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Assessment of the Toxicity Potentials of Spent Laptop Battery Wastes on Essential Soil Microbes and Plant Bioindicators

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BOU and ELO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BOU and JOO managed the analyses of the study. Authors BOU, COC and JOO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was undertaken to assess the toxicity potentials of spent laptop battery wastes on essential soil microbes and plant bio-indicators.
Study Design: Five treatments and the controls designs were set up in triplicates and incubated at 25 ± 2°C for 21 days. The five treatments and controls set ups were designated as 6.25%, 12.5%, 25%, 50%, 100% and CTRL.
Place and Duration of Study: Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University (COOU), Uli Anambra State, Nigeria during May, 2019 - July, 2019.
Methodology: The microbial growth inhibition was analysed using standard method of spread plate technique while growth indices and percentage seedling emergence were adopted for the seed growth inhibition.

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Results: The result revealed that fungal population was the most sensitive followed by bacterial population and then actinomycetes population in terms of toxic responses to the spent laptop battery samples soil contamination. The order of toxicity of the spent battery samples on the growth indices and percentage seedling emergence of *P. vulgaris* and *S. bicolor* were: Product B-bean (-18.89%) > Product A-sorghum (-32.22%) > Product A-bean (- 38.63%) > Product B- sorghum (- 45.77%) revealing that both *P. vulgaris* and *S. bicolor* are very good bio-monitoring models for spent product A and B battery pollution assessment.
Conclusion: Thus, strict and stringent measures on release of these electronic wastes in the environment are recommended.

Keywords: Bio-monitor; growth indices; microbial sensor; pollution; spent laptop battery.

1. INTRODUCTION

Laptops are electronic appliances used by man, in all human endeavours in order to make life and work easier. The high demand and utilization of these devices made some almost outdated and exhausted in its purposeful life [1]. The increased demand has led to the production of extremely large quantities of these wastes annually especially in Nigeria that has been reported as the top producer of these wastes in West Africa owing to its large population size [2].

The battery industry has ultimately made huge efforts to recover and restore the toxic parts of these electronic devices. In spite of these efforts, spent laptop batteries are still classified as harmful wastes and include: small sealed lead-acid batteries, alkaline batteries, silver button batteries, rechargeable nickel-cadmium batteries and mercury batteries [3].

Heavy metals such as lead, cadmium, nickel and metalloids from spent laptop battery wastes are mostly challenging soil pollutants due to their persistence in the environment [4]. Besides, it has been well known and established by several researchers that plants absorb these elements which don't have any recognized biotic role and are even acknowledged to be toxic at small concentrations or above certain threshold standards (heavy metals) leading to phytotoxicity of host plant species and may develop a health risk to man and animals [5,6]. Phytotoxicity test using seeds of *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) have been used by several researchers to monitor the effect of pollutants on seed emergence and growth indices [7,8]. These two botanical indicators were chosen in this study because they are staple food crops in Nigeria, easily responsive, economical and relatively easy to perform. They can be standardized and their quick signal to the presence of contaminants can be easily assessed. In addition, they are among the dicot and monocot plant test organisms recommended by the Organization for Economic Co-operation and Development (OECD) [9] for ecotoxicological testing.

Battery constituents has been reported to inhibit the growth and development of certain microbial groups such as bacteria, fungi and actinomycetes by impairing enzymatic activities such as nitrogenase that is widely known to participate in nitrogen fixation [10]. Other significant microbial processes in the soil ecosystem include: degradation and decomposition of resistant constituents of plant and animal tissues, humus formation and nutrient transformation, all which depends on the stability established by the different groups of microbes found in the soil ecosystem, which in turn are negatively influenced by the presence of high concentrations of these toxic wastes [11]. The microbial groups: bacteria, fungi and actinomycetes emphasized in this study were selected because they are three major and dominant microbial communities implicated in the processes previously described above in the soil ecosystem. They rapidly act in response to ecological changes and thus satisfactorily reflect natural changes induced by environmental pollution. Several researchers have reported them as strong indicators of soil fertility and toxicity [12,13]. Consequent upon these hazards reported, it is essential to quantify the likely ecological effects of these battery wastes in order to save the environment and to guide policymakers or researchers as there is paucity of information regarding its negative influence on both essential soil microbes and plant endpoints. Thus, this study was undertaken to assess the toxicity potentials of spent laptop battery wastes on essential soil microbes and plant bioindicators.

2. MATERIALS AND METHODS

2.1 Source and Preparation of the Spent Battery Samples

The spent Lenovo and Dell laptop batteries used in this study were bought from a Commercial Market in Nigeria. The two brands of laptop battery packs (renamed product A and B for competing interest issues) were prepared by forcefully opening of the battery cells under aseptic condition, into well labelled 1 L plastic containers [13].

2.2 Quantification of Heavy Metals

The heavy metal composition of the spent laptop battery samples was quantified using the standard method of American Public Health Association [14].

2.3 Soil Sample Collection

Sandy loamy soil samples were collected from the botanical garden of the Departmental of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University (COOU), Uli, Nigeria without incidence of such pollution. Soil samples were collected using sterile metallic spade at a depth of 15 cm and 2 m apart and mixed together in order to obtain a composite sample [13]. The composite soil samples were placed in sterile polyethylene bags and taken to the Microbiology Laboratory, Chukwuemeka Odumegwu Ojukwu University (COOU), Uli, Nigeria.

2.4 Toxicity Experimental Setup

The soil samples (pH = 7.30) were sieved, 500 g weighed and placed into 1 L sterile plastic containers with open lids. The test samples (products A and B) were prepared in 2 - fold dilutions (100%, 50%, 25%, 12.5% and 6.25%) using sterile distilled water as diluent with the negative control without test samples (0%). The soil samples were contaminated by adding aliquot of the prepared concentrations of the test sample suspensions through homogenous mixing to ensure even distribution of the test samples. The sample containers and their controls were labelled appropriately and the experiment was repeated in duplicates [7].

2.5 Source of Plant Seeds

The seeds of *Phaseolus vulgaris* (common bean) were purchased from COOU School Market, Uli Town Nigeria, while *Sorghum bicolor* (guinea corn) was purchased from Afor Egbu Local Market, Imo State, Nigeria. The seeds were placed in sterile polyethylene bags, transported to the Microbiology Laboratory, COOU Uli, and stored at laboratory temperature (25 ± 2°C) for not more than 24 h [8].

2.6 Viability Testing of Plant Seeds

Floatation method of Olubodun and Eriyamremu [7] was adopted for assessment of seed viability. The seeds of *Phaseolus vulgaris* and *Sorghum bicolor* were soaked inside a sterile bowel that was half - filled with sterile water and stirred such that the seeds that sunk (viable seeds) were selected while the seeds that floated on the water top were not selected and hence discarded.

2.7 Sowing of Plant Seeds

The method of Olubodun and Eriyamremu [7] was adopted for planting of the seeds. Three viable seeds of *Phaseolus vulgaris* and *Sorghum bicolor* were sown into each treated 500 g sandy loamy soil at a depth of 1 - 2 cm.

2.8 Microbial Quantification of the Treated Soil Samples

The spread plate method was used for the quantification of the rhizospheric microbial load at one-week intervals (0, 7, 14 and 21 days). One gram (1 g) of each treated soil sample and control around the plant roots was weighed aseptically and placed into 9 mL of sterile distilled water in glass test tubes. After serially diluting of the samples up to 10^{-3}, 0.1 mL aliquot was aseptically pipetted and dispensed on the surfaces of sterile Nutrient Agar (NA) (ketoconazole 0.025 %) plates, Potato Dextrose Agar (PDA) (chloramphenicol 0.005%) plates and Modified Glycerol Starch Casein Agar (MGSCA) (cycloheximide 50 µg/ mL); nystatin 25 µg/ mL) plates, respectively. The inoculants were evenly spread using a sterile glass rod and plates were incubated for 72 h at 25 ± 2°C for fungi; 24 h at 37 ± 2°C for bacteria and 96 h at 25 ± 2°C for actinomycetes [13,15]. The experiments were carried out in duplicates. After incubation, the colonies on the respective plates were counted and the mean values of total heterotrophic fungal counts, total heterotrophic bacterial counts and total heterotrophic actinomycetes counts were determined.

2.9 Assessment of Growth Parameters and Percentage Seedling Emergence

After 7, 14 and 21 days of planting, the growth parameters and the percentage seedling emergence in each treatment were evaluated as described by Olubodun and Eriyamremu [7] and Eze et al. [8].

2.10 Statistical Analysis

The results were analyzed using the two factor ANOVA and linear regression in order to compare means of the treatment groups and controls as well as the effect of the spent laptop battery wastes on the plant growth indices and microbial communities. The median effective concentration (EC_{50}), that concentration that inhibit 50% of the test organisms were determined from the linear regression equations. Statistical values less than the threshold value ($P < 0.05$) were regarded as significant.

3. RESULTS

3.1 Heavy Metal Profile

The result of the heavy metal constituents of the spent product A and B laptop battery wastes is presented in Table 1. The result showed that product A sample had the highest value of mercury (0.407 ppm) and nickel (21.898 ppm) while product B sample had the highest value of arsenic (0.119 ppm), cadmium (0.191 ppm) and lead (2.691 ppm), respectively.

3.2 Microbial Toxicity Profile

The results of the effects of product A battery concentrations on bacterial count, fungal count and actinomycetes count in *Phaseolus vulgaris* and *Sorghum bicolor* during the 21 days exposure period are presented in Tables 2, 3 and 4. From the results, 100% concentration had the lowest count (4.602 CFU/g) of bacteria while the control set up had the highest count (5.903 CFU/g) of bacteria in the product A exposed *P. vulgaris* after 21 days. Also, 100% concentration had the lowest count (5.000 CFU/g) of fungi while the control set up had the highest count (6.146 CFU/g) of fungi in the product A exposed *P. vulgaris* after 21 days. Furthermore, 100% concentration had the lowest count (5.000 CFU/g) of actinomycetes while the control set up had the highest count (5.602 CFU/g) of actinomycetes in the product A exposed *P. vulgaris* and *S. bicolor* after 21 days, respectively. Statistically, there was significant differences ($P < 0.05$) detected on dose effects and days of exposure in comparison to their controls.

The results of the effects of product B battery concentrations on bacterial count, fungal count and actinomycetes count in *Phaseolus vulgaris* and *Sorghum bicolor* during the 21 days exposure period are presented in Tables 5, 6 and 7. The results revealed that the highest count (5.954 CFU/g) of bacteria were observed in the control while the lowest count (5.000 CFU/g) were observed in the 100% concentrations of product B exposed *S. bicolor and P. vulgaris* after 21 days. Also, the highest counts (5.602 CFU/g) of fungi were observed in the control set up while the lowest count (5.301 CFU/g) of fungi were observed in the 100% concentrations of product B exposed *P. vulgaris* and *S. bicolor* after 21 days. Furthermore, the highest count (5.698 CFU/g) of actinomycetes were observed in the control setup while the lowest count (4.578 CFU/g) of actinomycetes were observed in the 100% concentrations of product B exposed *P. vulgaris* and *S. bicolor* after 21 days, respectively. Statistically, there was also significant differences ($P < 0.05$) detected on dose effects and days of exposure in comparison to their controls.

Table 1. Heavy metal constituents of the spent product A and B laptop battery wastes

Parameters	Metal (ppm)		WHO (1983)/FEPA (1991) standards in sediment (ppm)	WHO (1983)/FEPA (1991) standards in water (ppm)
	Product A	Product B		
Arsenic	0.111	0.119	-	0.100
Cadmium	0.000	0.191	0.030	0.005 – 0.010
Mercury	0.407	0.284	-	0.050
Lead	2.028	2.691	0.010	0.050
Nickel	21.898	13.874	0.020	0.100 – 0.200

WHO = World Health Organization; FEPA = Federal Environmental Protection Agency; ppm = Part per Million

Table 2. Effects of product A battery concentrations on bacterial count in *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) during the 21 days exposure period

Day	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
0	6.079	6.176	6.225	6.301	6.397	6.518	6.176	6.225	6.305	6.361	6.414	6.519
7	5.698	5.843	5.845	5.903	5.903	6.301	5.698	5.698	5.698	5.903	5.977	6.518
14	5.301	5.301	5.431	5.462	5.698	6.225	5.301	5.602	5.659	5.698	5.698	5.778
21	4.602	5.176	5.301	5.397	5.602	5.903	5.176	5.301	5.462	5.477	5.544	5.602

% = Percent

Table 3. Effects of product A battery concentrations on fungal count in *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (Guinea corn) during the 21 days exposure period

Day	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
0	5.903	6.000	6.000	6.146	6.812	6.875	5.903	5.954	5.954	6.000	6.176	6.305
7	5.698	5.954	5.607	6.079	6.301	6.812	5.698	5.788	5.845	5.929	5.963	6.301
14	5.474	5.477	5.602	5.903	6.000	6.301	5.477	5.698	5.845	5.903	5.954	6.146
21	5.000	5.397	5.477	5.602	5.602	6.146	5.477	5.477	5.544	5.544	5.602	6.076

% = Percent

Table 4. Effects of product A battery concentrations on actinomycetes count in *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) during the 21 days exposure period

Day	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
0	5.698	5.778	5.903	5.977	6.225	6.818	5.301	5.477	5.477	5.477	5.903	6.301
7	5.301	5.301	5.431	5.462	5.698	6.301	5.176	5.301	5.397	5.477	5.698	5.954
14	5.000	5.176	5.301	5.397	5.477	6.301	5.176	5.301	5.397	5.462	5.544	5.602
21	5.000	5.176	**5.176**	**5.301**	**5.477**	**5.602**	**5.000**	**5.176**	**5.301**	**5.397**	**5.505**	**5.602**

% = Percent

Table 5. Effects of product B battery concentrations on bacterial count in *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) during the 21 days exposure period

Day	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
0	6.000	6.255	6.301	6.342	6.544	6.778	6.004	6.079	6.176	6.225	6.322	6.544
7	5.301	5.698	5.903	5.991	6.000	6.322	5.301	5.477	5.954	5.954	6.000	6.301
14	5.301	5.477	5.477	5.602	5.698	6.176	5.176	5.301	5.477	5.477	5.903	5.995
21	5.000	5.397	5.477	5.477	5.698	5.602	5.000	5.301	5.397	5.397	5.602	5.954

% = Percent

Table 6. Effects of product B battery concentrations on fungal count in *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) during the 21 days exposure period

Day	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
0	5.845	5.903	5.954	6.000	6.079	6.322	5.778	5.875	5.903	6.000	6.176	6.322
7	5.301	5.342	5.903	5.903	6.041	6.301	5.544	5.778	5.903	5.977	6.060	6.176
14	5.301	5.301	5.431	5.602	5.698	6.176	5.477	5.698	5.903	5.944	5.977	6.176
21	5.301	5.301	5.462	5.477	5.477	5.602	5.301	5.301	5.431	5.462	5.477	5.602

% = Percent

Table 7. Effects of product B battery concentrations on actinomycetes count in *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) during the 21 days exposure period

Day	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
0	5.698	5.954	6.004	6.146	6.812	6.875	5.301	5.301	5.397	5.698	5.698	5.903
7	5.000	5.176	5.301	5.602	5.698	6.342	5.000	5.176	5.301	5.462	5.462	5.698
14	5.000	5.000	5.176	5.397	5.698	6.176	5.000	5.176	5.301	5.342	5.342	5.698
21	4.698	5.000	5.176	5.301	5.301	5.698	4.578	5.000	5.000	5.176	5.176	5.602

% = Percent

Table 8. Effects of different levels of product A battery samples on the growth indices of *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) after 7 days exposure

Parameter	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
No. of seeds germinated	1.00	2.00	2.00	2.00	3.00	3.00	2.00	2.00	2.00	2.00	3.00	3.00
Time of germination (day)	4.00	4.00	4.00	4.00	4.00	4.00	6.00	4.00	5.00	3.00	3.00	2.00
Root length (cm)	3.40	6.33	6.90	6.95	7.70	9.12	1.15	1.22	1.24	1.31	1.31	1.55
Shoot length (cm)	5.45	5.80	5.90	6.10	7.20	8. 15	1.40	1.55	2.30	2. 14	2.33	2.51
Leaf length (cm)	6.70	8.00	11.20	20.10	26.40	28.60	14.20	20.80	34.30	48.40	58.90	59.00
Leaf breadth (cm)	2.70	6.70	9.00	12.00	16.30	39.60	0.70	1.00	2.30	2.70	2.80	2.90
Leaf area (cm^2)	18.09	53.60	100.80	241.20	430.32	1132.56	9.94	20.80	78.89	130.68	164.92	171.10
Number of leaves	2.00	2.00	4.00	4.00	6.00	6.00	2.00	2.00	5.00	5.00	6.00	6.00
Percentage emergence of seedlings	33.33	66.67	66.67	66.67	100.00	100.00	66.67	66.67	83.33	83.88	100.00	100.00

% = Percent; cm = Centimetre; cm^2 = Centimetre square

Table 9. Effects of different levels of product B battery samples on the growth indices of *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) after 7 days exposure

Parameter	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
No. of seeds germinated	1.00	2.00	2.00	2.00	3.00	3.00	1.00	1.00	2.00	3.00	3.00	3.00
Time of germination (day)	4.00	4.00	4.00	4.00	4.00	4.00	6.00	4.00	3.00	3.00	3.00	3.00
Root length (cm)	3.40	6.33	6.90	6.95	7.70	9. 12	1.17	1.23	1.26	1.32	1.32	1.57
Shoot length (cm)	5.45	5.80	5. 90	6.11	7.20	8. 15	1.45	1.55	2.30	2.24	2.33	2.51
Leaf length (cm)	6.70	8.00	11.20	20.10	26.40	28.60	14.20	20.80	34.30	48.40	58.90	59.00
Leaf breadth (cm)	2.70	6.70	9.00	12.00	16.30	39.60	0.70	1.00	2.30	2.27	2.80	2.90
Leaf area (cm^2)	18.09	53.60	100.80	241.20	430.32	1132.56	9.94	20.80	78.89	101.11	164.92	171.10
Number of leaves	2.00	2.00	4.00	4.00	6.00	6.00	1.00	2.00	5.00	5.00	6.00	6.00
Percentage emergence of seedlings	33.33	66.67	66.67	66.67	100.00	100.00	33.33	33.33	66.67	100.00	100.00	100.00

% = Percent; cm = Centimetre; cm^2 = Centimetre square

Table 10. Effects of different levels of product A battery samples on the growth indices of *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) after 14 days exposure

Parameter	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
No. of seeds germinated	1.00	2.00	2.00	2.00	3.00	3.00	1.00	1.00	2.00	3.00	3.00	3.00
Time of germination (day)	4.00	4.00	4.00	4.00	4.00	4.00	6.00	4.00	3.00	3.00	3.00	3.00
Root length (cm)	3.40	6.33	6.90	6.95	7.70	9. 12	1.17	1.23	1.26	1.32	1.32	1.57
Shoot length (cm)	5.45	5.80	5. 90	6.11	7.20	8. 15	1.45	1.55	2.30	2.24	2.33	2.51
Leaf length (cm)	6.70	8.00	11.20	20.10	26.40	28.60	14.2	20.80	34.30	48.40	58.90	59.00
Leaf breadth (cm)	2.70	6.70	9.00	12.00	16.30	39.60	0.70	1.11	2.30	2.27	2.80	2.90
Leaf area (cm^2)	18.09	53.60	180.90	316.80	343.20	430.32	9.94	20.80	78.89	130.68	164.92	171.10
Number of leaves	2.00	2.00	4.00	4.00	6.00	6.00	1.00	2.00	5.00	5.00	6.00	6.00
Percentage emergence of seedlings	33.33	66.67	66.67	66.67	100.00	100.00	33.33	33.33	66.67	100.00	100.00	100.00

% = Percent; cm = Centimetre; cm^2 = Centimetre square

Table 11. Effects of different levels of product B battery samples on the growth indices of *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) after 14 days exposure

Parameter	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
No. of seeds germinated	1.00	2.00	2.00	2.00	3.00	3.00	1.00	1.00	2.00	3.00	3.00	3.00
Time of germination (day)	4.00	4.00	4.00	3.00	3.00	3.00	5.00	5.00	4.00	4.00	4.00	4.00
Root length (cm)	4.45	4.48	4.55	5.10	6.95.	7.30.	1.75	1.77	1.98	3.46	7.30	22.05
Shoot length (cm)	4.50	4.45	5. 10	5.33	7.00	7.20	2.45	2.82	3.26	3.62	3.96	4. 13
Leaf length (cm)	5.33	6.50	7.00	7.60	7.60	10.13	28.00	30.40	41.60	46.20	63.00	73.90
Leaf breadth (cm)	3.40	3.40	3.45	3.60	3.72	4. 11	2.30	2.80	4.10	6.80	7.80	10.00
Leaf area (cm^2)	18.12	22.10	24.15	27.36	28.27	41.63	64.4	85.73	170.56	314.16	496.08	739.00
Number of leaves	1.00	2.00	2.00	2.00	4.00	6.00	3.00	3.00	4.00	4.00	6.00	6.00
Percentage emergence of seedlings	33.33	33.33	66.67	66.67	66.67	100.00	33.33	33.33	66.67	83.33	100.00	100.00

% = Percent; cm = Centimetre; cm^2 = Centimetre square

Table 12. Effects of different levels of product A battery samples on the growth indices of *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) after 21 days exposure

Parameter	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
No. of seeds germinated	1.00	2.00	2.00	2.00	3.00	3.00	1.00	1.00	2.00	3.00	3.00	3.00
Time of germination (day)	4.00	4.00	4.00	4.00	4.00	4.00	6.00	4.00	3.00	3.00	3.00	3.00
Root length (cm)	3.40	6.33	6.90	6.95	7.70	9. 12	1.17	1.23	1.26	1.32	1.32	1.57
Shoot length (cm)	5.45	5.80	5. 90	6. 10	7.20	8. 15	1.45	1.55	2.30	2.24	2.33	2.51
Leaf length (cm)	6.70	8.00	11.20	20.10	26.40	28.60	14.20	20.80	34.30	48.40	58.90	59.00
Leaf breadth (cm)	2.70	6.70	9.00	12.00	16.30	19.60	0.70	1.00	2.30	2.27	2.80	2.90
Leaf area (cm^2)	18.09	53.60	100.80	241.20	430.32	560.56	9.94	20.80	78.89	109.87	164.92	171.10
Number of leaves	2.00	2.00	4.00	4.00	6.00	6.00	1.00	2.00	5.00	5.00	6.00	6.00
Percentage emergence of seedlings	33.33	66.67	66.67	66.67	100.00	100.00	33.33	33.33	66.67	100.00	100.00	100.00

% = Percent; cm = Centimetre; cm^2 = Centimetre square

Table 13. Effects of different levels of product B battery samples on the growth indices of *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn) after 21 days exposure

Parameter	*Phaseolus vulgaris* (common bean)						*Sorghum bicolor* (guinea corn)					
	Concentration (%)						Concentration (%)					
	100	50	25	12.5	6.25	Control	100	50	25	12.5	6.25	Control
No. of seeds germinated	1.00	1.00	2.00	2.00	2.00	3.00	2.00	2.00	3.00	3.00	3.00	3.00
Time of germination (day)	4.00	4.00	4.00	3.00	3.00	3.00	5.00	5.00	4.00	4.00	4.00	4.00
Root length (cm)	1.45	4.48	4.55	5.10	6.95	7.30	1.75	1.77	1.98	3.46	7.30	22.05
Shoot length (cm)	1.50	4.45	5. 10	5.33	7.00	7.20	2.45	2.82	3.26	3.62	3.96	4. 13
Leaf length (cm)	5.33	6.50	7.00	7.60	7.60	10.13	28.00	30.40	41.60	46.20	63.60	73.90
Leaf breadth (cm)	3.40	3.40	3.45	3.60	3.72	4. 11	2.30	2.80	4.10	6.80	7.80	10.00
Leaf area (cm^2)	18.12	22.10	24.15	27.36	28.27	41.70	64.40	85.12	170.56	314.16	496.08	739.00
Number of leaves	1.00	2.00	2.00	2.00	4.00	6.00	3.00	3.00	4.00	4.00	6.00	6.00
Percentage emergence of seedlings	33.33	33.33	66.67	66.67	66.67	100.00	33.33	33.33	66.67	83.33	100.00	100.00

% = Percent; cm = Centimetre; cm^2 = Centimetre square

The result of the 21 days median effective concentration (EC_{50}) of the spent product A and B laptop battery samples on the growth of bacteria, fungi and actinomycetes is demonstrated in Fig. 1. The result revealed that product A exposed *S. bicolor* had the highest EC_{50} (668.83 %) while product A exposed *P. vulgaris* had the least EC_{50} (245.50%) on bacteria; product A exposed *P. vulgaris* had the highest EC_{50} (1014.61%) while product A exposed *S. bicolor* had the least EC_{50} (104.12 %) on fungi; product A exposed *P. vulgaris* had the highest EC_{50} (520.21%) while product B exposed *S. bicolor* had the least EC_{50} (287.16%) after 21 days, respectively.

3.3 Plant Growth Toxicity Profile

The results of the effects of different levels of product A battery samples on the growth indices of *Phaseolus vulgaris* and *Sorghum bicolor* after 7 days, 14 days and 21 days exposure are shown in Tables 8, 10 and 12. The results revealed that the control set up had highest values of germinated seed (3), time of germination (4 days), root length (9.12 cm), shoot length (8.15 cm), leaf area (560 cm^2), leaf number (6) and percentage seed emergence (100.00%) in the product A exposed *P. vulgaris* while 100% set up had the lowest values of germinated seed (1), time of germination (6 days), root length (1.17 cm), shoot length (1.45 cm), leaf area (9.94 cm^2), leaf number (2) and percentage seed emergence (33.33%) in the product A exposed *S. bicolor* after 21 days, respectively.

Also, the results in Tables 9, 11 and 13 showed the effects of different levels of product B battery samples on the growth indices of *Phaseolus vulgaris* and *Sorghum bicolor* after 7 days, 14 days and 21 days exposure. From the results, the control set up had highest values of germinated seed (3), time of germination (4 days), root length (22.05 cm), shoot length (4.13 cm), leaf area (739 cm^2), leaf number (6) and percentage seed emergence (100.00%) in the product B exposed *S. bicolor* while 100% set up had the lowest values of germinated seed (1), time of germination (4 days), root length (1.45 cm), shoot length (1.50 cm), leaf area (18.12 cm^2), leaf number (1) and percentage seed emergence (33.33%) in the product A exposed *P. vulgaris* after 21 days, respectively. Statistically, significant differences ($P < 0.05$) was detected only on dose effects and not on days of exposure in comparison to their controls. Furthermore, the result of the 21 days median effective concentration (EC_{50}) of the spent laptop battery samples on the growth indices of *Phaseolus vulgaris* and *Sorghum bicolor* is illustrated in Fig. 2. It revealed that product B exposed *P. vulgaris* had the highest EC_{50} of - 18.87 % while product B exposed *S. bicolor* had the lowest EC_{50} of - 45.77%, respectively.

4. DISCUSSION

The significance of using microbes and plants in the assessment of chemical toxicity as well as risk assessment studies has been reported by different authors [7,8,13,16]. In this study, an attempt was made to evaluate the possible environmental and human health risks associated with indiscriminate release of spent laptop battery into the environment using soil bacteria, fungi, actinomycetes as well as plant *P. vulgaris* and *S. bicolor* bio-indicators.

The result in Table 1 showed that all the constituents of heavy metals analyzed (arsenic, cadmium, mercury, lead and nickel) in the spent laptop battery samples were not within the permissible limits of WHO [17] and FEPA [18] values. Our study corroborates with the published work of Douglas et al. [19] who reported higher contents of some of these hazardous metals in the spent battery samples they analyzed.

The abundance of the groups of microbes was negatively and significantly ($P < 0.05$) influenced by the doses of the spent product A and B laptop battery samples as well as the date of exposure (Tables 2 – 7). Higher doses of the analyzed spent laptop battery samples altered the soil's microbial balance compared to lower doses as demonstrated by the microbial counts of total heterotrophic bacteria, fungi and actinomycetes. The highest dose of 100% had the most significant inhibitory effect on heterotrophic bacteria count, heterotrophic fungi count and actinomycetes count in products A and B exposed *P. vulgaris* and *S. bicolor* after 21 days. The reason for the general inhibition in the microbial population could be traced to the presence of toxic metal components of the spent battery wastes which impaired the normal microbial morphology and physiology in the soil ecosystem. This observation is in line with previous study of Baldrian [12] who reported that toxic heavy metal components led to both physiological and morphologically changes in the microbial abundance and as a result influenced the species composition of that environment

negatively. There was general increase and more counts in all the microbial counts of the control samples throughout the study period and the possible reason could be due to the absence of the toxicants and the presence of nutrient such as phosphorus and nitrogen which are naturally present in the soil. Statistically, significant differences ($P < 0.05$) were observed in the microbial counts among the treatments, days of exposure and their controls. Similar observations were made by Douglas and Nwachukwu [13] with regards to bacterial set ups and their controls. On the other hand, median effective concentration (EC_{50}) denotes the concentration of a xenobiotic or chemical agent that will inhibit 50 % of a target population. It is generally reported that chemical agents with lower EC_{50} are more sensitive to a target population than those with higher EC_{50}. Therefore, in evaluation of the order of the toxicity or sensitivity of spent laptop battery samples (products A and B) on the microbial population: product A- bean (245.50% EC_{50}) > product B - sorghum (368.94 % EC_{50}) > product B - bean (460.75% EC_{50}) > product A - sorghum (668.83% EC_{50}) on the bacterial population count; product B - sorghum (104.12% EC_{50}) > product A - bean (316.59% EC_{50}) > product A - sorghum (750.56% EC_{50}) > product B - bean (1,014.61% EC_{50}) while product B - sorghum (287.16% EC_{50}) > product B - bean (318.40% EC_{50}) > product A - sorghum (484.184% EC_{50}) > product A - bean (520.21 % EC_{50}) on actinomycetes population count, respectively. These results implied that product A had the most toxic effect on bacterial population count while product B had the most toxic effect on fungal and actinomycetes population counts. Comparatively, fungal population was the most sensitive followed by bacterial population and then actinomycetes population. The reason for these differences could be attributed to the genetic make - up or mechanisms of the organisms especially plasmids which have been implicated more in heavy metal resistance of bacteria than fungi. The results are in line with previous studies of Baldrian [20]; Odokuma and Oliwe [21] and Douglas and Nwachukwu [13] who published that spent laptop battery waste was more toxic to fungal populations than bacterial populations when introduced into the soil and the reason was attributed to the complex genetic machinery, biochemistry and physiology of the bacterial cells.

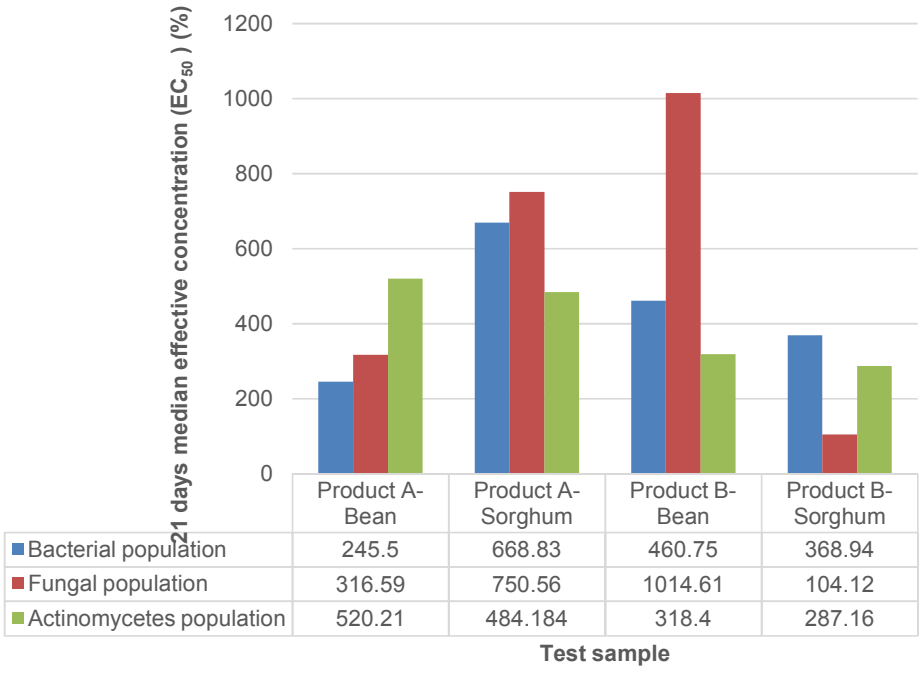

Fig. 1. 21 days median effective concentration (EC_{50}) of the spent product A and B laptop battery samples on the growth of bacteria, fungi and actinomycetes

Product A-Bean = Product A exposed Phaseolus vulgaris; Product A-Sorghum = Product A exposed Sorghum bicolor; Product B-Bean = Product B exposed Phaseolus vulgaris; Product B-Sorghum = Product B exposed Sorghum bicolor

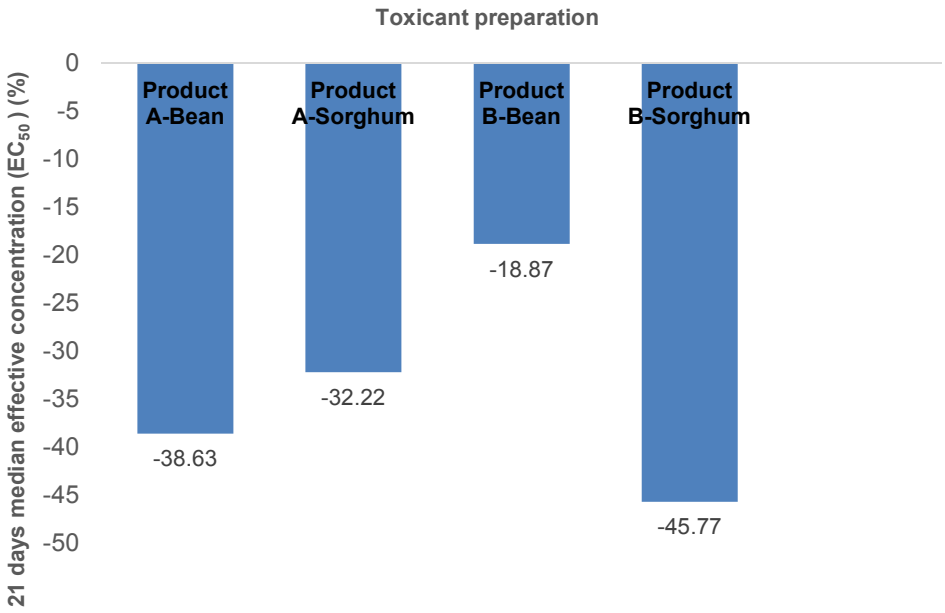

Fig. 2. 21 days median effective concentration (EC_{50}) of the spent product A and B laptop battery samples on the growth indices of *Phaseolus vulgaris* (common bean) and *Sorghum bicolor* (guinea corn)

Product A-Bean = Product A exposed Phaseolus vulgaris; Product A-Sorghum = Product A exposed Sorghum bicolor; Product B-Bean = Product B exposed Phaseolus vulgaris; Product B-Sorghum = Product B exposed Sorghum bicolor

The result in Tables 8 – 13 showed that the growth indices of *P. vulgaris* and *S. bicolor* were significantly ($P < 0.05$) retarded by the different doses of spent product A and B laptop battery samples. There was a 100% seedling emergence of the seeds of both *P. vulgaris* and *S. bicolor* in the test soil without spent battery sample doses while lesser percentage seedling emergence (83.33% – 33.33%) occurred in the test soils with varying spent battery sample doses. The more the spent battery sample dose exposure, the lesser the growth indices values for both *P. vulgaris* and *S. bicolor*, with 6.25% dose exposed test soils having the highest growth indices values. Statistically, significant differences ($P < 0.05$) were observed in the growth indices only among the doses and their controls and not in the days of exposure revealing that only doses inhibit the growth indices of *P. vulgaris* and *S. bicolor* greatly. The possible reasons for these reductions could be due to infiltration of the hazardous constituents of the spent battery samples into the *P. vulgaris* and *S. bicolor* seeds which may have impaired the embryos or could have acted as physical obstacles around the seeds hence lessening or preventing the movement of exchangeable gases and water into the seeds. Previous studies by Leita et al. [22], Hollenbach et al. [23], Parys et al. [24] and Weryszko – Chmielewska and Chwil [25] reported that a strong increase of phytohormone abscisic acid in both shoots and roots were initiated by heavy metals (Cd, Ni and Pb) leading to reduction of water potential and closure of stomata, subsequently restricting the gas exchange and hence the rate of transpiration. The reports of these researchers are in line with the results obtained in the present study. Furthermore, median effective concentration (EC_{50}) as shown in Fig. 2 demonstrated the order of toxicity of the spent battery samples on the growth indices of *P. vulgaris* and *S. bicolor* as: product B - Sorghum (- 45.77% EC_{50}) > product A - bean (- 38.63% EC_{50}) > product A - sorghum (-32.22% EC_{50}) > product B - bean (-18.89% EC_{50}). The results implied that *S. bicolor* was the most sensitive to spent product B battery sample while *P. vulgaris* was the most sensitive to spent product A battery sample and vice versa as lower EC_{50} value

represent higher sensitivity. This result further illustrates that *P. vulgaris* will be a good biomonitoring organism for spent product B battery pollution while *S. bicolor* will be a good biomonitoring organism for spent product A battery sample pollution. A study by Al-Qurainy [26] reported that *P. vulgaris* L. leaf area per plant, plant height, fresh and dry weight per plant were greatly inhibited at 150 mg/kg EC_{50} values of nickel (Ni) metal contaminated soil. Njoku et al. [27] reported that the EC_{50} for the percentage germination ranged from 5.50% in the 07/012 accession to 19.00% in the 07/182 accession of *S. bicolor* which is higher than the values obtained in the present study.

5. CONCLUSION

The present study revealed that fungal population was the most sensitive followed by bacterial population and then actinomycetes population in terms of toxic responses ($100 < EC_{50} < 1000$) to the spent product A and product B laptop battery soil contamination. The lower EC_{50} values obtained in this study made *P. vulgaris* test plant very good biomonitoring models for spent product A while *S. bicolor* test plant very good biomonitoring models for spent product B battery pollution assessment. Hence, prompt and proper measures for disposal of these electronic wastes are recommended for government, non - government and environmental policy makers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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