# Effects of Automobile Battery Wastes on Microbial Qualities of the Soil

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## Abstract

*The natural balance in the qualities of soil located in close proximity to automobile battery charging workshop has undergone alteration in the recent year due to pollution traceable to automobile battery wastes. This paper examines the effects of automobile battery wastes on the microbial qualities of the soil. The soil for this study was collected from a battery charger's workshop at 0-15cm depth in triplicates during the months of dry and wet seasons (August, September and October). An artificially contaminated soil which served as positive control (called amended soil) was also prepared by mixing 1.5kg of uncontaminated soil with 100g of battery waste while a fallow soil devoid of battery chargers' activities served as negative control. Morphological and biochemical tests performed on battery waste isolates revealed micro-organisms such as Chromobacter spp, Staphylococcus spp, Micrococcus spp, Streptococcus spp, Bacillus spp, Pseudomonas spp, Escherichia coli, Aspergillus niger, Fusarium spp, Geotrichum spp, Penicillium spp, Mucor spp and Rhizopus spp. The mean total heterotrophic bacterial (THB) count was $3.66\pm 2.62 \times 10^4$ cfu/g and the mean fungal count was $1.49\pm0.46\times 10^4$cfu/g in the contaminated soil. Meanwhile, in the negative control sample, THB and fungal counts were $5.10\pm3.89 \times 10^4$cfu/g and $4.47\pm0.20 \times 10^4$ cfu/g respectively. Statistical analysis shows that THB and fungal counts for negative control sample was significantly (P< 0.001) higher than that of the contaminated soil, which suggests that battery waste adversely affects microbial load and its activities in the soil.*
**Keywords:** battery, waste, soil, microorganisms, contaminated

## Introduction

A good quality soil is characterized by having adequate proportion of its components such as micro-organisms, soil water, soil air and mineral compounds. The type of activities prevalent in any given environment determines the type of contamination in that area [1].The biological activities in the soil are largely in the top soil which receives the greater impact from pollutants. Most environments in Nigeria and to a large extent in the whole of West Africa are subject to an increasing pollution from the discharge of different kinds of effluents resulting from anthropogenic activities which have become a major threatening factor to the quality of soil. According to a recent research, automobile battery wastes are common environmental pollutants [2].

Battery wastes have toxic effect on microorganisms which could ultimately affect the higher organisms which depend on microbes and their by-products for growth and development [3]. One of the most prominent soil contaminant is battery wastes. Varieties of battery are found based on the appliances that require it, for example, lead-acid batteries are found in automobiles. Some cars use more exotic starter batteries(2010 Porsche 911 GT3 RS which offer a lithium-ion battery as an option to save weight over a conventional lead-acid battery) [4]. Wastes from Automobile Battery Manufacturing companies (ABMC) are known to release a high percentage of heavy metals like lead (Pb) on soil, the resultant effect is unavoidably detrimental to the microbial population in the environment. ABMC has been reported in Nigeria to release about 6% upwards of lead [5].

Nigeria has three well-known automobile battery manufacturing companies namely, Exide battery in Ibadan, Oyo State(the biggest in West Africa in the I990s), , Union Battery Company in Nnewi, Anambra State and Metropolitan Battery in Ota, Ogun State which is still in operation. These companies dump their slags from smelting operations either in near-by bushes or on the premises. Enough work has not been done in the three sites to ascertain the pollution posed on the entire ecosystem by the automobile battery wastes. Hence in this work battery chargers' premises were used as case studies of such activities related to battery operation and its wastes especially on the microbial population. Most industrial wastes are often not well treated before disposal [6].The fertility of soil depends not only on its chemical composition but also on the qualitative and quantitative nature of microorganisms inhabiting it [7].

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## Materials and Methods
### Sampling area
The soil samples were obtained from three automobile battery wastes mechanic workshops in Adolor, Edaiken, and Uwelu areas of Benin City, Edo State and in a non-mechanic site at Isihor, Benin City.

The soil samples were collected by using soil auger up to a depth of 15cm. The soil samples were collected at 0-15cm depth from three points at interval of 10cm in each battery charger mechanic workshops. The collected samples were air dried, crushed with mortar and pestle to pass through 2mm sieve and stored in the sealed plastic bags at room temperature ($28\pm2^0$C) for 24h for further laboratory experiment. The soil samples were placed in polyethylene bags and labeled dry soil 1, dry soil 2, wet soil, amended soil and uncontaminated soil as described below in Table 1:

Table 1: Sample Collection at Designated Areas

| Sample | Depth collected | Description |
| --- | --- | --- |
| Dry soil 1 | 0-15 cm | Sample collected in dry season in mechanic workshop at Adolor, Benin City. |
| Dry soil 2 | 0-15cm | Sample collected in dry season in mechanic workshop at Edaiken, Benin City. |
| Wet soil | 0-15cm | Sample collected in wet season in mechanic workshop at Uwelu, Benin City |
| Amended Soil (Positive control) | 0-15 cm | 1.5 kg of uncontaminated soil mixed with 100 g of battery waste. |
| Control (Negative control) | 0-15 cm | Uncontaminated soil only, collected from a fallow bush in Isihor, Benin City |

### Isolation of microorganisms
Ten gram (10g), of soil samples from battery contaminated soil was weighed into sterile beaker and 90ml of sterilized distilled water was added. The stock suspension was subsequently serially diluted using ten-fold serial dilution up to $10^{-3}$. Aliquot of 0.1ml of the appropriate dilution from each contaminated soil was plated in nutrient agar for isolation of bacteria and potato dextrose agar for isolation of fungi. The nutrient agar plates were incubated at 37°C for 24-48 hours while the potato dextrose agar plates were incubated at room temperature (28°C) for 72 hours. After incubation, the numbers of discrete colonies were counted in terms of colony forming units. The viable count was obtained from this value by reference to the serial dilution used. The method described by [8] for estimating bacterial and fungal counts was used to enumerate the total viable counts of the isolates in units per gram (cfu/g).

### Isolation, characterization and identification of battery wastes utilizing bacterial and fungal isolates
The isolation of battery waste utilizing bacteria from the contaminated soil samples were performed in triplicate by plating out 0.1 ml of the samples on modified mineral salt medium to which 1% of the contaminated soil was added using the pour plate techniques [9]. Pure stock culture of battery waste utilizing bacteria were identified and characterized using the criteria in [10].

### Cultural characteristics
For the bacterial isolates, cultural characteristics were observed on Nutrient agar plates. The cultural characteristics include. Size, shape, surface, opacity, texture, elevation and pigmentation were determined by visual observation.

### Data analysis
Data from the laboratory were analyzed using Microsoft Excel 2013 and Statistical Package for Social Science (SPSS Inc. Cary, NC, USA, version 17.0). Analysis of variance (ANOVA) was carried out to test for significance in mean values across sampling points, $p<0.05$ is declared significant and Duncan test was used to determine the source of significance.

## Results and Discussion
### Total Heterotrophic Bacterial (THB) Counts
The mean THB counts in the battery wastes-contaminated soil samples designated as dry soil 1, dry soil 2 and wet soil were ($1.97\times10^4$, $2.7\times10^4$ and $6.3\times10^4$) cfu/g respectively. While the mean THB counts in uncontaminated (control) soil was $5.12\times10^4$ cfu/g. Hence, the THB counts in uncontaminated soil was comparably higher than contaminated soil samples though not significant (P>0.05). However, in the soil samples amended with battery wastes, the THB counts was $9.43 \times 10^4$ cfu/g and was slightly higher than control but not significantly different from the control sample(Table 2). The least mean THB counts was $1.97\times 10^4$ cfu/g in dry soil (at Adolor) while the highest mean THB counts were $9.43 \times10^4$ cfu/g in amended soil (Fig.1a). A further comparison of all

contaminated soil samples with the control soil showed that THB counts was less in the contaminated soil with a value of 5.10x $10^4$ cfu/g than in the control sample with 5.12x $10^4$ cfu/g as in (Fig. 1a).This indicates that battery wastes contamination reduces THB counts.

***Battery waste utilizing bacterial (BUB) counts***
The mean bacteria counts in the battery-wastes contaminated soil samples designated as dry soil 1, dry soil 2 and wet soil;2.l3 × $10^4$cfu/g, 2.53 ×$10^4$cfu/g and 2.87 ×$10^4$ cfu/g respectively were significantly higher than in the control soil sample 0.7 ×$10^4$cfu/g. The soil samples amended with battery waste had bacterial countsof1.27×$10^4$cfu/g which was also significantly (P<0.001) more than the control soil sample. Hence, the bacteria load counts in uncontaminated soil was significantly (P<0.001) less than contaminated and amended soil samples. The presence of more bacterial counts could be connected to contamination in the contaminated soil samples as many of bacteria aid biodegradation. The least mean bacteria count was 0.7 x$10^4$ cfu/g in the uncontaminated soil (control) while the highest mean THB count was 2.87 ×$10^4$ cfu/g wet soil at Uwelu (Fig.1a).

***Total Fungal Counts (TFC)***
The mean fungal viable counts in the battery wastes contaminated soil samples designated as dry soil I, dry soil 2 and wet soil were 0.73×$10^4$ cfu/g, 1.27×$10^4$ cfu/g and 2.5×$10^4$ cfu/g respectively. The mean fungal count in uncontaminated (control soil) was 4.47×$10^4$cfu/g. Hence, the fungal counts in uncontaminated soil was significantly higher than the contaminated and amended soil samples (P<0.00l). Even, in the soil samples amended with battery waste, the fungal count 3.4×$10^4$cfu/g was also significantly lower than the control soil (P<0.001). However, like in the bacteria count, there was no significant difference in fungal count recorded in the dry soil samples that were collected from different locations (Adolor and Edaiken) in Benin City (Table 2). The least mean fungal count was 0.73 ×$10^4$ cfu/g while the highest mean THB count was 4.47×$10^4$ cfu/g (Fig1a) but the average mean values of the soil samples was 1.96x $10^4$ cfu/g over the control value (Fig. 1b)

Comparatively, it is apparent that wet soil has significantly higher microbial density than dry soil in any battery contaminated soil especially in microbial and fungal count recorded (Fig 1a).

***Identification of bacterial and fungal isolates***
The bacterial isolates obtained from battery wastes contaminated soil samples were *Chromobacterspp*, *Staphylococcus spp*, *Micrococcus spp*, *Streptococcus spp*, *Bacillus spp*, *Pseudomonas spp* and *Escherichia coli*
The fungal organisms isolated from this study include*Penicillium* spp, *Mucor spp*, *Fusarium spp*, *Rhizopus* spp, *Geotrichium spp* and *Aspergillus spp*

Table 2: Effect of battery waste on microbial count in soil Qualities.

| Samples | THB | BUB | TFC |
|---|---|---|---|
| Uncontaminated soil | 5.10±3.89 | 0.70[d]±0.12 | 4.47[a] ±0.20 |
| Dry soil 1 | 1.97±0.33 | 2.13[b]±0.2 | 0.73[d]±0.09 |
| Dry soil 2 | 2.7±0.40 | 2.53[b]±1.20 | 1.27[d] ±0.15 |
| Wet soil | 6.33±0.75 | 2.87[a]±1.86 | 2.5[c] ±1.15 |
| Amended soil | 9.43±1.76 | 1.27[c] ±0.15 | 3.4[b]±2.516 |

Results are expressed in Mean ± SEM and value is in (cfu/g) x$10^4$
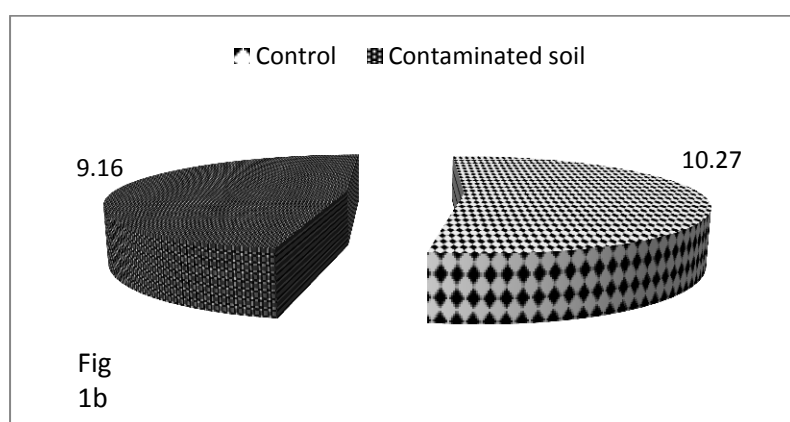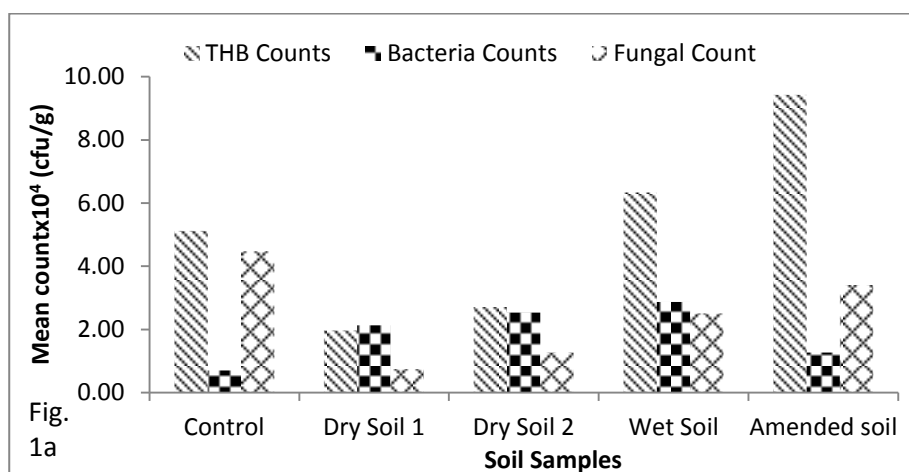Different letters across the column show that the mean are significant from each other

Fig.
1a



Fig
1b

Fig.1a-b. The mean microbial counts (x**10⁴**) in contaminated and control

Relatively low microbial counts were found in contaminated soil. Though, THB count in the contaminated soil ranged between $1.97\pm0.33x~10^4$ and $9.43\pm1.76x~10^4$ while the control has $5.10\pm3.89x10^4$cfu/g (as shown in Table 2). The mean THB counts were lower in the naturally contaminated soil than in uncontaminated soil sample (control). This result was in line with [13] who reported that there was a relatively low heterotrophic bacterial count observed in oil, and so attributed it to the toxic or un-favorable effect of oil contamination. In this case, it is applicable to battery wastes in the soil.

However, battery waste utilizing bacteria were significantly more in the contaminated soil an indication that they can adapt to toxic environment than other micro-organisms. However, wet contaminated soil has a higher potential to retain more bacteria than the dry contaminated soil (Table 2).

Fungal counts in all contaminated soil and amended soil samples were significantly lower (P<0.001) than uncontaminated (control) soil sample (Table 2). Fungal count $3.4x~10^4$cfu/gin the soil samples amended with battery waste, was also significantly lower (P<0.001) than the control soil; $4.47x~10^4$cfu/g. This indicates that fungal load is most abundant in the uncontaminated soil but reduces as the soil becomes more toxic. It was also observed that wet contaminated THB count soil has more fungal load than the dry contaminated soil samples. Oliveira and Pampulha [1] reported decrease in microbial loads of contaminated oil compared to uncontaminated oil and this result agrees with the report.

The bacterial isolates obtained from this study include *Pseudomonas spp*, *Bacillus spp*, *Streptococcus spp*, *Micrococcus spp*, *Staphylococcus spp*, *Chromobacter spp* and *Escherichia coli* (Table *2).* Meanwhile, the fungal organisms isolated from this study include *Penicillium* spp, *Mucor spp*, *Fusarium spp*, *Rhizopus* spp, *Geotrichium spp* and *Aspergillus spp* (Table 2). The presence of these isolated microorganisms in the battery contaminated soil ascertains that the bacterial and fungal species exhibit ability to tolerate the high level of heavy metals in contaminated soil samples.

### Conclusion

The result of this study suggests that battery wastes reduce microbial population and its activity. It is therefore detrimental to both agronomical crops and environmental public health.

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