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# Bacteriological and Physicochemical Assessment of Top Soils Collected from a Commercial Oil Palm Plantation and an Open Dumpsite

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**Abstract:** Standard bacteriological methods and physico-chemical procedures were utilized in the determination of the isolation and enumeration of the culturable heterotrophic bacterial flora, MCPA utilizing bacteria and physico-chemical profile of top soils collected from a variety of edaphic sites under varying anthropogenic usages. The mean heterotrophic bacterial count ranged from $3.5 \times 10^4$ - $1.15 \times 10^5$ cfu/g. No MCPA-utilizing bacterial counts were recorded for all the soils after seven days. The observed differences in the mean counts were not significant (*P>0.05).* Seven (7) tentatively identified bacterial isolates were characterized and they include *Bacillus alvei, Bacillus licheniformis, Bacillus polymxa, Achromobacter xylosoxidans, Aeromonas hydrophila, Klebsiella oxytoca* and *Pseudomonas mendocina*. *A. xylosoxidans* had the highest percentage frequency of occurrence while *P mendocina* was the least isolated soil borne bacterial specie. All the soils were both acidic and sandy. The ammonia-nitrogen and phosphate data varied from 6.12 - 17.80 mg/kg and 19.09 - 56.23 mg/kg respectively. The non-isolation of MCPA utilizing bacterial species from the soils using MCPA-MSA was reflective of the unsuitability of the culture medium in the preliminary recovery of this unique group of herbicide degrading bacteria.

**Keywords**: Top soils, MCPA utilizing, Bacteriological, Culturable, Counts, Anthropogenic usages

## Introduction

Modern agriculture has been reported to be heavily reliant on herbicide application for the weed control in crops and pastures in order to maximize yields and economic benefits so as to sustain an increasing world population (Zabaloy *et al.*, 2011). Buhlar *et al.* (2000) reported that despite modern control practices aimed at weed elimination, weed growth has continued to be a ubiquitous and recurrent threat for crop production due to its ability to shift in response to management practices and environmental conditions. It is desirable to use chemicals to control weeds during the season of application, but they should not remain in the soil long enough to affect subsequent crop growth. Hager and Nordby, (2007) opined that the length of time that an herbicide remains active in the soil is called soil persistence or soil residual life. The authors also reported that several physical, chemical and microbial factors play a key role in determining the persistence of applied herbicides in the environment. Herbicides are a group of compounds that, in spite of their benefits, may produce a wide range of toxic side effects which pose a potential threat to the environment (Kanissery and Sims, 2011). Graymore *et al.* (2001) indicated that the extensive use of herbicides can pose far-reaching consequences because of the potential runoff and leaching of these compounds through the soil leading to contamination of surface and ground water. Apart from this, literature suggests an array of possible toxicological implications of the persistent herbicides in the ecosystem (Buchanan *et al*., 2009). The environmental fate of herbicides is a matter of concern given that only a small fraction of the chemicals reach the target organisms (Pimentel, 1995), leading to potential impacts of residual herbicides in soil and water on human, animal and crop health (Zabaloy *et al.*, 2011).

Phenoxyalkanoic acids such as 4-chloro-2-methylphenoxyacetic acid (MCPA) are widely used in agriculture and forestry as herbicides against dicotyledonous weeds. Trade names for some products containing MCPA are Agritox™, Agroxone™, Chiptox™, Rhonox™ and Weed-Rhap™ (Wikipedia, 2013). The decomposition of MCPA in soil has been reported to be slow (Thorstensen and Lode, 2001). MCPA has been regarded as one of the most widely used pesticides for the control of broad-leaf weeds primarily in cereal and grass seed crops since World War II (Mortessen and Jacobsen, 2004). Although herbicides from this group are applied as salts or esters, they are readily hydrolyzed and found in their acidic forms in soils (Tadeo *et al*., 1996). MCPA, like many other xenobiotic compounds is not readily degraded in nature but it has been reported to be adsorbed weakly to soil, with a degradation half-lives usually in the range of 3-16 days (Thorstensen and Lode, 2001). However, adverse conditions such as acidic pH and low temperatures are known to increase its persistence in the soil (Thompson *et al*., 1984).

Phenoxyalkanoic acids degrading bacteria from different genera, such as, *Alcaligenes* (Don and Pemberton, 1985), *Flavobacterium* and *Pseudomonas* have been documented (Baelum *et al*., 2006). Majority of these genera were isolated from soils, sludge, waste water and lake water. The degradation of phenoxy herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D), MCPA, and related compounds has been studied extensively during the last 40 years, with the majority of the work being on 2,4-D (Baelum *et al*., 2006). In this study, the bacteriological and the selected physico-chemical status of top soils collected from a variety of sites under varying anthropogenic uses was evaluated. An objective of this research was to investigate the potential presence and distribution of culturable MCPA bacterial utilizers from these sites using a selective culture medium.

## Materials and methods

*Description of Sampled sites*: The sampled areas (Fig 1) were located in two Local Government Areas of Edo State; Ikpoba Okha and Ovia North-East LGA respectively. The locations were a commercial Oil Palm Plantation (located in Ikpoba Okha LGA), Edo State Municipal open waste dump site and fallow land close to the Biological Annex, Faculty of Life Science, University of Benin, Benin City (both located in Ovia North East LGA). Four (4) soils samples were sourced from the oil palm plantation, three (3) samples and a single (1) sample were collected from the dumpsite and the fallow land respectively. These areas are known to experience high intensity rainfall and the minimum and maximum temperature of the area ranged between $27^0$C and $31^0$C and with a rainfall between the months of April and November. The total annual rainfall ranged between 1700 and 2000 mm.

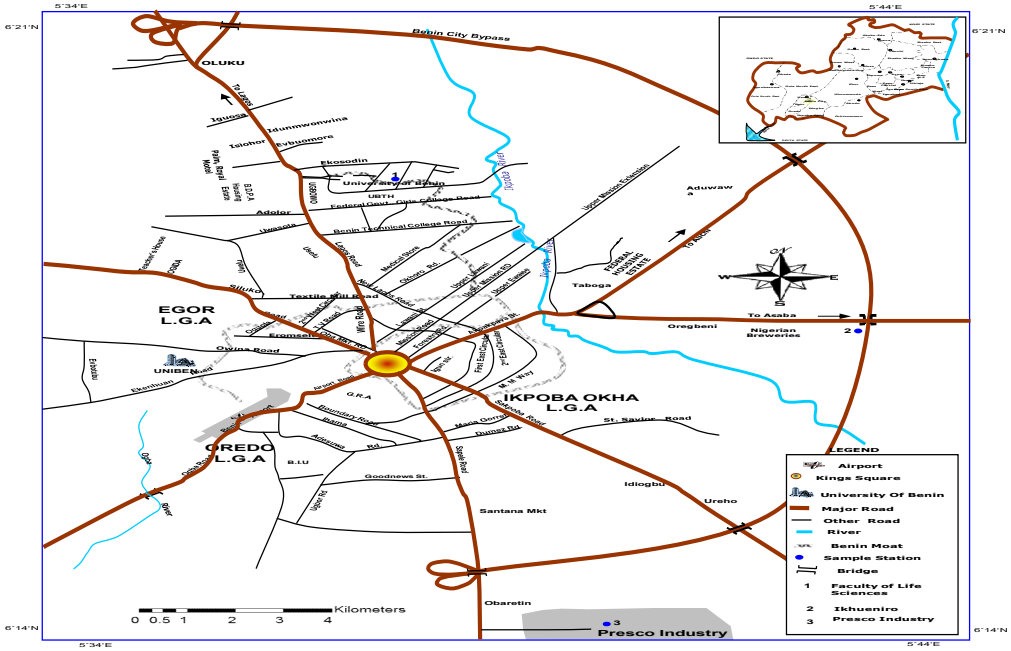


**FIG. 1. MAP OF BENIN METROPOLIS SHOWING SAMPLE STATIONS**

*Drawn by: Ukrakpor Ajiri, Department of Geography & Regional Planning, University of Benin, Benin City.*

*Collection of Soil Samples*: Top soils (0 – 15 cm) were collected with the aid of a clean soil auger. About 100g of the respective soils were bored from each sampling point and the top soils were collected in duplicates. The soils from the respective sampling points were collected in duplicates. The soils were transferred onto sterile labeled polyethylene bags and taken to the laboratory for bacteriological and physico-chemical analyses.

*Determination of the soil borne heterotrophic bacterial and MCPA-utilizing bacterial counts*: One (1) gram of the respective fresh soil samples were weighed and dissolved into 99 ml of sterile prepared peptone water diluent under aseptic conditions (Harley and Prescott, 2002). Serial fold dilutions were then made up to $10^{-5}$ and aliquots of each dilution were cultured on plates of Nutrient Agar for mean heterotrophic bacterial count by pour plate method (Sharma, 2009). Mineral salt agar (MSA) (Mills *et al*., 1978), modified by the addition of 1% ($^{w}/_{v}$) MCPA was used for the isolation of MCPA utilizing bacteria. The pH of the medium was adjusted to 7.2 with the aid of a calibrated Suntex pH meter SP 701(IMJI Temp., USA). For the selective isolation of MCPA utilizing bacteria, 1ml of an antifungal agent (Nystatin- 500mg in 50ml distilled water) was pipetted on the respective nutrient agar plates before the addition of the cool molten modified mineral salt agar (MMSA). Plating was done in duplicates, the agar plates were allowed to cool and incubated at 35 $^{O}$C for 48 h whilst the Modified Mineral salts agar plates were incubated at 35 $^{O}$C for 72 h. Resultant discrete bacterial colonies were enumerated and their colonial morphology was observed. The mean colony forming units per gram of the soil was derived according to a formula stated by Aneja (2003). Unique bacterial colonies were sub cultured onto freshly prepared nutrient agar plates and slants using sterile inoculating loop under aseptic conditions (Aneja, 2003). Appropriate physiological (cultural, morphological) and biochemical (Oxidase, indole, sugar fermentation, catalase, Vogues Praskeur.) tests were conducted to ascertain the identity of the isolates (Harley and Prescott, 2002; Aneja, 2003).

*Physicochemical analyses of the soil samples*: The physiochemical properties of the various soil samples were determined. With the exception of moisture content analysis, the respective soil samples were placed on large wooden trays and air-dried for 72 hr. Lumps of moist soil samples were broken by hand prior to air drying of the samples. The air dried samples were also sieved using a 2mm mesh. Parameters which included moisture content, pH and particle size distribution were ascertained using procedurees described by Kalra and Maynard (1991) and Skroch *et al*. (2006). The Ammonium-Nitrogen ($NH_4$-N), and Phosphate ($PO^{3-}_4$) content were evaluated using methods described by Onyeonwu (2000). The Total Organic Carbon (TOC) content of the respective samples was elucidated according to proceduree described by Bremmer and Mulvaney (1982).

*Statistical analysis*: The non parameteric statistical test; Kruskal Wallis Test was used to check for significant difference at 0.05 probability level for the soil bacterial counts. Ninety five percent (95%) probability was considered statistically significant.

## Results

The results of the bacteriological analysis of the soils are presented in Table 1. The mean heterotrophic bacterial count ranged from $3.5\times10^4$ cfu/g for soil B to $1.15\times10^5$ cfu/g for both soil E and the control soil respectively (Table 1). Nil MCPA-utilizing bacterial counts were recorded for all the soils after seven days (Table 1). The observed difference between the heterotrophic bacterial counts were statistically insignificant (*P>0.05*).

**Table 1:** Mean heterotrophic and MCPA utilizing bacterial counts of the top soils.

| | **Mean counts (cfu/g) ± Std. deviation** | |
|---|---|---|
| **Soils** | **THBC (cfu/g)** | **MCPAUBC (after 7 days)** |
| A | $^{a}2.0 \times 10^5 \pm 0.2$ | Nil |
| B | $^{a}3.5 \times 10^4 \pm 0.1$ | Nil |
| C | $^{a}0.8 \times 10^5 \pm 0.1$ | Nil |
| D | $^{a}4.9 \times 10^5 \pm 0.2$ | Nil |
| E | $^{a}1.15 \times 10^5 \pm 0.1$ | Nil |
| F | $^{a}7.5 \times 10^4 \pm 0.1$ | Nil |
| G | $^{a}6.5 \times 10^4 \pm 0.2$ | Nil |
| Control | $^{a}1.15 \times 10^5 \pm 0.1$ | Nil |

**Key**: THBC; Total heterotrophic bacterial count, MCPA-UBC; MCPA utilizing bacterial count, A – D: Top soils obtained from the commercial oil palm plantation, E – G; Top soils collected from municipal open dump site, Control: Top soil collected from a fallow site located in the University of Benin, Means preceded by alphabet "a "are not significantly different *(P>0.05)* from each other.

Seven (7) tentatively identified bacterial isolates were characterized from the soils (Fig. 2). The isolates were; *Bacillus alvei*, *Bacillus licheniformis*, *Bacillus polymxa*, *Achromobacter xylosoxidans*, *Aeromonas hydrophila*,

*Klebsiella oxytoca* and *Pseudomonas mendocina* (Fig. 2). Amongst the soil borne cultures, *A. xylosoxidans* had the highest percentage frequency of occurrence while *P mendocina* was the least isolated bacterial specie from the soil samples (Fig. 2).

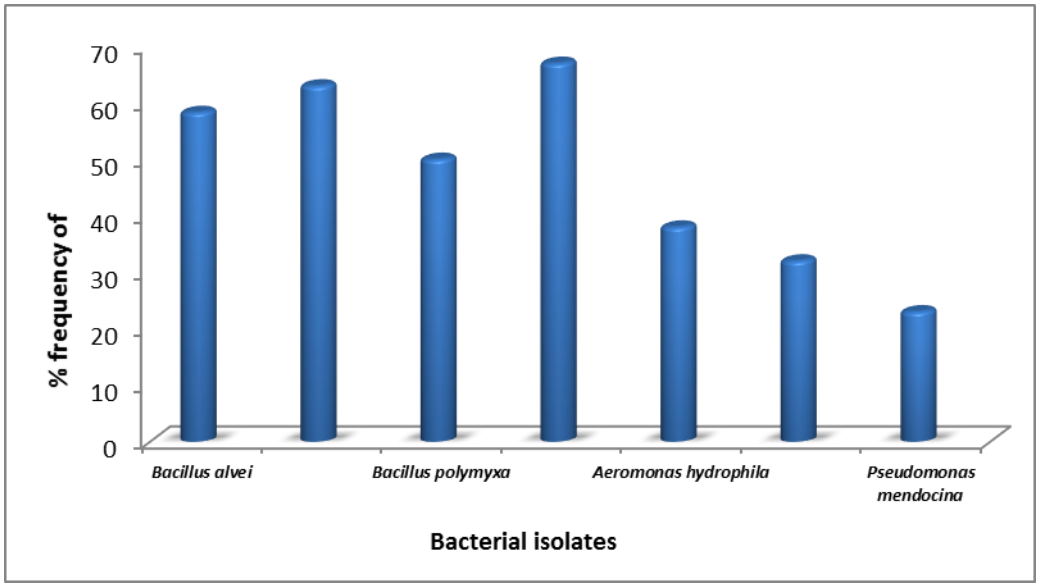


**Fig. 2:** Percentage cumulative frequency of isolation of the identified bacterial cultures

The results of the physicochemical evaluation of the soils are shown in Table 1. The pH values ranged from 5.2 for soil D to 6.9 for soil E (Table 2). The ammonia-nitrogen data varied from 6.12 mg/kg for soil D to 17.80 mg/kg for soil B (Table 2). The phosphate values ranged from 19.09 mg/kg to 56.23 mg/kg. The total organic carbon (TOC) concentrations ranged from 0.235% for soil D to 2.112% for soil E. Moisture content ranged from 1.5% for soil G to 2.8% for soil A. The clay and silt contents ranged from 2.0% for soil F to 3.5% for soil A and from 3.4% for soil G to 7.5% for soil A respectively. The sand value varied from 89% for soil A to 95.0% for soil G (Table 2)

**Table 2:** Physicochemical properties of the top soils obtained from the sampled areas

| Soil samples | pH | $NH_4$-N (Mg/kg) | $PO^{3-}_4$ (Mg/kg) | TOC (%) | Moisture (%) | Soil particle size | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Clay (%) | Silt (%) | Sand (%) |
| A | 6.7 | 13.78 | 56.23 | 1.081 | 2.8 | 3.5 | 7.5 | 89.0 |
| B | 6.4 | 17.80 | 44.12 | 0.832 | 2.5 | 2.6 | 6.5 | 91.3 |
| C | 5.8 | 11.34 | 30.23 | 0.563 | 1.8 | 2.1 | 5.2 | 92.7 |
| D | 5.2 | 6.12 | 19.09 | 0.235 | 1.7 | 2.2 | 4.0 | 93.8 |
| E | 6.9 | 17.05 | 50.54 | 2.112 | 2.3 | 2.3 | 4.4 | 93.3 |
| F | 5.9 | 8.34 | 24.90 | 0.978 | 1.8 | 2.0 | 4.4 | 93.6 |
| G | 5.7 | 9.28 | 48.04 | 0.893 | 1.5 | 3.4 | 3.4 | 95.0 |
| Control | 6.4 | 11.09 | 51.09 | 0.352 | 2.7 | 2.6 | 7.1 | 90.3 |

**Key:** TOC= total organic carbon, A-D: Top soils collected from the commercial oil palm plantation, E-G: Top soils obtained from municipal open dump site, Control: Top soil collected from a fallow site located in the University of Benin.

## Discussion

Soils collected from both the municipal open dumpsite (soil E) and the control had the highest culturable bacterial bio-load (Table 1). A similar observation was reported by Osazee *et al.* (2013) in respect to top soils obtained from four (4) open municipal dump sites located in Edo State. The authors opined that a possible reason for the high microbial bio-load of these soils was the increased availability of biodegradable organic and

inorganic substrates from the variety of municipal wastes continuously being dumped at these sites. All the preliminary MCPA utilizing bacterial counts using MCPA-MSA as selective culture medium were negative (Table 1). This trend could be as a result of the unfavorable micro-environment provided by the selective medium; MCPA-MSA. Soil microbial community structure and activity is largely dependent on the status of their soil habitat (Balser *et al*., 2010). All the soils were both acidic and sandy (Table 2). Odu *et al*. (1985), reported that soils with acidic pH levels tend to have an increased micronutrient solubility and mobility as well as increased heavy metal concentration Bhattacharya *et al*. (2002) observed that parameters such as; pH, TOC and particle size distribution are among several components of soil that are known to affect the availability, retention and mobility of metals. The sandy nature of the examined soils was in tandem with an earlier report by Osazee *et al.* (2013) which indicated that all the top soils collected from several open dumpsites and a fallow farmland within the University of Benin premises were sandy in texture. Oyedele *et al.*, (2008) reported that the textural attribute of a particular soil is primarily derived from the soil forming materials. Also several authors have reported that the particle size distribution and pH of soils are known to have a significant impact and influence on the diversity and activity of the microbial community present in the soil (Poll *et al*., 2003; Balser *et al*., 2010).

The bacteriological and selective physicochemical evaluation of top soils collected from edaphic locations under differing anthropogenic usages indicated the presence of viable heterotrophic bacterial bio-load and the absence of MCPA utilizing bacterial species. The study also revealed the acidic characteristics and the variations in the macro nutritional status of the analyzed soils

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