

# Metals bioleaching from electronic waste by *Chromobacterium violaceum* and *Pseudomonads sp*

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## Abstract

These days, electronic waste needs to be taken into consideration due to its materials content, but due to the heterogeneity of the metals present, reprocessing of electronic waste is quite limited. The bioleaching of metals from electronic waste was investigated by using cyanogenic bacterial strains (*Chromobacterium violaceum*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*). A two-step bioleaching process was followed under cyanide-forming conditions for maximum metals mobilization. Both single and mixed cultures of cyanogenic bacteria were able to mobilize metals from electronic waste with different efficiencies. In all the flasks in which high metal mobilizations were observed, the consequent biomass productions were also high. *Pseudomonas aeruginosa* was applied in the bioleaching process for the first time and this achieved its bioleaching ability of mobilization of metals from electronic waste. *Chromobacterium violaceum* as a single culture and a mixture of *C. violaceum* and *P. aeruginosa* exhibited maximum metal mobilization. *Chromobacterium violaceum* was capable of leaching more than 79, 69, 46, 9 and 7% of Cu, Au, Zn, Fe and Ag, respectively at an electronic waste concentration of 1% w/v. Moreover, the mixture of *C. violaceum* and *P. aeruginosa* exhibited metals leaching of more than 83, 73, 49, 13 and 8% of total Cu, Au, Zn, Fe, and Ag, respectively. Precious metals were mobilized through bioleaching which might be considered as an industrial application for recycling of electronic waste in the near future.

## Keywords

Bioleaching, electronic waste, cyanogenic bacteria, *Chromobacterium violaceum*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*

## Introduction

Consumer-oriented growth combined with rapid product obsolescence and technological advances are posing a new environmental challenge – the growing threat of ‘electronic waste’ or ‘e-waste’ that consists of obsolete electronic devices (Monika and Kishore, 2010). Proper management and safe disposal of electronic waste has become an emerging issue worldwide. According to UNEP, some 20 to 50 million metric tons of electronic waste are generated per year across the globe (Schwarzer et al., 2005). Electronic waste includes obsolete electrical and electronic items which are categorized into six categories such as (a) monitors (10%); (b) televisions (10%); (c) computers, telephones, and their peripherals (15%); (d) DVD/VCR players, CD players, radios and hi-fi sets (15%); (e) refrigerators (20%); and (f) washing machines, air conditioners, vacuum cleaners etc (30%) (Heberlein, 2006). Most of the electronic waste is a complex mixture of precious metals (Ag, Au, and Pt); base metals (Cu, Al, Ni, Si, Zn and Fe); toxic metals (Hg, Be, Cd, Cr (VI), As, Sb and Bi) along with halogens and combustible substances such as plastics and flame retardants (Robinson, 2009). Now, electronic waste may be measured as a ‘secondary ore’ or ‘artificial ore’ for the concentrations of precious metals richer than natural ores, which makes their recycling important from both economic

and environmental perspectives. However, complex composition and increasing volumes of e-waste, along with difficulties in treating it, are causes of concern (Tsydenova and Bengtsson, 2011; Gupta et al., 2008). So, recycling of electronic waste is an important subject not only from the point of waste management but also from the recovery aspect of valuable materials. However, recycling of electronic waste is still quite limited due to the heterogeneity of the materials present in the products and the complexity of the production equipment (Veit et al., 2005).

Pyrometallurgical (Lee et al., 2007) and hydrometallurgical (acid or caustic leaching of solid metals) (Park and Fray, 2009) are traditional metallurgical processes which are practised for the recovery of metals from electronic waste. Pyrometallurgical technology is employed by many leading recycling companies,

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such as Boliden, Dowa, Umicore and X-Strata in their recycling systems (Yu et al., 2009). Umicore is able to recycle seventeen metals, including seven precious metals (Hagelken, 2007). However, pyrometallurgical treatment raises concerns regarding possible formation of brominated and chlorinated di-benzo furans and dioxins in burning processes due to the presence of halogens in the plastic parts of electronic waste (Tsydenova and Bengtsson, 2011). Hydrometallurgical processes are also associated with risks of environmental impact due to the toxicity of the reagents used and the large amount of by-products generated (Scott, 2002). These processes are also expensive because more investment is necessary to set up highly efficient facilities (Yu et al., 2009), there is a high consumption of energy and they are not regarded as an economical way to extract valuable components from electronic waste (Bosecker, 1997; Krebs et al., 1996). The bioleaching process is now emerging as commercial exploitable technology applicable for metal extraction from electronic waste and low grade ores. Using bioleaching techniques, the efficiency of recovery of metals can be increased, as revealed in copper and gold mining where low grade ores are biologically treated to obtain metal values, which are not accessible by conventional treatments (mechanical and thermal) (Agate, 1996). Although, this process has been successfully applied for the leaching of metals from ores (Olson et al., 2003), data pertaining to its application for the extraction of electronic waste is still scanty. A few studies have been undertaken for the extraction of metals from electronic waste/printed circuit boards with mesophilic chemolithotrophic bacteria (*Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*) (Brandl et al., 2001; Choi et al., 2004), acidophilic moderately thermophilic bacteria (*Sulfobacillus thermosulfidooxidans* and *Thermoplasma acidophilum*) (Ilyas et al., 2007, 2010) and cyanogenic bacteria (*Chromobacterium violaceum* and *Pseudomonas fluorescens*) (Faramarzi et al., 2004; Ting et al., 2008). However, there is a dearth of exhaustive literature on extraction of metals from electronic waste by bioleaching process under alkaline conditions using cyanogenic bacteria. There are so many metals and metalloids such as Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ge, Mo, Tc, Ru, Rh, Pd, Ag, Cd, W, Re, Os, Ir, Pt, Au, Hg, Tl, Po, and U that form well-defined cyanides complexes with cyanide, which often show very good water solubility and exhibit high chemical stability (Blumer and Hass, 2000). Cyanide synthesis by bacteria is restricted to the proteobacteria *C. violaceum* and to fluorescent pseudomonades (*P. aeruginosa* and *P. fluorescens*) as well as certain fungi (*Marasmius oreades*, and *Clitocybe* sp.) (Knowles and Bunch, 1986). Glycine is the immediate metabolic precursor for cyanide biosynthesis in proteobacteria and pseudomonades has been proved by *in vivo* studies (Askeland and Morrison, 1983; Bunch and Knowles, 1982).

The present study was undertaken to evaluate the potential of single as well as mixed cultures of cyanogenic bacterial strains (*C. violaceum*, *P. aeruginosa* and *P. fluorescens*) for solubilization of metals from electronic waste; in particular (i) the biological mobilization of copper (Cu), gold (Au), zinc (Zn), iron (Fe)

and silver (Ag); (ii) the comparison of different cyanogenic strains for their ability to mobilize metals; (iii) the quantification of water soluble metal ions in bioleachable medium.

According to the authors' knowledge this is the first report on a bioleaching process using *P. aeruginosa* for mobilization of metals from electronic waste.

## Materials and methods

### *Procurement and metal content analysis of electronic waste*

Electronic waste, in the form of ground powder of printed circuit boards from personal computers, was obtained from a Bangalore-based electronic waste recycling unit known as E-Parisaraa (India). Before it was transported to the laboratory, no physical/mechanical separation process was used. For experimental use the ground electronic waste was further ground to fine powder (37 to 149  $\mu\text{m}$  particle size). The electronic waste sample was analysed for metal analysis content by the chemical leaching method (Ilyas et al., 2007). One gram of electronic waste sample was dissolved in 100 mL of aqua regia ( $\text{HNO}_3 : \text{HCl} = 1 : 3$ ) (Sheng and Estell, 2007) by refluxing in a round-bottom flask for 1 h at 100 °C. The solution was allowed to cool and the volume was made up to 100 mL with deionized water. After that, the solution was passed through glass fibre filters (PALL-GF-A/E-I) to guarantee particle-free suspensions and stored at 4 °C for further analysis. The concentrations of dissolved metal ions were determined by atomic absorption spectrometry (PerkinElmer Analyst 400) at the following wavelengths (nm): Ag (328.1), Au (242.8), Co (240.7), Cu (324.8), Cr (357.9), Fe (248.3), Ni (232.0), Se (196.0), and Zn (213.9). The finely ground electronic waste sample was sterilized by autoclaving at 121 °C for 15 min prior to bioleaching studies.

### *Two-step bioleaching studies*

For bioleaching studies, bacterial strains (*P. fluorescens* (MTCC-103) and *C. violaceum* (MTCC-2656)) were procured from the Institute of Microbial Technology, Chandigarh, India. The *P. aeruginosa* used for these studies, was previously isolated in the authors' laboratory (Awasthi et al., 2008). A two-step bioleaching process is believed to be appropriate to increase the metal leaching efficiency of micro-organisms from electronic waste (Mishra and Rhee, 2010). For more efficient metal mobilization, direct growth of micro-organisms in the presence of electronic waste is not advisable due to its toxic effects (Brandl et al., 2001). Micro-organisms were grown in the absence of electronic waste to produce biomass followed by the addition of different concentrations of electronic waste for metal mobilization for an additional time period of 7 days. Experiments on two-step bioleaching of metals from electronic waste powder were conducted in 250 mL baffled Erlenmeyer flasks containing 100 mL Luria Broth (LB) medium having 0.5 g glycine at pH 7.2. The flasks were autoclaved at 121 °C and 15 psi pressure for 15 min. For two-step bioleaching

studies with single cyanogenic bacterial strains, the experimental flasks were inoculated with biomass of 5.0 mL inoculum pellet (single culture) (O.D. = 1.0, 660 nm) aseptically and incubated at 30 °C temperature and 150 rpm. Likewise in the case of mixed cultures (*P. fluorescens* + *P. aeruginosa*; *P. aeruginosa* + *C. violaceum* and *P. fluorescens* + *C. violaceum*), biomass of 2.5 mL of inoculum pellet (O.D. = 1.0, 660 nm) from each bacterial strain was added to the experimental flasks. After 2 days of incubation, 1.0 g of sterilized electronic waste (1% w/v) was added to each flask under aseptic conditions. An un-inoculated control was run in parallel. Subsequently, the experimental flasks were incubated at 30 °C temperature and 150 rpm for another 7 days time period. The same two-step bioleaching experiments were performed with higher concentration of electronic waste (5 and 10% w/v) with *C. violaceum*, *P. aeruginosa* and *P. fluorescens*.

### Sampling and analysis of bioleachable samples

All flasks were weighed before sampling and any decrease in volume due to evaporation during incubation was compensated by adding a corresponding volume of sterilized distilled water. During the course of the bioleaching experiments, 5.0 mL of sample was taken out from each flask periodically to analyse the pH, total protein content and total metal ions content. The pH was measured by digital pH meter (Eutech pH 510). The samples were filtered through a Whatman no. 1 filter paper to remove solid particles and centrifuged at 10 000 rpm for 10 min to remove bacterial cells. The cell pellets were preserved to determine the total protein content while the supernatants were analysed for metal ions concentration of gold (Au), copper (Cu), zinc (Zn), iron (Fe) and silver (Ag) using an atomic absorption spectrometer (PerkinElmer Analyst 400). Before analysis on an atomic absorption spectrometer, the supernatants were filtered through glass fibre filter (PALL-GF-A/E-I) to guarantee particle free suspensions. The total protein content was determined by micro-biuret method (Itzhaki and Gill, 1964) after dissolving the cell pellet in 4.0 mol L<sup>-1</sup> NaOH solution using bovine serum albumin as standard.

### Statistical analysis

All experimental data were statistically interpreted with one-way and two-way analysis of variance (ANOVA). All statistical tests were performed at probability level  $P < 0.05$  using statistical package MSTAT/Minitab (Minitab Inc. USA, Version 13, 2004 for Windows®)

## Results and discussion

### Metal composition of electronic waste by chemical analysis

Chemical analysis of ground powder of printed circuit boards was carried out to determine the concentrations of various metals

**Table 1.** Metal analysis of electronic waste.

Metals	% w/w
Copper	12.06 ± 0.6
Iron	2.086 ± 0.03
Selenium	0.945 ± 0.014
Zinc	0.115 ± 0.004
Gold	0.084 ± 0.005
Silver	0.068 ± 0.008
Chromium	0.058 ± 0.005
Nickel	0.038 ± 0.004
Cobalt	0.0127 ± 0.017

present in it. The major metals were observed to be copper (12.06%), and iron (2.08%). Precious metals such as gold (0.08%) and silver (0.06%) were also present but in low concentration (Table 1). Heterogeneity was observed when a comparison was made among the concentrations of different metal ions obtained in these studies and those reported by other researchers (Brandl et al., 2001; Veit et al., 2002). This difference may be attributed, to some extent, to the analytical methods used, but on the basis of large differences in the concentrations of some metals, (e.g. Zn, Ni, and Fe) it seems to be mainly dependent upon the origin of the material.

### Metal bioleaching studies

In the same set of bioleaching experimental conditions, rate of bioleaching of Au, Cu, Zn and Fe appeared to be dependent upon their concentration in the electronic waste, and metals having high concentrations being leached out at higher rates. Better rates were observed when mixed culture of cyanogenic bacteria was used in bioleaching process (Table 2).

The maximum percentage of bioleachabilities of metals, after 7 days of bioleaching under the same set of experimental conditions with single and mixed cultures are shown in Figure 1. Among single bacterial cultures, *C. violaceum* exhibited the maximum bioleachability of all the metals, namely Cu (79.3% w/w), Au (69.3% w/w), Zn (46.12% w/w), Fe (9.86% w/w) and Ag (7.08% w/w) followed by *P. aeruginosa*. Among mixed cultures, the mixture of *P. aeruginosa* and *C. violaceum* exhibited maximum bioleachability, 83.46, 73.17, 49.11, 13.98 and 8.42% w/w of total Cu, Au, Zn, Fe and Ag, respectively. It was also observed that significantly high concentration of Cu (77.12% w/w) and Au (63.14% w/w) were leached out by the mixed cultures of *P. fluorescens* and *C. violaceum*. The Cu exhibited maximum leachability followed by Au, Zn, Fe and Ag. The precious metal Au leached out around 73.17% w/w by mixed cultures of *P. aeruginosa* and *C. violaceum* and 69.3% w/w by *C. violaceum* alone, which were the most noteworthy results. It was observed that mixed cultures of *P. aeruginosa* and *C. violaceum* exhibited more leaching capability for all metals than the other combinations of mixed cultures as well as the single cultures. This might be due to a higher tolerance to metal toxicity, formulation of stable metal complexes in the presence of electronic waste releasing

**Table 2.** Rates of bioleaching of metals from electronic waste with different types of bacteria under same set up of experimental conditions. The data presented in this table presents mean of those obtained from triplicate experiments at significant level ( $P < 0.05$ ) based on one-way ANOVA analysis.

Bacterial culture	Maximum rate of metal leaching ( $\text{mg L}^{-1} \text{day}^{-1}$ )			
	Gold	Copper	Zinc	Iron
<i>P. fluorescens</i>	0.25	19.336	0.054	0.476
<i>P. aeruginosa</i>	0.539	74.65	0.053	0.259
<i>C. violaceum</i>	0.716	110.79	0.064	0.268
<i>P. aeruginosa</i> + <i>P. fluorescens</i>	0.513	88.94	0.08	0.6
<i>P. aeruginosa</i> + <i>C. violaceum</i>	0.72	114.449	0.065	0.368
<i>P. fluorescens</i> + <i>C. violaceum</i>	0.651	103.956	0.046	0.421

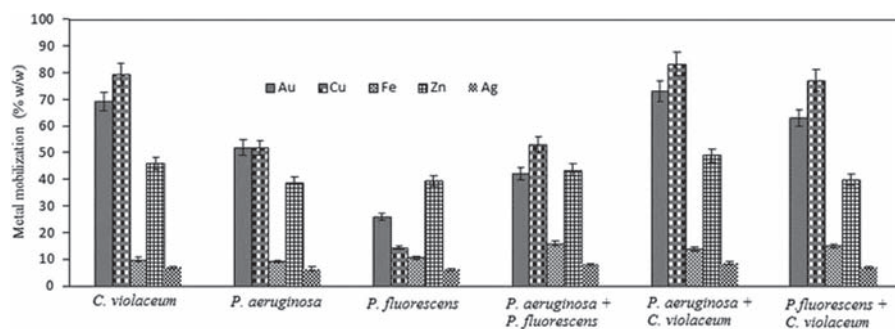
additional secondary metabolites for metal leaching and enhanced growth in comparison with other biological treatments (Brandl et al., 2003; Patil and Paknikar, 1999).

Although *C. violaceum* and *P. fluorescens* have been reported for their metal leaching capabilities (Campbell et al., 2001; Faramarzi et al., 2004; Ting et al., 2008), the use of *P. aeruginosa* in bioleaching of electronic waste was carried out for the first time in the present study. The *P. aeruginosa* contributed significantly to the leaching results of Au, Cu, and Zn mobilization with 52, 52 and 39% w/w, respectively, at 1% w/v of electronic waste concentration.

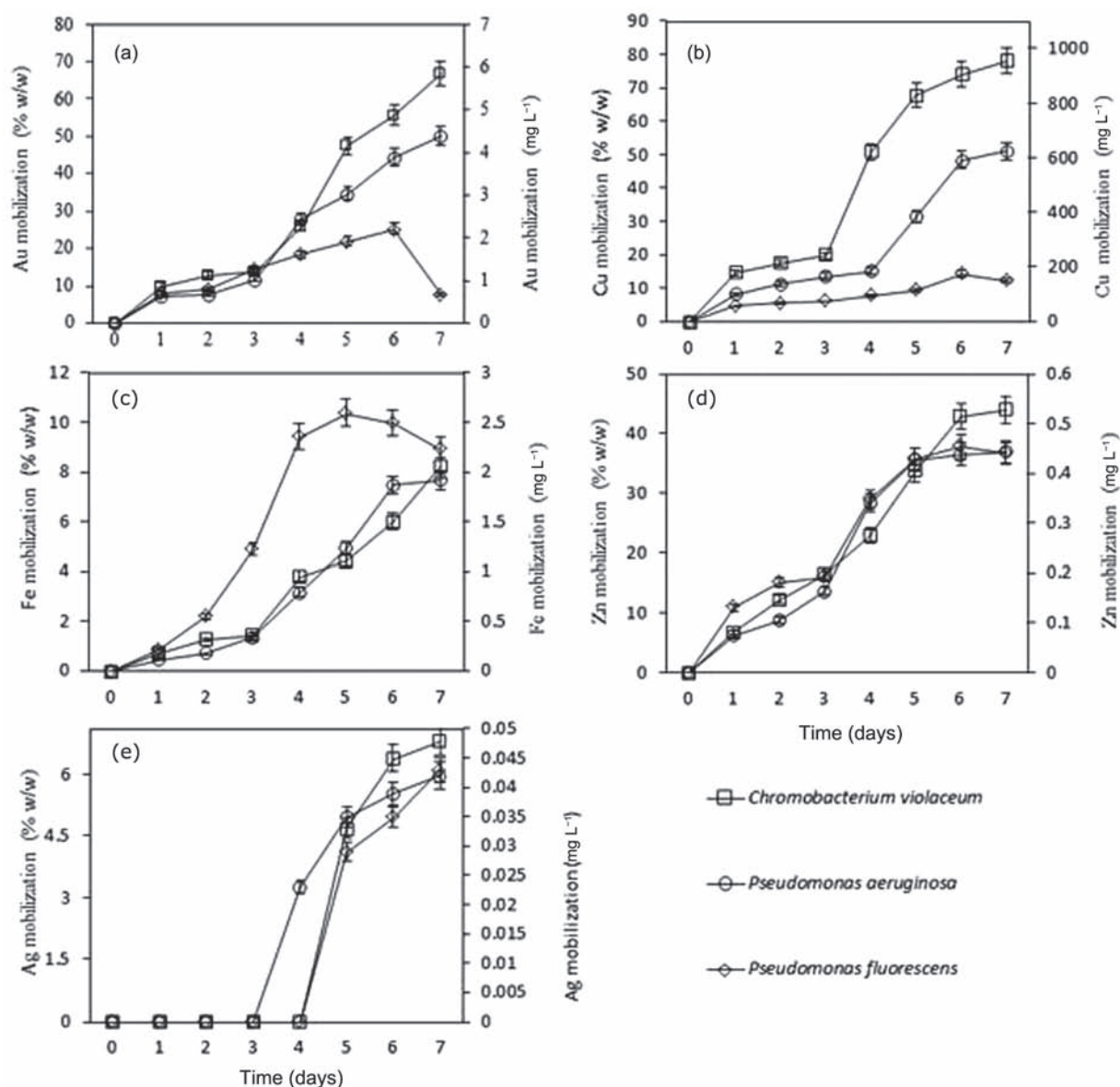
Figures 2 and 3 displays the trends of metal mobilization (%) and concentration (mg/L) of Au, Cu, Zn, Fe, and Ag with respect to single bacterial strains and mixed culture of these strains respectively during two-step bioleaching experiments. From these results it was observed that, especially when *P. fluorescens* alone or a mixed culture of *P. fluorescens* and *P. aeruginosa* was used for two-step bioleaching, the concentration of Au, Cu, Zn, Fe and Ag decreased with prolonged incubation time. This might be due to sorption processes onto biomass or biodegradation because metal cyanides serve as carbon or nitrogen source (Baxter and Cummings, 2006; Brandl et al., 2003). The leaching of Ag initiated after the third day of incubation and it was observed to be the least bioleachable metal at concentration of around 9% w/w with both single and mixed cultures in comparison with the other four metals (Au, Cu, Zn and Fe) after 7 days of incubation. Slow mobilization of Ag was observed when

individual strains were grown in the presence of electronic waste. After 3 days of incubation about 3.5% w/w of Ag was mobilized by *P. aeruginosa* whereas after 4 days of incubation *C. violaceum* and *P. fluorescens* mobilized Ag about 4.8 and 4.3% w/w, respectively. But in case of all mixed cultures, Ag was mobilized after two days of incubation and was about 2.7% w/w. After this the Ag concentration increased only slightly until the end of the incubation period in both single and mixed cultures. Mobilization of Ag by cyanogenic micro-organisms proved to be promising. However, the process has to be optimized by reducing inhibitory effects, for example by trapping the dicyanoargentate formed or by applying a sequential two-step process where biological cyanide formation is separated from metal mobilization.

Differences were observed in the bioleaching pattern of three single bacterial strains applied in presence of 1, 5 and 10% w/v electronic waste concentration (Figure 4). *Chromobacterium violaceum* was able to mobilize more metal concentrations in comparison with the other bacterial strains. Indeed, *C. violaceum* exhibited higher growth, a higher metal resistance and hence better metal leaching performance followed by *P. aeruginosa* which exhibited higher metal resistance and more growth than *P. fluorescens*. As the concentration of electronic waste increased from 1 to 10% w/v, the metal mobilization by these micro-organisms also decreased. *Chromobacterium violaceum* and *P. aeruginosa* mobilized Au 20.28% and 19.26%, respectively, at 10% w/v electronic waste



**Figure 1.** Percentage of metal solubilization after bioleaching of electronic waste with *C. violaceum*, *P. fluorescens*, *P. aeruginosa* and mixed cultures. The data presented in this figure presents mean of those obtained from triplicate experiments at significant level ( $P < 0.05$ ) based on one-way ANOVA analysis.



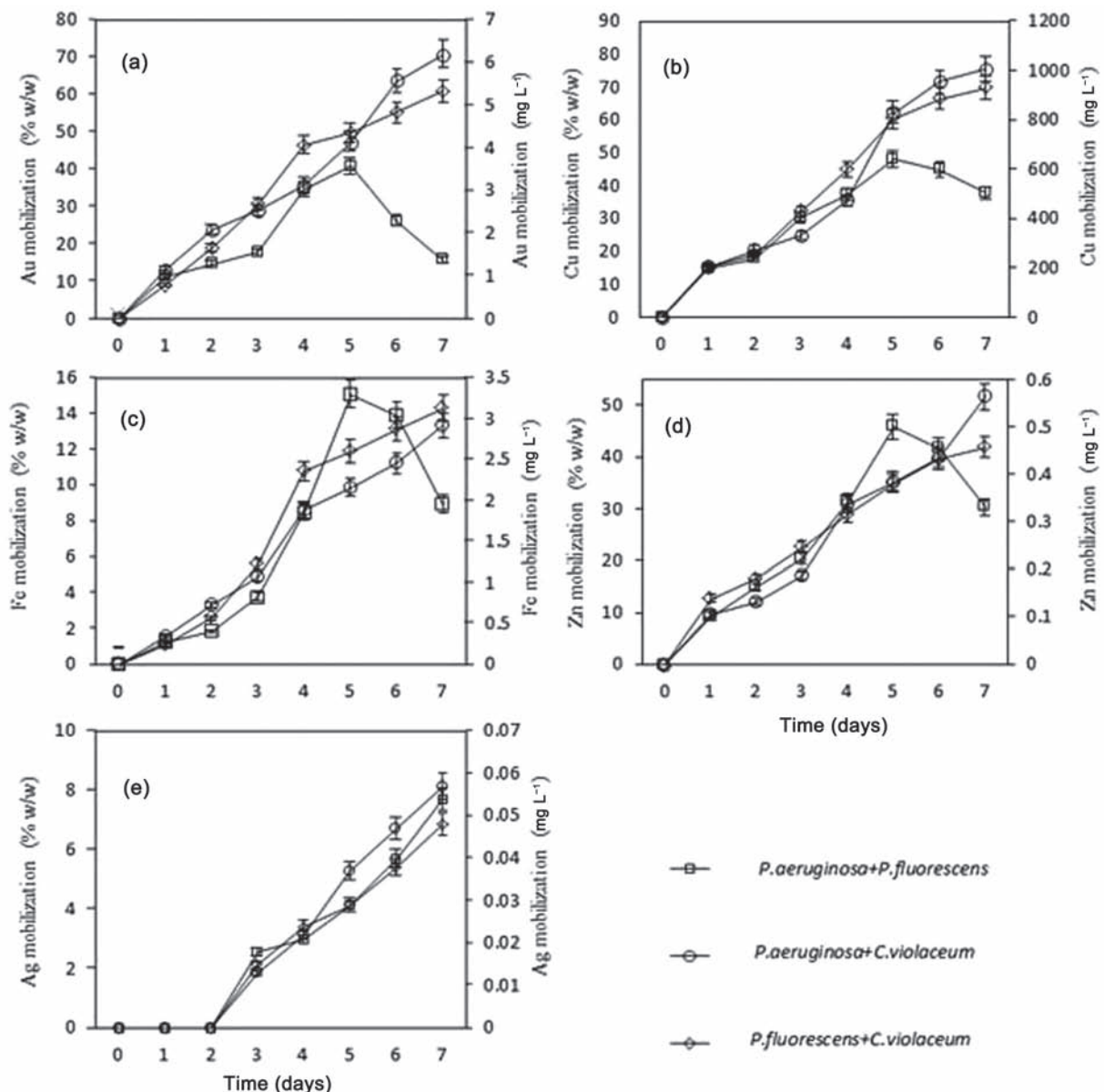
**Figure 2.** Trends of metal mobilization (%) and concentrations (mg L<sup>-1</sup>) during bioleaching of electronic waste by *C. violaceum*, *P. fluorescens* and *P. aeruginosa*: (a) Au, (b) Cu, (c) Fe, (d) Zn and (e) Ag. The data presented in this figure presents the mean of those obtained from triplicate experiments at significant level ( $P < 0.05$ ) based on two-way ANOVA analysis.

concentration. Perhaps this is due to the toxic effect of electronic waste and decreased metal tolerance of micro-organisms (Brandl et al., 2001).

The maximum leachability (0.8%) was exhibited by Cu in the case of un-inoculated control which was run parallel with two-step bioleaching experiments. Under the same set of experimental conditions for other metal ions, the percentage leachabilities were 'considerably' less good as above-mentioned metal-leaching value. It was seen that, the media used for micro-organisms was insignificant for the leaching of metals from electronic waste. The bioleaching data from the above studies was specifically applicable to the above-mentioned concentration of metal (chemical leaching) present in the electronic waste procured from E-Parisaraa Company (after processing).

### *pH change during bioleaching of metals from electronic waste*

In the present study, the pH profile of two-step bioleaching medium having 1% w/v concentration of electronic waste was studied with single cyanogenic bacterial strains as well as with mixed cultures. Figure 5(a) indicates the trends in changes of pH of the medium during bioleaching of electronic waste with single cyanogenic bacterial strains and Figure 5(b) shows the change in pH during bioleaching with mixed cultures. Using *P. fluorescens* with electronic waste for two-step bioleaching, the pH was increased linearly from 7.2 to 9.2 during the incubation period of 7 days. In the case of mixed cultures of *P. aeruginosa* and *P. fluorescens*, the pH was increased from 7.2 to 7.96 after 2 days of incubation and then increased gradually up to 9.3 until



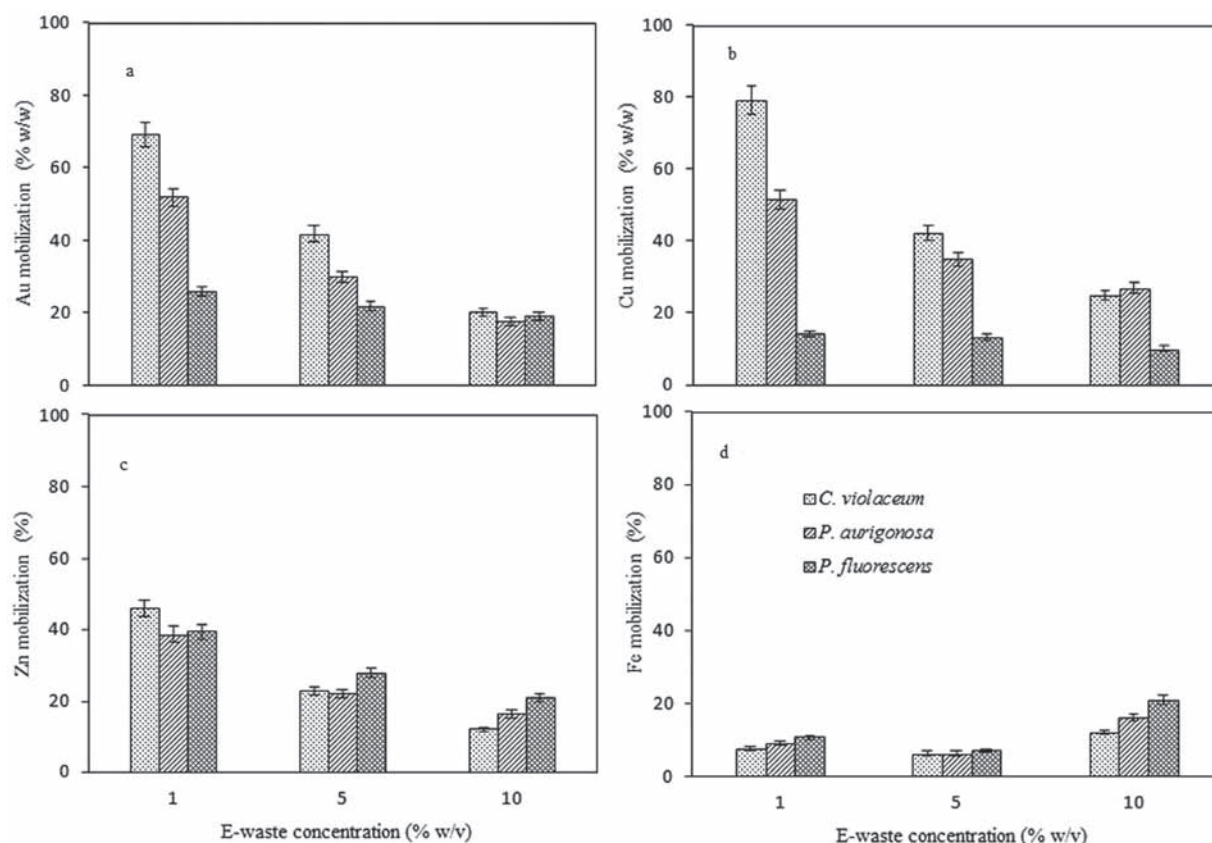
**Figure 3.** Trends of metal mobilization (%) and concentrations (mg L<sup>-1</sup>) during bioleaching of electronic waste by different mixed cultures (*P. aeruginosa* + *P. fluorescens*; *P. aeruginosa* + *C. violaceum*; and *P. fluorescens* + *C. violaceum*): (a) Au, (b) Cu, (c) Fe, (d) Zn and (e) Ag. The data presented in this figure presents the mean of values obtained from triplicate experiments at a significant level ( $P < 0.05$ ) based on two-way ANOVA analysis. There was no significant difference between mixed bacterial cultures for Zn mobilization at  $P < 0.05$  based on two-way ANOVA analysis.

the seventh day of incubation whereas in the case of the *P. fluorescens* culture, the pH was observed to be 7.48. Increase in pH was only observed when the electronic waste was subjected to two-step bioleaching studies. In earlier literatures (Campbell et al., 2001; Mascher et al., 2003), it was mentioned that cyanide was the cause of increase of pH. At physiological pH 7.0, cyanide is present mainly as HCN due to its pK<sub>a</sub> value of 9.3 and is, therefore, volatile. In the presence of salts this value decreases to approximately 8.3 and the volatility is reduced (Faramarzi et al., 2004). Hence, the pH increased by the formation of HCN and formulates complexes with metals. Use of cyanogenic bacteria for the mobilization of metals from solid under alkaline conditions can be of industrial interest since metal chemistry (e.g.

solubility, mobility, sorption, precipitation, formation of secondary minerals) especially in the presence of cyanide aids in recovery of metals more easily (by sorption onto activated carbon) compared to acidic conditions (Brandl et al., 2008; Ibragimova et al., 2007).

#### Determination of microbial growth during two-step bioleaching

Microbial growth was determined as total cellular protein as mentioned in methods. It may be mentioned that these results provide a rough estimate of the growth and do not give an exact measurement of the biomass in the flask, as the readings were



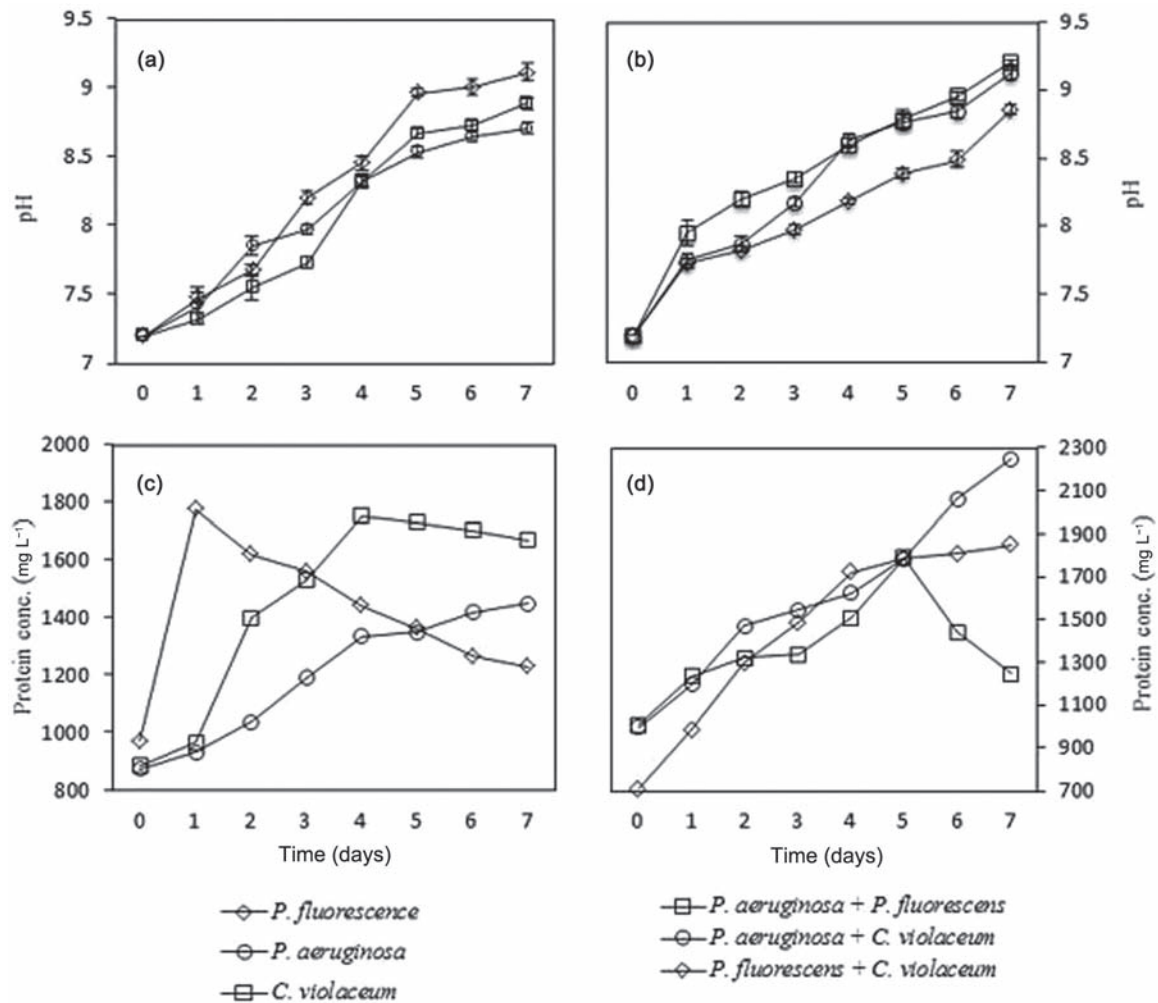
**Figure 4.** Leaching of metals from electronic waste by *C. violaceum*, *P. fluorescens* and *P. aeruginosa* during bioleaching experiment with different concentrations of electronic waste: (a) Au, (b) Cu, (c) Zn and (d) Fe. The data presented in this figure presents the mean of those obtained from triplicate experiments at significant level ( $P < 0.05$ ) based on two-way ANOVA analysis. There was no significant difference between single bacterial strains and between interactions for Fe mobilization at  $P < 0.05$  based on two-way ANOVA analysis.

only for planktonic cells, whereas cells attached to the particular matter were not accounted for (Ilyas et al., 2007). The growth (determined as total cellular protein) of *C. violaceum*, *P. fluorescens* and *P. aeruginosa* is shown in Figure 5(c) and the trend of increase in growth of mixed cultures (*P. aeruginosa* + *P. fluorescens*, *P. aeruginosa* + *C. violaceum* and *P. fluorescens* + *C. violaceum*) in Figure 5(d) during the two-step bioleaching with 1% w/v electronic waste concentration at 30 °C temperature and 150 rpm. The results indicate the increase in growth with respect to metal bioleaching for all bacterial strains as well as mixed cultures in the presence of electronic waste. The metal bioleaching capability was associated with bacterial growth as they released secondary metabolites which mobilize metals from electronic waste (Brandl et al., 2008). In the case of *P. fluorescens* the growth was suddenly increased on the second day and decreased gradually towards the seventh day. This may be due to a shortage of carbon source or the toxic effect of secondary metabolites (Brandl et al., 2003; Patil and Paknikar, 1999). The same was also observed in the case of mixed cultures of *P. fluorescens* and *P. aeruginosa*. Furthermore, the growth plot for mixed cultures should not be regarded as a true growth curve due to presence of two different types of bacteria (Ilyas et al., 2007). Growth curve in the case of mixed cultures showed the higher rate of biomass

production than the individual bacterial strains in presence of electronic waste.

## Conclusions

Results from this study revealed that in an alkaline condition using cyanogenic bacterial strains such as *C. violaceum*, *P. fluorescens* and *P. aeruginosa* and their mixed culture had capability to leach out metals (gold, copper, iron, zinc and silver) from electronic waste. Higher metal bioleaching capabilities were achieved by using combinations of mixed cultures of cyanogenic bacterial strains. *Pseudomonas aeruginosa* was used for the bioleaching study of electronic waste for the first time and in combination with *C. violaceum* exhibited higher metal leaching capabilities than other combinations. Precious metals such as gold were mobilized by cyanogenic bacteria which can be developed as an industrial application of bioleaching in alkaline conditions (biocyanidation). Heterogeneity was observed in the mobilization of metals by bioleaching from electronic waste. These results may vary with different growth conditions applied, concentration of electronic waste used and depending on microorganism used. Extensive optimization studies are required to study the parameters influencing the bioleaching of metals from electronic waste.



**Figure 5.** pH change and growth curve (total cellular protein) during bioleaching with electronic waste: (a) trends of pH change by single bacterial strains, (b) trends of pH change by mixed cultures, (c) growth curve of single bacterial strains, and (d) growth curve of mixed cultures. The data presented in this figure presents the mean of values obtained from triplicate experiments at significant level ( $P < 0.05$ ) based on two-way ANOVA analysis.

Issue of heterogeneity of the electronic waste should be addressed at first hand to make the process of bioleaching cost-effective and successful at industrial scale.

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