase	+	+	+	+	–	–
Indole production	–	–	–	+	–	+
Methyl-red (MR) test		–	–	+	–	+
Voges-Proskauer (VP) test	+	–	+	–	+	+
Nitrate reduction	+	+	+	–	+	–

microorganisms in e-waste soils, the operational taxonomic units representing that *Bacillus* were the most abundant and highly capable to adjust to the pollutants. *Bacillus* may produce extremely quiescent endospores in response to ecological stress (Earl et al. 2008).

Micrococcus genus is designated as a gram-positive, non-spore-forming, non-motile, little circular bacterial species. The members of this genus have been isolated from different territories like soil, air, stimulated sludge, eternally cold samples, animal skin, waste from dairy industries, and plants tissues (Liu et al. 2000, 2007; Kloos et al. 2009; Zhao et al. 2009; Zhang et al. 2010a; Chittpurna et al. 2011; Rieser et al. 2013). Almost all the members of the genus *Micrococcus* are yellow and developed at pH 10. The specific features of this genus are an aerobic progression, high content of fatty acid (iso-C15: 0 and anteiso-C15: 0), catalase-positive cells, more G + C content (66.3–73.3 mol%), and the occurrence of MK-8 and MK-8 (H2) as the prime respiratory quinones (Wieser et al. 2002; Stackebrandt et al. 2009).

Kocuria is designated as gram-positive mainly organized in couples, quadruplicates, small chains, cubical bundles of eight and unpredictable groups, and belongs to *Actinobacteria* phylum, *Actinobacteria* class, *Actinomycetales* order, *Micrococcineae* suborder, and *Micrococcaceae* family. Miroslav Kosur, a Slovakian microbiologist, first recognized and termed this bacterium. Further 18 species of *Kocuria* were recognized based on phylogenetic studies (Kandi et al. 2016).

Bacillus velezensis was isolated in 1998 as gram-positive, PGPR (plant growth-promoting rhizobacteria) which has been first sequenced in 2007. This bacterium was designated as a spore-forming and plant-associated rhizobacteria (Reva et al. 2004) and acknowledged as a colleague of the *Bacillus subtilis* species complex (Fritze 2004), mainly comprised of *Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus licheniformis* (Gordon et al. 1973). *Bacillus amyloliquefaciens* was first isolated in 1987 and some additional bacterial strains were also originated belonging to this species (Borriss et al. 2002). Two subspecies *Bacillus amyloliquefaciens* and *Bacillus amyloliquefaciens* were distinguished with the help of an increasing number of genome sequences (Borriss et al. 2011). *Bacillus amyloliquefaciens* was presented as a later

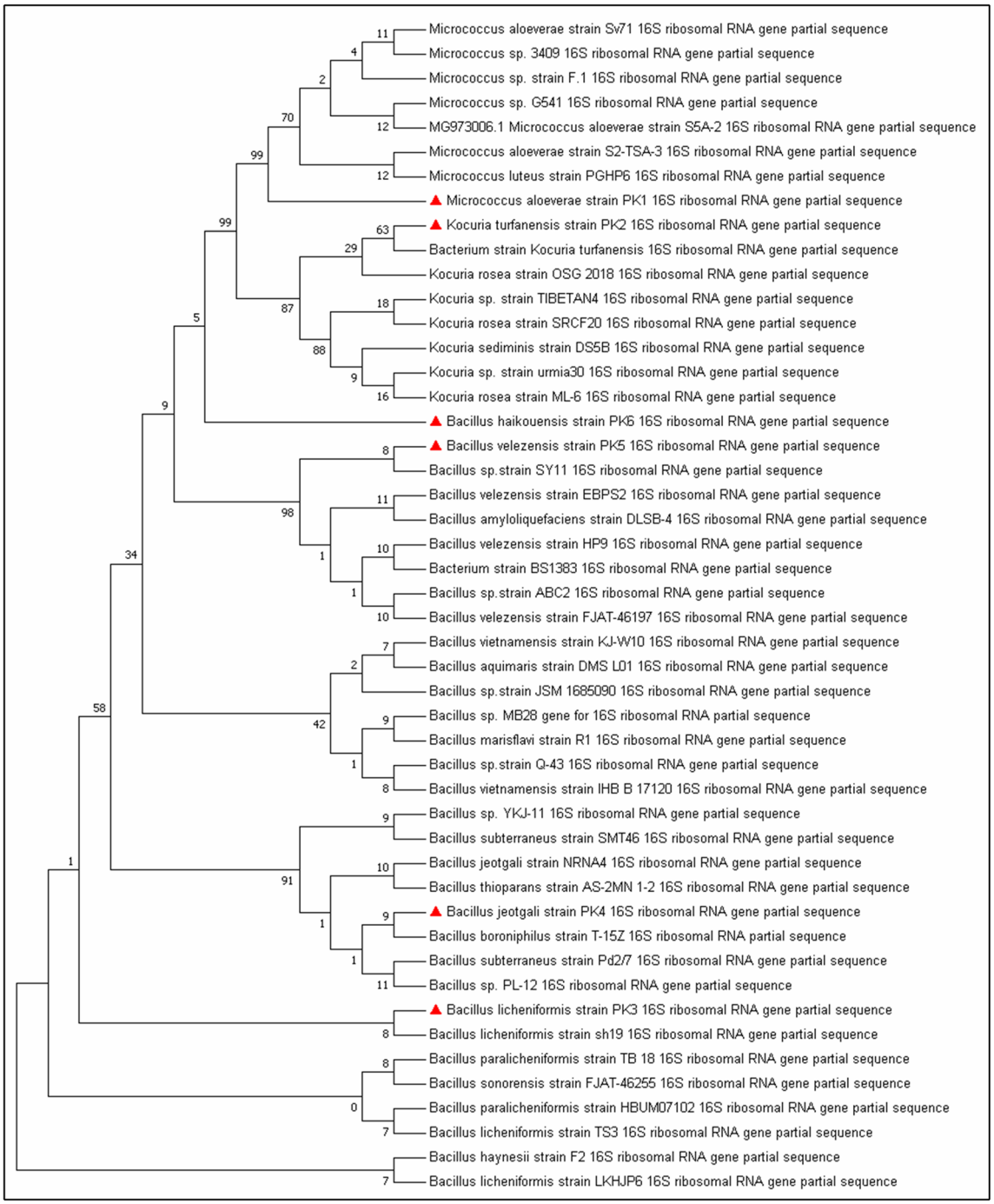


Fig. 3 Neighbor-joining phylogenetic tree based on 16 S rRNA gene sequences showing the relationship among 6 strains and some of their closest phylogenetic relatives

heterotypic substitute of *Bacillus velezensis* conferring comprehensive phylogenomic exploration (Dunlap et al. 2016).

To understand the ecology of dumping sites, it is necessary to explore the link between compositions of bacterial community and different ecological features. According to many previous research studies, the inadequate quantity of clone sequenced was excessively low to understand the collective profile of the bacterial community structure of dumping sites (Song et al. 2015). Another research study also showed the isolation and molecular characterization of various bacterial colonies based on 16S rRNA sequencing from e-waste contaminated area and confirmed the presence of *Bacillus subtilis*, *Bacillus licheniformis,* and *Bacillus badius* and reported the identified strain as *Bacillus licheniformis* with accession no. CP000002.3 (Gayatri and Shailaja 2016).

Liu et al. (2015) described the ecological effects of e-waste recycling on a variety of bacterial populations found in contaminated soil in Guiyu, China; the native bacterial groups were summarized by 16S rRNA sequencing and replica library exploration. The findings revealed major modifications in bacterial taxonomic alignment between the reference soil and polluted soil, with *Acidobacteria, Proteobacteria, Bacteroidetes*, and *Firmicutes* controlling the electronic waste–affected communities.

The bacterial diversity in the soil nearby the e-waste reprocessing plant in Taizhou, China, was examined by denaturing gradient gel electrophoresis (DGGE). The findings revealed that as the distance from the recycling workshop is increasing, the PCBs' content is decreasing. A steady variation in soil bacterial diversity was also perceived along the PCBs' contamination gradient. Additionally, the majority of *Bacteroidetes* and *Proteobacteria* were observed according to sequence study, and several extremely analogous sequences of microbes were also found to be associated with the catabolism of organic compounds and PCBs (Tang et al. 2013).

The findings of the present research work are also supported by an earlier study based on different biochemical characterizations of isolated bacterial species from electronic waste–contaminated soil of an integrated waste management industry in Coimbatore, India in which the isolate was designated as a gram-positive and spore-forming *Bacillus* sp. (Geethanjali et al. 2016).

The presence of several microbial species like *Staphylococcus*, *Pseudomonas*, *Proteus, Bacillus*, *Klebsiella*, and *Streptococcus* indicates that microorganisms are not only abundant in the environment but also nourished the soil. Numerous types of enzymes like DNase, staphylolysin, hyaluronidases, streptokinase, and staphylokinase produced by these microorganisms help in degrading the waste constituents at dumping sites (Chuku 2004).

Impacts of soil properties and pollutants on bacterial composition and diversity

The impacts of e-waste pollution on the microbial community structure and their composition at several recycling sites have been reported by various recent research studies (Liu et al. 2015). The effects of organic compounds and heavy metals are very complex in the bacterial community in a natural environment. Various physical, chemical, and biotic factors reveal this complexity (Battin et al. 2008). The soil microbial activity and composition are closely linked to soil potency and ecological features. The microbial community composition is mainly affected by the heavy metals' toxicity and diverse levels of PAHs (Wang et al. 2007; Zhang et al. 2010b). Taxonomic examination specified that b-proteobacteria and *Firmicutes* were plentiful microbial families in PAH-contaminated soils.

Microbial community structure may be affected by several ecological variables. The seasonal variation was described to be insignificant while comparing with soil physicochemical properties by earlier research studies (Krave et al. 2002; Fierer and Jackson 2006). The mobility of heavy metals might be modified by soil pH and organic matter (Calugaru et al. 2016; Pakzad et al. 2016), which may disturb the microbial community structure significantly. Organic carbon was associated with the presence of organic contaminants comprising PCBs, PBDEs, and PAHs (Calugaru et al. 2016; Jiang et al. 2016), additionally influencing microbial access to these toxins and their biological toxicity.

Conclusion

The analysis of e-waste contaminated soil has shown a significant positive relationship between the pairs of some physicochemical properties of the soil. The organic carbon content of the soil was low because of the high temperature and higher aeration in the soil which raises the oxidation rate of organic matter. The correlation coefficient between heavy metals revealed a significant positive correlation. The 16S rRNA gene sequencing has delivered an inclusive appearance at the configuration and variety of microbial populations in the soil polluted by electronic waste, which indicted that collective contamination of metals and toxic biological compounds has a significant influence on the microbiota of soil. The present study revealed that the contamination of heavy metals influences bacterial activities in the soil. The present research study demonstrated that the microbial community structure specifically 16S rRNA gene sequencing indicated the presence of different bacterial species such as *Micrococcus aloeverae*, *Kocuria turfanensis*, *Bacillus licheniformis*, *Bacillus jeotgali*, *Bacillus velezensis*, and *Bacillus haikouensis* as dominant lineages in the soil microbial assemblies. Due to the growing environmental concerns of e-waste, the possible hazards of diversified pollution to the quality and health of soil should be studied further. Such extensive knowledge would contribute to a deeper understanding of the ecological risk assessment of e-waste.

Acknowledgments The authors are also thankful to the Central University of Gujarat for providing the instrumentation facilities. The authors are also grateful to the Gujarat State Biotechnology Mission (GSBTM), Department of Science and Technology (DST), Gandhinagar, Government of Gujarat, India, for bacterial identification. The comments and suggestions by the reviewers have improved the quality of the research article. Hence the authors express heartfelt gratitude to the reviewers for their inputs.

Funding information The research work was supported by the University Grants Commission, New Delhi, India, under the scheme of Rajiv Gandhi National Fellowship (F1-17.1/2014-15/RGNF-201415-SC-UTT-69661).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Animal studies This article does not contain any data based on the experiments performed on animals.

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