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# Tolerance of Some Soil Fungi to the Content of Deep Cycle Battery and Their Bioremediation Potential

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***Authors' contributions***

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

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## ABSTRACT

**Aims:** The purpose of this study was to isolate and screen soil fungi that are able to tolerate the contents of spent deep cycle battery (inverter), and to test for their bioremediation potential.
**Place and Duration of Study:** Sample: Department of Microbiology, Rivers State University, between June 2019 and February 2020.
**Methodology:** Soil samples were collected from a mechanic village while spent inverter batteries were obtained from a waste vendor. The battery was forced open to extract its contents of the battery. Using standard microbiological techniques, fungi were enumerated and characterized. Stock solution of the battery content was prepared by dissolving the inverter battery content in sterile deionized water. This stock solution was used to carry out the screening test on the fungal isolates to ascertain the fungi that can tolerate the contents of the spent battery.
**Results:** Total heterotrophic fungal counts for the polluted and unpolluted soil were 6.0 x $10^3$ cfu/g and 7.5 x $10^4$cfu/g respectively. The fungal isolates identified from the polluted soil samples were members of the genera *Rhizopus, Mucor, Aspergillus, Penicillium,* and *Candida,* while, the isolates identified from the unpolluted soil sample includes: *Candida* sp, *Aspergillus niger*, *Penicillium* sp, *Aspergillus fumigatus*, *Aspergillus flavus*, *Mucor* sp, *Yeast*, *Fusarium* sp and *Aspergillus* sp. After

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the screening, total heterotrophic fungal counts for the soil ranged from 1.0 x $10^2$cfu/g to 9.5 x $10^2$cfu/g. Two fungi of the genera: *Rhizopus* and *Mucor* had the highest counts during 72 hours of incubation for the screening test. The results obtained from this study indicated that species of *Aspergillus, Penicillium,* and *Candida* were the most inhibited by the contents of the spent battery while *Rhizopus* and *Mucor* spp were more tolerant to the contents of the inverter. *Rhizopus* and *Mucor* spp were therefore, adopted in the bioremediation of soil contaminated with contents from the battery. It was observed that *Rhizopus* and *Mucor* spp in a consortium had the highest percentage of heavy metal removal (or uptake) in the following order: Cadmium (66.66%) > Lead (38.15%) > Zinc (26.83%) > Nickel (20.83).
**Conclusion:** These organisms can be used in the bioremediation of soil polluted with metals from spent deep cycle batteries.

*Keywords: Fungal isolates; soil; inverter battery content; Rhizopus; Mucor.*

## 1. INTRODUCTION

As a result of man's quest to make his environment more conducive for living, as well as advances in information and communications industries, various electronic devises are manufactured, and used. This has led to large scale generation of waste, called electronic or e-waste at the end of their life span [1]. Most of its components are harmful to the environment and the living things in it [2]. When these wastes are improperly disposed of they may pollute water bodies; and also they may contaminate soil and seep into groundwater. Owing to poor power problem, there is an increased demand for various charged batteries which are used in diverse electronic gadgets, including inverters, cell phones, laptops, television, refrigerators etc [3].

Batteries and other energy storage devices store energy so that it can be used when needed. In a stand-alone power system, the energy stored in batteries can be used when energy demand exceeds the output from renewable energy sources like solar and wind [4]. Battery types can be divided into two basic categories namely, the primary batteries (e.g. Mercury oxide, Lithium, silver oxide, Zinc-carbon) which are disposable and the secondary batteries (e.g. Lithium-ion, Nickel-metal hydride, Nickel-Cadmium, Lead-acid) which are rechargeable [5]. Lithium-ion batteries are about half as toxic to humans as Lead-acid batteries, and less toxic than nickel-cadmium batteries. Nickel-metal hydride batteries are the least toxic to humans [6].

Deep cycle batteries are rechargeable batteries that could be drained of most of their power and recharged repeatedly. Deep cycle batteries are composed of thick solid lead plates. Because the plates are thicker there is less surface area producing less current. But this current can be produced and maintained for longer periods [7].

Inverters are a class of deep cycle battery that turns energy from one form to another. An inverter is an electronic device or circuitry that converts direct current (DC) to alternating current (AC) [8].

Inverter batteries are classified based on the chemistry of their cells. The four major categories include; Nickel-Cadmium (NiCad), Lithium-ion, Nickel-metal hydride and the Lead-Acid (L-A) (A1 Power Technologies, 2019) [9]. The L-A batteries are the most common type and are more hazardous and prone to leaks than the other battery types [10]. Lead-Acid batteries contain 60-75% elemental lead, lead dioxide and a sulphuric acid solution electrolyte. These heavy metal elements make them toxic, and improper disposal can be hazardous to the environment (Green Earth Battery Recycling), microorganisms, animalsand human health [1].

Heavy metals are elements that exhibit metallic properties such as ductility, malleability, conductivity, cation stability, and ligand specificity with relatively high density and high relative atomic weight and an atomic number greater than 20 [11]. Some heavy metals that are toxic to soil organisms, plants and humans include: mercury, lithium, cadmium, chromium, lead, nickel, selenium, and silver. These heavy metals are toxic even at very low concentrations [11].

Bioremediation is a known biological technique which relies on microorganisms and plants to alter heavy metals bioavailability in the environment and can be enhanced by addition of organic amendments to soils [12]. It is effective,

economical and environmentally friendly compared to other remediation techniques [13]. Microbial activities are essential to how pollutants in the ecosystem are transformed which is reflected in biogeochemical cycles and food webs. The mode in which microorganisms respond to toxicants in an ecosystem will partially, if not majorly, determine the fate of that ecosystem when the assimilative capacity has not been exceeded [2].

This study was conducted to evaluate the tolerance of soil fungi to the content of deep cycle battery as well as their bioremediation potential in the removal of selected heavy metals from a contaminated soil.

## 2. METHODOLOGY

### 2.1 Sampling

Polluted soil samples were collected from e mechanic village located at Odo lane, Rumuochita beside Kesioru playground in Obio-Akpor Local Government, Rivers State, Nigeria. The coordinates are 4º50ˈ46" N and 6º59ˈ9" E. Also, uncontaminated soil samples were collected from the school farm in Rivers State University which is void of electronic waste contamination. The soil samples were collected into black polyethylene bags using soil auger. Spent inverter battery was obtained from a dumpsite worker at a major dumpsite at Location road, off Ada George road, Port Harcourt.

### 2.2 Physicochemical and Heavy Metals Analysis of the Soil Samples

The physicochemical parameters were determined using Standard Methods according to APHA [14]. The following parameters were analyzed: Sulphate ($SO_4^{2-}$), Phosphate ($PO_4^{3-}$), Electrical conductivity, pH, Temperature, Moisture Content and Total Organic Carbon. Zinc (Zn), Nickel (Ni), Lead (Pb) and Cadmium (Cd) were the heavy metals analysed in this study [15].

### 2.3 Analysis of Some Heavy Metals Present in the Battery

The inverter battery content was analyzed for the levels of some heavy metals and their quantity (in mg/kg) using the API-RP45 method (American Petroleum Institute Recommended Practices). The heavy metals analyzed were Zinc, Nickel, Lead and Cadmium, using an Atomic Absorption Spectrophotometer (AAS) calibrated daily with specific metallic standard [15].

### 2.4 Enumeration of Total Heterotrophic Fungi

The standard plate count method was used in enumerating fungi in the soil samples. Serial tenfold dilution was made from soil samples from both the mechanic village and the school farm. One gram (1g) of each the soil sample was transferred into a test tube containing 9ml sterile diluent (normal saline). Subsequent serial dilutions were carried made up to $10^{-4}$. Using a sterile pipette, 0.1ml amounts of dilutions $10^{-1}$, $10^{-3}$ and $10^{-4}$ were inoculated in duplicate on to freshly prepared sterile Sabouraud dextrose agar (SDA) plates, to which 0.2ml of 0.5% ampicillin was added to prevent bacterial growth, and incubated at 25°C for 2-5 days. The fungal colonies were counted; average counts of the duplicate plates were recorded. Discrete colonies were subcultured unto freshly prepared SDA plates to get pure fungal isolates, which were preserved on SDA slants [1].

#### 2.4.1 Identification of fungal isolates

The fungal isolates were identified based on macroscopic examination of the colonies such as: colour of colony, shape, and surface appearance. The microscopic examination was by the wet mount method as described by Cheesebrough, [16]. From the pure culture plates a small portion of the isolate was picked using a sterile inoculating needle, placed on a clean grease free slide, to make a smear with lactophenol. Thereafter, the slide was covered with cover slip and viewed under the microscope at X10 and X40 to check the hyphae (septate and non-septate) and fruiting body according to Barnett and Hunter [17].

### 2.5 Preparation of Stock Solution

The stock solution of the inverter battery was prepared using the method of Odokuma and Akponah[18] and Kpormon and Douglas [2] with slight modifications. In this method, 4g of the inverter battery content was dissolved in sterile 100 ml deionized water.

**Table 1. Experimental setups for bioremediation treatments**

| S/N | Set up label | Treatments |
|---|---|---|
| 1 | CONTROL | 2.5 kg Soil + 125 ml Battery Content |
| 2 | SI/FA | *Rhizopus* sp. + 2.5 kg soil + 125 ml battery content |
| 3 | SI/FB | *Mucor* sp. + 2.5 kg soil + 125 ml battery content |
| 4 | SI/FA/FB | *Rhizopus* sp. + *Mucor* sp + 2.5 kg soil + 125 ml inverter content |

#### 2.5.1 Preliminary screening test

The method of Odokuma and Akponah [18] was adopted, where 9ml of contents from the inverter battery stock solution was dispensed into sterile labeled test tubes (test tubes were labeled according to the isolates to be screened). One milliliter (1ml) of 48 hours old fungal cultures in Sabouraud dextrose broth were transferred into respective labeled test tubes containing 9ml of the battery content. Another set of test tubes which contained only 9ml of normal saline and 1ml of the respective inoculum served as the positive control while an uninoculated 10ml test tube containing the battery content only served as the negative control. The inoculated test tubes were incubated at 25°C for 48 hours. After incubation, aliquots of 0.1ml from the different test tubes were drawn and inoculated on fresh Sabouraud dextrose agar plates using the spread plate method. Inoculated plates were incubated at 25°C for 48 hours. Fungal isolates that were able to proliferate after screening, as seen in Table 1 were adopted for the bioremediation set up [18].

#### 2.5.2 Production of inoculum for bioremediation experiments

The screened fungal isolates preserved in SDA slants were subcultured to obtain 72hour old cultures. One milliliter of each of the screened fungal isolates was separately inoculated into 1000 ml of freshly prepared sterile Sabouraud Dextrose Broth in a 1500 ml Erlenmeyer flask. Incubation followed at 25°C for 72hours [18].

### 2.6 Bioremediation Experimental Setup

Bioaugmentation was performed using pure cultures of *Rhizopus* sp and *Mucor* sp, where unpolluted soil sample was prepared for the bioremediation process and subdivided into the various experimental setups, 2.5 kg each, in which was added with 125 ml of stock solution from inverter content and 25 ml of fungal culture [19]. This was done for each set up using *Rhizopus* and *Mucor* spp as bioaugmenting organisms individually and in a consortium as described in Table 1. After mixing properly, microcosms were kept at ambient temperature in green house. Sterile distilled water was used to water every 5days to maintain water holding capacity of 50% and properly tilled for proper aeration and mixing using a sterile hand trowel. Samples were taken out every seven days to monitor the levels of the metals for 28 days. Table 1, shows the various experimental set ups.

#### 2.6.1 Percentage (%) heavy metal uptake evaluation

The percentage (%) heavy metal removal is calculated as follows:

% Toxin or Heavy Metal Removal = $100 \times [(C_0 - C_l) / C_0]$

Where $C_0$ = initial concentration
$C_l$ = final concentration

### 2.7 Data Analysis

The data gathered in this study were subjected to statistical analysis. The data were properly arranged in the Microsoft excel (2016 version), the means and standard deviations were computed using the SPSS (Version 22).

## 3. RESULTS AND DISCUSSION

The total heterotrophic fungal counts obtained for the polluted and unpolluted soil samples were $6.0 \times 10^3$ cfu/g and $7.5 \times 10^4$cfu/g respectively. The results of microbiological analyses conducted on the soil samples showed that the unpolluted soil had a higher fungal population, when compared to the polluted soil. This may be as a result of the presence of the pollutant in the soil. When there is pollutant in an environment it puts a selective pressure on the organisms present, those that are able to with stand the pollutant grow while others die off. Thereby, affecting the fungal diversity and populations of the soil polluted soil [1].

The fungal isolates identified from the polluted soil samples were members of the genera

*Rhizopus, Mucor, Aspergillus, Penicillium,* and *Candida.* The results are presented in Table 2. The fungal isolates identified from the unpolluted soil sample includes: *Candida* sp, *Aspergillus niger*, *Penicillium* sp, *Aspergillus fumigatus*, *Aspergillus flavus*, *Mucor* sp, *Yeast*, *Fusarium* sp and *Aspergillus* sp*.* The results are also presented in Table 3. These results shown that pollution affects fungal population and diversity.

**Table 2. Cultural and morphological characteristics of fungal isolates from polluted soil**

| Isolates | Macroscopy | Microscopy | Probable Identity |
|---|---|---|---|
| A | White cottony growth with blackish spores, yellow reverse | Non septate hyphae with non-septate sporangiophores bearing sporangia | *Rhizopus* spp |
| B | White fluffy growth, white reverse | Aseptate hyphae bearing long sporangiosphore, presence of bug | *Mucor* spp |
| C | Blue-green powdery growth, pale yellow reverse | Septate hyphae with smooth-walled conidiophores bearing conidia in chains | *Aspergillus* spp |
| D | White to grey-green flat cottony growth, pale yellow reverse | Septate hyphae with long conidiophores bearing conidia in chains | *Penicillium* spp |
| E | Smooth creamy colonies | Branched pseudohyphae with blastoconidia in small clusters | *Candida* spp |

**Table 3. Cultural and morphological characteristics of fungi from the unpolluted soil**

| Isolates | Macroscopy | Microscopy | Prob. ID |
|---|---|---|---|
| A. | Cream large round | Oval budding blastoconidia | *Candida* spp |
| B. | Black spores surrounded by cream background, brown reverse | Septate hyphae with aeseptateconidiosphore bearing conidia | *Aspergillus niger* |
| C. | Green powdery surface surrounded by white lawn, brown reverse | Septate hyphae with septate conidiophores bearing conidia | *Penicillium* spp |
| D. | Black-brown suede surface, black reverse | Septate hyphae with aeseptateconidiosphore and scattered conidia | *Aspergillus fumigates* |
| E. | Light green lawn surrounded by white lawn-like growth | Septate hyphae with aeseptateconidiosphore bearing conidia | *Aspergillus flavus* |
| F. | Fluffy white cottony, white reverse | Aeseptate hyphae bearing sporangiospores | *Mucor* spp |
| G. | white small round | Oval budding blastoconidia | *Yeast* |
| H. | White cottony lawn like growth, with reverse yellow colour | Septate hyphae, with presence of banana shaped septate conidia | *Fusarium* spp |
| I. | Black spores surrounded by cream background, brown reverse | Septate hyphae with aeseptateconidiosphore bearing conidia | *Aspergillus* spp |

Table 4, shows the selected heavy metals analysis of the inverter content which showed that Lead concentration was 1.577 mg/kg, Nickel was 0.292 mg/kg, Cadmium was 0.059 mg/kg, Zinc was <0.005 mg/kg. This confirms that the Luminous inverter battery used in this study is a Lead-Acid battery (L-A battery), not a Nickel-Cadmium battery (NiCad battery) or Mercury battery. The Intervention Values for Micropollutants for a Standard Soil in Nigeria is Lead: 530 mg/kg, Nickel: 210 mg/kg, Zinc: 720 mg/kg and Cadmium: 380 mg/kg [20]. This implies that currently Lead-Acid inverters are not likely to pose any threat of heavy metal pollution to our environment.

**Table 4. Heavy metal content of the inverter battery**

| Heavy Metals | Battery Content(mg/kg) |
|---|---|
| Zinc | <0.005 |
| Nickel | 0.292 |
| Lead | 1.577 |
| Cadmium | 0.059 |

Results of the preliminary screening for the tolerance of the fungal isolates to the contents of the battery (Table 5), showed that two of the isolates (*Rhizopus* and *Mucor* spp) were able to tolerate the toxic contents of the inverter while the other three isolates (*Aspergillus, Penicillium,* and *Candida*) were inhibited by the contents of the inverter, hence showed no growth. *Rhizopus* and *Mucor* spphave been found to be among the list of fungi to have the highest metal adsorption capacities as reported by Zaidi et al., [21]. This may also be due to the fact that apart from the presence of heavy metals, some other components contained in the battery content may be toxic, which completely inhibited the growth of *Aspergillus, Penicillium,* and *Candida* spp. These fungi may lack the mechanisms required to tolerate the toxic contents of the inverter battery. Microorganisms found in polluted environment possess astonishing metabolic pathways which tolerate and possibly utilizes various toxic compounds as a source of energy for growth and development, through respiration, fermentation, and cometabolism [22]. According to Ayangbenro and Babalola (2017) [23], the majority of heavy metals disrupt microbial cell membranes, but microorganisms can develop defense mechanisms that assist them in overcoming the toxic effect and also transform pollutants in the environment.

The mechanism of heavy metal uptake in fungi (living fungal cells) is basically metabolism-independent uptake, which involves adsorption processes such as ionic, chemical and physical uptake. A variety of ligands located on the fungal cell walls are known to be involved in metal chelation [24].

The results of the physicochemical parameters for the polluted and unpolluted soil samples are presented in Table 6. The results showed that the pH of the polluted and unpolluted soils used in this study were 8.5 and 7.5 respectively. The polluted soil has a higher pH than the unpolluted soil which is agreement with the results obtained by Klimek and Niklinska[25] who observed that the pH of polluted soils is significantly higher than that of unpolluted soils. As seen from the results of the total heterotrophic fungal counts, the unpolluted soil is still capable of supporting a wider range of soil fungi.

The results of electrical conductivity of the polluted and unpolluted soils are 0.51µS/cm and 0.05µS/cm respectively. Soil electrical conductivity (EC) is a measure of the amount of salts in soil (salinity of soil) [26]. It is an important indicator of soil health and can serve as an indirect indicator of the moisture content and water-soluble nutrients available for plant removal such as nitrate, sulphate and phosphate [26]. Thus, this confirms the results of the moisture content of polluted and unpolluted soils (6.21% and 11.48% respectively), meaning a lower moisture content for the polluted soil with a high electric conductivity. Soil microbial activity declines as EC increases [26]. This impacts important soil processes such as respiration, residue decomposition, nitrification and dentrification [26]. According to the United States Department of Agriculture (USDA, 2013), When EC readings are less than 1 dS/m, soil are considered non-saline and do not impact soil microbial processes while EC readings greater than 1 dS/m means the soil are considered saline and impact important microbial processes, such as nitrogen cycling, production of nitrous and other N-oxide gases, respiration, and decomposition; increased nitrogen losses; populations of plant-parasitic nematodes can increase [27]. The both soil samples showed EC readings less than 1 dS/m meaning the soil microbial processes are not impacted despite the presence of the metals.

The phosphate ($PO_4^{3-}$) levels of the polluted and unpolluted soils are 0.22 mg/kg and 0.86 mg/kg

respectively. According to Ademola et al., [28], phosphate presents itself as a nutrient to soil microflora and readily sequesters metals and reduces their bioavailability via the formation of insoluble metal phosphate species. This explains why the phosphate level in the polluted soil is lower than that of the unpolluted soil. The results for nitrate ($NO_3^{2-}$) of the polluted and unpolluted soils (26.00 mg/kg and 33.00 mg/kg respectively) are in line with the findings made by Tanee et al., [29]; with nitrate values of 23.04 mg/kg and 61.76 mg/kg for polluted and unpolluted soils respectively and Ataikiru et al., [30], with nitrate values of <0.001 mg/kg and 15.42 mg/kg for polluted and unpolluted soils respectively, showing that most heavy metal polluted soils usually have lower nitrate concentrations compared to non-polluted soils. In polluted sites, the limiting nutrients, (nitrate and phosphate) which are essential for biodegradation to occur are usually released to the microorganisms involved, thereby causing a reduction in the concentration of these nutrients (Ataikiru et al. [30]. The sulphate ($SO_4^{2-}$) of the polluted and unpolluted soils (14.37 mg/kg and 36.26 mg/kg respectively) is an indicator that the fungi present in the soil might be involved in inorganic nutrition [31].

### 3.1 Bioremediation Potential of Screened Fungal Isolates

The treatments for the bioremediation (bioaugmentation) process are described in Table 1. The results of the individual heavy metal uptake by the isolates are presented in Tables 7 - 10. The results revealed that the concentration of Zinc in the contaminated soil was reduced greatly by the combination of *Rhizopus* and *Mucor* spp used in a consortium when compared to their individual performances. This was also observed in the reduction of the concentrations of the other metals - Nickel, Lead and Cadmium. *Rhizopus* and *Mucor* spp in a consortium showed heavy metal percentage removal in the following order: Cadmium (66.66%) > Lead (38.15%) > Zinc (26.83%) > Nickel (20.83). This is observed in Figs. 1 to 4. This implies that a combination of potent microorganisms in a consortium has higher efficiency in the removal of heavy metal compared to single species, and the final removal efficiency for the consortium could be reached in a considerably shorter time [32].

**Table 5. Fungal counts after screening**

| Isolates | With toxicant (cfu/ml) | Without toxicant (cfu/ml) |
|---|---|---|
| *Rhizopus* sp | $2.1x10^2$ | $1.0 x10^2$ |
| *Mucor* sp | $2.3x10^2$ | $1.0 x10^2$ |
| *Aspergillus* sp | No growth | $1.5 x10^3$ |
| *Penicillium* sp | No growth | $9.0x10^2$ |
| *Candida* sp | No growth | $9.5x10^3$ |

**Table 6. Physicochemical properties of the soil samples**

| Parameters | Unit | Polluted soil | Unpolluted soil |
|---|---|---|---|
| Nitrate ($NO_3^{2-}$) | mg/kg | 26.00 | 33.00 |
| Sulphate ($SO_4^{2-}$) | mg/kg | 14.37 | 36.26 |
| Phosphate ($PO_4^{3-}$) | mg/kg | 0.22 | 0.86 |
| Electrical conductivity | µS/cm | 0.51 | 0.05 |
| pH | | 8.6 | 7.5 |
| Temperature | °C | 29.2 | 29.3 |
| Moisture Content | % | 6.21 | 11.48 |
| Total Organic Carbon | mg/kg | 0.38 | 0.35 |
| Zinc (Zn) | mg/kg | 6.80 | 0.83 |
| Nickel (Ni) | mg/kg | 3.00 | 0.71 |
| Lead (Pb) | mg/kg | 3.82 | 0.54 |
| Cadmium (Cd) | mg/kg | 1.00 | 0.03 |

**Table 7. Rate of metal uptake of zinc in the setup**

| Setup Identity | Unit | Initial concentration | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|---|---|
| CONTROL | mg/kg | 0.83±0.02$^{a}$ | 0.83±0.01$^{a}$ | 0.78±0.01$^{d}$ | 0.74±0.01$^{f}$ | 0.70±0.01$^{de}$ | 0.69±0.01$^{e}$ |
| SI/FA | mg/kg | 0.83±0.02$^{a}$ | 0.80±0.01$^{a}$ | 0.74±0.01$^{abc}$ | 0.71±0.01$^{ef}$ | 0.70±0.01$^{de}$ | 0.65±0.01$^{e}$ |
| SI/FB | mg/kg | 0.83±0.02$^{a}$ | 0.82±0.01$^{a}$ | 0.75±0.01$^{bcd}$ | 0.73±0.01$^{f}$ | 0.72±0.01$^{e}$ | 0.66±0.01$^{de}$ |
| SI/FA/FB | mg/kg | 0.83±0.02$^{a}$ | 0.82±0.01$^{a}$ | 0.72±0.01$^{ab}$ | 0.67±0.01$^{cd}$ | 0.62±0.01$^{b}$ | 0.60±0.01$^{c}$ |

*Means with similar superscripts within columns show no significant difference at P ≥ 0.05*

**Table 8. Rate of metal uptake of nickel in the setup**

| Setup Identity | Unit | Initial concentration | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|---|---|
| CONTROL | mg/kg | 0.72±0.01$^{a}$ | 0.72±0.01$^{a}$ | 0.70±0.01$^{d}$ | 0.70±0.01$^{e}$ | 0.68±0.01$^{f}$ | 0.66±0.01$^{e}$ |
| SI/FA | mg/kg | 0.72±0.01$^{a}$ | 0.70±0.01$^{a}$ | 0.67±0.01$^{cd}$ | 0.64±0.01$^{d}$ | 0.61±0.01$^{e}$ | 0.57±0.01$^{d}$ |
| SI/FB | mg/kg | 0.72±0.01$^{a}$ | 0.70±0.01$^{a}$ | 0.67±0.01$^{cd}$ | 0.64±0.01$^{d}$ | 0.60±0.01$^{de}$ | 0.58±0.01$^{d}$ |
| SI/FA/FB | mg/kg | 0.72±0.01$^{a}$ | 0.72±0.01$^{a}$ | 0.65±0.01$^{abc}$ | 0.62±0.01$^{cd}$ | 0.56±0.01$^{bc}$ | 0.57±0.01$^{d}$ |

*Means with similar superscripts within columns show no significant difference at P ≥ 0.05*

**Table 9. Rate of metal uptake of lead in the setup**

| Setup Identity | Unit | Initial concentration | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|---|---|
| CONTROL | mg/kg | 0.54±0.01$^{a}$ | 0.54±0.01$^{a}$ | 0.47±0.01$^{d}$ | 0.47±0.01$^{f}$ | 0.48±0.01$^{e}$ | 0.47±0.01$^{c}$ |
| SI/FA | mg/kg | 0.54±0.01$^{a}$ | 0.55±0.01$^{a}$ | 0.55±0.01$^{e}$ | 0.38±0.01$^{d}$ | 0.34±0.01$^{c}$ | 0.35±0.01$^{b}$ |
| SI/FB | mg/kg | 0.54±0.01$^{a}$ | 0.54±0.01$^{a}$ | 0.48±0.01$^{d}$ | 0.43±0.01$^{e}$ | 0.40±0.01$^{d}$ | 0.35±0.01$^{b}$ |
| SI/FA/FB | mg/kg | 0.54±0.01$^{a}$ | 0.54±0.01$^{a}$ | 0.51±0.01$^{d}$ | 0.38±0.01$^{d}$ | 0.35±0.01$^{c}$ | 0.33±0.01$^{b}$ |

*Means with similar superscripts within columns show no significant difference at P ≥ 0.05*

**Table 10. Rate of metal uptake of cadmium in the setup**

| Setup Identity | Unit | Initial concentration | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|---|---|
| CONTROL | mg/kg | 0.03±0.01$^{a}$ | 0.03±0.01$^{a}$ | 0.03±0.01$^{a}$ | 0.03±0.01$^{a}$ | 0.02±0.01$^{a}$ | 0.02±0.01$^{a}$ |
| SI/FA | mg/kg | 0.03±0.01$^{a}$ | 0.03±0.01$^{a}$ | 0.03±0.01$^{a}$ | 0.02±0.01$^{a}$ | 0.02±0.01$^{a}$ | 0.01±0.00$^{a}$ |
| SI/FB | mg/kg | 0.03±0.01$^{a}$ | 0.03±0.01$^{a}$ | 0.03±0.01$^{a}$ | 0.03±0.01$^{a}$ | 0.02±0.01$^{a}$ | 0.01±0.00$^{a}$ |
| SI/FA/FB | mg/kg | 0.03±0.01$^{a}$ | 0.02±0.01$^{a}$ | 0.03±0.01$^{a}$ | 0.03±0.01$^{a}$ | 0.02±0.01$^{a}$ | <0.01±0.00$^{a}$ |

*Means with similar superscripts within columns show no significant difference at P ≥ 0.05*

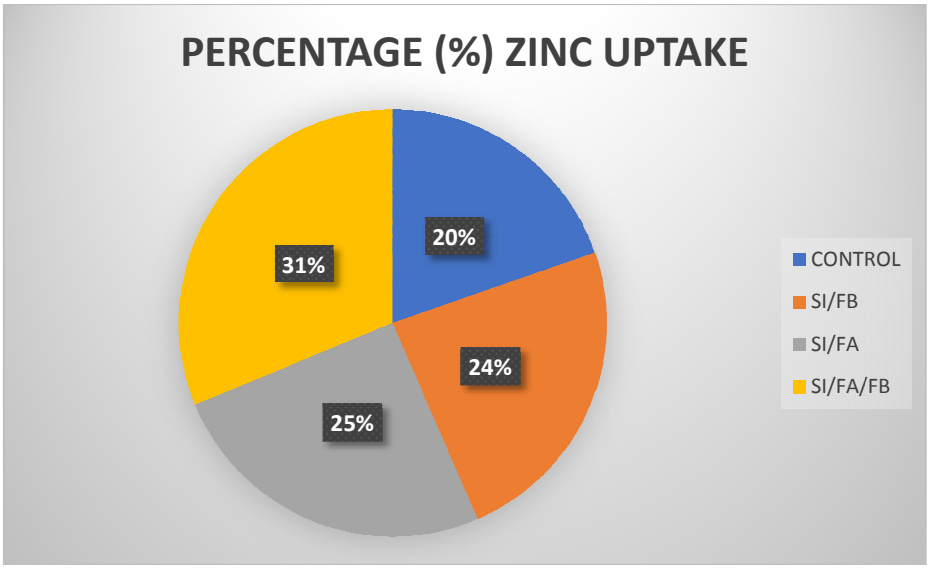


**Fig. 1. Percentage zinc removal**

*Key: SI/FA = Rhizopus sp. + 2.5 kg soil + 125 ml inverter content; SI/FB = Mucor sp. + 2.5 kg soil + 125 ml inverter content; SI/FA/FB = Rhizopus sp. + Mucor sp + 2.5 kg soil + 125ml inverter content*

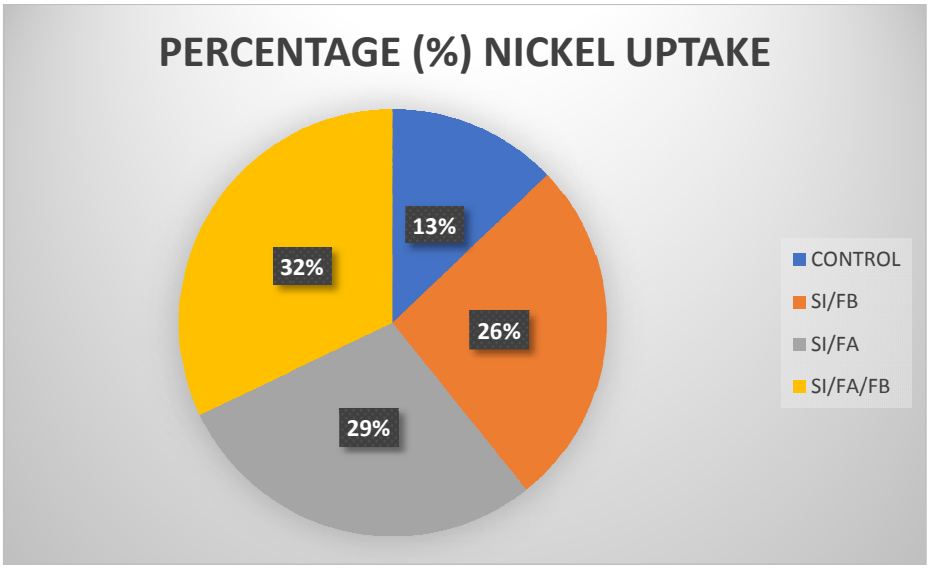


**Fig. 2. Percentage nickel uptake**

*SI/FA = Rhizopus sp. + 2.5 kg soil + 125 ml inverter content; SI/FB = Mucor sp. + 2.5 kg soil + 125 ml inverter content; SI/FA/FB = Rhizopus sp. + Mucor sp + 2.5 kg soil + 125ml inverter content*

The increase in the microbial load of the control was not significant (at P ≥ 0.05) while a significant increase was observed in the fungal load of the set up containing a combination of *Rhizopus* + *Mucor* spp in a consortium ($2.67\pm0.38^{a}<3.78\pm0.03^{a}<3.89\pm0.27^{ab}<3.93\pm0.21^{a}<4.17\pm0.06^{a}$) load during the 28 days compared to the individual performances of the fungi. This implies that the spent inverter content was inhibitory to some of the fungal species in the control while the fungi adopted for the study as well as some fungal species present in the soil were able to tolerate the spent battery content, thereby increasing their fungal counts. A similar observation was made by Ataikiru et al., [30], in their research on Bioremediation of

Bonny light crude oil polluted soil by bioaugumentation using yeast isolates. They observed a significant increase in the fungal load of microcosm A of which *Rhizopus* and *Mucor* spp were inclusive ($6.21\pm0.15^{a}$< $7.70\pm0.12^{a}$< $7.28\pm0.25^{ac}$) over a period of 28 days, based on monitoring fortnightly.

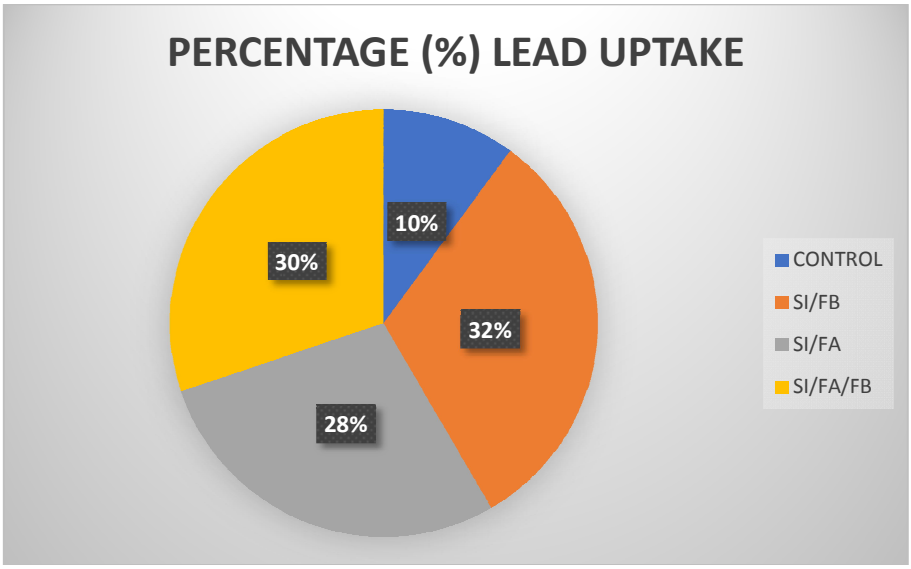


**Fig. 3. Percentage lead uptake**

*Key: SI/FA = Rhizopus sp. + 2.5 kg soil + 125 ml inverter content; SI/FB = Mucor sp. + 2.5 kg soil + 125 ml inverter content; SI/FA/FB = Rhizopus sp. + Mucor sp + 2.5 kg soil + 125 ml inverter content*

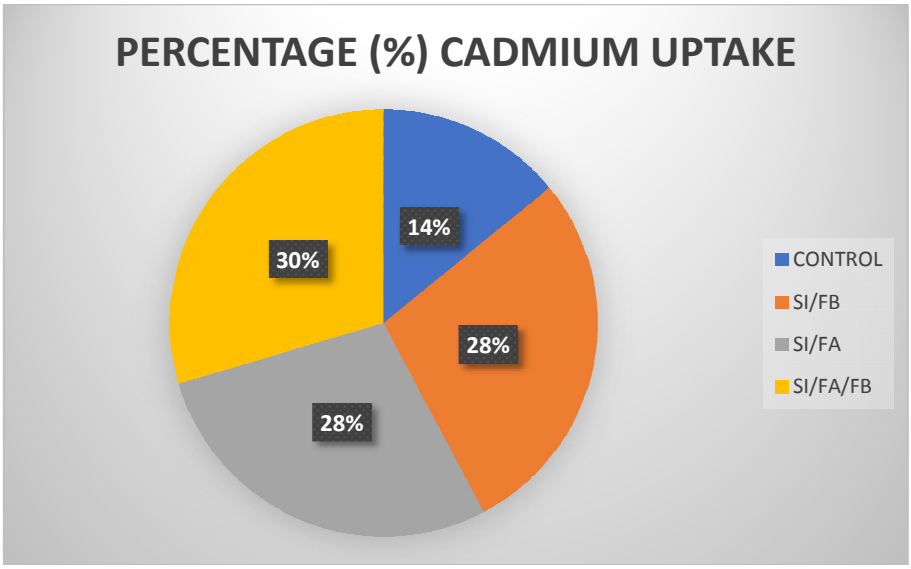


**Fig. 4. Percentage uptake of cadmium**

*Key: SI/FA = Rhizopus sp. + 2.5 kg soil + 125 ml inverter content; SI/FB = Mucor sp. + 2.5 kg soil + 125 ml inverter content; SI/FA/FB = Rhizopus sp. + Mucor sp + 2.5 kg soil + 125ml inverter content*

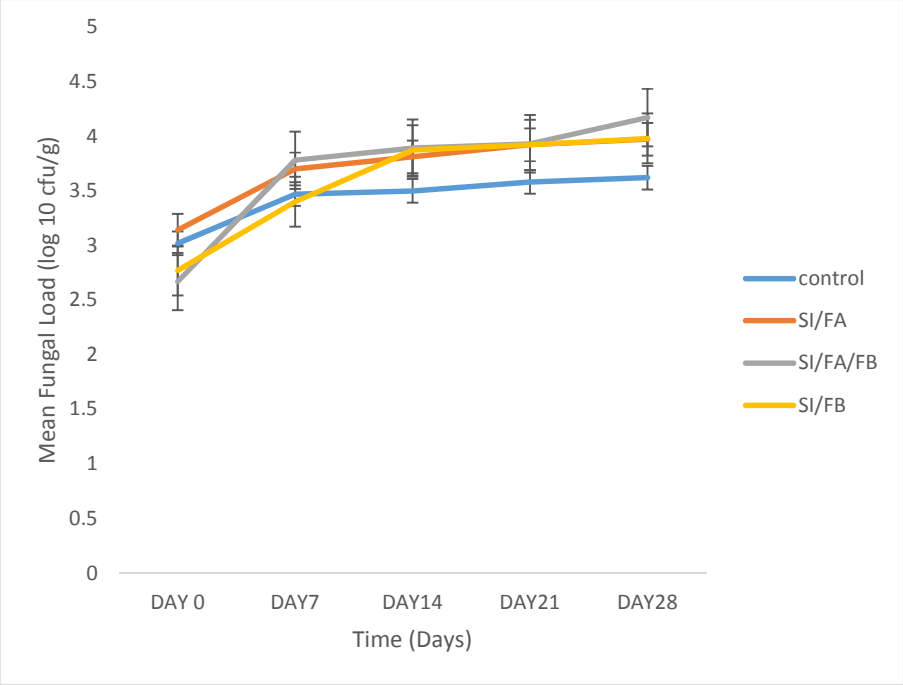


**Fig. 5. Mean fungal counts (cfu/g) of the bioremediation setup during the 28 days**

*Key: SI/FA = Rhizopus sp. + 2.5 kg soil + 125 ml inverter content; SI/FB = Mucor sp. + 2.5 kg soil + 125ml inverter content; SI/FA/FB = Rhizopus sp. + Mucor sp + 2.5 kg soil + 125 ml inverter content*

## 4. CONCLUSION

Soil fungi play very important role in the soil structure, production of humus, decomposition, nutrient recycling and organic matterproduction of the soil. *Mucor* and *Rhizopus* spp were among the indigenous fungal species identified in the polluted soil that have the ability to tolerate the contents of deep cycle battery. The fungal isolates - *Mucor* and *Rhizopus* spp in a consortium were found to be more efficient in the removal of the selected heavy metals during the 28days monitoring period, yielding a higher percentage of heavy metal uptake compared to their individual performances. Therefore, since *Mucor* and *Rhizopus* spp were able to tolerate contents of deep cycle batteries, they could be used as bioaugumenting organisms to bioremediate soil polluted with contents from spent deep cycle batteries.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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