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Bioleaching of metals from waste printed circuit boards using bacterial isolates native to abandoned gold mine

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Abstract In the present study, native bacterial strains isolated from abandoned gold mine and *Chromobacterium violaceum* (MTCC-2656) were applied for bioleaching of metals from waste printed circuit boards (WPCBs). Toxicity assessment and dose–response analysis of WPCBs showed EC_{50} values of 128.9, 98.7, and 90.8 g/L for *Bacillus* sp. SAG3, *Bacillus megaterium* SAG1 and *Lysinibacillus sphaericus* SAG2, respectively, whereas, for *C. violaceum* EC_{50} was 83.70 g/L. This indicates the viable operation range and technological feasibility of metals bioleaching from WPCBs using mine isolates. The influencing factors such as pH, pulp density, temperature, and precursor molecule (glycine) were optimized by one-factor at a time method (OFAT). The maximum metal recovery occurred at an initial pH of 9.0, a pulp density of 10 g/L, a temperature of 30 °C and a glycine concentration of 5 g/L, except for *L. sphaericus* which showed optimum activity at initial pH of 8.0. Under optimal conditions the metals recovery of Cu and Au from WPCBs were recorded as 87.5 ± 8% and 73.6 ± 3% for *C. violaceum* and 72.7 ± 5% and 66.6 ± 6% for *B. megaterium*, respectively. Kinetic modeling results showed that the data was best described by first order reaction kinetics, where the rate of metal solubilization from WPCBs depended upon microbial lixiviant production. This is the first report on bioleaching of metals from e-waste using bacterial isolates from the gold mine of Solan, HP. Our study demonstrated the potential of bioleaching for resource recovery from WPCBs dust, aimed to be disposed at landfills, and its effectiveness in extraction of elements those are at high supply risk and demand.

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Introduction

The higher obsolescence rate of electrical and electronic equipment's (EEE) have increased electronic-

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waste (e-waste) load globally. In 2019, world has generated 53.6 Mt (7.3 kg per capita) of e-waste of which 17.4% (9.3 Mt) was documented to be properly collected and recycled, whereas 82.6% (44.3 Mt) remains undocumented (Baldé et al. 2017; Forti et al. 2020). Further, it is expected to keep on increasing up to 74.7 Mt by 2030, with an increase of 2 Mt/annum (Forti et al. 2020). Globally, China is at the top to generate maximum e-waste i.e., 10.1 Mt in 2019. In India, the estimated amount of e-waste generated in 2019 was 3.2 Mt as compared to the waste generated in 2016 i.e., 2.0 Mt. E-waste is mixture of metals and persistent organic pollutants (POP's), which have detrimental impacts on the environment/ human health, if processed improperly (Chakraborty et al. 2017; Pradhan and Kumar 2012; Tansel 2017). Among the e-waste, printed circuit boards (PCBs) are the major bearers of metals [approximately 30% (w/w)] and account for 3% weight of total electronic scrap (Kaya 2016). E-waste contains approximately 69 metals from periodic table including the precious metals like platinum, gold and silver and palladium and rare earth metals. According to recent estimates, in 2019, the value of secondary raw material of e-waste is worth 57 billion US Dollar, of which, values of Cu, and Au corresponds to 10,960, and 9481 million US Dollar, respectively (Forti et al. 2020). The significant amount of material values present in e-waste attracts the attention of recyclers. Numerous countries are continuously framing and updating policies, legislation or regulations for e-waste management. As on October 2019, 78 countries are covered by legislation, policy and regulation; making the e-waste recycling mandatory (Forti et al. 2020). The recycling of e-waste will not only reduces the burden from primary resources but also creates jobs and save the landfill space required to dispose off the material (Kumar et al. 2017). At present, physico-mechanical, pyrometallurgical and hydrometallurgical methods are commonly used to recycle and recover metals from WPCBs. During mechanical treatment a large amount of valuable and rare earth metals are lost or ends up as dust in output fractions, generally not involved in metallurgical refining. In addition, currently employed metallurgical methods have high energy and chemicals demand, material loss during recovery and are not safe because of release of significant amounts of toxic gases, acidic waste water, and hazardous substances in to the environment (Priya and Hait 2017; Sahni et al. 2016). These limitations pressingly urge for intensive research to set environmentally sound and sustainable treatment system for e-waste recycling.

In this context, biological processes are preferred choice over traditional methods which, not only extract metals but simultaneously mitigate the pollution. Bioleaching is a clean, environmentally compatible, energy efficient and cost-effective process of metals recovery from e-waste (Brandl et al. 2001; Ilyas 2013; Kumar et al. 2017). Bioleaching has been successfully used to recover metals from low-grade ores and complex sources like e-waste, which cannot be accessible by traditional metallurgical methods (Brandl et al. 2008; Kumar et al. 2017; Priya and Hait 2017). The bioleaching studies are more focused on autotrophic microbes and are merely on heterotrophic microorganisms. Some of the commonly exploited bioleaching microorganisms include sulfur and iron oxidizing chemolithotrophic acidophiles such as *Ferrobacillus*, *Acidithiobacillus*, *Leptospirullum*, and heterotrophs such as species of *Chromobacterium*, *Pseudomonas, Sulfolobus*, and *Bacillus* (Priya and Hait 2017). During the process of bioleaching, microorganisms follow various routes namely acidolysis, complexolysis, and redoxolysis to solubilize metals from e-waste. Cyanogenic bacteria (e.g., *C. violaceum, B. megaterium, P. aeruginosa, and P. fluorescens*) produce hydrogen cyanide as a secondary metabolite and form solubilized metal-cyanide complex from metal-containing solids/wastes such as low-grade ore and e-waste (Arshadi and Mousavi 2015; Natarajan and Ting 2014). Among heterotrophic cyanogenic microorganisms, *C. violaceum* has been widely exploited for recovering metals from e-waste (Brandl et al. 2008; Natarajan and Ting 2014; Pradhan and Kumar 2012; Sahni et al. 2016). Though *C. violaceum* has been widely studied and shows great bioleaching ability, it is not suitable to be employed in industrial bioleaching applications. *C. violaceum* is an opportunistic pathogen and mostly found in tropical and subtropical regions. The special need for growth conditions restricts the scope of its industrial application in recovering metals from e-waste (Jujun et al. 2014, 2015). Therefore, it will be of great significance to search/explore new microbial strains for the industrial bioleaching process of recovering metals from e-waste (Jujun et al. 2014; Priya and Hait 2017).

There are growing evidence that microorganisms isolated from native gold resources/gold mines have potential to dissolve/bioleach Au from soil or other secondary sources like e-waste (Kaksonen et al. 2014). Therefore, in this study, bacterial diversity of an abandoned gold mine was characterized for bioleaching application. To improve the bioleaching efficiency, the following parameters were selected with attention to previous work: (1) pH (Chi 2011); (2) pulp density (Pradhan and Kumar 2012); (3) temperature (Jujun et al. 2014); (4) precursor molecule (glycine) (Brandl et al. 2008); and (5) EC_{50} (Kumar et al. 2017) using native and reported cyanogenic bacteria. The purposes of this study were (1) to find out suitable bacterial strains capable of solubilizing Cu and Au from WPCBs (2), to find effective concentration (EC_{50}) for metal bioleaching (3), and to determine optimum conditions for the bioleaching process.

Materials and methods

WPCBs characterization

The WPCBs used in the study was supplied by Exigo Recycling, Panipat, Haryana, India. Recyclers collect the discarded electronic equipments from different states/parts of the country. The WPCBs obtained from shredding, pulverization and other mechanical operations (dismantling and segregation) was used for bioleaching of Cu and Au at Jaypee University Information Technology, Waknaghat, Solan. The metal content analysis of WPCBs dust was carried as per protocol given in Kumar et al. (2017), and analyzed using atomic absorption spectrophotometry [(AAS) (AAnalyst 400, PerkinElmer)] at 324.8 and 242.8 nm wavelengths for Cu and Au, respectively. The elemental composition of WPCBs has previously been reported as 23.39 and 0.08 mg/g of Cu and Au, respectively (Kumar et al. 2017). The significant proportions of precious metals present in WPCBs dust attracts the interest of recyclers as it can be a potential secondary resource of these metals. However, simultaneous presence of numerous metals in WPCBs cause difficulties in their selective extraction.

Soil sampling

Soil samples (top soil i.e. 0–15 cm) were collected from two different sites (side walls and floor) of an abandoned gold mine (Fig. 1). The abandoned gold mine is situated at village Makdoa (30°50′56″N and 77°10′12″E) which is 15 Km from Solan town of Himachal Pradesh, India. The mine is situated along the banks of Kewal River and was closed down years ago due to the scanty quantity of Au. Soil and water samples within periphery (10–15 m) of mine were also collected and pooled. The collected samples were aseptically transported to the laboratory in zip lock bags and stored at – 20 °C until further analysis.

Isolation and screening of bacterial isolates for their e-waste bioleaching potential

For microbial isolation, the soil sample collected from the abandoned gold mine was enriched in Luria broth (LB) medium (100 mL in 250 mL Erlenmeyer flask) containing sterilized WPCBs. One gram of the representative soil sample was inoculated in LB medium supplemented with 1% pulp density of WPCBs (particle size $\leq$150 µm). The experimental flasks were incubated at 30 °C in an incubator shaker (Thermo Scientific MaxQ 8000) at 150 rpm for 6 days. After 6 days, 5 mL of inoculum [2×10^8 (Colony Forming Unit) CFU/mL] was transferred to the 2nd flask containing fresh medium supplemented with 2.5% WPCBs followed by incubation under same set of conditions for next 6 days. The sequential enrichment was continued for a period of 30 days at an increasing concentration of WPCBs i.e., 5, 7.5 and 10%, respectively. After 30 days, samples from the flask containing 10% pulp density were serially diluted and spread plated on nutrient agar (NA) plates. The plates were incubated at 30 °C for 2–4 days and observed for the appearance of colonies after every 24 h. Morphologically distinct colonies were selected and repeatedly sub-cultured to ensure the purity of the bacterial isolates (Kumar et al. 2015). To avoid microbial contamination, an un-inoculated control was incubated in parallel. The mine isolates were screened for their metal bioleaching potential from WPCBs using a two-step bioleaching process.

Characterization and phylogenetic analysis of gold mine isolates

The identification of bacterial isolates was carried using 16S rDNA sequencing technique. Bacterial DNA was extracted using Wizard genomic DNA purification kit (Promega) as per manufacturer

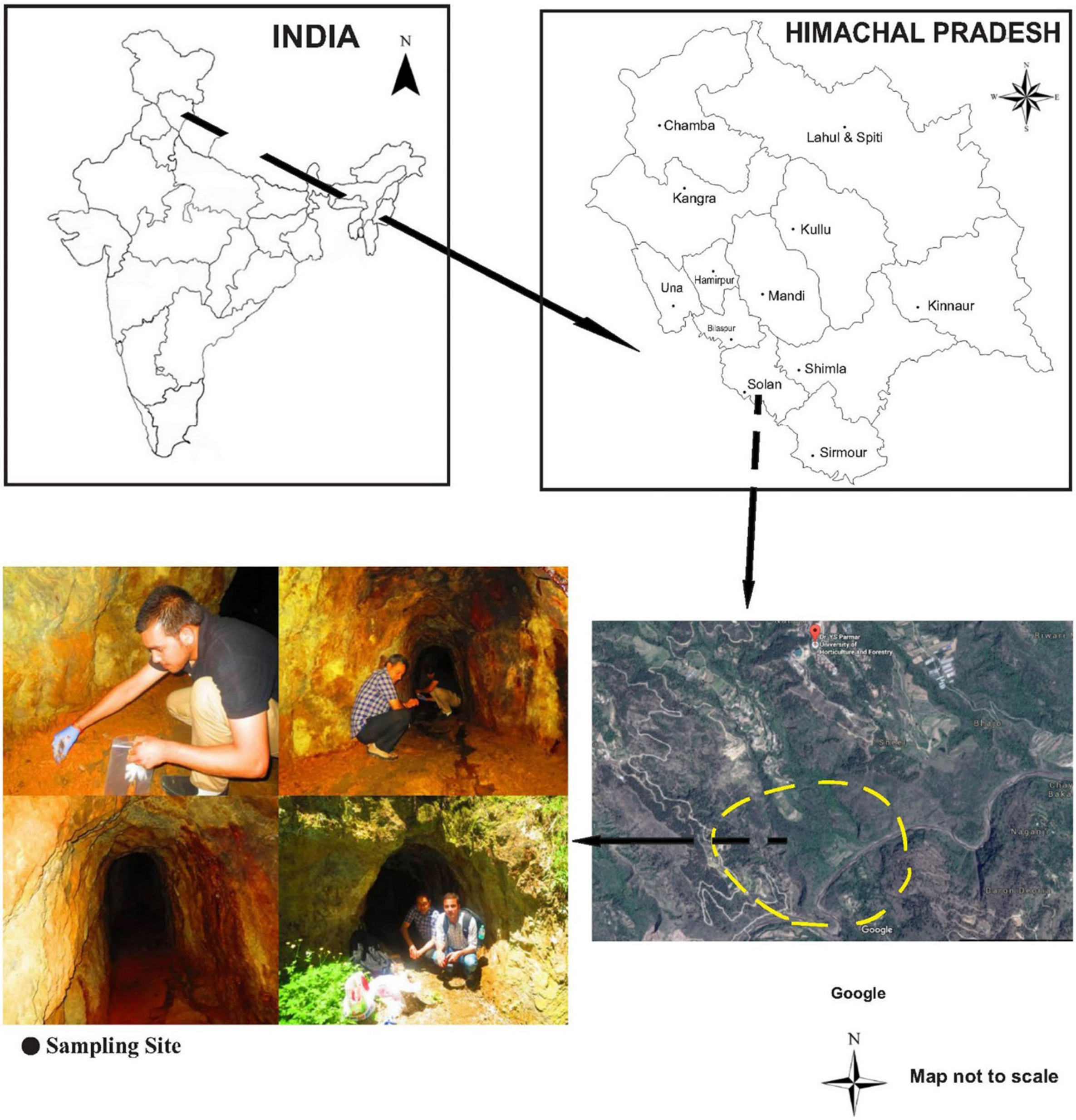

Fig. 1 Location map and snapshot of abandoned gold mine (sampling site) of district Solan of Himachal Pradesh

instruction. The Polymerase Chain reaction (PCR) amplification of 16S rRNA gene was done using universal primers 27F and 1492R. PCR reaction of 20 μL volume contained 50 ng of template genomic DNA, 5 pmole of forward and reverse primers, and PCR master mix (promega). The PCR reaction started with initial denaturation at 95 °C for 5 min followed by 35 cycles at 95 °C for 1 min, 51.8 °C for 1 min and 72 °C for 1 min and having a final extension at 72 °C for 7 min. The amplified PCR product (1465 bp) was separated by 1.5% agarose gel electrophoresis and then sequenced (Xcelris Labs Limited, India) using Sanger sequencing method. The nucleotide basic local alignment search tool (BLAST) of the National Center for Biotechnology Information, MD, USA, was used for similarity search of the sequences. The alignment was done with the help of ClustalW program. Evolutionary distances were computed by Juke-Cantor method and a phylogenetic tree was constructed using the neighbor-joining method by MEGA v 6 (Tamura et al. 2013). The topology of the tree was estimated by bootstrap resampling analysis for 1000 replicates (Felsenstein 1985).

Toxicity assessment and dose–response analysis

The mine isolates were subjected for toxicity assessment and dose-response analysis to guarantee the feasibility of bioleaching. The toxicity tolerance was determined using CFU count method. The bacterial cells were exposed to 1, 5, 10, 15, 20, 30, 40 and 50% pulp density of WPCBs. Experimental flasks were inoculated with $1–2\times10^5$ CFU/mL (approx.) along with sterilized WPCBs at their respective concentrations (Institute 2013). The flasks were incubated at 30 °C and 150 rpm for 24 h. After 24 h, the samples were serially diluted and spread plated on NA followed by enumeration of bacteria in terms of CFU/mL. For dose-response curve, the percent inhibition response was calculated as per Eq. (1):

$$\%IR = \frac{A - B}{A} \times 100 \tag{1}$$

where *IR* is Inhibition Response; *A* is bacterial growth in absence of e-waste, and *B* is bacterial growth in presence of e-waste.

Dose-response curves were generated using software GraphPad Prism version 6 (GraphPad Software, Inc., La Jolla, California). Percent IR data at respective pulp density was subjected for nonlinear regression followed "*log (agonist) vs. normalized response-variable slope*" in GraphPad Prism version 6 (Li et al. 2015). The EC_{50} values (statistical estimated values about the quantity of a substance/ pollutant/ chemical resulting in 50% growth reduction within a specified time period) were calculated on the basis of dose-response data.

WPCBs bioleaching study

According to previous reports, the bioleaching of Cu and Au using cyanogenic microorganisms depends upon WPCBs pulp density, temperature, initial pH and type of precursor used (Arab et al. 2020; Kumar et al. 2018; Thakur and Kumar 2020). Therefore, influence of these parameters on bioleaching of Cu and Au was investigated using a two-step bioleaching process. The selected bacterial isolates were assayed at different temperature (25, 30, 35, and 40 °C), glycine concentration (2.5, 5.0, 7.5 and 10.0 g/L), pH (7, 8 and 9) and pulp density (1, 5, and 10%) to obtain maximum bioleaching of Cu and Au, respectively.

The two step bioleaching process was employed to leach metals from WPCBs. In the process, bacterial cells were inoculated with an inoculum size of 5.0 mL (approximately 2×10^8 CFU/mL) followed by incubation at 30 °C and 150 rpm for 48 h. After 48 h of incubation, the sterilized (121 °C, 15 psi, and 30 min) WPCBs (1% w/v) of particle size $\leq$ 150 µm were added to the experimental flasks, aseptically. The flasks were again incubated under the same set of conditions for additional time period of 7 days. An appropriate control containing medium and sterilized WPCBs was run in parallel. After 7 days, content of all the flasks was filtered and centrifuged (Eppendorf, 5804 R) at 7000 rpm for 10 min to remove the bacterial cells and residual WPCBs from the medium. The supernatant obtained was further filtered through glass fiber filter of 0.45 µm (PALL-GF-A/E-I) to warrant particle free suspension. The metal ions dissolution was analyzed using AAS. Other than metal ions, the bacterial isolates were tested for production of cyanide ion during their growth in the LB medium. The samples were sent for analysis to Jeedimetla Effluent Treatment Ltd. Hyderabad, India and tested using standard method ($4500CN^{-}E$). The change in final pH of the medium was also analyzed through a portable digital pH meter (Eutech pH Testr30).

Statistical analysis

All the experiments were performed in duplicate sets along with an appropriate control. A two-way analysis of variance (ANOVA) was used to interpret the significance of data at probability value (p) < 0.05 through GraphPad Prism version 6 (GraphPad Software, Inc., La Jolla, California).

Results and discussion

Isolation, characterization and screening of native bacterial isolates

The aim to isolate native bacteria from abandoned gold mine is to exploit the privileges of microorganisms adapted to toxic/hazardous environments where metals may constitute a significant portion of soil. The microbes present in such environments are in direct contact with hazardous substances and regulate their metabolic activity in accordance to their inhabitation for their survival. In this study, a total of eight morphologically distinct bacterial strains were isolated on nutrient agar with different morphological characteristics (data not shown). Genomic DNA of each bacterial strain was PCR amplified to obtain 16S rRNA gene sequences and classified according to their similarity with the available 16S rRNA sequences in the GenBank database. The results of BLASTN search revealed closest sequence identities from the GenBank database with a 97–100% similarity. Based on the 16S rRNA sequencing data, bacterial strains were identified as *B. megaterium* SAG1; *L. sphaericus* SAG2; *Bacillus* sp. SAG3; *B. amyloliquefaciens* SAG4; *Bacillus* sp. SAG5; *Brevibacterium frigoritolerans* SAG6; *Chryseomicrobium amylolyticum* SAG7 and *B. safensis* SAG8, respectively. The 16S rRNA gene sequence has been submitted and available under accession no: KU163234-KU163241 in the GenBank database. The phylogenetic analysis of selected eight bacterial strains was performed using the neighbor-joining method with 1000 bootstrap sampling. The results revealed the evolution of bacterial strains from one phylogenetic group of Firmicutes (Fig. 2; represented by a triangle). The isolated bacterial strains showed 50.23–99.73% 16S rRNA gene sequence similarity values among themselves. In the present study, *Bacillus* and *Bacillus*-derived genera were found to be dominant in the soil of abandoned gold mine. The prevalent existence of *Bacillus* genera from the gold mine, as well as other soil samples, has been well reported by various researchers (Kumar et al. 2015; Rastogi 2009a; Rastogi et al. 2009b).

All the isolated bacterial strains (eight in number) were screened for their Cu and Au bioleaching capabilities from e-waste. Out of eight isolates, three bacterial strains i.e., *B. megaterium* SAG1; *L. sphaericus* SAG2; *Bacillus* sp. SAG3 showed good bioleaching abilities (Fig. 3). It has been reported that, the microorganisms native to low grade ores/pollution sites play important role in biogeochemical cycling of Au and other metals (Kaksonen et al. 2014), for example, Ghosh et al. (2018) isolated bacterial strains indigenous to low grade Mn-ore and subsequently applied for bioleaching of Mn under optimized conditions of sucrose concentration (2 g/100 mL), pulp density (2%), initial pH 6.5, and 30°C incubation temperature for 20 days. Their result showed 70% and 67% recovery of Mn using *Acinetobacter* sp. MSB5 and *Lysinibacillus* sp. MSB11, respectively. Another study by Arab et al. (2020) investigated bioleaching of metals using cyanogenic microorganisms indigenous to an e-waste landfill. Their results reported 96.73% recovery of Cu by isolate S22. Kumar et al. (2017) reported 68.5 and 33.8% recovery of Au and Ag from e-waste using *P. balearica*, indigenous to e-waste recycling site. The selected microorganisms (*B. megaterium* SAG1, *L. sphaericus* SAG2, and *Bacillus* sp. SAG3) along with one standard bacterium *C. violaceum* (MTCC-2656) previously procured from Institute of Microbial Technology (IMTECH), Chandigarh, India were used in further toxicity assessment, optimization, and kinetic modeling studies. Although, the bioleaching potential of *C. violaceum* and *B. megaterium* have been well documented, heterogenity of e-waste lead to variable results in bioleaching of metals (Arshadi and Mousavi 2015; Arshadi et al. 2016; Brandl et al. 2008; Pradhan and Kumar 2012).

Toxicity assessment and dose response analysis

Bacterial cells of *B. megaterium* SAG1, *L. sphaericus* SAG2 and *Bacillus* sp. SAG3 were exposed to 1, 5, 10, 15, 20, 30, 40 and 50% of e-waste pulp density. The toxicity assessment was studied by reduced cell growth compared to that of control. To get an estimate about e-waste toxicity, quantitative dose response curves were generated (Fig. 4). The R^2 values for dose response curves of *Bacillus* sp. SAG3, *B. megaterium* SAG1 and *L. sphaericus* SAG2 were 0.95, 0.92 and 0.88, respectively. The mine isolate *Bacillus* sp. SAG3 exhibited higher tolerance to e-waste toxicity than *B. megaterium* and *L. sphaericus*. The EC_{50} values for *Bacillus* sp. SAG3, *B. megaterium* SAG1 and *L. sphaericus* SAG2 were Log 2.1 (128.9 g/L), 1.9 (98.7 g/L) and 1.9 (90.8 g/L), respectively. The 95% confidence intervals for *Bacillus* sp. SAG3, *B. megaterium* SAG1 and *L. sphaericus* SAG2 were 113.9-145.9, 71.3-115.8 and 81.9–118.9 of WPCBs in culture medium (g/L), respectively. The toxicity assessment of *C. violaceum* ($EC_{50} =$ Log 1.9 (83.70 g/L); $R^2 = 0.91$) was already reported in our

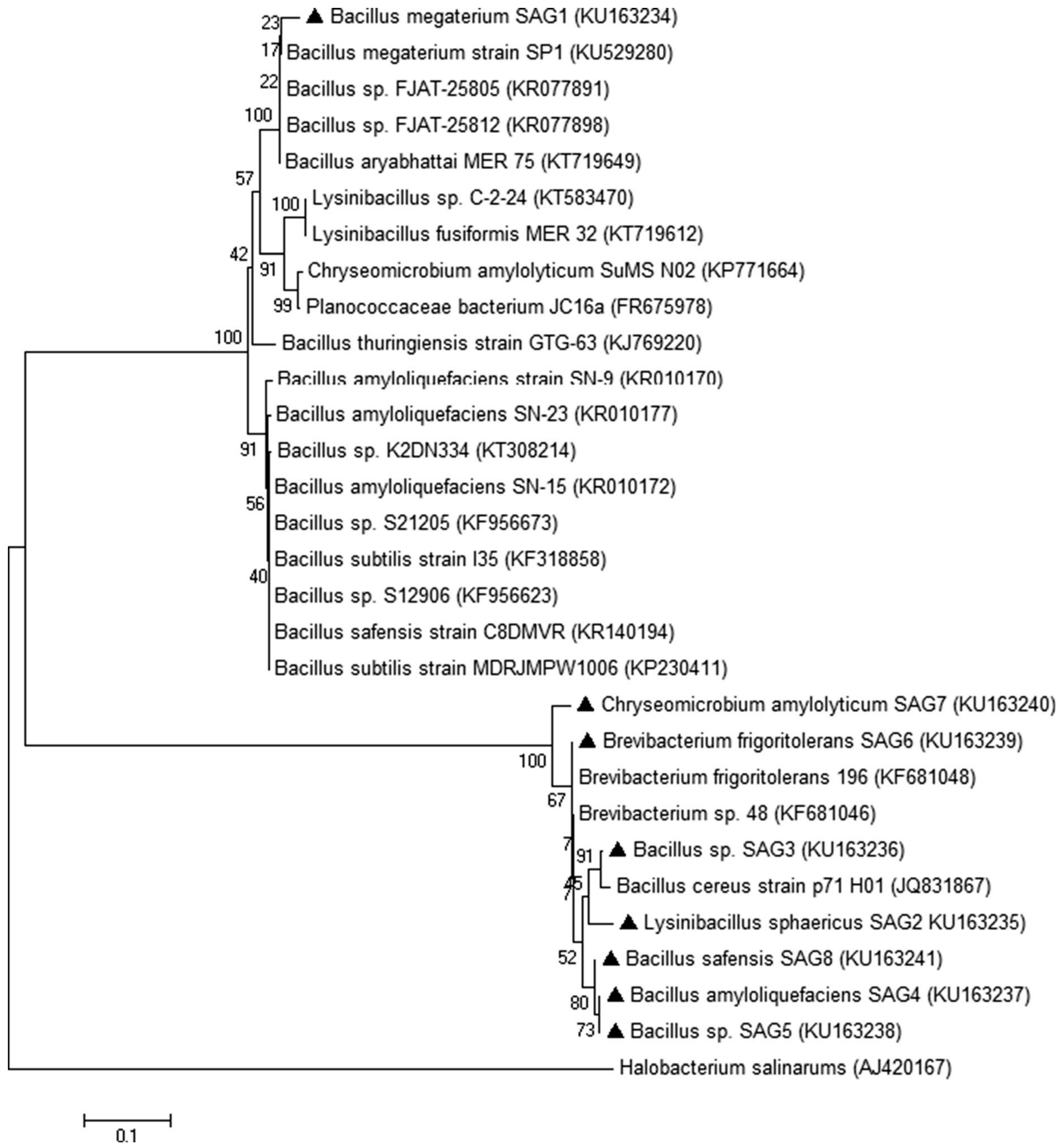

Fig. 2 Neighbour-joining phylogenetic tree showing relationship between 16S rRNA gene sequences retrieved from abandoned gold mine with reference sequences in GenBank. The *scale bar* represents 0.1 substitutions per nucleotide position. Numbers at the node are the bootstrap values (%). *Halobacterium salinarums* (AJ420167) was selected as outgroup. Triangle represents gold mine isolates

previous study by Kumar et al. (2017). It was observed that mine isolates showed highest tolerance to e-waste toxicity than *C. violaceum*. This may be attributed to their habitat "gold mine", which have developed metabolic machinery to resist the effect of toxic metals ions. Further, it should be noted that the EC_{50} may vary with growth conditions, type of WPCBs/e-waste and bacterial strain used. The results of toxicity

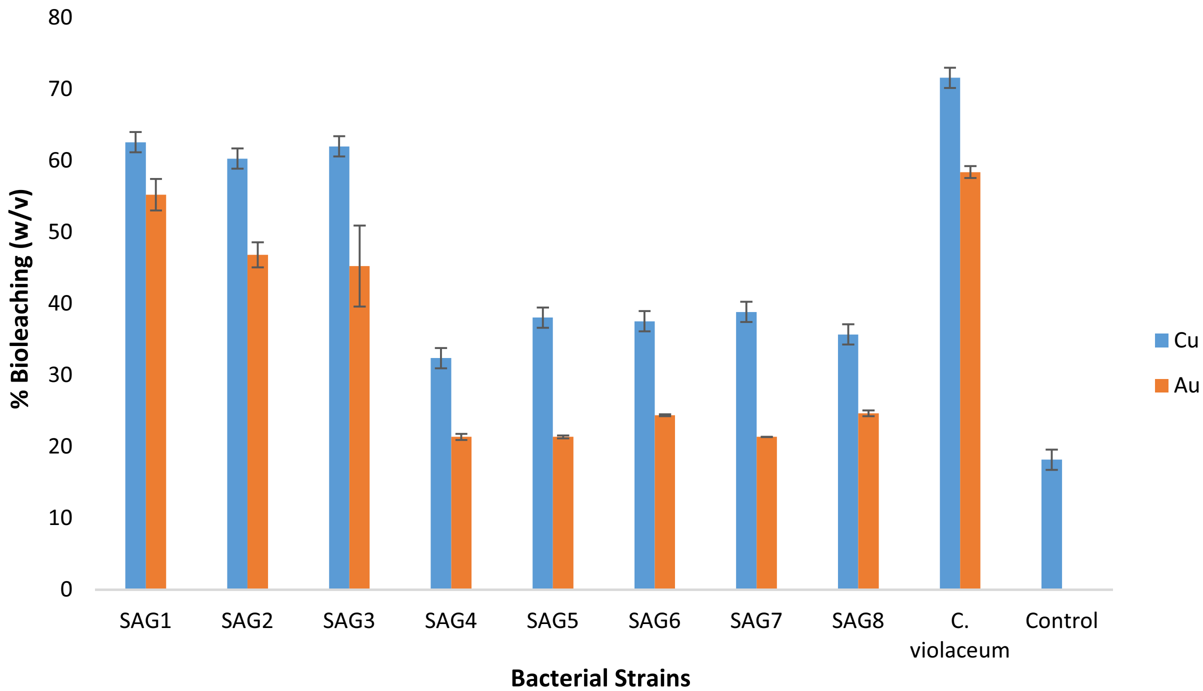

Fig. 3 Percent bioleaching of Cu and Au from waste WPCBs by cyanogenic bacterial strains. The presented data is the average of those obtained from duplicate experiments. The results were significant at a $p < 0.05$ based on two-way ANOVA analysis

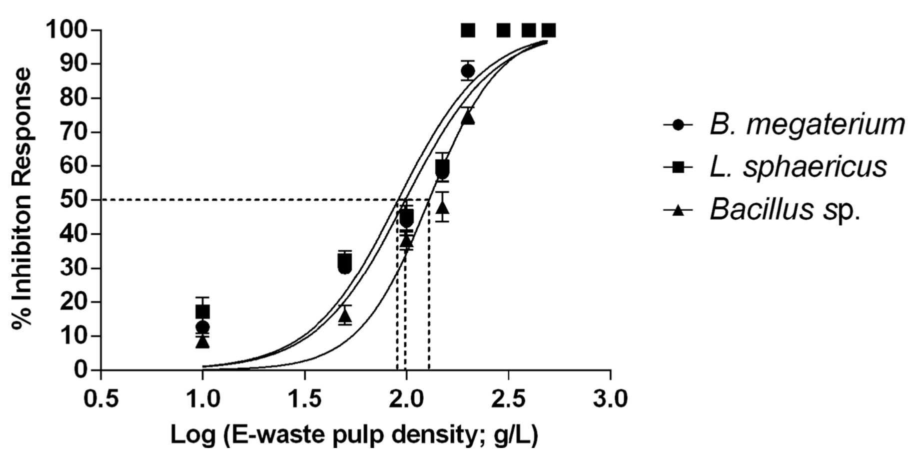

Fig. 4 Dose–response curves of WPCBs toxicity on native mine isolates predicted using equation "*log (agonist) versus normalized response–variable slope*" of GraphPad Prism 6

assessment depicts viable operation range for metals bioleaching from WPCBs, suggesting the industrial possibility of bioleaching using mine isolates.

WPCBs bioleaching Study

In industrial production, processes optimization is a topic of central importance and small improvements can be decisive for commercial success. Various physical and microbiological parameters influence the effectiveness of the bioleaching process (Amiri et al. 2011). Optimization via improvements and manipulation of these parameters can increase the overall productivity (Reddy et al. 2008); hence it is a critical component. Therefore, to increase the bioleaching efficiency (using cyanogenic bacteria), the following key parameters were selected i.e., (1) pH; (2) pulp density; (3) temperature; and (4) precursor molecule, as discussed in detail below.

Effect of pulp density

Pulp density is a key factor in the bioleaching process. A batch leaching was carried out to investigate the influence of pulp density in the shake flasks at a concentration of 1, 5, and 10%, respectively. Other factors, such as pH (8.0), temperature (30°C) and glycine concentration (5 g/L) were kept constant. From the results, it was observed that all the bacterial strains were able to mobilize maximum of Cu and Au from WPCBs at 1% pulp density under shake flask conditions. Further, increase in WPCBs pulp density from 5 to 10% resulted in a significant decrease in metals mobilization (Fig. 5). Among the tested strains, *B. megaterium* was able to mobilize 62.6 and 57.7 of Cu and Au at 1% pulp density. The mobilization of Cu and Au was decreased to 18.1 and 9.1% at 5% pulp density and 5.1 and 4.5%, at 10% pulp density (Fig. 5a, b). Similarly, *L. sphaericus* and *Bacillus* sp. also showed higher metals mobilization at 1% pulp density. Our results are in accordance with Pradhan and Kumar (2012), who reported higher mobilization of Cu (79%) and Au (69%) from e-waste at 1% pulp density compared to 5% and 10% pulp density using *C. violaceum*. Natarajan and Ting (2014) performed bioleaching of pretreated and untreated electronic scrap material (ESM) at pulp densities of 0.5, 1, 2 and 4%, respectively, using *C. violaceum*. Their results showed higher mobilization of Cu and Au at 0.5% pulp density with both untreated and pretreated ESM. Another study by Işıldar et al. (2016) reported higher recovery of Au (44%) from WPCBs using *P. putida* at 1% pulp density. The mine isolates *B. megaterium*, *L. sphaericus* and *Bacillus* sp. were able to leach the significant amount of metals from WPCBs, however; *C. violaceum* showed higher mobilization of Cu (71.6%) and Au (58.42%) under the same set of conditions. The low bioleaching at higher pulp densities may be because of lethal effects of metallic and non-metallic components of WPCBs on bacteria or oxygen mass transfer limitation (Marra et al. 2018); which is a challenge in the industrial implementation of the process economically.

Effect of temperature

The microbial growth takes places at a wider range of temperature, and microorganism's exhibit optimum bio-activities at a specified narrow range of temperature. It is reported that the cyanogenic

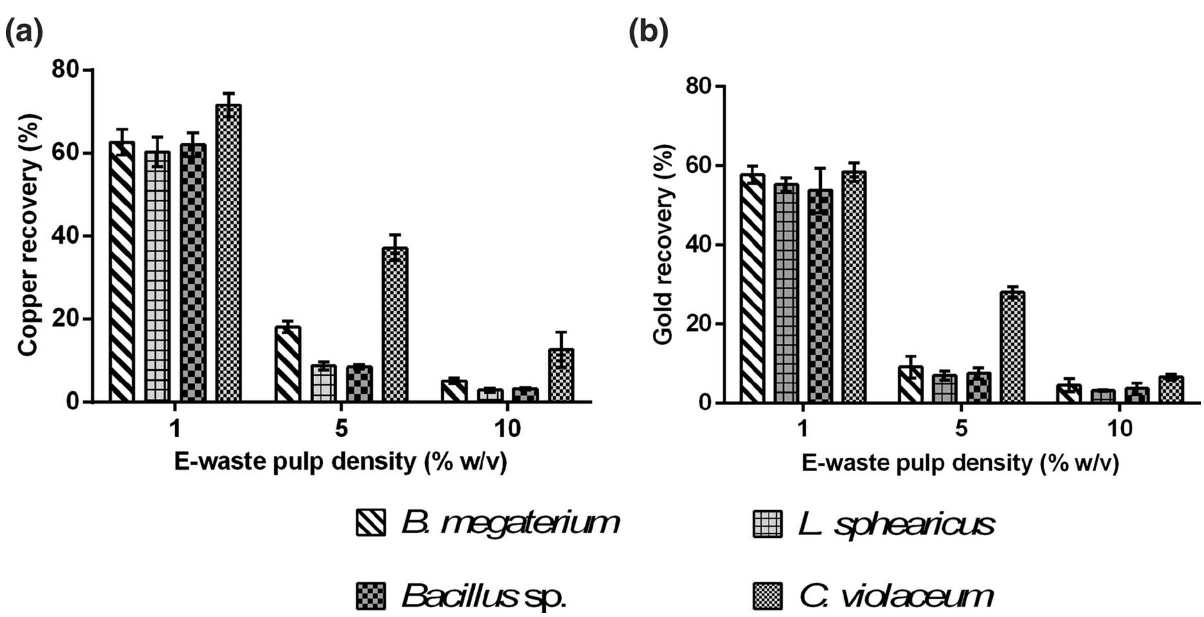

Fig. 5 Influence of WPCBs pulp density on percent bioleaching of metals by cyanogenic bacterial strains: **a** influence on Cu recovery (%), **b** Influence on Au recovery (%).The presented data in this figure is the average of those obtained from duplicate experiments

microorganisms produce maximum cyanide at a temperature range of 15–35 °C, indicating a temperature dependent characteristics of cyanogenic microbes (Arab et al. 2020; Jujun et al. 2014). Therefore, effect of temperature on bioleaching of metals was investigated at a range from 25 to 40 °C. The results revealed that the maximum metal (Cu and Au) mobilization occurred at 30 °C, whereas minimum was at 40 °C. This corresponds to the fact that temperature variation influenced the degree of secondary metabolite (HCN) formation and maximum cyanide production occurs at a temperature range of 25–30 °C (Castric 1975). The percentage of metals (Cu, and Au) mobilized by different bacteria at 30 °C were measured in the following order: *C. violaceum* (71.7% and 58.9%) > *B. megaterium* (66.1% and 57.2%) >*L. sphaericus* (64.9% and 56.1%) > *Bacillus* sp. (61.78% and 50.9%), respectively (Fig. 6a, b). Thus 30 °C temperature was considered as optimum, which is in accordance with former bioleaching studies (Arshadi and Mousavi 2015; Brandl and Faramarzi 2006). The 30 °C temperature offers advantages of low energy requirement and may reduce the cost of the process.

Effect of glycine concentration

The cyanide production by cyanogenic microorganisms depends not only on carbon source but also on amino acids like glycine present in medium. Glycine is a known immediate direct precursor molecule of cyanide, which forms HCN by oxidative decarboxylation (Brandl et al. 2008). The addition of glycine in the production medium can enhance cyanide production. Thus, influence of different glycine concentrations (2.5, 5.0, 7.5 and 10 g/L) on bioleaching of Cu and Au from WPCBs was investigated. It was noted that glycine response varied from bacteria to bacteria in terms of metal mobilization. The maximum mobilization of Cu and Au by *B. megaterium* (Cu-65.5% and Au-56.8%), *L. sphaericus* (Cu-64.2% and Au-55.8%) and *Bacillus* sp. (Cu-63.4% and Au-52.4%) occurred at 5.0 g/L glycine concentration. Further, increase in glycine concentration from 5.0 to 10.0 g/L resulted in decreased metal mobilization except for *C. violaceum*. This is because increased glycine concentration in the solution causes the toxicity stress to bacterial cells which leads to reduced growth as well as low lixiviant production; and hence, a decreased metals recovery (Arshadi and Mousavi 2015; Faramarzi et al. 2004). The *C. violaceum* showed maximum mobilization of 76.9% and 60.8%, for Cu and

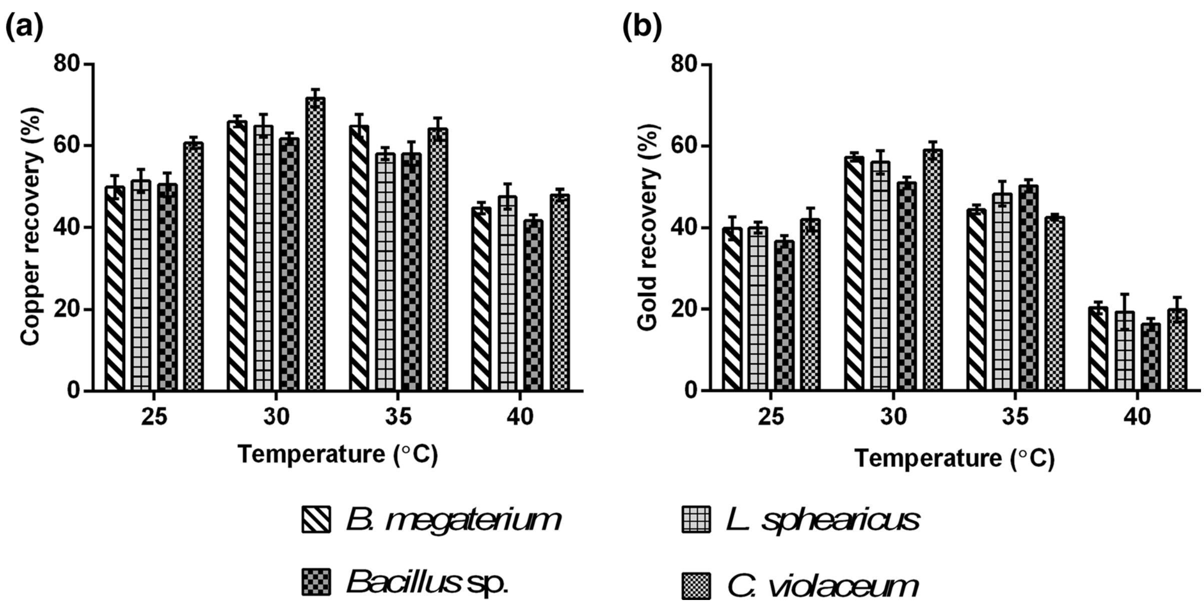

Fig. 6 Influence of temperature on percent bioleaching of metals by cyanogenic bacterial strains: **a** influence on Cu recovery (%), **b** Influence on Au recovery (%). The presented data in this figure is the average of those obtained from duplicate experiments

Au, respectively, at a glycine concentration of 7.5 g/L (Fig. 7a, b). However, the metal leaching efficiency was statistically not significant when glycine concentration increased from 5.0 to 7.5 g/L. Our results are in good agreement with Arab et al. (2020) and Shin et al. (2013) which reported 5 g/L glycine concentration as optimum during bioleaching by cyanogenic bacterial. Faramarzi et al. (2004) determined a range of 8–10 g/L glycine concentration as optimum for highest cyanide production as well as recovery of tetracyanonickelate (43.5% mobilization of Ni) using *C. violaceum*. At 10 g/L of glycine concentration, a decrease in the bioleaching of metals was observed.

Effect of pH

Since pKa of cyanide is 9.3, conducting metal dissolution reaction under alkaline condition increases the total cyanide ions available for bioleaching (Natarajan and Ting 2014). According to Chi (2011) the cyanide producing ability of *C. violaceum* increased with pH value ranging from 7 to 9, and further increase in pH resulted in decreases in cyanide production (Arab et al. 2020). Therefore, metal mobilization ability of bacterial strains were tested at different pH (7, 8 and 9) in LB medium. It was observed that maximum metal mobilization took place at a pH of 9.0 followed by 8.0, whereas the minimum was observed at a pH of 7.0 except for *L. sphaericus*, where maximum metal mobilization was observed at a pH of 8.0 (Fig. 8a, b) The low mobilization at pH 7 is because equilibrium shifts to produce HCN gas (Eq. 2), which is volatile and less soluble in water. However, at pH (9), equilibrium favors formation of more cyanide ions (CN^-) and increases its availability for metals solubilization/complexation (Natarajan and Ting 2015).

$$HCN \leftrightarrow H^+ + CN^-. \tag{2}$$

Out of four bacterial strains, *C. violaceum* showed 87.5%, and 73.6% mobilization of Cu and Au, respectively. Whereas, among the mine isolates, *B. megaterium* showed the maximum dissolution of Cu (72.7%) and Au (66.6%) during bioleaching of WPCBs. Our results are in accordance with Arab et al. (2020) who reported increase in cyanide production from a pH 7 to 9 by an isolate N37. In addition researchers also monitored bacterial growth during bioleaching experiments, which revealed inhibition of microbial growth with increase in pH. The maximum cell growth took place near to pH of 7.4 (Tran et al. 2011). Several researchers investigated the

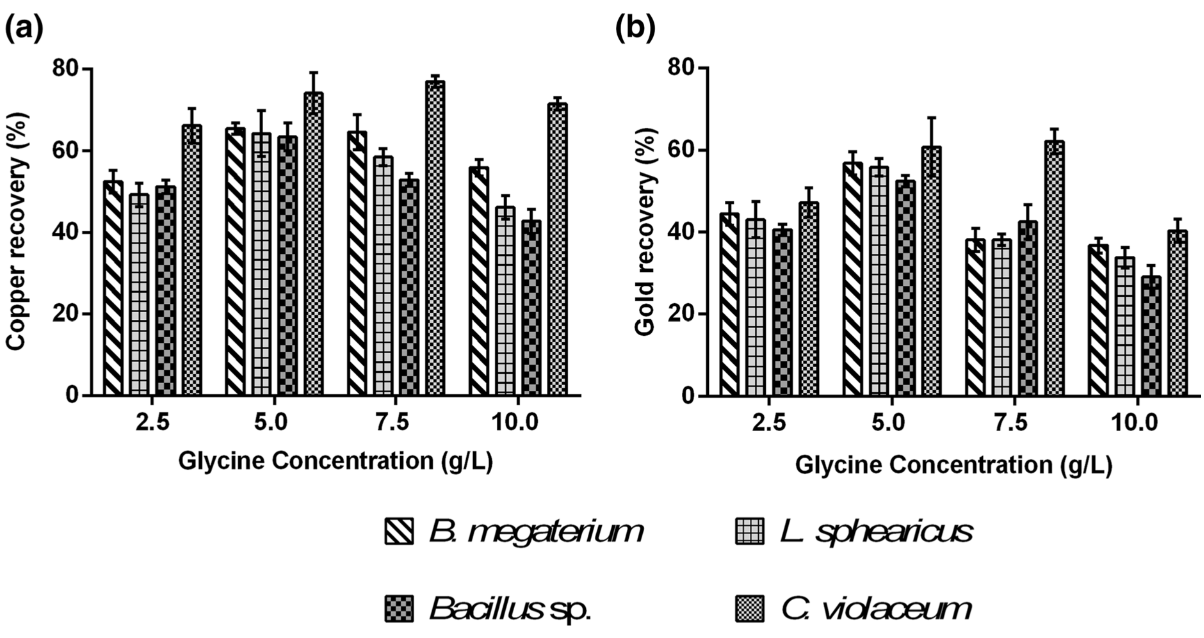

Fig. 7 Influence of glycine concentration on percent bioleaching of metals by cyanogenic bacterial strains: **a** influence on Cu recovery (%), **b** influence on Au recovery (%). The presented data in this figure is the average of those obtained from duplicate experiments

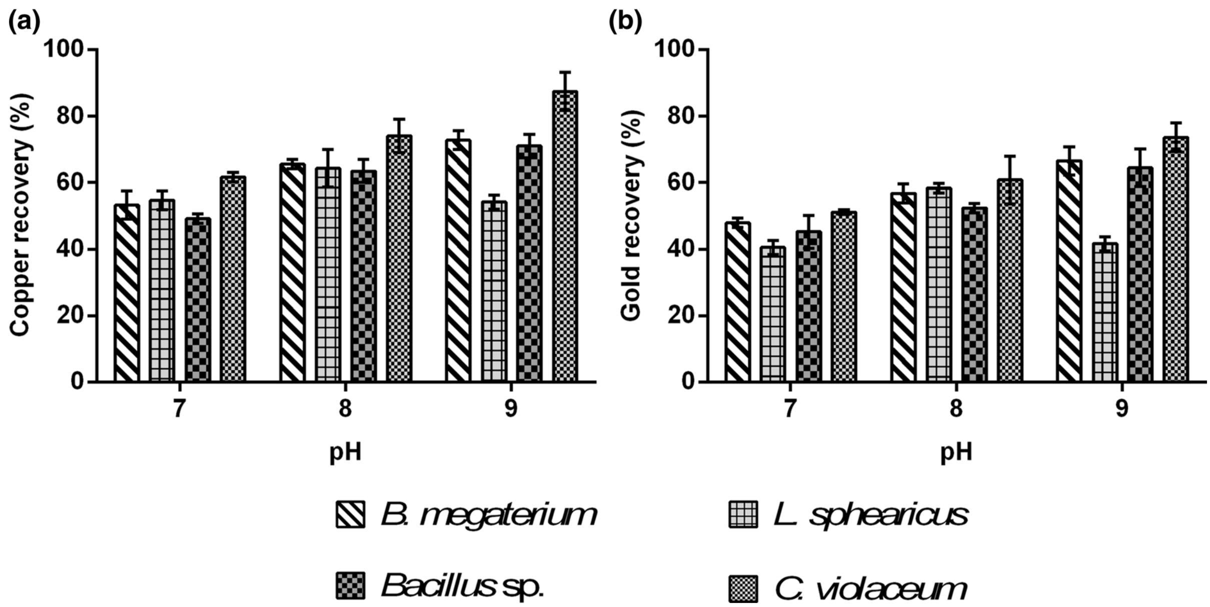

Fig. 8 Influence of initial pH on percent bioleaching of metals by cyanogenic bacterial strains: **a** Influence on Cu recovery (%), **b** Influence on Au recovery (%). The presented data in this figure is the average of those obtained from duplicate experiments

effect of pH on bioleaching of metals using cyanogenic microorganisms from PCBs; for example, Chi (2011) performed bioleaching of Cu and Au from waste mobile phone PCBs using *C. violaceum*. They reported that the dissolution of Cu and Au increased from 4.9 to 11.4% and 7.78 to 10.8% in 8 days with the increase in pH from 8–10 and 8–11, respectively. Natarajan and Ting (2014) reported the highest recovery of Au (22.5%) at pH 9.5 using mutated *C. violaceum*. In another study, they reported 30% and 95.7% of Au and Cu recovery using spent medium leaching at pH 10 (Natarajan and Ting 2015). The bioleaching of precious metals from e-waste using abandoned mine isolates at alkaline pH can be of industrial interest. .

Bioleaching under optimal conditions

After optimization, bioleaching experiments were performed on the optimized parameters i.e., pulp density of 1%, glycine concentration of 5 g/L, a temperature of 30 °C, and a pH of 9.0, except for *L. sphaericus*, where optimized pH was 8.0. The experiments were carried for a time period of seven days, and metal ions were analyzed after 24 h of time interval. The *C. violaceum* showed maximum mobilization of Cu (87.5±8%) and Au (73.6±3%) after 7 days of bioleaching compared to other bacterial strains used in our study (Fig. 9). The higher mobilization of metals by *C. violaceum* may be attributed to its higher cyanide producing abilities compared to other cyanogenic microorganisms. Overall, Cu bioleaching was the maximum among all the bacterial strains tested. Preferred Cu leaching compared to Au may be the result of higher Cu content in the WPCBs which might have consumed more cyanide ions (Chi 2011; Natarajan and Ting 2014). Among the mine isolates, *B. megaterium* showed the highest metal mobilization (Cu-72.7±5% and Au-66.6±6%). The type of e-waste, its metal composition, bacterial strain employed with optimal process conditions to be used are the governing parameters for the industrial success of the bioleaching process.

Other than metal ions analysis, change in final pH during bioleaching was also analyzed. It was observed that before e-waste addition, pH dropped from the initial pH 9.0 (data not shown). This may be due to acidification during microbial growth by secretion of organic acids (Sahni et al. 2016). However, after e-waste addition during two step bioleaching, increase in the pH was observed. The pH of the leachate increased gradually until 7 days of incubation period by all bacterial strains. The pH of medium changed from initial 9 to 9.48, 9.45, and 9.26 for *C. violaceum*, *B.*

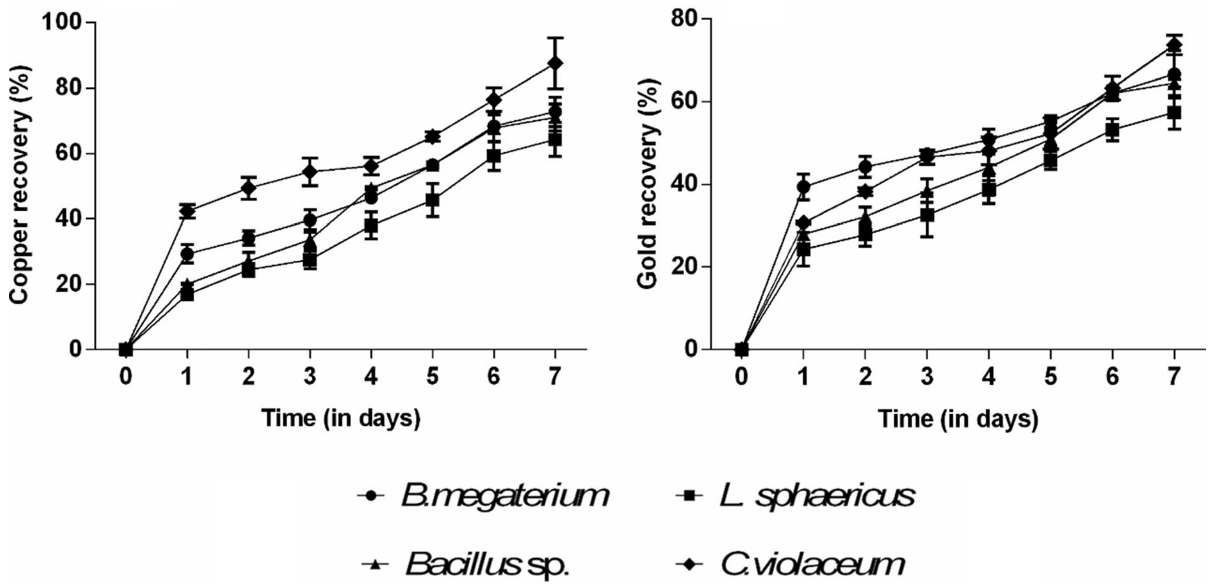

Fig. 9 Bioleaching of metals under optimal conditions from WPCBs by cyanogenic bacterial strains: **a** % Cu mobilization; and **b** % Au mobilization. The data presented in this figure presents the mean of values obtained from duplicate experiments

megaterium and *Bacillus* sp., respectively, whereas, in case of *L. sphaericus* pH increased from 8 to 9.1 during 7 days of the incubation period. According to literature, increase in pH may be due to the alkaline nature of WPCBs (Arshadi et al. 2016), and formation of cyanide and metal cyanide complexes causing production of OH^- in the medium (Arab et al. 2020).

Kinetic modeling

The kinetic modeling was performed for bioleaching metals from WPCBs using different cyanogenic microorganism's viz., *B. megaterium, L. sphaericus, Bacillus* sp. and *C. violaceum*. It was observed that metal bioleaching increased with time up to 7th day and then reached to saturation level. The bioleaching data was plotted against time and with fitting zero, first and second order reaction kinetic model. The linear plot suggested that bioleaching process followed first order reaction kinetics (Fig. 10) and showed the highest R^2 value (Table 1). To plot first order kinetic reaction model, the following Eq. (3) was used considering boundary conditions (time) t = 0–7 days and (metals concentration) [A] = 0–7 days:

$$\ln[A]_t = -kt + \ln[A]_0 \tag{3}$$

where ln = natural log; $[A]_t$ and $[A]_0$ = concentration of metals leached at time t and 0 days, respectively.

According to Gharabhagi et al. (2013) the reaction rate for a liquid-solid reaction is typically controlled by: (1) diffusion of the reactant (HCN in case of cyanogenic bacteria) from liquid film to the solid product (WPCBs); (2) reactant diffusion through solid product layer; and (3) chemical reaction in the studied system at the surface of the solid or product layer (Mishra et al. 2009). The kinetics of bioleaching process showed that the metal concentrations increased with time till it reached saturation. Bioleaching of metals viz. Cu and Au from WPCBs using cyanogenic microorganisms depends on bacterial cyanide production. Cyanogenic microorganisms like *C. violaceum*, *Pseudomonas* and *Bacillus* sp. produce HCN as secondary metabolite during the stationary phase of their growth. All the four bacterial strains used in the study showed presence of cyanide ions in the LB medium, hence confirming the mechanism of bioleaching as cyanide based. The dissolution of Au can be represented as shown in Eq. (4):

$$4Au + 8CN^- + O_2 + 2H_2O = 4Au(CN)_2^- + 4OH^- \tag{4}$$

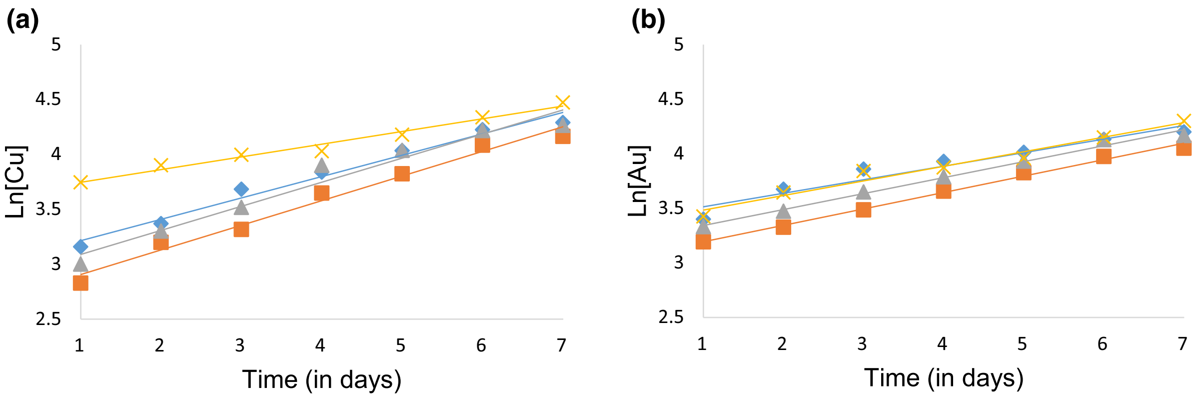

Fig. 10 Kinetic modeling results showing first order reaction kinetics during bioleaching of metals from WPCBs for: **a** Cu; and **b** Au

Table 1 Bacterial strains representing line of best fit and correlation coefficient for first order kinetics

Bacterial strain	Line of best fit and correlation coefficient	
	Cu	Au
B. megaterium	Y = 0.1948x + 3.0192, R^2 = 0.97	Y = 0.1238x + 3.3902, R^2 = 0.93
L. sphaericus	Y = 0.2246x + 2.6805, R^2 = 0.98	Y = 0.1506x + 3.041, R^2 = 0.99
Bacillus sp.	Y = 0.2194x + 2.8694, R^2 = 0.95	Y = 0.1465x + 3.1942, R^2 = 0.98
C. violaceum	Y = 0.1158x + 3.6297, R^2 = 0.97	Y = 0.134x + 3.3475, R^2 = 0.96

The E_h–pH diagramme show that Cu forms various complexes with cyanide like $[Cu(CN)_2^-]$, $[Cu(CN)_3^-]$ and$[Cu(CN)_4^-]$, depending upon the pH conditions (Chi 2011). The dissolution of Cu with cyanide can represented as per Eq. (5):

$$4\mathrm{Cu} + 8\mathrm{CN}^- + \mathrm{O}_2 + 2\mathrm{H}_2\mathrm{O} = 4[\mathrm{Cu(CN)}_2^-] + 4\mathrm{OH}^- \quad (5)$$

In the same set of experimental conditions bioleaching rate (mg/L/day) was calculated and was found to be dependent on metal (Cu and Au) concentration present in the e-waste. Metals with higher concentration in the WPCBs (e.g. Cu-23.4 mg/g) are bioleached at faster rates as compared to Au (Table 2). Similar findings have been reported in previous studies (Pradhan and Kumar 2012).

Table 2 Evaluation of bioleaching parameters with different bacterial strains under same set of experimental conditions

Bacterial cultures	Metal leaching rate*(mg/L/day)	
	Cu	Au
B. megaterium	24.4	0.075
L. sphaericus	21.5	0.071
Bacillus sp.	23.8	0.074
C. violaceum	29.3	0.082

Data represented is the average of those obtained from duplicate experiments

*Metals leaching rate (mg/L/day) = $\frac{A}{T}$; where A is maximum metal bioleaching concentration, T is time (7 days)

Conclusion

Three native mine bacterial isolates i.e., *B. megaterium* SAG1, *L. sphaericus* SAG2, *Bacillus* sp. SAG3 as well as *C. violaceum* were able to extract Cu and Au from WPCBs. From the toxicity assessment and dose–response analysis, it can be concluded that native microbial strains from contaminated environments/toxic mines/low grade ores can resist higher levels of pollutants/ toxic compounds present in e-waste and have potential to grow under unfavorable conditions. The maximum recovery of metals was attained by optimization of initial pH, pulp density, temperature, and glycine concentration. Bioleaching of Cu and Au from WPCBs resulted in 87.5 ± 8% and

73.6 ± 3% recovery by *C. violaceum* and 72.7 ± 5% and 66.6 ± 6% by *B. megaterium*. Kinetic modeling results showed that bioleaching followed first order reaction kinetics, where the rate of metal solubilization from WPCBs depends on microbial lixiviant production. The present study is useful to treat WPCBs dust, destined to landfilled, will not only avoid the loss of valuable/ rare resources but also limits the environmental impact; thus promotes circular economy.

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Declarations

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

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