

A Review on Impact of E-waste on Soil Microbial Community and Ecosystem Function

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ABSTRACT: The ever increasing pile-up of electronic waste in dumping sites, especially in developing countries such as China, Pakistan, India and several African countries, might have caused a significant alteration in the microbial community of the contaminated sites. This change in the microbial population may have significant impact to the soil ecology function. The major pollutants of electronic waste are heavy metals like cadmium, lead, nickel, mercury, hexavalent chromium, arsenic and persistent organic pollutants like polychlorinated biphenyls and polybrominated diphenyl ethers. In general, the toxic pollutants reduce the normal soil microbial biota but give rise to increase in the heavy metal resistant and organic pollutants remediating microbes. With the development of culture- independent approach as a tool for studying microbial diversity, the microbial community structures in toxic waste contaminated sites have been revealed gradually. Studies on the microbial community structure of electronic waste contaminated sites show that there are significant differences between the contaminated and the non-contaminated sites. Soil pH in the e-waste contaminated sites of various regions has been reported in a wide range varying from pH 4 to pH 12. However, the predominant phyla so far identified in the electronic waste contaminated sites, based on studies through culture independent approach, are *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, *Crenarchaeota* and *Bacteroidetes* accounting for more than 80% of the total sequence reads on an average. The genera like *Pseudomonas*, *Bacillus*, *Clostridium*, *Rhodococcus*, *Achromobacter* and many unclassified bacteria are the common types in the contaminated sites.

Keywords: Electronic waste, Microbial community, Microbial diversity, Heavy metals, Organic pollutants.

INTRODUCTION

Electronic waste (e-waste), which is also known as waste electrical and electronic equipment (WEEE), is a major type of waste especially in the developing countries due to unregulated way of disposal and many illegal recycling sites (Perkins et al., 2014). Over a period of time, due to accumulation of e-waste materials in the dumping sites, the surrounding environment gets contaminated

through leaching of harmful materials into the soil and water supply (Kiddee et al., 2013). This gives rise to bioaccumulation creating health hazards and disturbance in the ecosystem function (Zhang et al., 2010; Pinto, 2008). Various investigations have shown that the soil, air and water have been seriously polluted by heavy metals and organic pollutants due to improper disposal and recycling processes in many of the developing countries leading to many

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negative effects on human being and the environment (Awasthi et al., 2016). The major concern about e-waste is that, in the developing countries, people are unaware about the harmful effects of e-waste and also there is lack of strict legislation for the management of e-waste. Therefore, illegal recycling units, improper handling and disposal of electronic waste still exist in many of the developing countries (Saritha et al., 2015). So far, there are very less reports on the study of the microbial community structure in the e-waste contaminated sites and in the present scenario it is very much necessary to investigate further and get a clear knowledge about the microbial diversity and functions in these contaminated sites. Also, understanding the effect of harmful pollutants on the environment and management thereof, considering the wastes as a source of wealth, is of prime interest in the recent years (Singh et al., 2017). This review article focusses on the discussion of e-waste pollutants and their effect on soil microbial population and ultimately on the ecosystem function and sustainability of plant growth in the contaminated sites. Also, the emergence of novel microorganisms capable of bioremediation potential is discussed and the methods to study the microbial diversity are summarized.

E-waste pollutants

The major activities at e-waste recycling centres are dismantling, repair and burning. The main chemical components of e-waste are persistent organic pollutants (POPs) like polybrominated diphenyl ethers (PBDE), polychlorinated biphenyls (PCB), dioxins, polyaromatic hydrocarbons (PAH), tetrabromobisphenol-A and heavy metals (Bhattacharya and Khare, 2016; Grant et al., 2013). These pollutants, if present in high concentrations, have toxic effects and alter the soil microbial community (Zhang et al., 2013). Microbes play the major role in important biogeochemical cycles and transformations

of metals and minerals. Therefore a decline in the microbial diversity of soil may result in significant disturbances of soil ecosystem function (Colombo et al., 2016).

Heavy metals such as cobalt, copper, iron, manganese, molybdenum, nickel, zinc and vanadium are required in minute quantities but they may also prove harmful at very high concentrations. On the other hand, heavy metals like lead, cadmium, hexavalent chromium, mercury and arsenic are not beneficial to all organisms and serve as potential threat to the environment (Tchounwou et al., 2012). The maximum permissible limits of various heavy metals for soil are given in Table 1. Presence of heavy metals at alarming levels in soil and water samples have been shown in various studies of e-waste contaminated sites. For e.g. in a study involving various active e-waste sites of Delhi NCR, India, the heavy metals which were found to be present at high levels in the soil samples were Pb, Cd and Cr. Cadmium was present at about 15 times higher than the standard value of agricultural soil taken in the study. The water samples were contaminated with Cd, Pb and Cr at a concentration more than 20 times higher than the potable water limits (Panwar and Ahmed, 2018). In another study of Delhi NCR, it was found that Pb was present 80 times higher than the control while Ni was present 4 times higher than the control. Soil samples from the e-waste sites also showed presence of significant amount of As, Hg and Cd as compared to the control uncontaminated site where these heavy metals were not detected (Pradhan and Kumar, 2014). Excessive release of heavy metals through leaching was detected in freshwater samples collected from rivers around Guiyu, China, where illegal e-waste processing is commonly done. Dissolved Ag, Cd, Cu and Ni were detected as 0.344, 0.547, 87.6 and 93.0 $\mu\text{g L}^{-1}$ respectively in the water samples which is far beyond the potable limits (Wong et al., 2007). The

Table 1. Maximum permissible limits of heavy metals in soil

	Lead (mg/kg)	Cadmium (mg/kg)	Chromium (mg/kg)	Mercury (mg/kg)	Arsenic (mg/kg)	Nickel (mg/kg)
Maximum permissible limit for soil	250-500 ^a ; 100 ^c ; 300 ^b	3 ^{a,b,c}	100 ^c ; 150 ^b	8-11 ^d (methyl mercury); 80-170 ^d (Inorganic mercury)	20 ^c	50 ^c ; 75-150 ^{a,b}

(a) Indian standard (Awasthi, 2000); (b)European Union (2002); (c)Ewers, 1991; (d) Environment Agency, Bristol, 2009

extent of metal pollution also depends on how the metal exists in the soil. Metals present as separate entities or as free ion in high concentration tend to cause pollution problems compared to metals which exist in combination with other soil components. Also, soil properties like soil pH, organic matter, clay content, iron oxide content and surface area of soil particles also affect the metal availability in the soil (Chibuike and Obiora, 2014; Giller et al., 1998).

Depending on the metal, the risk of heavy metal pollution also depends on the pathway of exposure either through uptake by plants or bioaccumulation by microorganisms (McLaughlin, 2006; Dai et al., 2004). For example, cadmium (Cd) is considered a highly toxic metal pollutant because of its high mobility, bioaccumulation in lower organisms and easy transfer to higher trophic levels in the food chain (Xie et al., 2016). Lead (Pb) is another persistent heavy metal which is known to persist in the environment for long time. Studies showed that long term exposure to such persistent metals harms the soil microbial activity, especially the microbial respiration (Sobolev and Begonia, 2008) and causes a decrease in soil microbial biomass (Giller et al., 1998). It has also been reported that heavy metal exposure decreased the amount of bacteria and fungi with incubation time and also fungi are more tolerant to these exposures (Wang et al., 2010; Rajapaksha et al., 2004).

Organic pollutants present in the e-waste like PBDE and PAH cause environmental pollution substantially because of their highly resistant nature and tendency to remain in the environment for many years. According to the Canadian environmental guidelines by the Canadian

Council of ministry of environment, the set permissible limits for total PCBs in soil are 0.5 mg kg⁻¹, 1.3 mg kg⁻¹ and 33 mg kg⁻¹ for agricultural, residential and industrial soils (Canadian Council of Ministers of the Environment, 1999). The environmental impact of e-waste dumping and processing is on a large scale having its effect on plants as well. There are various reports in which high levels of organic pollutants (PCB and PBDE) and heavy metals (Pb and Cd) have been detected in the crops and vegetables growing in e-waste processing sites (Cayumil et al., 2016; Pradhan and Kumar, 2014).

Effect of e-waste pollutants on soil microbial activities

Considering the effect of soil heavy metal contamination on microbial respiration, there has been reports that microbial respiration and soil organic N mineralization rate appears affected only at very high concentration of metals. At low concentrations, there is a positive relationship between the heavy metals and soil respiration. Although results show a wide variation among various studies, it appears that soil organic matter accumulation is enhanced as a result of heavy metal pollution (Ghorbani et al., 2002). Also, numerous environmental variables like soil pH, soil organic matter, organic pollutants and presence of Zn can affect the microbial community. Thus, soil properties can have co-effects with the presence of contaminants on the microbial community (Jiang et al., 2017). On the other hand, heavy metal contamination may also affect the number and diversity of soil microorganisms and the toxicity of

these metals to the microorganisms depends on soil physical and chemical properties (Chibuike and Obiora, 2014).

There are reports that in heavy metal contaminated soils, microbial biomass and N₂ fixation reduced to a great extent (Lenart-Boroń and Piotr Boroń, 2014) but there are some plants and microorganisms (especially present in the rhizosphere of these plants) which are unaffected (Konopka et al., 1999; Burd et al., 1998). Also, in one report, it was shown through culture dependent studies that soil samples from three e-waste dumpsites in Nigeria had higher bacterial and fungal counts than the control sample and the most frequent bacterial and fungal species were *Bacillus* spp. and *Aspergillus* spp. respectively (Taiwo et al., 2018). Now it is better understood from various reports that although heavy metal and organic pollutants contamination reduces the normal soil microbial biota, it also enhances the emergence of microbial populations with resistant and biodegradability properties (Jiang et al., 2017; Xie et al., 2016).

Studies show that there are significant differences in the microbial composition between e-waste contaminated (heavy metals, polychlorinated biphenyls and polybrominated diphenyl ethers) and non-contaminated soils. Laboratory studies on the effect of PBDE along with heavy metals like Cu and Pb showed reduced microbial diversity (Chen et al., 2015; Zhang et al., 2012). Effect of PBDE congeners (BDE 15 and BDE 209) on soil microbial community showed that at higher concentrations the soil microbial community structure was altered (Liu et al., 2010). Bacterial counts in the microcosm amended with BDE 15 were higher than that with BDE 209. The dominant types of bacteria were *Pseudomonas*, *Bacillus* and uncultured bacteria (Liu et al., 2010). Effect of BDE 209 on microbial diversity in Pearl River estuary, China, indicated that high concentration of BDE 209 favored increase in the microbial

diversity. Further BDE 209 stress induced laboratory experiments showed that the community structure changes were directly related to the concentration of BDE 209 and also on the incubation time showing different effects of BDE209 on microbial community (Wu et al., 2013). The study demonstrated that with longer period of incubation (17 to 35 days) and under 100 mg/kg BDE 209 stress, *Fermicutes* and other uncultured bacteria were dominant showing greater tolerance to BDE 209. The predominance of *Proteobacteria* and *Actinobacteria* in PCB contaminated soils has also been shown by studying the effect of extracellular organic matter from *Micrococcus luteus* (known to enhance biphenyl biodegradation) on the microbial community of PCB-contaminated soils (Su et al., 2015).

A number of *in situ* studies on the effect of e-waste pollutants on soil microbial diversity are now gradually being reported. With 16S rRNA high-throughput sequencing, presence of *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Crenarchaeota* and as dominant phyla accounting for 80% of the total sequence reads have been reported. The genera like *Acinetobacter*, *Pseudomonas*, and *Alcanivorax* which are known to be organic pollutants degrading bacteria have been reported in the e-waste contaminated sites of Guiyu, China (Liu et al., 2015). Earlier report on the e-waste contaminated sites of Guiyu, China, showed that PAH contamination led to bacteria community changes in species diversity but it was found that bacterial diversity was not decreased compared to the non-contaminated site possibly due to the increase in POPs degrading bacteria, for example uncultured *Clostridium* sp. and *Massilia* sp. (Zhang et al., 2010). In another report, bacterial community structure of polluted sediment showed characteristic feature which was different from the unpolluted sediment sample, mainly reflected by the dominant position of

epsilon-*Proteobacteria* and *Chloroflexi* in the bacterial flora (Zhou et al., 2011). Investigations around an e-waste recycling workshop in the Taizhou e-waste recycling area of China also showed the predominance of *Proteobacteria* and *Bacteroidetes* and it was found that the PCB concentration in the soil decreased with increasing distance from the recycling workshop and soil bacteria diversity gradually increased (Tang et al., 2013). In a similar study, through sequencing of 16S rRNA gene amplicons, occurrence of *Firmicutes*, *Chloroflexi* and *Crenarchaeota* as most abundant phyla with relatively lower abundance of *Proteobacteria* and *Actinobacteria* was reported in the highly heavy metal contaminated samples of Xiangjiang river, China (Yin et al., 2015). In one of the most recent studies, the impact of e-waste recycling activities on soil microbial community was studied for different sites in China and Pakistan using molecular techniques and ecological network analysis tools (Jiang et al., 2017). The study revealed that the predominant phyla in these sites are *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Chloroflexi*, *Acidobacteria*, and *Bacteroidetes*. *Bacillus* was found to be the most abundant genus. A comparison of results of studies from various e-waste contaminated regions showing similarity of the effect of e-waste pollutants on soil microbial composition is shown in Table 2.

Reduced soil enzyme activities of urease, catalase and saccharase have been shown in laboratory studies of the combined effect of PBDE and Cu (Zhang et al., 2012). Chen et al., (2015) showed that combined effect of PBDE and Pb can decrease microbial biomass and stimulate microbial basal respiration. Jiang et al., (2017) showed that in e-waste contaminated soils studied over long distances in China and Pakistan, the important pollutants determining the soil microbial composition and diversity are PAHs, PBDEs and heavy metals (Cu, Zn and Pb). The constructed ecological network of soil microbial communities clearly

delineated the co-occurrence, competition and antagonism of microorganisms across soils in response to pollutants and soil properties. The microbial community profile showed that it was more sensitive to PAHs and PBDEs than soil pH, as toxic waste contamination is a short-term effect whereas the soil pH might be stable for long-time. Zn was identified as the most important factor affecting the microbial community which is in accordance with previous study reports that Zn can damage microbial activities and inhibit soil enzyme activities (Jiang et al., 2017).

The organic pollutants of e-waste i.e. POPs may give rise to increase in the microbial functional gene diversity and abundance. Proper functioning of the carbon (C), nitrogen (N), phosphorus (P) and sulfur (S) cycles may take place if electron acceptors like nitrate, sulphate and ferric ions are present. This has been studied in several bioremediation studies (Xu et al., 2015; Yang et al., 2013). Functional gene microarray studies showed that in heavily metal contaminated soils, genes involved in metal homeostasis like *chrR* (chromium detoxification), *metC* (mercury detoxification), *merB* (mercury detoxification), and *silaffin* gene (silicon biosynthesis) were abundant (Yin et al., 2015). Among the organic remediation genes, aromatics degradation genes (e.g., *xyiL*, *benD*, *nhh*), polycyclic aromatics degradation, herbicides and pesticides related compound degradation have been found to be abundant (Yin et al., 2015). Chlorinated organic pollutants have been found to negatively affect soil protein concentration and expression in case of studies on PCBs contaminated agricultural soil samples from Taizhou, China (Zhang et al., 2013). It was shown that large molecular weight proteins were less expressed than low molecular weight proteins (<40 kD).

There is a strong correlation between soil microorganisms and plants growing in the

contaminated sites. The symbiotic relationship between plants and microorganisms has been employed for the phyto- or microbial remediation of heavy metal and organic pollutants contaminated soils and is a promising alternative for remediation (Teng et al., 2015). The microorganisms present in the root ecosystem respond to the pollutant along with the plant growing in the area and is also species specific for the plant. In one study involving the rhizospheric effects on the microbial community of e-waste contaminated soils, it was shown that the total phospholipid fatty acid (PLFA) concentration was higher in the rhizospheric soils than in the non-rhizospheric soils giving information of the active microbial communities. There were also higher ratios of fungal-to-bacterial

PLFAs and Gram-positive bacteria showed lower relative abundance in rhizospheric soils. The rhizospheric effect on the total microbial biomass was shown to have greatest positive effect in *Colocasia esculenta* L. and these effects were shown to be varied among different plants (Song et al., 2018). It has been shown that organic pollutants like PBDE contamination in the soil had an important effect on the microbial N transformation and is also plant species-specific. High PBDE decreased the microbial nitrification activity but stimulated the denitrification activity in a 12-month microcosm experiment using two species of mangrove plants. The response was shown to be plant species-specific and also dependent on soil nitrate concentration (Chen et al., 2016).

Table 2. Comparison of the results of studies on soil microbial communities in various e-waste contaminated regions of different countries

S. No.	E-waste site	pH of the soil	Pollutants	Soil Microbial Community (in decreasing order)	References
1.	Alaba International Market, Lagos State (N 6°27'22", E 3°11'16.2"), Nigeria	7 to 8	Cu (1.1×10^3 - 6.4×10^3 mg/kg), Pb (1.1×10^3 - 4.9×10^3 mg/kg), Zn (1.0×10^3 - 2.2×10^3 mg/kg), Ni (0.05×10^3 - 2.2×10^3 mg/kg), Cd (2.1×10^3 - 13.6×10^3 mg/kg), Cr (9.1×10^3 - 39.1×10^3 mg/kg)	Proteobacteria, Firmicutes, Actinobacteria, Chloroflexi, Acidobacteria, Planctomycetes and Bacteroidetes	Jiang et al., 2019
2.	Guiyu town (23°18'-23°25'N and 116°19'-116°23'E), China	4 to 7	Pb (0.4-20 mg/kg), Zn (3-384 mg/kg), Cu (7-1236 mg/kg), PAH (821-4900 µg/kg), PCB (6-142 µg/kg), PBDE (2500-10111 µg/kg)	Proteobacteria, Bacteroidetes, Acidobacteria, Chloroflexi and Firmicutes	Liu et al., 2018; Zhang et al., 2009
3.	Guiyu, (N 116°37', E 23°33' and N 116°35', E 23°34'), China	4 to 7	As (5 mg/kg), Cd (0.3 mg/kg), Cu (378 mg/kg), Ni (90 mg/kg), Pb (76 mg/kg), Zn (126 mg/kg)	Proteobacteria, Acidobacteria, Bacteroidetes, Firmicutes	Deng et al., 2018)
4.	Karachi, 24550 N, 67300 E; Multan, 30°14' N, 71°29'E and Lahore, (31°32' N, 74°20' E), Pakistan	7.2 to 12.6	Cu (3000 to 3130 mg/kg), Pb (290 to 1670 mg/kg), Zn (895 to 3030 mg/kg), PAH (2180 to 2940 ng/g), PCB (473 to 8780 ng/g), PBDE (49 to 1010 ng/g)	Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Candidate division TM7	Jiang et al., 2017
5.	Pearl River Delta (Site A1 (N 23°05'12.12", E 113°26'00.00") in Guangzhou Channel, site A2 (N 22°35'34.80, E 113°48'14.40) in Shiziyang Channel, and site A3 (N 22°07'12.00, E 114°00'36.00) in Lingding Bay, China	6 to 8.6	PBDE congeners (0.04 to 94.7 ng/g)	Proteobacteria, Firmicutes, Actinobacteria	Wu et al., 2013

Bioremediation and biotransformation of e-waste contaminants

Increase in the microbial functional diversity with increase in the level of heavy metal pollution can be attributed by the stimulatory effects of bioavailable heavy metals on the bacterial growth (Kenarova and Boteva, 2015). Thus, a wide variety of heavy metal resistant microorganisms have been reported from various ecological niches. Mechanisms employed by microorganisms for remediating heavy metal contamination are biosorption, bioleaching, precipitation and transformation. Examples are Iron- and Sulphur oxidizing bacteria like *Thiobacillus* species which can be used for bioleaching, *Pseudomonas* and *Arthrobacter* species for biosorption of heavy metals like Pb, Cu and Cd, sulphate reducing bacteria for metal precipitation in the form of sulfides. Fungi like *Aspergillus niger* and *Penicillium* species also have great advantage to be used in bioleaching because they can tolerate high concentrations of heavy metals (Gadd, 2012; Patel and Kasture, 2014). Moreover, acidophilic group of bacteria like *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, and *Sulfolobus sp.* have the ability for bioleaching of heavy metals from the wastes (Needhidasan et al., 2014).

Similarly, microorganisms having potential degrading property of PCBs are being reported. The common types of bacteria having resistant properties are reported from proteobacteria like *Burkholderia*, *Pseudomonas*, *Sphingomonas*, *Clostridium* and *Rhodococcus* species (Borja et al., 2005; Furukawa, 2000; Pieper, 2005; Tillmann et al., 2005). Organisms closely related to the green non-sulphur bacteria isolated by culture independent methods are known to dehalogenate chlorinated compounds and use the energy liberated for their growth (Abraham et al., 2002). These organisms can be used to develop microbial consortia having novel degrading properties.

Microbial degradation of PBDE can be

either aerobic or anaerobic. The reductive debromination of PBDE in anaerobic condition requires electron donors and the microbial community structure is dependent on the PBDE concentration (Qiu et al., 2012). The products formed by anaerobic degradation are less brominated congeners whereas hydroxylated brominated diphenyl ethers and brominated phenols or brominated catechols are formed in the aerobic degradation of PBDE. Some examples of PBDE degraders are *Sphingomonas sp.*, *Trametes versicolor*, *Rhodococcus*, *Burkholderia xenovorans*, *Pseudonocardia dioxanivorans*, *Lysinibacillus fusiformis*, *Pseudomonas stutzeri* and *Bacillus sp.* (Xia et al., 2013). *Micrococcus luteus* has been reported to be very efficient (100% removal efficiency) in degrading biphenyls (Su et al., 2013). *Ochrobactrum sp.* is another bacterium isolated from e-waste recycling site and is reported to have more than 90% removal of tetrabromobisphenol (An et al., 2011).

Remediation using combined biological and physical means such as high temperatures and ultrasonication followed by soil washing using oil-water solvent system has been proved to be very effective. The washed soil after treatment with PBDE degrading bacteria *Rhodococcus sp.* strain RHA1 led to maximum clean-up of the mixed-contaminated sites of PBDE and heavy metals (Ye et al., 2015). It was reported that after the remediation using this method the microbiological counts, biomass C, N, and functioning diversity was gradually increased.

There have been reports about the study of uptake of PBDE by plants in e-waste contaminated sites using ryegrass, maize and pumpkin. It was found that the lower brominated PBDEs were more readily dissipated than the higher brominated PBDEs (Huang et al., 2011). Some plants which can take up heavy metals in high concentration are *Pteris vittata*, *Populus nigra* and *Amaranthus* for arsenic (As),

Brassica campestris, *Brassica carinata*, *Brassica juncea* and *Brassica nigra* for lead (Pb) and *Brassica juncea* L. Czern. for mercury (Hg), cadmium (Cd), chromium (Cr) (Patel and Kasture, 2014; Tangahu et al., 2011). These plants have the potential for phytoremediation of soils and wastewater. At present phytoremediation technology is not used as an effective method due to some limitations like time-consuming, root depth, age of plant etc. and further research is required in order to minimize the limitations. Rhizobial interactions with plants have been proved to be a promising option for the remediation of contaminated soils as it enhances plant growth, stimulates the activity of biodegrading bacteria and also helps in the availability of pollutants to the plant roots (Teng et al., 2015).

Microbial recovery of precious metals from e-waste pollutants

PCBs contain several valuable metals with quantities higher than those in some ores. Therefore, apart from treatment of e-waste to avoid environmental contamination, microbial recovery of precious metals from e-waste is of major interest. Microbial recovery of precious metals or biometallurgy comprise of two steps: bioleaching and biosorption. Due to its low cost, low environmental impact and low energy consumption, this technique is considered favourable. It has been found to be feasible for the extraction of Cu, and precious metals, Au and Ag. Microbial species capable of recovering precious metals from discarded printed circuit boards (PCB) have potential valuable applications. For example, two step bioleaching process, using *Acidithiobacillus ferrivorans* and *Acidithiobacillus thiooxidans* in the first step and *Pseudomonas fluorescens* and *Pseudomonas putida* in the second step, showed efficient recovery of Copper and gold from PCB (Isildar et al., 2016). Also there are reports on indigenous bacteria tolerant to e-waste toxicity like *Pseudomonas balearica* SAE1, isolated

from an e-waste recycling facility, which has properties of bioleaching. It can recover gold and silver from PCB (Kumar et al., 2017). A novel copper oxidase from *Proteus hauseri* ZMd44 has been studied for its applications in metal recovery and bioremediation. The gene encoding the copper oxidase was cloned and expressed in *E. coli*. It was found that the enzyme had great potential for Au adsorption and can be used in the biorecovery of gold or as a biosensor (Tan et al., 2017). Efficient bioleaching by a consortium of bacterial free culture supernatant reported 100% copper recovery in 2 h from 5 g/L PCBs. In this method, the limitation of toxicity of PCBs to bacteria has been resolved. The bacteria used were iron-sulphur oxidizing bacteria *Leptospirillum ferriphilum* and *Sulfobacillus thermosulfidooxidans* (Wu et al., 2018).

Methods for studying soil microbial diversity

The soil contains a diverse variety of bacteria and fungi which are non-culturable and therefore researchers have been developing new methods to discover the microbial world of the soil and study their functions in the ecosystem. In 1 g of soil it is estimated that the number of species of bacteria per gram of soil vary between 2000 and 8.3 million and only 1% of the soil bacterial population can be cultured by standard laboratory techniques (Roesch et al., 2007). Similarly, about 1,500,000 species of fungi are estimated to be present in the world, but most of the fungi cannot be cultured in the laboratory using standard protocols (Kirk et al., 2004). Biochemical based methods used to study microbial diversity are plate counts, community level physiological profiling (CLPP), phospholipid fatty acid (PLFA) and Fatty acid methyl ester (FAME) analysis (Weber and Legge, 2010; Quideau et al., 2016). These methods give highly reproducible results but cannot detect non-culturable microorganisms and are very specific. Various molecular based methods

have been developed which gives valuable information about the microbial community structure of a particular site. Popular molecular-based approaches are denaturing and temperature gradient gel electrophoresis (DGGE and TGGE), single strand conformation polymorphism (SSCP), Amplified ribosomal DNA restriction analysis (ARDRA) or restriction fragment length polymorphism (RFLP), Terminal restriction fragment length polymorphism (T-RFLP), Ribosomal intergenic spacer analysis (RISA)/automated ribosomal intergenic spacer analysis (ARISA). These methods have the limitations of PCR biases but methods like DGGE/TGGE, T-RFLP and ARISA give a clear picture of community profiles and can be used for comparing differences in microbial communities (Kirk et al., 2004).

Metagenomics, which is the direct extraction of DNA from environmental samples, cloning and analysis of the genomic DNA isolated from the entire community, has grown to be one of the best techniques for understanding the functional diversity of microbial communities. In this regards, a number of improved sequencing technologies have been developed which provides reads of sufficient length for proper assembly and study of the genetic diversity of microorganisms. Improved methods of analysis of metagenomics DNA such as the targeted approach of specific genes can give deep insight into the functional and evolutionary patterns of important genes of various microorganisms from various ecosystems (Suenaga, 2012). High throughput sequencing (HTS) has been commonly used nowadays in biodiversity studies. Microarray technology helps determine the dynamics and activities of hundreds to thousands of microbial populations in complex environments simultaneously. Microarrays for microbial ecology research can be divided into five types: (i) Phylogenetic oligonucleotide arrays (POAs) in which probe is based on

ribosomal rRNA genes, (ii) Functional gene arrays (FGAs) based on functional genes, (iii) Community genome arrays (CGAs) based on whole genome, (iv) Metagenomic arrays (MGA) based on environmental DNA and (v) Whole-genome open reading frame arrays (WGA) based on open reading frames in whole genome (Gentry et al., 2006). Although microarray is a sensitive technology, it needs improvement in its technology such as in obtaining pure samples, data analysis and automation in order to use it as a standard tool in microbial ecology research.

Gene microarrays such as GeoChip which targets specific genes involved in microbial functions have been used in a number of studies. For example, GeoChip 4 uses conventional gene families involved in carbon, nitrogen, sulphur and phosphorous cycles, organic remediation, metal reduction and antibiotic resistance, and also genes involved in various environmental stress responses, bacteriophages and virulence processes (Tu et al., 2014).

Gene microarray methods may have the limitations of non-specific binding of the DNA fragments to the probes. In an improved strategy for the study of microbial functional diversity, a technique called capture metagenomics was developed in which probes were designed to target multiple functional genes coding for carbohydrate-active enzymes that are involved in organic matter degradation. The probes were designed using web-based tools and efficiently targets a number of genes involved in a particular pathway. This method was able to detect non-specific binding and had higher resolution than the other approaches as it was able to sequence a large number of genes for the analysis of the functions of microbial communities in different environments (Manoharan et al., 2015).

In recent years, there has been a rapid development in both molecular and statistical methods for the study of

microbial community structure and this has made the analysis of results challenging. Detailed discussion about the advantages and disadvantages of various statistical analyses and integration of HTS-data of marker genes for the analysis of microbial diversity and composition is given by Balint et al., (2016).

Considering the present state of knowledge, the study of microbial community structure and its functions should be done based on both biochemical and molecular methods in order to obtain a clear picture of the microbial community.

CONCLUSIONS

The heavy metals and toxic organic pollutants present in e-waste affect the soil microbial population and alter the microbial functional gene diversity. With the improvement in techniques to study microbial diversity, especially through culture-independent approach, present studies show that the microbial functional diversity increases with the emergence of microorganisms which are able to resist the effect of toxic pollutants. Metagenomics and microarray technology have proven to be an efficient tool to study microbial community and functional diversity in the contaminated sites. More investigations are required to finally draw a conclusion on how the microorganisms respond to the increase in e-waste contaminants in the soil and to understand the role of dominant microorganisms in ecosystem function and the major pathways of degradation of organic pollutants in the e-waste. In addition, rhizobial interactions with plants having phytoremediation capability need to be explored in order to finally find efficient means of remediating contaminated soils.

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