

Bacterial Remediation of e-waste Contaminated Soils: Recent Strategies and Future Perspectives

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Abstract

This paper presents a review on the alarming production and detrimental effects of electronic waste a source of toxic material such as heavy metals/metalloid concentrations in soil as environmental media in electronic-waste (e-waste) recycling site. Incomplete combustion of e-waste management and recycling of waste electrical and electronic equipment (WEEE) in open air and dumping of processed materials are the major sources of various toxic chemicals. By comparing with existing data available in India and also guidelines adopted in different countries, it is obvious that the environment is highly contaminated by these toxic chemicals derived from the recycling processes. It provides a living for the urban and rural poor, but causes severe risks for humans and the local environment. In order to safeguard the environment and human health, detailed investigations are urgently needed. The techniques presently used for the clean-up of soils contaminated by heavy metals are mainly based on tracking the exposure, by using different microorganisms which may bring remediation of the soil. This paper has special focus on recent advances and future perspectives

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Keywords: Electronic waste; Environmental media; Heavy metals, organic compounds; Genetically Engineered Bacterial Remediation

Introduction to E waste

Electronics and information technology (IT) is a fast growing segment of Indian industry. Driven by innovation, fads, and the universal success of consumer electronics, the high consumption of these products leads to growing amounts of e-wastes or waste electrical and electronic equipment (WEEE). The global volume of e-waste defined herein as any unwanted product with one or more circuit boards has been increasing by approximately 4 percent per year (1, 2).

However, e-waste is more problematic than ordinary municipal waste because it contains potentially toxic materials such as heavy metals and flame retardants, which threaten environmental quality and public health if they are not handled properly. An unknown volume of e-waste (up to 80 percent of total annual generation by some estimates (3) is shipped to

developing countries, where inadequate handling can have devastating ecological and human health consequences (4) The accumulation of heavy metals, PAHs(polycyclic aromatic hydrocarbons) in air, water, sediment and soil has been reported by several studies (5).

E-wastes contain some valuable and reusable materials, e.g., base metals (copper) and precious metals (gold and palladium), so e-waste recycling maybe viewed as a tremendous business opportunity for some developing countries (6). On the other hand, e-wastes also include and/or generate a number of harmful substances during manufacturing like heavy metals (lead, cadmium, and mercury) and organic compounds (PAHs), polybrominated diphenyl ethers (PBDEs), dioxin-like compounds such as polychlorinated and polybrominated dibenzofurans (PCDFs and PBDFs) (3). However, due to lack of facilities, high labor costs, and tough environmental regulations, rich countries tend not to recycle e-waste. Instead, it is either land filled, or exported from rich countries to poor countries, where it may be recycled using primitive techniques and little regard for worker safety of environmental protection (7)

Although the per capita waste production in populous countries like China and India is still relatively small, these countries are already huge producers of WEEE. These countries tend to also have the fastest growing markets for electrical and electronic equipment (EEE), ones that are far from saturation. From 1993 to 2000, the number of PC users in China increased 1052% whilst the average growth throughout the world was much lower at 181%. During the same period, India showed an increase of 604%. From 1996 to 2002, the number of mobile phone users in China rose to 200 million (8). Due to these developments, countries like India face a fast increasing load of WEEE, originating both inland and through illegal imports (9)

It is well known that India has recycling centers of WEEE, especially printed circuit boards (PCBs). The greater part of E-waste around the world has been recycled in either China or India. Such waste generated in the eastern half of the USA, in the Europe, in Central East Asia, and in Southeast Asia generally is exported to India, while what is generated in the western half of the USA, and in Japan, Korea, and Southeast Asia is exported to China. (10, 6 and 11).

Since the 1990s India has been another center of E-waste recycling. According to the Central Pollution Control Board (CPCB), every year 146,000t of e-waste is recycled in India (12).The GTZ (Deutsche Gesellschaft für Technische Zusammenarbeit), however, estimated the total amount of E-waste in India at around 500,000 t in 2015. (13).

It has been reported by the Basel Action Network (BAN), WA, and Toxics Link, India, that 1.38 million personal computers (PCs) will be obsolete from the business sector and individual households in India. At the same time, around 1050 tonnes of electronic scrap is being produced by manufacturers and assemblers in a single calendar year in India. Reports of the import of 30 metric tonnes (MT) of e-waste at Ahmadabad, a town in India, have also been published.

There is no reliable database of the total amount of e-waste in India. At the same time, no scientific evidence exists regarding the application of a standard approach and methodology to

estimate e-waste in India. The review of the impact of e-waste contaminants on the Environment is discussed in the next section.

E waste contaminants and their Environmental toxicity

E-waste contaminants	Source	Toxicity
Cadmium	Switches, solder joints, rechargeable batteries, UV stabilizers and cathode ray tubes	damage to the kidneys and bone structure Cadmium and its compounds are known human carcinogens, primarily through inhalation of contaminated fumes and dusts.
Lead (Pb)	major component of solders, cathode ray tubes, lead-acid batteries and stabilizers in some PVC cables	toxic to humans, as well as to animals and plants, irreversible effects on the nervous system particularly the developing nervous system in children
Phthalates	PVC, children's toys and childcare articles	Toxic to the reproductive system
Antimony (Sb)	flame retardant,	Can lead to severe skin problems and other health effects. Antimony trioxide is recognized as a possible human carcinogen
PCBs (polychlorinated biphenyls)	PVC, electrical transformers and capacitors	suppression of the immune system, liver damage, cancer promotion, damage to the nervous system, behavioral changes and damage to both male and female reproductive systems
Chlorobenzenes	combustion of the chlorinated plastic PVC	Hexachlorobenzene effects the central nervous system, endocrine disruptor and a possible human carcinogen
PAH (Polycyclic aromatic hydrocarbons)	Organic waste at Re-cycling sites.	Genotoxic, risk of lung, skin and bladder cancer
PBDEs (polybrominated diphenyl ethers)	brominated flame retardants, casings and components of many electronic goods	interfering with normal brain development in animals, suspected endocrine disruptors, interfere hormones involved in growth and sexual development
Triphenyl phosphate (TPP)	flame-retardants and casings of computer monitors	TPP is acutely toxic to aquatic life and a strong inhibitor of a key enzyme system in human blood and dermatitis in some individuals and is a possible endocrine disruptor
Beryllium	motherboards and connectors	human carcinogen
Americium	smoke detectors	carcinogenic

Adapted from Green peace(44)

E-waste Re-cycling and the need for Bacterial Remediation

E-waste recycling involves the disassembly and destruction of the equipment to recover new materials (14). Recycling can recover 95% of the useful materials from a computer and 45% of materials from cathode ray tube monitors (15). In rich countries, such as Japan, high tech recycling operations function well with little environmental impact (16). Valuable metals may be recovered from e-waste by pyro-hydro- and bio-metallurgical processes. Pyrometallurgical

processing includes incineration of the matrix and smelting of the target metals. The efficacy of this process depends on investment. Shoddy operations have the potential to emit dangerous compounds into the environment. (14)

Leung (4) reported that, the workers engaged in e-waste recycling operations are subjected to toxicity risks. There are chances for the contaminant to spread to nearby civilizations by wind particulate matter. Pyrometallurgical process, which results in waste-acid, rich in heavy metals, are usually discarded onto the soil or into nearby waterways.

E-waste contaminants can enter aquatic systems via leaching from dumpsites where processed or unprocessed E-waste may have been deposited. Similarly, the disposal of acid following hydrometallurgical processes into waters or onto soils, as well as the dissolution or settling of airborne contaminants, can also result in the contamination of aquatic systems. (46). Reports (17, 18, 19 and 20) clearly indicate water contamination from e-waste re-cycling sites.

Many e-waste contaminants are spread into the air via dust. This is a major exposure pathway for humans through ingestion, inhalation and skin absorption (21). Reports (22, 23, and 24) prove e-waste aerial contamination.

Translocation of e-waste contaminants from soil to plants especially crops like rice and sorghum has been reported by many workers (3).

Soils at an e-waste recycling slum in Bangalore contained up to 39 mg/kg Cd, 4.6 mg/kg In, 957 mg/kg Sn, 180 mg/kg Sb, 49 mg/kg Hg, 2850 mg/kg Pb, and 2.7 mg/kg Bi (25). These concentrations are some one-hundredfold higher than those found at a nearby control site in the same city.

Reports of Cui and Zhang (14), Brandl et al (26) and Creamer et al (27) clearly show bioremediation with the help of soil bacteria is an attractive method, due to its low-cost and high specificity for the target e-waste toxicants. The authors reported that the bacteria like *Thiobacillus* could facilitate metal leaching from electronic scrap and *Desulfovibrio desulfuricans* used in the recovery of gold, platinum and copper from e-waste.

For bioremediation to be successful, the bioremediation methods depend on having the right microbes in the right place with the right environmental factors for degradation to occur. The right microbes are bacteria or fungi, which have the physiological and metabolic capabilities to degrade the pollutants. Bioremediation is a scientifically intensive procedure which must be tailored to the site-specific conditions, which means one has to do treatability studies on a small scale before the actual clean-up of the sites. (28).

Recent strategies of Bacterial remediation of stable E-waste contaminants

Metal bioremediation by use of microbial surfactants:

Microbial compounds that exhibit particularly high surface activity and emulsifying activity are classified as biosurfactants.

According to Herman et al .(29) toxic metals such as lead and cadmium have stronger affinities for rhamnolipids than for many of the soil components to which they are bound in contaminated soils. Studies have shown that this surfactant complexes preferentially with toxic metals. Considerable work has been done on rhamnolipids biosurfactants produced by various *Pseudomonas aeruginosa* strains capable of selectively complexing cationic metal species such as Cd, Pb, and Zn.

Mulligen (30) evaluated the feasibility of using surfactin, rhamnolipid, and sophorolipid for the removal of heavy metals, Cu and Zn, from sediments.

A strategy suitable for effective remediation would be, to stimulate biosurfactants produced by indigenous population or use commercial biosurfactants produced by organisms found to be already present at the contaminated site.

Biomolecular engineering in the enzymatic degradation of PAH's

In the past several years, several oxido-reductases such as laccases and cytochrome P450 monooxygenases (CYPs) have been exploited for the enzymatic degradation of PAHs. In a study undertaken by (31), the laccase gene from *Myceliophthora thermophila* (MtL), expressed only in *Asperigillus oryzae*, was transformed into *Saccharomyces cerevisiae* and subjected to directed evolution. The enzyme from MtL holds great potential for the bioremediation of PAHs due to its high thermal stability that enables it to work at the elevated temperatures needed to increase the solubility of highly recalcitrant PAHs.

Aerobic and Anaerobic dechlorination of PCBs

Polychlorinated biphenyls (PCBs) are a class of compound in which the aromatic biphenyl carbon skeleton carries between one and 10 chlorine atoms. Due to industrial developments 209 possible PCB congeners, are produced.

PCBs are degraded aerobically and anaerobically. As a general rule, highly chlorinated congeners (which are highly stable and highly hydrophobic) are good substrates for anaerobic degradation, possibly via chlororespiration (the initial use of the PCB as an electron acceptor), but are poor substrates for aerobic degradation. Dechlorination is a progressive process that converts higher-chlorinated congeners to lower-chlorinated forms, or more hydrophobic congeners to less hydrophobic forms. Lower-chlorinated congeners are, in turn, poor substrates for anaerobic dechlorination, but are good substrates for aerobic degradation, in which they act primarily as electron donors(32).

Culture-independent community fingerprinting methods have recently been applied to cultures exhibiting reductive dehalogenation. *Burkholderia*, *Pseudomonas* and *Sphingomonas*, *Rhodococcus globerulus* can degrade PCBs aerobically can actually mineralize the substrate.

Dutta (33) characterized microbial communities mediating *para*- or *meta*-dechlorination of PCBs, and found sequences related to members of the genus *Clostridium* to be abundant.

Aerobic Degradation of Polybrominated Diphenyl Ethers (PBDEs)

Polybrominated diphenyl ethers (PBDEs) are a class of widely used flame retardants that have recently been detected in environmental samples, diverse biota, human blood serum, and breast milk at exponentially increasing concentrations. Currently, little is known about the fate of these compounds, and in particular, about the microbial potential to degrade them. A hybrid treatment reported by Young and Kumarasamy (34) is highlighted in which through reductive debromination followed by biological oxidation. The reductive debromination is accomplished by nanoscale zero-valent iron and biological oxidation by diphenyl ether degrading bacteria *Sphingomonas* sp.

Nanomolar degradation of Chlorobenzenes.

Burkholderia sp. strain PS12 (35), the only microorganism presently known to utilize 1, 2, 4, 5-tetrachlorobenzene (1,2,4,5-TeCB) aerobically as the sole source of carbon and energy. Chlorinated benzenes are converted by PS12 and PS14 by an aromatic ring dioxygenase and a dihydrodiol dehydrogenase to chlorocatechols as central intermediates, which are degraded via the modified *ortho*-cleavage pathway to 3-oxoadipate. The results demonstrate that *Burkholderia* sp. strain PS14 exhibits a very high affinity for chlorobenzenes at nanomolar concentrations.

Remediation of toxic metals by Integrated Microbial process

Communities of Sulfur-oxidizing bacteria were utilized in the bio-leaching of toxic metals, sulfuric acid (insoluble sulfides) when produced as a metabolic product. An industrially contaminated soil was also efficiently leached and approximately 69% of the main toxic metals present, Cu, Ni, and Mn, were removed after 175 days. The leachate that resulted from the action of sulfur-oxidizing bacteria on contaminated soil was stripped of metals using an anaerobic bioreactor containing a mixed culture of sulfate-reducing bacteria which precipitated soluble metal species as solid metal sulfides. More than 98% of the metals were removed from solution with the exception of Mn, Ni, and Pb, where 80–90% was removed. The metal content of the resultant effluent liquor was low enough to meet European criteria for discharge into the environment. (36).

Future Perspectives

The rational design approach for bioremediation typically involves either the construction of a single microorganism in which desirable biodegradation pathways or enzymes from different organisms are brought together to perform specific reactions using recombinant DNA technology (whole cell level), or the engineering of enzymes with desired characteristics using site-directed mutagenesis (protein level). The capability of rational design is rapidly improving due to recent advances in enabling technologies such as X-ray crystallography and bioinformatics. Biomolecular engineering can be practice to produce novel bio-substrates to increase the efficiency of microbial bio-degradation.

With the aid of biotechnology and genetic engineering, the symbiotic relationship between bacteria and plants has been exploited for in situ bioremediation of a wide range of organic

pollutants such as trichloroethylene (37), toluene (38), polychlorinated biphenyls (45), etc. The current strategy to utilize the recombinant rhizobacteria, which may colonize as a rhizosymbiont, offers the advantage of removing the metal bound on the rhizobacteria while harvesting the plants. This self-sustainable rhizobacterial population is likely to provide both long-term growth plant protection and removal of the heavy metals.

Many heavy metal contaminated sites are co-contaminated with organic pollutants such as trichloroethene (TCE) and other toxic compounds (39). Brim et al. (40) have reported that the engineered *Deinococcus radiodurans* strain, cloned with *tod* and *xyl* genes of *Pseudomonas putida*, is capable of complete degradation of organic contaminants. They also suggested that as such, the *Tod*/ *Xyl* strain could provide a model for examining the reduction of metals coupled to organic contaminant.

A similar strategy of combining complementary metabolic activities can be used for the development of microorganisms capable of mineralizing PCBs by combining an oxidative pathway for (chloro)biphenyl transformation (encoded by the *bph* genes) into (chloro)benzoate with a chlorobenzoate degradative pathway. Several hybrid strains have been engineered in recent years by conjugative matings (41) of appropriate organisms or by introduction of the *bph* genes into chlorobenzoate degraders.

The recent advances in the area of recombinant DNA technologies have paved the way for conceptualizing “suicidal genetically engineered microorganisms” (S-GEMS) to minimize such anticipated hazards and to achieve efficient and safer decontamination of polluted sites (42). The GEM contains two different sets of killer–anti-killer genes. In one set, the killer has been integrated into the genome and cognate anti-killer into a plasmid, while in the other set the positions of the killer–anti-killer have been reversed. Anti-killer genes are inducible by the pollutant. In the presence of the pollutant both the anti-killer genes are induced and the product inactivates the cognate killer; as soon as the pollutant depleted, the anti-killer genes are shut down and the killer exterminates the GEM; in a rare case of plasmid transfer, the plasmid-encoded killer gene eliminates the recipient immediately; self-termination of SGEMs occurs when the concentration of pollutants falls below a certain range that can be sensed by it

Conclusion

Additional advances are expected with the use of new techniques. For example, genomic approaches are set to revolutionize many aspects of biology and will undoubtedly make an impact in the arena of environmental biotechnology. Whole genome transcription profiles have already been screened to uncover potentially useful metal detoxification strategies in *E. coli* (43). Given the imminent availability of complete genome sequences for several environmentally relevant microorganisms, this type of approach will surely prove useful for determining the precise mechanisms of environmentally relevant metal–microbe interactions. Thus engineered microorganisms can be a promising source for the degradation of broad spectrum e waste

pollutants. Much of the bioremediation aspects of e-waste degradation have been employed in developed countries like China and USA. India being one of the largest e-waste recycling centre, it is of crucial need, to develop novel bioremediation protocols.

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