



Enhanced Biodegradation and Release Pattern of Heavy Metal from Spent Laptop Batteries using *Pseudomonas* and *Bacillus* Species in Freshwater

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Authors' contributions

This work was carried out in collaboration between both authors. Author RRN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RRN and BCN managed the analyses of the study. Author BCN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate Enhanced-Biodegradation and Release Pattern of Heavy Metal from Spent Laptop Batteries using *Pseudomonas* and *Bacillus* species in Freshwater

Study Design: The study employs experimental design, statistical analysis of the data and interpretation.

Place and Duration of Study: Freshwater was collected from Isiokpo River, Isiokpo town in Ikwerre L.G.A, Rivers state, Nigeria within co-ordinates 5° 02'14"N and 6° 54'50"E, These samples were transported with ice pack to the Microbiology Laboratory of the Rivers State University, for analyses within 6 hours. Spent laptop batteries were obtained from Ogbunabali Laptops Shopping Complex Garrison area of Port Harcourt, Nigeria. Three sets of the brand of battery (HP, Dell and Acer).

Methodology: A total of twelve (12) experimental set-up with three controls (each of the three laptop batteries in freshwater without augmenting organisms) while the other nine were enhanced with augmenting microbes. In step 1, Stock toxicant solution was prepared by soaking the spent Laptop batteries (of average weight of 300-400g, in two (2) liters of sterilized freshwater in a

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sterile trough with vented top for aeration, of dimension 12 x 7.5 x 6cm separately for each set-up, The soaking (Toxicant preparation) lasted for 3 months (about 92 days). In step 2, Five hundred milliliters (500ml) of each set-up was transferred into sterile Conical flask plugged with cotton wool perforated for aeration; each was inoculated with five milliliter (5 ml) of the test organisms (*Pseudomonas* and *Bacillus* species broth, singly and consortium) separately and monitored for duration 0, 30, 60 and 90 days respectively using the spread plate techniques. The bacterial cultures were incubated at 37°C for 24 hours while fungal cultures were incubated for three (3) days at 35°C. Parameters monitored were Heavy metal (Lithium, Cadmium, Chromium, iron and Lead) concentration, Total Heterotrophic Bacteria, Total Heterotrophic Fungi, Lithium Utilizing Bacteria, Cadmium Utilizing Bacteria, Chromium Utilizing Bacteria, Iron Utilizing Bacteria, Lead Utilizing Bacteria, Lithium Utilizing Fungi, Cadmium Utilizing Fungi, Chromium Utilizing Fungi, Iron Utilizing Fungi, Lead Utilizing Fungi.

Results: Enhanced biodegradation and release pattern of heavy metal from spent laptop batteries using *Pseudomonas* and *Bacillus* species in freshwater was evaluated and the concentration of heavy metals (Lithium, Cadmium, Chromium, iron and Lead) found associated with the spent laptop batteries (HP, Dell and Acer) were increasing simultaneously with time in all set-ups from day 1 to day 90. The control (without augmenting microbes) has the highest concentration of heavy metals from day 1 to 90 followed by set-ups augmented with *Bacillus* specie followed by set-ups augmented with *Pseudomonas* specie, least in set-ups augmented with the consortium of the 2 isolates. The changes in concentration of heavy metals during biodegradation of which Cd < Cr < Li < Fe < Pb respectively, for all spent laptop batteries used in this study. Percentage (%) degradation potential of the consortium of *Bacillus* species and *Pseudomonas* species for Li-HP, Li-Dell, and Li-Acer shows a higher percentage (%) release of 22.68%, 37.63% and 24.22% respectively as compared to the individual strains of for *Bacillus* species and *Pseudomonas* species. With *Pseudomonas* species having 10.03%, 18.65%, and 11.24%, followed by *Bacillus* species having 8.46%, 12.49%, and 7.20% for Li-HP, Li-Dell, and Li-Acer respectively.

Conclusion: The study identifies the degradability potential of *Bacillus* and *Pseudomonas* species to degrade spent laptop battery in freshwater. it shows that the consortium was able to degrade the batteries better than the individual strains. It is recommended that spent laptop batteries discharged into aquatic environment should be enhanced with broth culture of eco-friendly species of *Pseudomonas* and *Bacillus* for quick degradation.

Keywords: Enhanced biodegradation; spent laptop batteries; heavy metals; pseudomonas; bacillus.

1. INTRODUCTION

With the current trends in technological advancement, and newest discoveries in information and communication technology, laptops have become a part of everyday life [1]. Batteries are used as backup or direct power to IT systems and networks and these uninterrupted powers are very important for proper functioning of the IT equipment and networks [2]. The battery industry has put in great efforts overtime to recycle and replace toxic components of these electronic materials. Even with these efforts spent batteries still fall under hazardous waste and they include; "rechargeable nickel-cadmium batteries, silver button batteries, mercury batteries, small sealed lead-acid batteries, and alkaline batteries" [3,4].

The term 'heavy metal' is somewhat imprecise, but includes most metals with an atomic number greater than 20, and excludes alkali metals,

alkaline earths, lanthanides and actinides. Metals are introduced in aquatic systems as a result of the weathering of soils and rocks, from volcanic eruptions, and from a variety of human activities involving the mining, processing, or use of metals and/or substances that contain metal pollutants. The most common heavy metal pollutants are arsenic, cadmium, chromium, copper, nickel, lead and mercury [5].

The most common metal pollution in freshwater comes from mining companies. They usually use an acid mine drainage system to release heavy metals from ores, because metals are very soluble in an acid solution. After the drainage process, they disperse the acid solution in the groundwater, containing high levels of metals [6]. Heavy metals are released into freshwater ecosystem when the pH in water falls, metal solubility increases and the metal particles become more mobile. That is why metals are more toxic in soft waters. Metals can become

'locked up' in bottom sediments, where they remain for many years. Streams coming from draining mining areas are often very acidic and contain high concentrations of dissolved metals with little aquatic life. Both localized and dispersed metal pollution cause environmental damage because metals are non-biodegradable. Unlike some organic pesticides, metals cannot be broken down into less harmful components in the environment [6].

The increased demand results in the production of very large amount of these wastes annually. These wastes are referred to as E-waste or electronic waste, which includes computers (laptops), mobile phones, television sets, office equipment, refrigerators etc. Due to the population of Nigeria, the domestic consumption of these devices is on the increase which in turn leads to the growing volumes of these wastes. And Nigeria is the highest producer of this waste in West Africa [7]. Introducing these wastes in large volumes, without proper environmental management system in place, these wastes could impact the environment (including microorganisms, plants and animals), the populace and the economy at large [7]. Most times, people prefer to buy new electronic device when their old device go bad, rather than repairing a faulty one, even when their devices have reusable parts [8].

The electronic waste, contain components which are hazardous this may pose serious environmental concerns when they are disposed off without adequate treatment. And batteries are grouped as hazardous waste which makes their disposal regulated. Most of the batteries in use are classified as either secondary: Nickel-cadmium, nickel metal hydride, lithium-ion, and lead-acid batteries are rechargeable, and are more heavily used in commercial settings than the primary batteries, mostly non-rechargeable [3,9].

When introduced into the environment, chemical components from the batteries regardless of the type lead to the selective pressure of species which are resistant to their harmful effects. Soil contamination with batteries limits the microbial biodiversity, but it also increases the abundance of some bacteria species which are more resistant to changes in the environmental homeostasis [10]. Battery components can inhibit the growth of certain microorganisms by interfering with enzymatic activity, like the inhibition of *Nitrogenase* actively involved in

Nitrogen fixation [11]. The inhibition of *Nitrogenase* actively can reduce the amount of nitrogen available for plants, thus reducing crop yield. Other important microbial processes in the soil like: nutrient transformation, degradation and decomposition of resistant components of plant and animal tissues, bioremediation, humus formation, surface blooming to reduce erosion losses, all which depends on the equilibrium found among the different groups of microorganisms present in the soil environment, which are in turn affected when high concentrations of these toxic waste are present [12]. When the toxic components are found in the soil, they affect the presence of the soil microbial enzymes which are required for the above processes and for organic matter turnover. Key enzymes affected in the soil are dehydrogenase (play a very essential role in the process of organic matter oxidation [13].

Laptop battery as a global electronic product, results in daily huge spent battery waste generation, which is posing disposal problem in the environment recently [1].

With the current trends in technological advancement and the increase in demand implies that, there is need for more efficient method of waste management. The electronic waste contains recalcitrant and toxic materials which pose distinct environmental and public health challenges in aquatic environments. Using bio-degrading organisms *Pseudomonas* and *Bacillus* species as an enhancer in biodegradation of spent laptop battery will reduce exposure to the hazardous compound which may pose serious environmental concerns when they are disposed of without adequate treatment thereby cubing the challenge of electronic-industrial wastes management.

Therefore, this study was to evaluate the biodegradation of spent laptop batteries enhanced with bio-augmenting organisms *Pseudomonas* and *Bacillus* species in freshwater Ecosystem.

2. MATERIALS AND METHODS

2.1 Sample Collection and Study Area

Freshwater was collected from Isiokpo River, Isiokpo town in Ikwere L.G.A, Rivers state, Nigeria within co-ordinates 5^o 02'14"N and 6^o 54'50"E, with a ten (10) litres sterile container, the freshwater sample was taken in ice pack to

the Microbiology Laboratory of Rivers State University, Port Harcourt, Nigeria for analyses within 24 hours.

2.2 Source of Laptop Batteries

Spent laptop batteries were obtained from Ogbunabali Laptops Shopping Complex Garrison area of Port Harcourt, Nigeria. Three sets of the brand of battery (HP, Dell and Acer).

2.3 Source of Test Organisms

Bacillus and *Pseudomonas* species were isolated from same freshwater habitat of the study area.

2.4 Media Used - *Pseudomonas* Agar Base

Composition of the medium for selective isolation of *pseudomonas* species: Gelatin peptone 16.0g/l, Casein hydrolysate 10.0g/l, Potassium sulphate 10.0g/l, Magnesium chloride 1.4g/l, Agar 11.0g/l [14].

Pseudomonas agar base were prepared and autoclaved at 121°C at 15psi for 15 minutes after which it was allowed to cool to about 40°C and poured on the petri-dishes. Then, the medium was allowed to solidify before putting it into the hot air oven to dry the moisture. Aliquot (0.1ml) of the water samples was transferred onto the petri-dishes in duplicates respectively. It was uniformly spread with sterile glass spreader (spread plate method) and incubated at room temperature (30± 2°C) for 24-48hrs. After incubation, bluish green, circular, convex colonies appeared and Gram staining of the colonies revealed Gram negative rods indicative of *Pseudomonas* sp. After incubation, the bacterial colonies that grew on the plates were sub-cultured into fresh nutrient agar plates using the streak plate technique to obtain pure culture of the bacterial isolates as adopted by Nrior and Kpormon [13].

Discrete colonies on the plates were aseptically transferred into 10% (v/v) glycerol suspension, well labelled and stored as stock cultures for preservation [14].

2.5 Media Used - *Bacillus* Selective Agar Base

Composition of the medium for selective isolation of *bacillus* species: Peptone 1.0g/l, Mannitol 10.0g/l, Sodium chloride 2.0g/l, Magnesium

sulphate 0.1g/l, Disodium hydrogen phosphate 2.5g/l, Potassium dihydrogen phosphate 0.25g/l, Bromothymol blue 0.12g/l, Sodium pyruvate 10.0g/l, Agar 15.0g/l [14].

Bacillus agar base were prepared and autoclaved at 121°C at 15psi for 15 minutes after which it was allowed to cool to about 40°C and poured on the petri-dishes. Then, the medium was allowed to solidify before putting it into the hot air oven to dry the moisture. Aliquot (0.1ml) of the water samples was transferred onto the petri-dishes in duplicates respectively. It was uniformly spread with sterile glass spreader (spread plate method) and incubated at room temperature (30± 2°C) for 24-48hrs. After incubation, the colonial characteristics appeared irregular, large, flat, undulated, rough gray white colonies and Gram staining of the colonies revealed Gram positive rods indicative of *bacillus* sp.

After incubation, the bacterial colonies that grew on the plates were sub-cultured into fresh nutrient agar plates using the streak plate technique to obtain pure culture of the bacterial isolates as adopted by Nrior and Kpormon [13].

Discrete colonies on the plates were aseptically transferred into 10% (v/v) glycerol suspension, well labelled and stored as stock cultures for preservation [15].

2.6 Preparation of Broth Cultures and Standardization of Inoculums

A loopful of the test organism from the pure culture was inoculated into sterile nutrient broth in 500ml conical flask separately for each of the organism, and incubated at 37°C for 24-48 hours. After incubation Aliquot (1ml) of the 24h culture was transferred into fresh sterile broth (10ml), incubated for 24h (to ensure that actively growing organisms were used for toxicity test) and preliminary standard Inoculum determined [16].

2.7 Biodegradation Set-up

A total of twelve (12) experimental set-up with three controls (each of the three laptop batteries in freshwater without augmenting organisms) while the other nine were enhanced with augmenting microbes. In step 1, Stock toxicant solution was prepared by soaking the spent Laptop batteries (of average weight of 300-400g, in two (2) liters of sterilized freshwater in a sterile

trough with vented top for aeration, of dimension 12 x 7.5 x 6cm separately for each set-up, The soaking (Toxicant preparation) lasted for 3 months (about 92 days). In step 2, After which 500ml of each set-up was transferred into sterile Conical flask plugged with cotton wool perforated for aeration; each was inoculated with five milliliter (5 ml) of the test organisms (*Pseudomonas* and *Bacillus* species broth, singly and consortium) separately and monitored for duration 0, 30, 60 and 90 days respectively using the spread plate techniques. The bacterial cultures were incubated at 37°C for 24 hours while fungal cultures were incubated for three (3) days at 35°C (Table 1) [17,18].

2.8 Analytical Evaluation of Toxic Release and Biodegradation of Heavy Metals

The percentage (%) biodegradation rate will be calculated from the formula adopted by Nrior et al. [19]. As follows:

Step 1:

Amount of total pollutant degraded equals to Initial concentration of pollutant (Day 1) minus final concentration of pollutant at end of experiment (last day).

Step 2:

Percentage (%) biodegradation equals to amount of pollutant remediated divided by initial concentration of pollutant (Day 1) multiplied by 100.

$$\text{Thus; } Bc = Ic - Fc \text{-----Eqn. 1}$$

Where,

Bc = Amount of pollutant Biodegraded
Ic = Initial concentration of pollutant (Day 1)
Fc = Final concentration of pollutant end of experiment (Last day)

$$\% \text{ Biodegradation} = \frac{Bc \times 100}{Ic} \text{ (Nrior \& Mene, [20]) -----Eqn. 2}$$

2.9 Amount and Percentage (%) of Heavy Metals Released in Freshwater

Step 1:

Amount of heavy metals released (HM_A) equals to final concentration of heavy metals (HM_F) at final day (Day 90) minus initial concentration of

heavy metals (HM_I) at the beginning of the experiment (day 1). [NOTE: Toxicant (metals) concentration released into water increased with time]

Step 2:

Percentage (%) of heavy metals released equals to amount of heavy metals divided by final concentration of heavy metals (HM_F) at Day 90 multiplied by 100.

Amount of heavy metals released (HM_A) (Nrior and Kpormon [13])

$$HM_{\Sigma A} = HM_F - HM_I \quad \text{Equation 1}$$

Percentage (%) of heavy metals released (HM_R)

$$HM_{\%R} = \frac{HM_F - HM_I}{HM_F} \times 100 \text{ OR } HM_{\%R} = \frac{HM_{\Sigma A}}{HM_F} \times$$

$$100 \quad \text{Equation 2}$$

Where;

HM_{ΣA} = Amount released
HM_{%R} = Percentage release
HM_F = Final Concentration
HM_I = Initial Concentration

2.10 Monitoring of the Biodegradation Potential and Release Pattern of Heavy Metals

Each of the biodegradation experimental set-ups was incubated on the laboratory bench at room temperature. Each set-up was agitated manually daily to aid for aeration and even distribution of the toxicant. The biodegradation potential of the respective test organisms was monitored for 90 days at a constant interval of 30 days beginning from the first day. Total heterotrophic Bacteria (THB) and Total Heterotrophic Fungi (THF) were carried out and physicochemical analysis of the freshwater sample was also done [13].

2.11 Monitoring of the Physicochemical and Microbiological Parameters

The biodegradation process was monitored for 90 days at a constant interval of 30 days using the following Physiochemical parameter; Total dissolved Solid (TDS), Hydrogen concentrations (pH) and Total Hydrocarbon Content (THC). While the following Microbiological parameters;

Total heterotrophic Bacteria, (THB) Total Heterotrophic Fungi (THF), Hydrocarbon Utilizing Bacteria (HUB) and Hydrocarbon Utilizing Fungi (HUF) will be also monitored. [13,21].

2.12 Total Heterotrophic Bacteria (THB)

Total heterotrophic bacteria will be enumerated using spread plate method. An aliquot (0.1ml) dilution was used from each of the set-ups and was aseptically transferred unto properly dried nutrient agar plates in duplicate, spread evenly using flamed bent glass rod and incubate at 37°C for 24 hours. After incubation, the bacterial colonies that grew on the plates will be counted and average taken. Total Heterotrophic Bacteria (THB) Counts were then taken and expressed as colony forming unit per milliliter using the equation below as adopted by Nrior and Kpormon [13].

$$\text{THB (cfu/ml)} = \text{Number of Colonies} / (\text{Dilution} \times \text{Volume plated (0.1 ml)})$$

2.13 Total Heterotrophic Fungi (THF)

The total Heterotrophic fungi in each of the setups was enumerated using spread plate method. An aliquot (0.1 ml) of the dilution was aseptically transferred unto properly dried Sabouraud Dextrose Agar plates containing antibiotic (250 Tetracycline) to inhibit bacterial growth, it was spread evenly using bent glass rod and incubate at 35°C for 3-5 days (This incubator temperature when using Sabouraud Dextrose Agar gives optimal clear growth in 3 days but

ambient temperature of 28±0.2°C in South South Nigeria stays for 5 days for optimal growth) [22].

Fungal colonies that grew on the plate was counted and expressed as colony forming unit per milliliter using the below equation: [20,23]

$$\text{THF (cfu/ml)} = \text{Number of colony} / (\text{Dilution} \times \text{Volume plated (0.1 ml)})$$

2.14 Determination of Heavy Metals

The Samples were treated with hydrochloric/nitric acid mixture by standing for 3 h at room temperature, followed by boiling under reflux for 2 h/ heating until a white fume appears with the mixture becoming clearer. The extract is then clarified and made up to volume with nitric acid. Elements are determined by spectrometry [16].

Procedure: 10ml of the mixture of nitric acid and hydrochloric acid in a ratio of 1:3 was added unto the reaction vessel containing 50mL sample and heated using hot plate inside a fume hood until white fume is observed and allowed to cool [16].

The reaction vessel was allowed to stand so that most of any insoluble residue settles out of suspension. (Contents of the absorption vessel were added to the reaction vessel, via the condenser, rinsing both the absorption vessel and condenser with a further 10 ml of nitric acid (0.5 mol/l). The relatively sediment-free supernatant was decanted carefully onto a filter paper, collecting the filtrate in a 100 ml volumetric flask. All the initial filtrate was allowed

Table 1. Biodegradation experimental set-up modules

S/N	SET-UP CODE	Diluent (Freshwater) (ml)	Toxicant (Spent Laptop Batteries) (g)	<i>Pseudomonas</i> Broth (ml)	<i>Bacillus</i> Broth (ml)
1	FW+HP (CTRL)	300ml	HP	-	-
2	FW+Dell (CTRL)	300ml	Dell	-	-
3	FW+Acer(CTRL)	300ml	Acer	-	-
4	FW+HP+Pse	297ml	HP	3ml	-
5	FW+Dell+Pse	297ml	Dell	3ml	-
6	FW+Acer+Pse	297ml	Acer	3ml	-
7	FW+HP+Bac	297ml	HP	-	3ml
8	FW+Dell+Bac	297ml	Dell	-	3ml
9	FW+Acer+Bac	297ml	Acer	-	3ml
10	FW+HP+Pse+Bac	297ml	HP	1.5ml	1.5ml
11	FW+Dell+Pse+Bac	297ml	Dell	1.5ml	1.5ml
12	FW+Acer+Pse+Bac	297ml	Acer	1.5ml	1.5ml

to pass through the filter paper, then the insoluble residue was washed onto the filter paper with a minimum of nitric acid (0.5 mol/l). The filtrate is then collected with the first (initial filtrate). The extract thus prepared were then used for the determination of heavy metals using Atomic Absorption Spectrophotometer (AAS) one of the spectrometric determination methods. Note: ISO 11047 was used as a guideline for the determination of Cd, Cr, Cu, Pb and Li [16].

3. RESULTS AND DISCUSSION

Physiochemical parameters of the freshwater ecosystem were done and the results are shown in Table 2. pH is one of the parameters which determine the suitability of water for various purposes. In this study pH value was 5.82 which indicates that the water is capable of enhancing the growth of both Bacteria and Fungi. The permissible TDS limit in freshwater ecosystem is 500g/l and this study has a value of 9.00g/l, this shows the water is likely to support freshwater organisms [24]. Electrical conductivity and total hardness had values of 30.00 Su/cm and 15.50mg/l was found to be within the permissible limit. Chloride which is an important physiochemical parameter had higher concentration which indicates high degree of organic pollution [25]. In this study chloride was found to be 6.60mg/l which was within permissible limit freshwater ecosystem. Nitrate, sulphate, calcium, magnesium, total iron, lead, BOD, and COD were found to be 4.45mg/l,

52.8mg/l, 0 7.70 mg/l, 1.10 mg/l, 0.38 mg/l, 0.12 mg/l, 1.55 mg/l and 6.09 mg/l respectively which was within permissible limit freshwater ecosystem [26].

Evaluation of the concentration of heavy metals (Lithium, Cadmium, Chromium, iron and Lead) were increasing simultaneously with time in all set-ups from day 1 to day 90. The control has the highest concentration of heavy metals from day 1 to 90 followed by set-ups augmented with *Bacillus* specie followed by set-ups augmented with *Pseudomonas* specie, followed by set-ups augmented with the consortium of the 2 isolates [18].

3.1 Heavy Metals Analysis

The result of concentration of heavy metal during biodegradation and heavy metal release monitoring are presented in Tables 3-4 and Figs. 1-4. The result shows that the concentrations of heavy metals (Lithium, Cadmium, Chromium, Iron and Lead) were increasing with time simultaneously in all set-ups from day 1 to day 90. This might be as a result of heavy metal concentration from the spent laptop batteries altering the physico-chemical parameters of the water bodies. This study also shows the changes in concentration of heavy metals during biodegradation where $Cd < Cr < Li < Fe < Pb$ respectively, for all spent laptop batteries used in this study, Pb, Fe and Li were highly

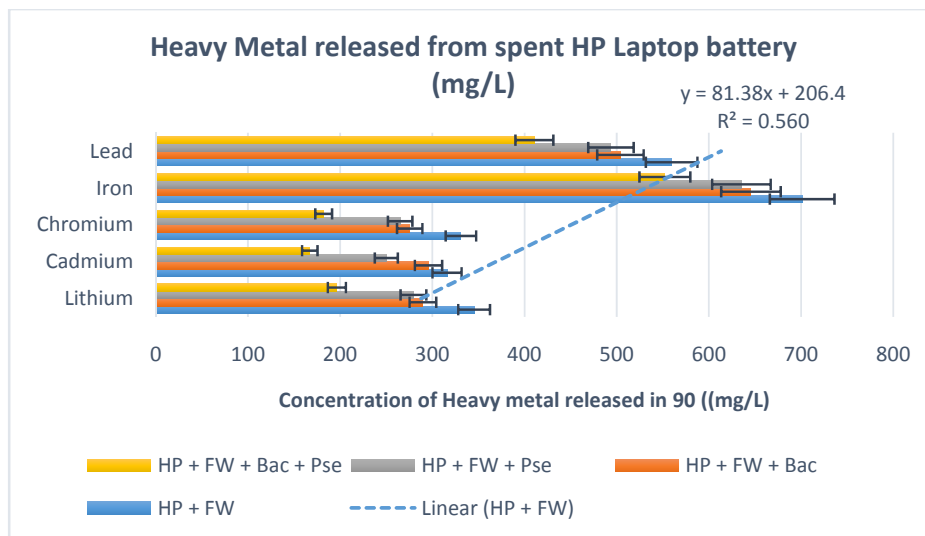


Fig. 1. Changes in concentration and amount of heavy metals (Li, Cd, Cr, Fe, Pb) (mg/l) released from spent HP batteries during biodegradation of spent laptop batteries in freshwater

concentrated, this is because they remain in the environment for a long time. The effect of Lead in the freshwater may lower the biodegradation potential of the test organism (*Bacillus* and *Pseudomonas*). The high concentration of these

heavy metals from spent laptop batteries in the set-ups with days shows that the toxicant exhibited more toxic effect on the individual strains of bacteria and the consortium of both isolate [27].

Table 2. Results of physiochemical parameters of the freshwater ecosystem

S/N	Parameter	Freshwater	WHO Standard/Unit
1	Colour	16.00	15 Hazen units
2	pH	5.82	6.5 - 8.5
3	conductivity	30.00	1000 uS/cm
4	turbidity	1.00	5 NTU
5	Total hardness	15.50	100 mg/l
6	Total alkalinity	13.60	200mg/l
7	chloride	6.60	250mg/l
8	Total suspended solid	59.00	30mg/l
9	Total dissolved solid	9.00	500mg/l
10	Total solid	68.00	500mg/l
11	Nitrate	4.45	10mg/l
12	Sulphate	52.80	250mg/l
13	Calcium	7.70	70mg/l
14	Magnesium	1.10	30mg/l
15	Total iron	0.38	30mg/l
16	Lead	0.12	15mg/l
17	Mineral oil	<0.01	15mg/l
18	BOD	1.55	30mg/l
19.	COD	6.09	250mg/l

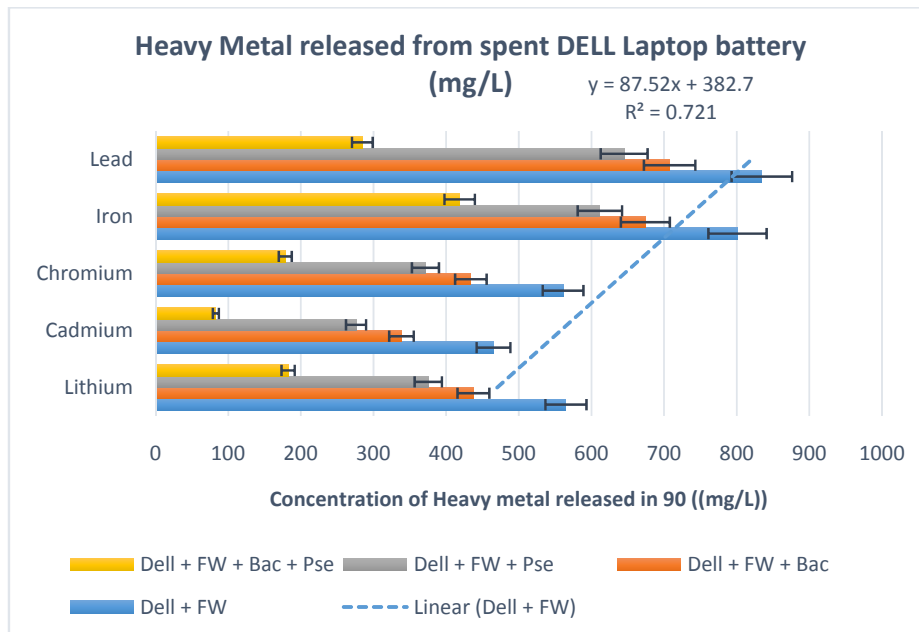


Fig. 2. Changes in concentration and amount of heavy metals (Li, Cd, Cr, Fe, Pb) (mg/l) released from spent DELL batteries during biodegradation of spent laptop batteries in freshwater

Table 3. Changes in concentration of heavy metals (Li, Cd, Cr, Fe, Pb) (mg/l) during biodegradation and heavy metal release monitoring from spent laptop batteries in freshwater

HP - Lithium Conc. (mg/L)					DELL - Lithium Conc. (mg/L)					ACER - Lithium Conc. (mg/L)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
Li-HP + FW	311.20	511.6	639.09	656.51	Li-Dell + FW	451.62	871.65	999.14	1016.56	Li-Acer + FW	465.05	757.05	884.54	901.96
Li-HP + FW + Bac	311.20	456.05	583.54	600.96	Li-Dell + FW + Bac	451.62	744.70	872.19	889.61	Li-Acer + FW + Bac	465.05	692.1	819.59	387.01
Li-HP + FW + Pse	311.20	445.75	573.24	590.66	Li-Dell + FW + Pse	451.62	682.10	809.59	827.01	Li-Acer + FW + Pse	465.05	655.65	783.14	800.56
Li-HP + FW + Bac + Pse	311.20	362.7	490.19	507.61	Li-Dell + FW + Bac + Pse	451.62	489.10	616.59	634.01	Li-Acer + FW + Bac + Pse	465.05	538.6	660.09	683.51
HP - Cadmium Conc. (mg/L)					DELL - Cadmium Conc. (mg/L)					ACER - Cadmium Conc. (mg/L)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
Li-HP + FW	111.78	162.22	295.94	424.71	Li-Dell + FW	322.27	522.27	655.74	787.46	Li-Acer + FW	307.67	407.67	541.39	673.11
Li-HP + FW + Bac	111.78	106.67	240.39	407.61	Li-Dell + FW + Bac	322.27	395.32	529.04	660.76	Li-Acer + FW + Bac	307.67	342.72	476.44	608.21
Li-HP + FW + Pse	111.78	96.37	230.09	361.86	Li-Dell + FW + Pse	322.27	332.72	466.44	598.21	Li-Acer + FW + Pse	307.67	306.27	439.99	571.76
Li-HP + FW + Bac + Pse	111.78	13.32	147.04	278.81	Li-Dell + FW + Bac + Pse	322.27	139.72	273.44	405.21	Li-Acer + FW + Bac + Pse	307.67	189.22	322.94	454.71
HP - Chromium Conc. (mg/L)					DELL - Chromium Conc. (mg/L)					ACER - Chromium Conc. (mg/L)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
Li-HP + FW	132.22	262.22	395.94	463.16	Li-Dell + FW	262.21	622.27	755.99	823.21	Li-Acer + FW	307.67	507.76	641.39	703.61
Li-HP + FW + Bac	132.22	206.67	340.39	407.61	Li-Dell + FW + Bac	262.21	495.32	629.04	696.26	Li-Acer + FW + Bac	307.67	442.72	576.44	643.66
Li-HP + FW + Pse	132.22	196.37	330.09	397.31	Li-Dell + FW + Pse	262.21	432.72	566.44	633.66	Li-Acer + FW + Pse	307.67	406.27	539.99	607.21
Li-HP + FW + Bac + Pse	132.22	113.32	247.04	314.26	Li-Dell + FW + Bac + Pse	262.21	239.72	373.44	440.66	Li-Acer + FW + Bac + Pse	307.67	289.22	422.94	490.16
HP - Iron Conc. (mg/L)					DELL - Iron Conc. (mg/L)					ACER - Iron Conc. (mg/L)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
Li-HP + FW	116.72	316.72	450.44	817.86	Li-Dell + FW	376.77	672.77	810.49	1177.91	Li-Acer + FW	262.17	562.17	695.89	1063.31
Li-HP + FW + Bac	116.72	261.17	394.89	762.31	Li-Dell + FW + Bac	376.77	549.82	683.54	1050.96	Li-Acer + FW + Bac	262.17	497.22	630.94	998.36
Li-HP + FW + Pse	116.72	250.87	384.59	752.01	Li-Dell + FW + Pse	376.77	487.22	620.94	988.36	Li-Acer + FW + Pse	262.17	460.77	594.49	992.63

Li-HP + FW + Bac + Pse					Li-Dell + FW + Bac + Pse					Li-Acer + FW + Bac + Pse				
HP – Lead Conc. (mg/L)					DELL - Lead Conc. (mg/L)					ACER - Lead Conc. (mg/L)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
Li-HP + FW	331.40	606.4	848.58	891.01	Li-Dell + FW	416.45	966.45	1208.63	1251.06	Li-Acer + FW	351.85	851.85	1094.03	1136.44
Li-HP + FW + Bac	331.40	261.17	394.89	762.31	Li-Dell + FW + Bac	416.45	839.5	1081.68	1124.11	Li-Acer + FW + Bac	351.85	786.9	1029.08	1071.51
Li-HP + FW + Pse	331.40	250.87	384.59	752.01	Li-Dell + FW + Pse	416.45	776.91	1019.08	1061.51	Li-Acer + FW + Pse	351.85	750.45	992.63	1035.06
Li-HP + FW + Bac + Pse	331.40	167.82	301.54	668.96	Li-Dell + FW + Bac + Pse	416.45	583.91	826.08	868.51	Li-Acer + FW + Bac + Pse	351.85	633.42	875.58	918.01

Key: Li – Lithium, Cd – Cadmium, Cr – Chromium, Fe – Iron, Pb – Lead, FW - Freshwater, Bac- bacillus, Pse- Pseudomonas

Table 4. Changes in concentration and amount of heavy metals (Li, Cd, Cr, Fe, Pb) (mg/l) released during biodegradation of spent laptop batteries in freshwater

Amount of heavy metals (mg/l) released HM _{ΣA} in freshwater						
	Lithium	Cadmium	Chromium	Iron	Lead	Total
HP + FW	345.31	315.93	330.94	701.14	559.61	2252.93
HP + FW + Bac	289.76	295.83	275.39	645.59	504.06	2010.63
HP + FW + Pse	279.46	250.08	265.09	635.29	493.76	1923.68
HP + FW + Bac + Pse	196.41	167.03	182.04	552.24	410.71	1508.43
Dell + FW	564.94	465.19	561	801.14	834.61	3226.88
Dell + FW + Bac	437.54	338.49	434.05	674.19	707.66	2591.93
Dell + FW + Pse	375.39	275.94	371.45	611.59	645.06	2279.43
Dell + FW + Bac + Pse	182.39	82.94	178.45	418.59	284.6	1146.97
Acer + FW	436.91	365.44	395.95	800.14	784.61	2783.05
Acer + FW + Bac	371.96	300.54	335.99	736.19	719.66	2464.34
Acer + FW + Pse	335.51	264.09	299.54	730.46	683.48	2313.08
Acer + FW + Bac + Pse	218.46	147.04	182.49	582.69	566.16	1696.84

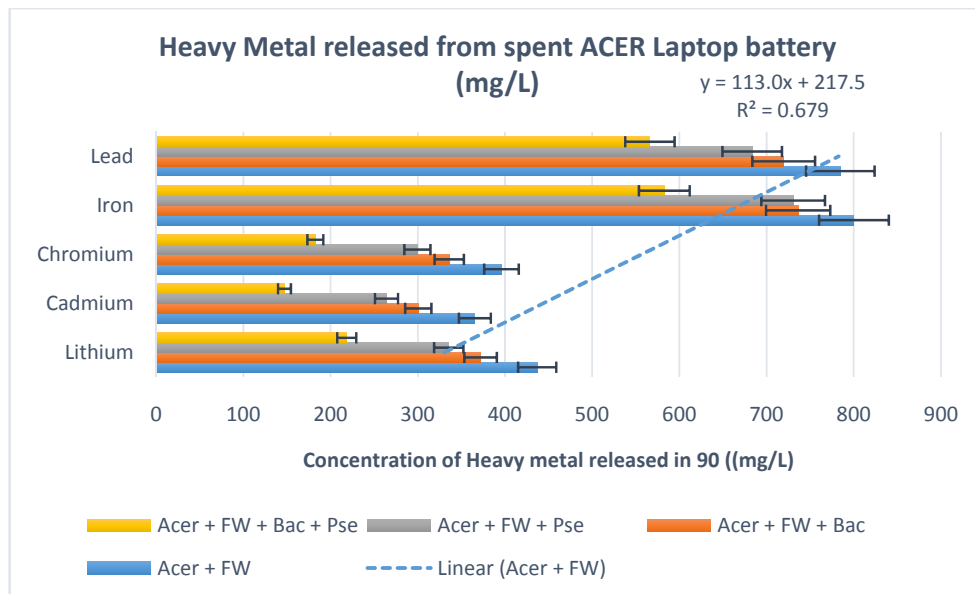


Fig. 3. Changes in concentration and amount of heavy metals (Li, Cd, Cr, Fe, Pb) (mg/l) released from spent ACER batteries during biodegradation of spent laptop batteries in freshwater

However, the set-up augmented with consortium of 2 isolate (*Bacillus* and *Pseudomonas*) for all the heavy metals had the lowest heavy metal pollution. This indicates that the consortium was able to degrade the heavy metal better than the individual strains. This being corroborated by Fritsche and Hofrichter [28].

Hasan et al. [29] reported the need for mixed cultures or consortium in degrading recalcitrant environmental contaminants since each organism would possess varying enzymatic

capacities. However, these results were not consistent with studies carried out by Jing et al. [30] and Khalid et al. [31], who from their results showed that although the mixed cultures work better to degrade different components of the environmental pollutants; the degradative potential of some bacteria are best harnessed as individual strain. This study also revealed that HP spent batteries are less toxic to the test organism than Dell and Acer. According to Sanders et al. [32] the site of action of any toxicant depends on the nature of the toxicant and environment.

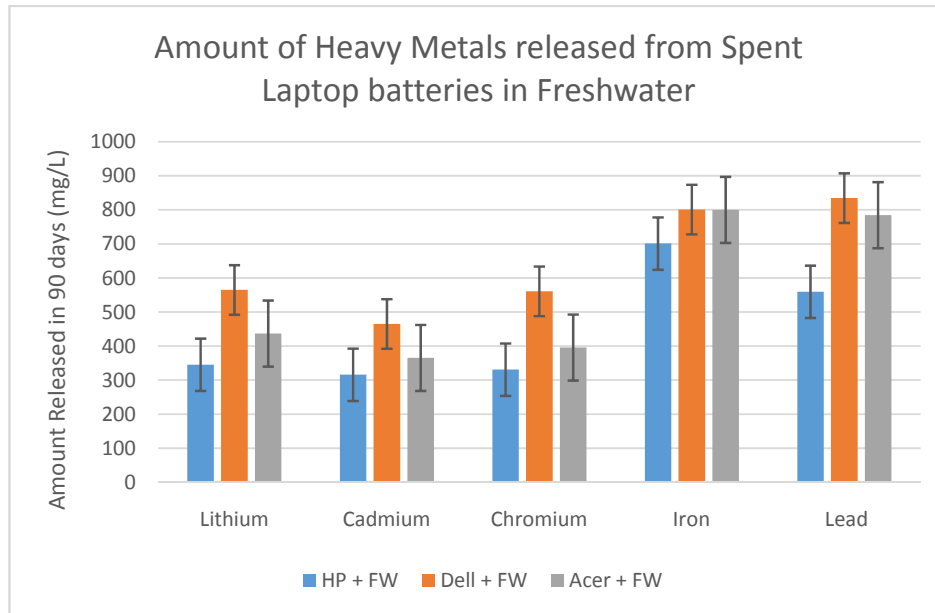


Fig. 4. Comparative concentration and amount of heavy metals (Li, Cd, Cr, Fe, Pb) (mg/l) released from spent HP batteries during biodegradation of spent laptop batteries in freshwater

3.2 Microbiological Analyses

Microbiological analyses were carried on 12 experimental set-ups at a constant interval of 30 days for 90 days in order to evaluate the changes in heavy metal utilizing bacteria and to monitor the release pattern of heavy metal from spent laptop batteries in freshwater as well as the degradability potential of the test organism (*Bacillus* and *Pseudomonas*) and their synergetic effect.

The results from the heavy metal utilizing bacteria and fungi during biodegradation showed similar trend but the mean value of the heavy metal utilizing bacteria for Lithium, Cadmium, Chromium, iron and Lead had higher values as shown in Tables 5-6. Comparative mean values of the heavy metal utilizing fungi for Lithium, Cadmium, Chromium, iron and Lead as shown in Table 7 respectively. For controls, set-ups augmented with *Bacillus* sp., *Pseudomonas* sp. and consortium of the two (2) isolates. The relative occurrence of specific genera of bacteria could be used to ascertain the biodegradation potential of an environment [33]. This fact clearly emphasizes the thought that the significance of occurrence may be due to the fact that *Bacillus* specie, *Pseudomonas* specie are more adapted to survival and biodegradation capabilities in freshwater environment [18].

3.3 Monitoring Released Pattern of Heavy Metals

The percentage (%) of heavy metals released from spent laptop battery in freshwater using *Bacillus* species and *pseudomonas* and a consortium of both organisms were monitored for 90 days at a constant interval of 30days. The percentage (%) of heavy metals (Lithium, Cadmium, Chromium, iron and Lead) released from HP battery in the experimental set-up indicates that iron has the highest percentage (%) followed by Cadmium, Chromium, lead and Lithium (Table 8). The percentage (%) of heavy metals (Lithium, Cadmium, Chromium, iron and Lead) released from Dell battery in the experimental set-up shows that iron has the highest percentage (%) followed by Chromium, lead, Cadmium and Lithium.

The percentage (%) of heavy metals (Lithium, Cadmium, Chromium, iron and Lead) released from Acer battery in the experimental set-up indicates that iron still has the highest percentage (%) followed by lead Chromium, Cadmium and Lithium. However, percentage (%) degradation potential of *Bacillus* species and *pseudomonas* species and a consortium of both organisms on heavy metals released from spent laptop batteries in freshwater at day 90 for all experimental set-up are shown in table.

Table 5. Microbiological - changes in heavy metals utilizing bacteria (LiUB, CdUB, CrUB, FeUB, PbUB) (Log10 CFU/ml) during Biodegradation and heavy metal release monitoring from spent laptop batteries in freshwater

HP - Lithium Utilizing Bacteria (LiUB) (Log10 CFU/ml)					DELL - Lithium Utilizing Bacteria (LiUB) (Log10 CFU/ml)					ACER - Lithium Utilizing Bacteria (LiUB) (Log10 CFU/ml)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
HP-Li + FW	2.939	2.929	2.708	2.398	Dell-Li + FW	2.623	2.653	2.322	2.114	Acer-Li + FW	2.672	2.591	2.176	1.845
HP-Li + FW + Bac	2.939	3.332	3.176	2.903	Dell-Li + FW + Bac	2.623	3.281	3.083	2.785	Acer-Li + FW + Bac	2.672	3.250	3.076	2.771
HP-Li + FW + Pse	2.939	3.303	3.146	2.982	Dell-Li + FW + Pse	2.623	3.342	3.210	2.763	Acer-Li + FW + Pse	2.672	3.336	3.155	2.806
HP-Li + FW + Bac + Pse	2.939	3.455	2.149	2.919	Dell-Li + FW + Bac + Pse	2.623	3.435	3.134	2.919	Acer-Li + FW + Bac + Pse	2.672	3.210	2.934	2.708
HP - Cadmium Utilizing Bacteria (CdUB) (Log10 CFU/ml)					DELL - Cadmium Utilizing Bacteria (CdUB) (Log10 CFU/ml)					ACER - Cadmium Utilizing Bacteria (CdUB) (Log10 CFU/ml)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
HP-Cd + FW	2.857	2.826	2.477	2.255	Dell-Cd + FW	2.580	2.519	2.176	1.778	Acer-Cd + FW	2.505	2.415	2.041	1.699
HP-Cd + FW + Bac	2.857	3.146	2.944	2.833	Dell-Cd + FW + Bac	2.580	3.146	2.924	2.699	Acer-Cd + FW + Bac	2.505	3.097	2.863	2.531
HP-Cd + FW + Pse	2.857	3.217	3.029	2.875	Dell-Cd + FW + Pse	2.580	3.025	2.792	2.491	Acer-Cd + FW + Pse	2.505	3.255	3.176	2.987
HP-Cd + FW + Bac + Pse	2.857	3.299	3.041	2.708	Dell-Cd + FW + Bac + Pse	2.580	3.301	3.004	2.792	Acer-Cd + FW + Bac + Pse	2.505	3.207	2.919	2.623
HP - Chromium Utilizing Bacteria (CrUB) (Log10 CFU/ml)					DELL - Chromium Utilizing Bacteria (CrUB) (Log10 CFU/ml)					ACER - Chromium Utilizing Bacteria (CrUB) (Log10 CFU/ml)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
HP-Cr + FW	2.708	2.623	2.398	2.279	Dell-Cr + FW	2.672	2.602	2.301	1.954	Acer-Cr + FW	2.653	2.602	2.398	2.080
HP-Cr + FW + Bac	2.708	3.987	2.724	2.519	Dell-Cr + FW + Bac	2.672	3.000	2.740	2.462	Acer-Cr + FW + Bac	2.653	3.207	2.949	2.623
HP-Cr + FW + Pse	2.708	3.279	3.097	2.826	Dell-Cr + FW + Pse	2.672	3.193	2.987	2.708	Acer-Cr + FW + Pse	2.653	2.987	2.724	2.052
HP-Cr + FW + Bac + Pse	2.708	3.414	3.107	2.690	Dell-Cr + FW + Bac + Pse	2.672	3.243	2.996	2.763	Acer-Cr + FW + Bac + Pse	2.653	3.431	3.137	2.732
HP - Iron Utilizing Bacteria (FeUB) (Log10 CFU/ml)					DELL - Iron Utilizing Bacteria (FeUB) (Log10 CFU/ml)					ACER - Iron Utilizing Bacteria (FeUB) (Log10 CFU/ml)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
HP-Fe + FW	2.732	2.699	2.591	2.301	Dell-Fe + FW	2.778	2.724	2.568	2.204	Acer-Fe + FW	2.690	2.580	2.204	2.041
HP-Fe + FW + Bac	2.732	3.109	2.881	2.653	Dell-Fe + FW + Bac	2.778	3.210	3.004	2.653	Acer-Fe + FW + Bac	2.690	3.068	2.785	2.544
HP-Fe + FW + Pse	2.732	3.236	3.045	2.716	Dell-Fe + FW + Pse	2.778	3.140	2.914	2.591	Acer-Fe + FW + Pse	2.690	3.097	2.903	2.681
HP-Fe + FW + Bac + Pse	2.732	3.425	3.114	2.806	Dell-Fe + FW + Bac + Pse	2.778	3.425	3.114	2.806	Acer-Fe + FW + Bac + Pse	2.690	3.320	3.076	2.716
HP - Lead Utilizing Bacteria (PbUB) (Log10 CFU/ml)					DELL - Lead Utilizing Bacteria (PbUB) (Log10 CFU/ml)					ACER - Lead Utilizing Bacteria (PbUB) (Log10 CFU/ml)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90

HP - Lithium Utilizing Bacteria (LiUB) (Log10 CFU/ml)					DELL - Lithium Utilizing Bacteria (LiUB) (Log10 CFU/ml)					ACER - Lithium Utilizing Bacteria (LiUB) (Log10 CFU/ml)				
HP-Pb + FW	2.623	2.301	2.041	1.778	Dell-Pb + FW	2.580	2.431	2.079	1.602	Acer-Pb + FW	2.531	2.342	2.000	1.477
HP-Pb + FW + Bac	2.623	3.004	2.778	2.505	Dell-Pb + FW + Bac	2.580	2.959	2.960	2.255	Acer-Pb + FW + Bac	2.531	3.021	2.748	2.176
HP-Pb + FW + Pse	2.623	2.949	2.756	2.462	Dell-Pb + FW + Pse	2.580	3.041	2.763	2.505	Acer-Pb + FW + Pse	2.531	3.176	2.959	2.699
HP-Pb + FW + Bac + Pse	2.623	3.193	2.978	2.623	Dell-Pb + FW + Bac + Pse	2.580	3.342	3.107	2.839	Acer-Pb + FW + Bac + Pse	2.531	3.021	2.740	2.568

Key: Li – Lithium, Cd – Cadmium, Cr – Chromium, Fe – Iron, Pb – Lead, FW - Freshwater, Bac- bacillus, Pse- Pseudomonas

Table 6. Microbiological - Changes in heavy metals utilizing fungi (LiUF, CdUF, CrUF, FeUF, PbUF) (Log10 CFU/ml) during biodegradation and heavy metal release monitoring from spent laptop batteries in freshwater

HP - Lithium Utilizing Fungi (LiUF) (Log10 CFU/ml)					DELL - Lithium Utilizing Fungi (LiUF) (Log10 CFU/ml)					ACER - Lithium Utilizing Fungi (LiUF) (Log10 CFU/ml)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
HP-Li + FW	2.555	2.041	1.845	1.670	Dell-Li + FW	2.279	2.114	2.000	1.903	Acer-Li + FW	2.097	2.041	1.477	1.778
HP-Li + FW + Bac	2.555	1.903	1.845	1.602	Dell-Li + FW + Bac	2.279	2.114	2.000	1.778	Acer-Li + FW + Bac	2.097	2.230	1.903	2.301
HP-Li + FW + Pse	2.555	2.279	1.954	2.000	Dell-Li + FW + Pse	2.279	2.230	1.903	1.954	Acer-Li + FW + Pse	2.097	2.279	2.079	2.000
HP-Li + FW + Bac + Pse	2.555	2.114	2.041	1.778	Dell-Li + FW + Bac + Pse	2.279	2.255	1.954	1.954	Acer-Li + FW + Bac + Pse	2.097	2.041	1.778	1.699
HP - Cadmium Utilizing Fungi (CdUF) (Log10 CFU/ml)					DELL - Cadmium Utilizing Fungi (CdUF) (Log10 CFU/ml)					ACER - Cadmium Utilizing Fungi (CdUF) (Log10 CFU/ml)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
HP-Cd + FW	2.301	2.176	1.602	1.903	Dell-Cd + FW	2.322	2.176	2.000	1.602	Acer-Cd + FW	2.041	2.114	1.778	1.699
HP-Cd + FW + Bac	2.301	2.079	2.041	1.845	Dell-Cd + FW + Bac	2.322	1.699	1.954	1.699	Acer-Cd + FW + Bac	2.041	2.146	1.000	1.000
HP-Cd + FW + Pse	2.301	2.230	2.041	2.146	Dell-Cd + FW + Pse	2.322	2.204	1.954	1.845	Acer-Cd + FW + Pse	2.041	2.000	1.845	1.778
HP-Cd + FW + Bac + Pse	2.301	2.903	2.114	1.477	Dell-Cd + FW + Bac + Pse	2.322	2.079	1.699	1.699	Acer-Cd + FW + Bac + Pse	2.041	2.301	2.255	1.845
HP - Chromium Utilizing Fungi (CrUF) (Log10 CFU/ml)					DELL - Chromium Utilizing Fungi (CrUF) (Log10 CFU/ml)					ACER - Chromium Utilizing Fungi (CrUF) (Log10 CFU/ml)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
HP-Cr + FW	2.146	1.845	1.602	1.477	Dell-Cr + FW	2.176	1.954	1.778	1.699	Acer-Cr + FW	1.954	1.845	1.699	1.477
HP-Cr + FW + Bac	2.146	2.114	2.041	1.903	Dell-Cr + FW + Bac	2.176	1.903	2.079	1.602	Acer-Cr + FW + Bac	1.954	2.079	1.845	2.000
HP-Cr + FW + Pse	2.146	2.146	2.000	1.903	Dell-Cr + FW + Pse	2.176	1.114	1.778	1.699	Acer-Cr + FW + Pse	1.954	1.954	1.699	1.602
HP-Cr + FW + Bac + Pse	2.146	1.602	1.845	1.699	Dell-Cr + FW + Bac + Pse	2.176	2.079	1.903	2.477	Acer-Cr + FW + Bac + Pse	1.954	2.041	2.000	1.778
HP - Iron Utilizing Fungi (FeUF) (Log10 CFU/ml)					DELL - Iron Utilizing Fungi (FeUF) (Log10 CFU/ml)					ACER - Iron Utilizing Fungi (FeUF) (Log10 CFU/ml)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
HP-Fe + FW	2.322	2.114	1.778	1.845	Dell-Fe + FW	1.954	1.845	1.301	1.477	Acer-Fe + FW	2.079	2.079	1.954	1.903

HP - Lithium Utilizing Fungi (LiUF) (Log10 CFU/ml)					DELL - Lithium Utilizing Fungi (LiUF) (Log10 CFU/ml)					ACER - Lithium Utilizing Fungi (LiUF) (Log10 CFU/ml)				
HP-Fe + FW + Bac	2.322	1.778	1.903	1.845	Dell-Fe + FW + Bac	1.954	1.477	1.845	1.301	Acer-Fe + FW + Bac	2.079	2.255	2.079	1.602
HP-Fe + FW + Pse	2.322	1.954	1.903	1.778	Dell-Fe + FW + Pse	1.954	1.845	1.477	1.477	Acer-Fe + FW + Pse	2.079	2.079	1.954	1.778
HP-Fe + FW + Bac + Pse	2.322	1.778	2.000	1.845	Dell-Fe + FW + Bac + Pse	1.954	2.230	2.000	2.041	Acer-Fe + FW + Bac + Pse	2.079	1.845	1.699	1.602
HP – Lead Utilizing Fungi (PbUF) (Log10 CFU/ml)					DELL - Lead Utilizing Fungi (PbUF) (Log10 CFU/ml)					ACER - Lead Utilizing Fungi (PbUF) (Log10 CFU/ml)				
Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90	Exp. Set-up	Day 1	Day 30	Day 60	Day 90
HP-Pb + FW	2.079	1.778	1.477	1.301	Dell-Pb + FW	2.041	1.954	1.903	1.301	Acer-Pb + FW	2.000	1.903	1.699	1.301
HP-Pb + FW + Bac	2.079	2.079	2.114	1.778	Dell-Pb + FW + Bac	2.041	2.000	2.146	1.903	Acer-Pb + FW + Bac	2.000	2.230	2.000	1.602
HP-Pb + FW + Pse	2.079	2.114	1.845	1.699	Dell-Pb + FW + Pse	2.041	2.146	1.602	1.903	Acer-Pb + FW + Pse	2.000	2.230	1.845	1.778
HP-Pb + FW + Bac + Pse	2.079	1.477	2.041	1.602	Dell-Pb + FW + Bac + Pse	2.041	2.114	2.079	2.000	Acer-Pb + FW + Bac + Pse	2.000	1.176	2.079	1.778

Key: Li – Lithium, Cd – Cadmium, Cr – Chromium, Fe – Iron, Pb – Lead, FW - Freshwater, Bac- bacillus, Pse- Pseudomonas

Table 7. Changes in Mean value of Heavy Metals Utilizing Bacteria (HMUB)(Log10 CFU/ml) and Heavy Metals Utilizing Fungi (HMUF)(Log10 CFU/ml) during biodegradation of spent laptop batteries in freshwater

Experimental Set-up	Heavy Metals Utilizing Bacteria (HMUB) (Log10 CFU/ml)					Heavy Metals Utilizing Fungi (HMUF) (Log10 CFU/ml)				
	LiUB	CdUB	CrUB	FeUB	PbUB	LiUF	CdUF	CrUF	FeUF	PbUF
HP + FW	2.744	2.604	2.502	2.581	2.186	2.028	1.996	1.768	2.015	1.659
HP + FW + Bac	3.088	2.945	2.985	2.844	2.728	1.976	2.067	2.051	1.962	2.013
HP + FW + Pse	3.093	2.995	2.978	2.932	2.698	2.197	2.180	2.049	1.989	1.934
HP + FW + Bac + Pse	2.866	2.976	2.980	3.019	2.854	2.122	2.199	1.823	1.986	1.800
Dell + FW	2.428	2.263	2.382	2.569	2.173	2.074	2.025	1.902	1.644	1.799
Dell + FW + Bac	2.943	2.837	2.719	2.911	2.689	2.043	1.919	1.940	1.644	2.023
Dell + FW + Pse	2.985	2.722	2.890	2.856	2.722	2.092	2.081	1.692	1.688	1.923
Dell + FW + Bac + Pse	3.028	2.919	2.919	3.031	2.967	2.111	1.950	2.159	2.056	2.059
Acer + FW	2.321	2.165	2.433	2.379	2.088	1.848	1.727	1.744	2.004	1.726
Acer + FW + Bac	2.942	2.749	2.858	2.772	2.619	2.133	1.731	1.970	2.003	1.958
Acer + FW + Pse	2.992	2.981	2.604	2.843	2.841	2.114	2.013	1.802	1.972	1.963
Acer + FW + Bac + Pse	2.881	2.814	2.989	2.951	2.715	1.904	2.111	1.943	1.806	1.758

Key: Cd – Cadmium, FW- Freshwater, Bac- bacillus, Pse- Pseudomonas

Table 8. Percentage (%) biodegradation potential of bacillus and pseudomonas on heavy metals released from spent laptop batteries in freshwater at day 90

Heavy metals from spent laptop batteries	Percentage (%) Biodegradation potential		
	<i>Bacillus sp.</i>	<i>Pseudomonas sp.</i>	<i>Bacillus + Pseudomonas</i>
Li- HP	8.46	10.03	22.68
Li-DELL	12.49	18.65	37.63
Li-ACER	7.20	11.24	24.22
Cd-HP	4.70	15.41	34.81
Cd-DELL	16.09	24.03	48.54
Cd-ACER	9.64	15.06	32.45
Cr-HP	11.99	14.21	32.15
Cr-DELL	15.42	23.03	46.47
Cr-ACER	8.52	13.70	30.34
Fe-HP	6.79	8.05	18.21
Fe-DELL	10.78	16.09	32.48
Fe-ACER	6.11	6.65	20.54
Pb-HP	6.23	7.39	16.71
Pb-DELL	10.15	18.75	30.58
Pb-ACER	5.72	8.92	19.22

Percentage (%) degradation potential of the consortium of *Bacillus* species and *Pseudomonas* species for Li-HP, Li-Dell, and Li-Acer shows a higher percentage (%) release of 22.68%, 37.63% and 24.22% respectively as compared to the individual strains of for *Bacillus* species and *Pseudomonas* species. With *Pseudomonas* species having 10.03%, 18.65%, and 11.24%, followed by *Bacillus* species having 8.46%, 12.49%, and 7.20% for Li-HP, Li-Dell, and Li-Acer respectively. The degradation potential of the consortium of both *Bacillus* species and *Pseudomonas* species for Lithium, Cadmium, Chromium, iron and Lead for all three batteries shows similar trend with those of the Lithium with higher values compared to the individual strains of *Bacillus* species and *Pseudomonas* species respectively. The results obtained in this study revealed that the experimental set-up augmented with *Bacillus* species and *Pseudomonas* species has higher percentage degradation potential to degrade the batteries.

4. CONCLUSION AND RECOMMENDATION

The results obtained in this research revealed that spent laptop batteries toxicant have the ability to change environmental condition as the high concentration of these heavy metals from spent laptop batteries toxicant exhibited more toxic effect on the individual strains of bacteria and lesser on the consortium, since mixed cultures had the highest percentage degradation which offers the advantage to be used in bioremediation activities therefore, the use of mixed cultures serve as an alternative solution to detoxify contaminants and is being used as an effective means of mitigating heavy metals and other toxic compounds which could affect the ecosystem negatively. The study was able to identify the degradability potential of *Bacillus* and *Pseudomonas* species of spent laptop battery in freshwater.

From this research, it shows that mixed cultures of *Bacillus* and *Pseudomonas* species were the best in reducing the concentration of heavy metals from spent laptop battery in freshwater

it is highly recommended that; Proper waste management and regulation policies should be put in place by government to reduce the hazardous effect of the waste on the environment. Also, there is need to use standardized products for easy disassembling

and used of readily biodegradable materials for effective recycling.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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