



***Selenastrum capricornutum* Prinz, *Zea mays* L. and *Phaseolus vulgaris* L. Biomonitor: Natural Monitors of Spent Phone Battery Toxicity**

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Authors' contributions

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

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ABSTRACT

Aims: This study was undertaken to monitor the different effects of spent Toshiba and Dell laptop battery on the growth and germination indices of *Selenastrum capricornutum* Prinz, *Zea mays* L. and *Phaseolus vulgaris* L.

Study Design: Five treatments and the controls designs designated as 6.25%, 12.5%, 25%, 50%, 100% and CTRL were set up in triplicates and incubated at 25 ± 2°C for 72 h and 5 days.

Place and Duration of Study: Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University (COOU), Uli Anambra State, Nigeria during May, 2019 - December, 2019.

Methodology: The algal growth inhibition was analyzed using spectrophotometric method while growth and germination indices were adopted for the seed growth inhibition.

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Results: The result revealed that the positive control $K_2Cr_2O_7$ had the lowest ErC_{10} (-86.65 mg/L) and the highest ErC_{90} (130.92 mg/L) while the spent Dell battery sample had the highest ErC_{10} (10.18 mg/L) and spent Toshiba battery sample had the lowest ErC_{90} (75.52 mg/L) on *Selenastrum capricornutum* growth rate, respectively. Boric acid standard toxicant had the highest EC_{50} (-12.62 mg/kg and -14.30 mg/kg) on *Z. mays* and *P. vulgaris* while Toshiba and Dell battery samples had the lowest EC_{50} (-73.03 mg/kg and -22.37 mg/kg) on *Z. mays* and *P. vulgaris*, respectively.

Conclusion: The study revealed that both *S. capricornutum*, *Z. mays* L. and *P. vulgaris* L. were very good bio-monitoring models for spent Toshiba and Dell laptop battery contamination assessment; and are therefore recommended for E-wastes bioassays.

Keywords: *Biomonitoring; E-waste contamination; growth response; Toshiba and Dell laptop battery; public health education.*

1. INTRODUCTION

In Nigeria, the local consumption of electronic and electrical devices is rapidly growing leading to continuous generation of large hazardous e-wastes [1]. The results of the survey of components 1 and 2 of the E-Waste Africa Project revealed that Nigeria is the highest producers of e-wastes in West African regions. These e-waste bulks, along with the absence of specific environmentally sound management systems have various effects on the environment, general public and the economic system in Nigeria [1].

Besides, nearly all the recycling processes such as the open burning of cables and battery parts induced severe productions of toxic substances such as heavy metals and dioxins. Moreover, these e-wastes contain a wide range of hazardous metals like copper (Cu), silver (Ag), nickel (Ni), mercury (Hg), lead (Pb), chromium (Cr) and cadmium (Cd) that are lost if not recovered in an early stage of waste treatment [1]. Thus, heavy metals are significant ecological contaminants, and their harmfulness is a problem of great concern for environmental, nutritional and toxicological reasons [2].

Toxic substances and environmental contaminants are evaluated using different test organisms and bioassays. Microalgae are significant members of the producing trophic level in the aquatic ecosystem as they play vital roles as carbon and nitrogen fixers in the environment. As a result, they are considered of ecological importance in the assessment of environmental contaminants. They are applied as model aquatic bio-indicators due to their metabolic diversity and high sensitivity to the presence of toxic substances. Additionally, microalgae are obtainable throughout the year, simple to grow with quick growth rate and can be

maintained under suitable laboratory conditions thereby making them model organisms for ecotoxicological investigations [3]. *Selenastrum capricornutum* Prinz currently known as *Pseudokirchneriella subcapitata* is a model planktonic fresh water green microalga species suggested by ISO [4] for toxicological investigations and this necessitates its use as test microalga in this study.

Biomonitoring methods using plant indicators have attractive and common approaches in detection of toxic levels of toxic substances in the aquatic and terrestrial environments [5]. As a result of their immobile nature, plants are mostly selected for toxicity testing as their nature evade the fear of advanced identification of point - source contamination frequently encountered with use of animals [6]. An indicator plant is characterized by amassing one or more designated pollutants, having low response to the amassed pollutants, wider spread in several surrounding environments, and express a relationship between metal accumulation and input into the environment [5]. *Zea mays* L. (Maize), and *Phaseolus vulgaris* L. (common beans) were selected in this study because they are staple cereal and leguminous crops of temperate and tropical regions, have variable environmental tolerant characteristics, commonly available in different environments and possess diverse pollutant accumulating abilities. In addition, they were recognized among the monocots and dicots bio indicators for toxicity evaluation of chemical substances by OECD [7]. In a study by Oladele et al. [8], it was revealed that lead and zinc compounds inhibited both seed germination and seedling growth parameters of *Zea mays* L. Al-Qurainy [9] reported that *Phaseolus vulgaris* L. seed growth and development were inhibited by the different concentrations of nickel and aluminum compounds.

In Nigeria, there are few literatures on biotoxicity assays of spent laptop battery. Also, the literatures available didn't focus studies on microalgae and plant as potential bio-monitors for spent laptop battery toxicity assessment and hence necessitates this study. This study was undertaken to monitor the different effects of spent Toshiba and Dell laptop battery on the growth and germination indices of *Selenastrum capricornutum*, *Zea mays* L. and *Phaseolus vulgaris* L. It is hope that valuable data obtained from the study would propose the likely toxins present in the phone battery wastes with a view of demonstrating the ability of such substances to induce growth and germination inhibitions in the cell populations and seeds of *Selenastrum capricornutum*, *Zea mays* L. and *Phaseolus vulgaris* L.

2. MATERIALS AND METHODS

2.1 Sample and Specimen Source

The spent laptop batteries samples (Toshiba and Dell) were bought from Emeka Offor Plaza, Main Market Onitsha, Anambra State, packed into clean polyethylene bags and transported to Microbiology Laboratory Chukwuemeka Odumegwu Ojukwu University (COOU) Uli for further analysis. The pure culture of the green microalga *Selenastrum capricornutum* was purchased from Carolina Biological Supply Company North Carolina, USA. The Garden soil was collected from Botanical garden of the Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University (COOU), Uli, Nigeria without incidence of such pollution. They were collected using sterile metallic spade at a depth of 15 cm and 2 m apart, mixed together in order to obtain a composite sample, packed into clean sterile polyethylene bags and transported to the Microbiology Laboratory, COOU Uli, [10]. The seeds of *Zea mays* L. and *Phaseolus vulgaris* L. were purchased from COOU School Market, Uli Town Nigeria. The seeds were placed in sterile polyethylene bags, transported to the Microbiology Laboratory, COOU Uli and stored at laboratory temperature ($25 \pm 2^\circ\text{C}$) for not more than 24 h [11].

2.2 Preparation of the Sample

The spent laptop batteries samples were prepared by forcefully opening the batteries under the aseptic conditions and battery contents were released into well labelled 1L

round bottomed plastic containers [12]. The composite garden soil was sieved with metal mesh of size 10 mm to remove stones and debris and oven – dried using electric oven (Gallenkamp, USA) at 105°C for 24 h [13].

2.3 Quantification of Heavy Metal

The standard method of APHA [14] was implemented for quantification of the heavy metal composition of the spent laptop battery samples.

2.4 Growth Response Bioassay

2.4.1 Algal growth inhibition response test

The algal growth inhibition test was conducted in line with the method of ISO [4] with little modifications. The algal growth medium was prepared by adding four sterile nutrient stock solutions labelled 1, 2, 3 and 4 in to sterile distilled water. The prepared growth medium was dispensed into 25 mL test tube sex posed to different dilutions (6.25 mg/L, 12.5 mg/L, 25 mg/L, 50 mg/L and 100 mg/L) of the spent Toshiba and Dell laptop battery samples with 0 mg/L designated as negative control. Similar treatments were also conducted for the reference chemical potassium heptaoxidichromate (IV) ($\text{K}_2\text{Cr}_2\text{O}_7$) designated as positive control. Triplicate and six replicate batches of both spent Toshiba and Dell laptop battery concentrations and control were prepared by adding 1 mL pure culture broth of *Selenastrum capricornutum* containing 5×10^4 cells/mL. The pH of the experimental setups was monitored at the beginning and end of experiment. The test tubes were incubated at $25 \pm 2^\circ\text{C}$ under constant fluorescent white lamps for 72 h. The cell density in each test batches test sample and controls were evaluated spectrophotometrically at 0, 24, 48 and 72 h intervals. The values obtained were used to determine the growth rates expressed as percentage growth inhibition and ErC_{10} = 10% effective growth rate concentration, ErC_{50} = 50% median effective growth rate concentration, ErC_{90} = 90% effective growth rate concentration were calculated from the percentage growth inhibition values.

2.4.2 Plant toxicity response assay

The modified technique of Emami et al. [13] was implemented in investigating the toxic effects of the spent laptop battery samples on the seed germination and growth indices. In this study, 54 cleaned Petri dishes were filled with 30 g of un-

polluted sterile sieved garden soil. Ten millilitres of sterile distilled water were dispensed on the surfaces of Whatman No.1 filter papers placed on the soil in order to moisten them. Dilutions 6.25 mg/kg, 12.5 mg/kg, 25 mg/kg, 50 mg/kg and 100 mg/kg of the spent laptop battery sample were prepared, 20 mL pipetted and slowly added on surface of the filter paper. Similar treatments were also conducted for the reference chemical boric acid designated as positive control while sterile distilled water was used and designated as negative control. Ten seeds of the selected test plant species: *Zea mays* L. and *Phaseolus vulgaris* L. were placed on top of the filter paper at equal distance to each other. The experiment was conducted in triplicates. The plates were incubated at $25 \pm 2^\circ\text{C}$ for 5 days in darkness after being covered and well labelled with the respective sample names. The length of each roots and shoots were measured and the number of germinated seeds, germination index as well as the dry biomass weight were determined after 5 days of incubation.

2.5 Biostatistical Study

The findings obtained were analyzed using Graph Pad Prism Version 8.0. The values were expressed as mean standard error and later subjected to two approach analysis of variance (ANOVA) using Dunnett multiple comparison test to compare the mean of experimental groups against control groups with equation =

$$D_{\text{Dunnett}} = t_{\text{Dunnett}} \frac{\sqrt{2MS_{S/A}}}{n}$$

Linear regression analysis was used to determine the toxic effects of the spent laptop battery samples on the bio-monitor at 95 percentage (%) confidence levels such that values lower than 0.05 probability levels were considered statistically significant [10].

3. RESULTS

3.1 Heavy Metal Profile

The result of the heavy metal composition of the spent laptop batteries is presented in Table 1: From the result, Toshiba laptop battery had the highest values of arsenic (0.167 ppm), mercury (1.532 ppm), lead (6.405 ppm) and nickel (22.802 ppm) while Dell laptop battery had the

highest values of cadmium (0.019 ppm), respectively.

3.2 Algal Exposure Assessment

The result of the growth inhibition response of *Selenastrum capricornutum* in the presence of spent phone battery samples (Fig. 1) showed that the spent Toshiba phone battery sample had the highest percentage growth inhibition of 88.09% while $\text{K}_2\text{Cr}_2\text{O}_7$ sample had the lowest percentage growth inhibition of 68.81% at 100 mg/L concentration. There were significant differences ($F = 4.936$; $P = 0.016$) among the treatment samples and control. In Fig. 2, the result of the 72 h effective growth rate concentration (ErC) of spent phone battery samples and potassium dichromate on *Selenastrum capricornutum* population is shown. The result revealed that the positive control $\text{K}_2\text{Cr}_2\text{O}_7$ had the lowest 10% effective growth rate concentration (ErC_{10}) (-86.65 mg/L) and the highest 90% effective growth rate concentration (ErC_{90}) (130.92 mg/L) while the spent Dell battery sample had the highest ErC_{10} (10.18 mg/L) and spent Toshiba battery sample had the lowest ErC_{90} (75.52 mg/L), respectively.

3.3 Plant Exposure Assessment

The results of the inhibitory effects of spent Toshiba laptop battery, spent Dell Laptop battery and boric acid on *Zea mays* L. and *Phaseolus vulgaris* L. growth indices are demonstrated in Figs. 3, 4 and 5. In Fig. 3a, there was rise in growth indices of *Z. mays* L. as the concentration of the spent laptop battery decrease with concentration 100 mg/kg of Toshiba laptop battery sample having the least values of root length (5.70 cm), shoot length (3.20 cm), germinated seed (6.00), root length percentage (40.71%), germinated seed percentage (60.00%), germination index (24.43%) and dry weight (0.32 g). Similarly, in *P. vulgaris* L., the 100 mg/kg of Toshiba Laptop battery sample had the least values in root length (2.50 cm), shoot length (0.80 cm), germinated seed (4.00), root length percentage (41.67%), germinated seed percentage (44.44%), germination index (18.52%) and dry weight (0.02 g) (Fig. 3b). In Fig. 4a, there was similar rise in growth indices of *Z. mays* L. As the concentration of the spent laptop battery decrease with concentration 100 mg/kg of Dell laptop battery sample having the least values of root length (2.40 cm),

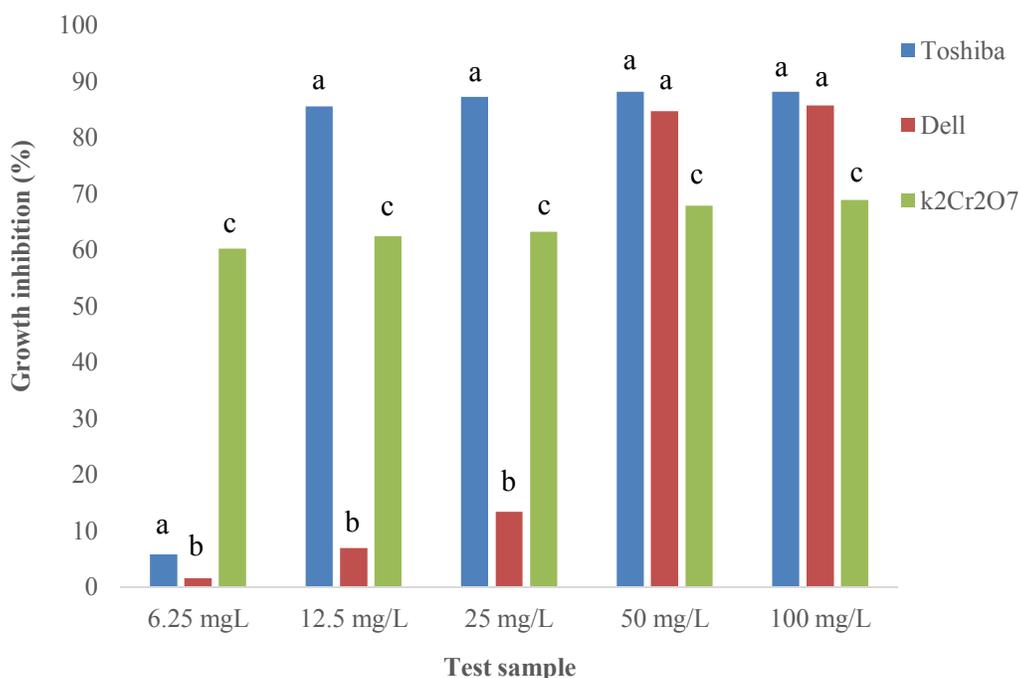


Fig. 1. Growth inhibition response of *Selenastrum capricornutum* in the presence of spent phone battery samples

Legend: $K_2Cr_2O_7$ = Positive control Potassium dichromate; mg/L = milligram per litre; % = Percent; Columns of different letters denotes that they statistically significant ($F = 4.936$; $P = 0.016$)

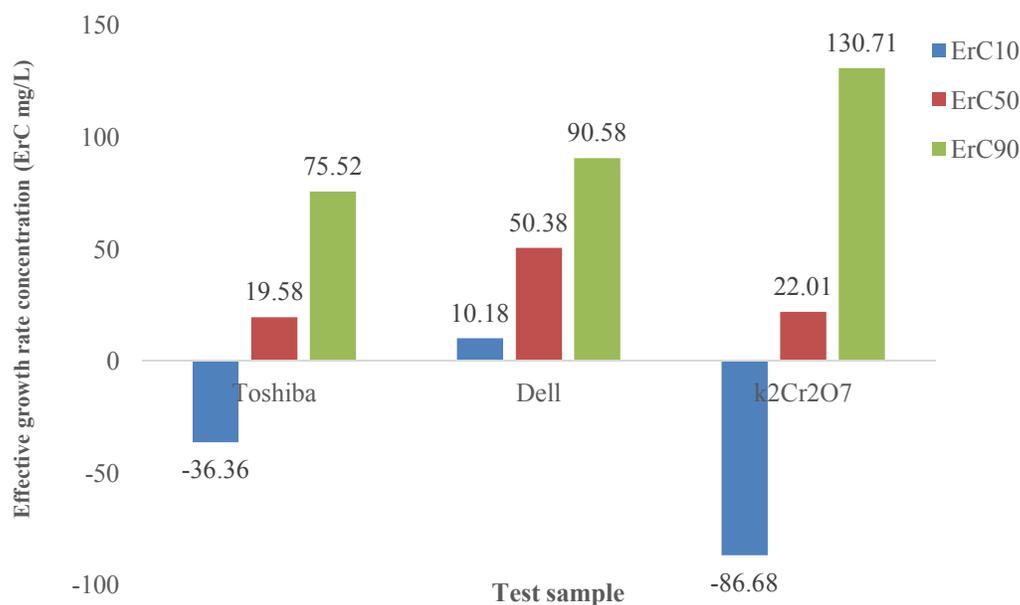


Fig. 2. 72 h effective growth rate concentration (ErC) of spent phone battery samples and potassium dichromate on *Selenastrum capricornutum* population

Legend: $K_2Cr_2O_7$ = Positive control potassium dichromate; ErC_{10} = 10% effective growth rate concentration; ErC_{50} = 50% median effective growth rate concentration; ErC_{90} = 90% effective growth rate concentration

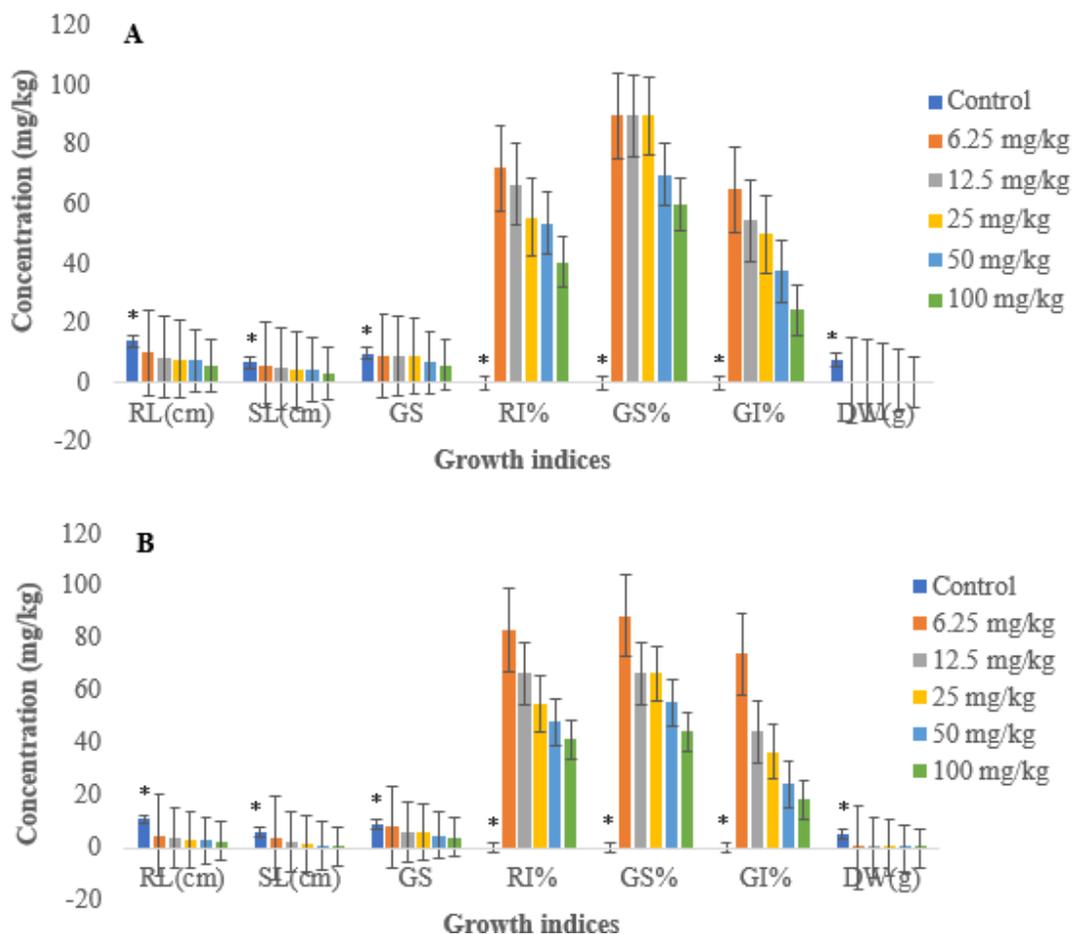


Fig. 3. Inhibitory effect of spent Toshiba laptop battery on (A) *Zea mays* L. (maize) and (B) *Phaseolus vulgaris* L. (common beans) growth indices

Legend: mg/kg = Milligram per kilogram; cm = Centimetre; g = Gram; % = Percent; RL = Root length; SL = Shoot length; GS = No. of germinated seed; GS % = Percentage germinated seed; RL % = Percentage root length; GI % = Percentage germination index; DW = Dry weight; Columns are mean \pm SEM of 3 replicates; Columns with asterisks are significantly different (Dunnet test, $P < 0.05$) while columns without asterisk are not significantly different (Dunnet test, $P > 0.05$); Error bar = Standard error in mean

Table 1. Heavy metal composition of the spent laptop batteries sample

Parameters	Metal concentration (ppm)		WHO (1983)/FEPA (1991) standards in sediment (ppm)	WHO(1983)/FEPA (1991)standardsin water(ppm)
	Toshiba	Dell		
Arsenic	0.167	0.119	NA	0.100
Cadmium	0.000	0.019	0.030	0.005 – 0.010
Mercury	1.532	0.357	NA	0.050
Lead	6.405	4.171	0.010	0.050
Nickel	22.802	11.491	0.020	0.100 – 0.200

shoot length (1.60 cm), germinated seed (7.00), root length percentage (17.14%), germinated seed percentage (70.00%), germination index (12.90%) and dry weight (0.32 g). Similarly, in *P. vulgaris* L., the 100 mg/kg of Dell Laptop battery

sample had the least values of root length (1.2 cm), shoot length (1.20 cm), germinated seed (5.00), root length percentage (20.00%), germinated seed percentage (55.56%), germination index (11.11%) and dry weight (0.06

g) (Fig. 4b). In Fig. 5a, there was reduction in growth indices of *Z. mays* L. As the dose of the standard toxicant boric acid increase with 100 mg/kg of boric acid concentration having the least values of root length (2.40 cm), shoot length (1.60 cm), germinated seed (7.00), root length percentage (17.14%), germinated seed percentage (70.00%), germination index (12.00%) and dry weight (0.10 g). Similarly, in *P. vulgaris* L., the 100 mg/kg of boric acid standard had the lowest values of root length (1.2 cm), shoot length (1.20 cm), germinated seed (4.00), root length percentage (20.00%), germinated seed percentage (44.44%), germination index (8.89%) and dry weight (0.02 g) (Fig. 5b). The

result in Fig. 6 illustrated the 5-day median effective concentration (EC_{50}) (mg/kg) of spent laptop battery and boric acid samples on the growth indices of *Zea mays* L. and *Phaseolus vulgaris* L. From the result, boric acid standard toxicant had the highest EC_{50} (-12.62 mg/kg and -14.30 mg/kg) on *Z. mays* L. And *P. vulgaris* L. while Toshiba and Dell battery samples had the lowest EC_{50} (-73.03 mg/kg and -22.37 mg/kg) on *Z. mays* L. and *P. vulgaris* L., respectively. Two factor ANOVA with Dunnett comparison test revealed significant differences ($P < 0.05$) among the treatment groups of the spent laptop battery samples, boric acid standard toxicant and their controls.

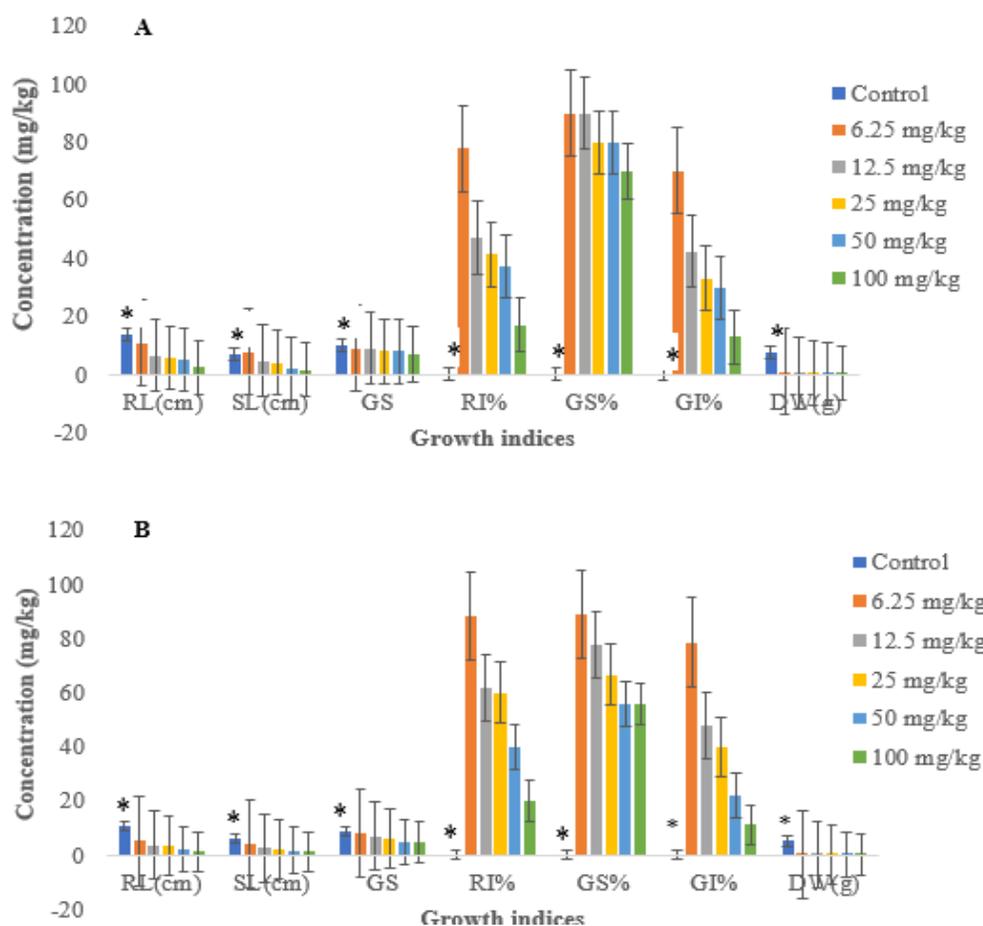


Fig. 4. Inhibitory effect of spent Dell laptop battery on (A) *Zea mays* L. (maize) and (B) *Phaseolus vulgaris* L. (common beans) growth indices

Legend: mg/kg = Milligram per kilogram; cm = Centimetre; g = Gram; % = Percent; RL = Root length; SL = Shoot length; GS = No. of germinated seed; GS % = Percentage germinated seed; RL % = Percentage root length; GI % = Percentage germination index; DW = Dry weight; Columns are mean \pm SEM of 3 replicates; Columns with asterisks are significantly different (Dunnett test, $P < 0.05$) while columns without asterisk are not significantly different (Dunnett test, $P > 0.05$); Error bar = Standard error in mean

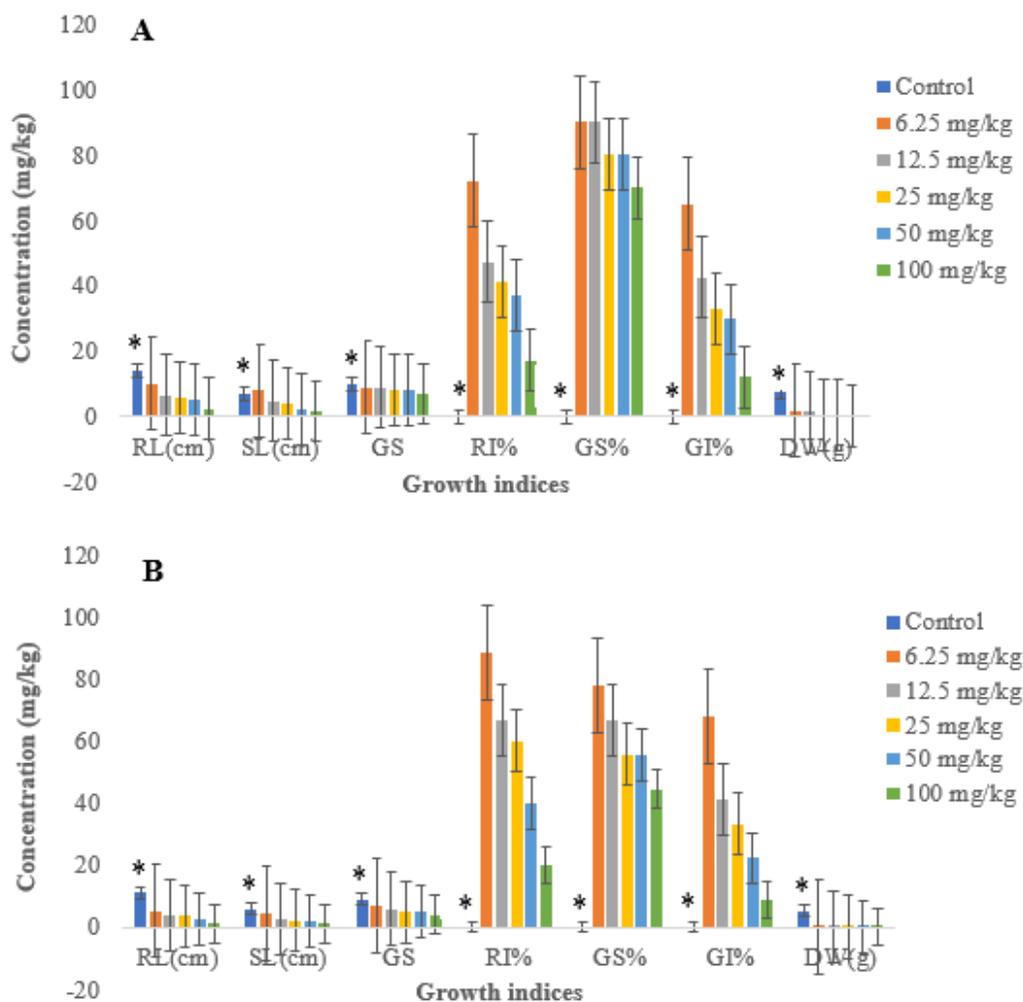


Fig. 5. Inhibitory effect of positive control boric acid on (A) *Zea mays* L. (maize) and (B) *Phaseolus vulgaris* L. (common beans) growth indices

Legend: mg/kg = Milligram per kilogram; cm = Centimetre; g = Gram; % = Percent; RL = Root length; SL = Shoot length; GS = No. of germinated seed; GS % = Percentage germinated seed; RL % = Percentage root length; GI % = Percentage germination index; DW = Dry weight; Columns are mean \pm SEM of 3 replicates; Columns with asterisks are significantly different (Dunnet test, $P < 0.05$) while columns without asterisk are not significantly different (Dunnet test, $P > 0.05$); Error bar = Standard error in mean

4. DISCUSSION

In the present study, the result in Table 1 indicated that virtually all the heavy metal analyzed from the spent phone battery samples were above the WHO [15] and FEPA [16] limits. This result is similar to the publication of Darko [17] and Douglas et al. [12] who reported higher concentrations of these hazardous metals in the electrodes of spent battery samples.

Growth inhibition is one of the significant biomarkers applied in the eco toxicological assay as the toxic impacts are revealed as reduction in

specific growth rate relative to the control. The results in Fig. 1 demonstrated a significant reduction in the growth rate which advanced as the time of exposure (0- 72 h) and concentrations of the spent laptop battery samples (0-100 mg/L) advanced. Likewise, higher inhibitions on the growth rate of *S. capricornutum* were induced by the spent laptop battery samples than the positive control sample $K_2Cr_2O_7$. The reasons for these differences in growth rate inhibitions could be due to the high metalloid contents and various hazardous materials that make up the spent phone battery samples and were therefore considered harmful to algal growth. The

observation is line with the publication of Arunakumara and Xuecheng [18] who reported that several biochemical and physiological processes in microalgae are altered as result of heavy metal entry through either active transport or protein chelating endocytotic process. Moreso, the result in Fig. 2 revealed the order of toxicity of the test samples to *S. capricornutum* growth rate thus: Toshiba battery ErC_{50} (19.58 mg/L) > positive control $K_2Cr_2O_7$ ErC_{50} (22.01 mg/L) > Dell battery ErC_{50} (50.38 mg/L), respectively. The implications of these results in tested battery samples were very acutely toxic to minor acutely toxic according to Verma [19] general guidelines for classification of toxicity. The average growth rate (0.150), coefficient of variation (4.70%) and pH (1.10) of control replicates in this study met the validity criteria of ISO [4] which states that the average growth rate, variation coefficient (CV) and pH in the control replicates should be atleast $1.40 d^{-1}$, not more than 5.00% and 1.50 relative to the pH of the growth medium. Previous study by Hussein [20] reported an EC_{50} values of 0.75% for Co (II) and 0.89% for Zn (II) on *S. capricornutum*. Horvatic et al. [21] reported EC_{50} values of 0.33 – 9.4 mg/L with an average of 2.06 mg/L for mercury, copper, iron and cobalt

metals on the growth rate inhibition of *Raphidocelis subcapitata* also known as *S. capricornutum*. The findings of these authors were comparable to the results of this study as the range of EC_{50} values falls within the results.

Plant bioassays such as evaluation of seed germination, root length, plant shoot and biomass have long been applied in monitoring of pollutant substrates or soils. The result in Figs. 3 – 5 demonstrated that there was significant inhibition of *Z. mays* L. and *P. vulgaris* L. growth indices by different concentrations of spent laptop battery and boric acid standard samples as well as duration of exposure. The possible reasons could be due to the impairment of water and gaseous transports by the harmful heavy metal components of the test samples and the eventual seeds' death. In general, significant reductions in growth indices were observed on the treated set ups than the untreated set ups (negative controls). Similar observations were made by Oladele et al. [8] who reported that the growth parameters (stem height, root length, leaf area, fresh and dry weight) of *Z. mays* L. when compared to control experiment was significantly inhibited by compounds of lead (Pb) and

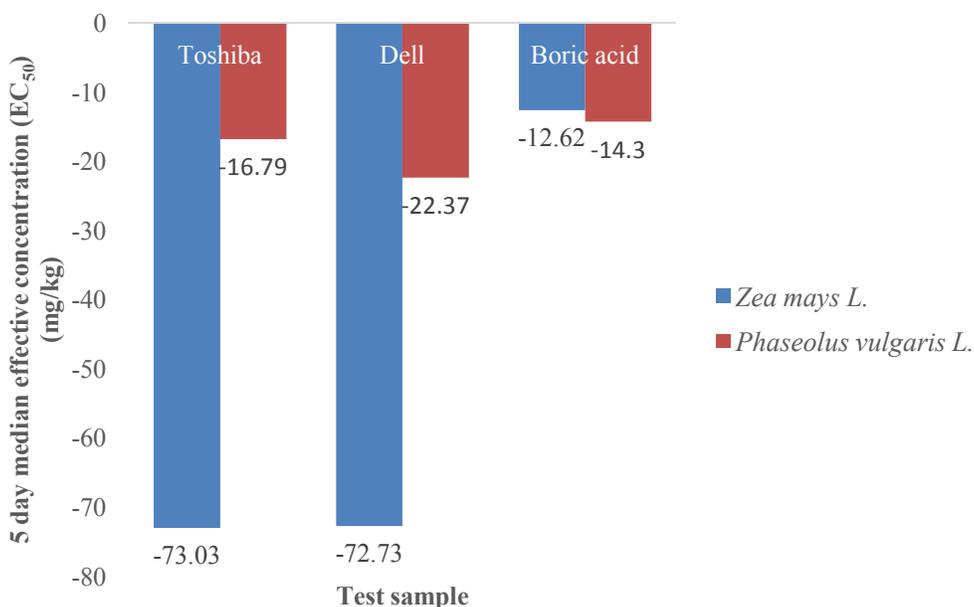


Fig. 6. 5 day median effective concentration (EC_{50}) (mg/kg) of spent laptop battery and boric acid samples on the growth indices of *Zea mays* L. (maize) and *Phaseolus vulgaris* L. (common beans)

Legend: mg/kg = Milligram per kilogram; EC_{50} = Median effective concentration; Error bar = 95 % confidence limit

zinc (Zn). Furthermore, median effective concentration (EC₅₀) refers to the concentration of a test substance that will inhibit or harm 50% population of a test organism. On a general note, the higher the EC₅₀ values, the more the tolerance of a test organism to a chemical substance. The investigation of EC₅₀ as demonstrated in this study in Fig. 6 showed that both *Z. mays* L. and *P. vulgaris* L. test seeds were more sensitive to the spent laptop battery samples than boric acid standard sample. On the other hand, the order of toxicity to *Z. mays* L. revealed thus: Toshiba battery sample > Dell battery sample > boric acid while the order of toxicity to *P. vulgaris* L. revealed thus: Dell battery sample > Toshiba battery sample > boric acid. This result implies that both *Z. mays* L. and *P. vulgaris* L. seeds are good bioindicators for monitoring spent Toshiba laptop battery, spent Dell laptop battery and boric acid pollution in the soil ecosystem. Heavy metals (manganese, lead, cadmium, chromium and cobalt) treatment of maize seeds led to the decreased dry matter and seed yield [22]. Al-Qurainy [9] reported that concentration 150 mg/kg of nickel (Ni) and aluminum (Al) metal contaminated soil induced grave impacts on *P. vulgaris* L. leaf area per plant, plant height, fresh and dry weight per plant, respectively. These metallic compounds studied by these authors were implicated in Table 1 of our study and could be responsible for the observed inhibitory effects in this study.

5. CONCLUSION

The present study revealed that the tested Toshiba and Dell battery samples were very acutely toxic to minor acutely toxic in *S. capricornutum*. The order of toxicity to *Z. mays* L. revealed that Toshiba battery sample > Dell battery sample > boric acid while in *P. vulgaris* L. it was in the order of Dell battery sample > Toshiba battery sample > boric acid. Both *S. capricornutum*, *Z. mays* L. and *P. vulgaris* L. were very good bio-monitoring models for spent Toshiba and Dell laptop battery contamination assessment. Thus, adequate regulatory framework should be established to deal effectively with e-wastes problems.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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