



## Ecological Risk and Bacteriological Assessment of Top Soil Samples from Lairage Locations in Benin Metropolis, Nigeria

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### Abstract

*This study assessed the ecological risk and bacteriological qualities of soil samples from lairage locations in Benin metropolis. Standard analytical procedures and pour plate technique were employed in the determination of the physicochemical, heavy metal and heterotrophic microbial flora of the topsoils. Ecological risk was assessed adopting standard mathematical equations. The pH values range from 6.04 – 7.73, electrical conductivity range from 931 – 3100 ( $\mu\text{S}/\text{cm}$ ), percentage organic carbon mean values were 7.29 % -14.6 % and ammonium nitrogen values ranged from 5.21 mg/kg – 24.5 mg/kg. Iron had the highest concentration range from 322.3 – 573.2 - 573.2 mg/kg. Manganese levels ranged from 2.92 - 29.6 mg/kg; range concentrations for copper, chromium and lead were 0.28 -3.10 mg/kg, 0.19 – 1.50 mg/kg and 0.11-2.18 mg/kg respectively. Pollution load index results of this studied soil samples were greater than 100, thus indicating that heavy metal pollution require immediate remediation action. Potential ecological risk index for individual heavy metals showed slight degree of ecological risk as values were less <40, with lead having ecological risk value of 10.62. The total heterotrophic bacterial and fungal counts range from 2.3 – 16.1 ( $\times 10^4$  cfu/g) and 0.6 – 3.6 ( $\times 10^4$  cfu/g) respectively. The predominant microbial isolates were species of Micrococcus, Bacillus, Pseudomonas, Escherichia, Staphylococcus, Klebsiella and Enterobacter Aspergillus, Mucor, Penicillium and Trichoderma. This study hereby strengthens the concern that administrators and owners of lairages should monitor and manage heavy metals pollution.*

## 1. Introduction

Facilities where animals are kept for sales, abattoir and treatment are regarded as lairage [1]. Lairages are designed to ensure that animals are kept in batches for proper identification by owners and animal welfare monitors and avoid stress on the animals [1, 2].

Soil is a naturally occurring unconsolidated or loose material on the surface of the earth, capable of supporting life. It is a small ecosystem consisting of both living and no-living matter [3]. Soil fertility is determined by the organic and inorganic matter in the soil, the proliferation of various microflora that play vital roles in maintaining the nutritional balance of the soil is aided. The top soil is where most of the organic matter and microorganisms activities take place, and also where most of the earth's biological soil activity occurs [4]. Soil is a basic component of the environment, composing of active and dynamic system of chemical, physical and biological activities [5], which may emanate from anthropogenic or natural origin. Heavy metals in soil can

be redistributed in different chemical forms of variable bioavailability, mobility and toxicity [6, 7]. Aoyama and Tanaka [8] reported that heavy metal pollution do not occur only in soil around mining ores and smelters environment but also in agricultural soils that had been exposed to sewage sludge fertilizers and heavy metal-based pesticides. The usability of land can be limited by heavy metal contamination and other chemical components that are toxic to biological entities [9]. The release of substances with toxic components into the environment is a reflection of severe environmental challenge and a source of human health exposure due to toxicants which include heavy metals. There had been rise in environmental pollution due to amplified steady waste generation of domestic, industrial and agricultural origin [10]. Heavy metals have a great ecological significance and due to their toxicity and accumulative behavior. This study was aimed at assessing the ecological and microbiological quality soil samples collected from lairage location in Benin City metropolis, Nigeria.

## 2. Methodology

### 2.1 Study locations and sample collection

The studied soil samples were collected from two lairage locations (Figures 1 and Plates 1) with GPS coordinates of Latitude 6°22'54.5" N; Longitude 5°37'32.9" E (Benin-technical College cattle market), Latitude 6°19'37.8" N; Longitude 5°34'45.4" E (Asoro hill Cattle market) in Benin City, Nigeria. The samples were air dried for five days and thereafter crushed to small particles to allow free passage through a 2mm sieve. The resultant homogenous samples were analyzed for physicochemical and microbiological qualities [11].

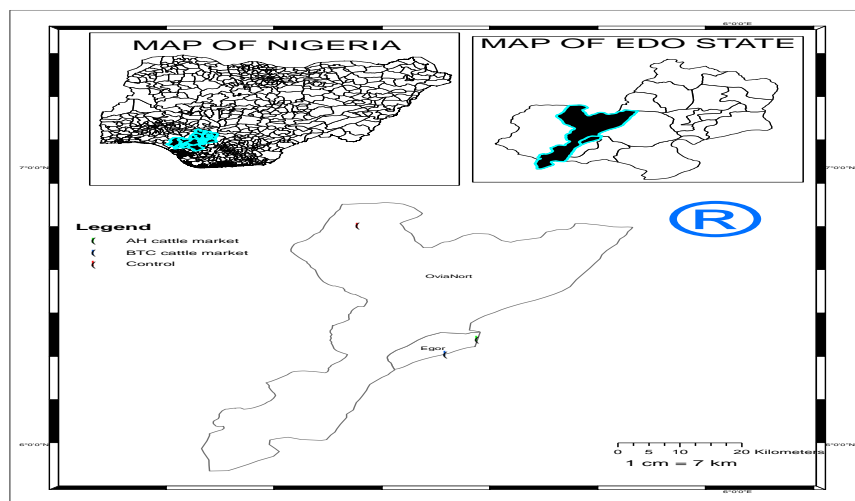


Figure 1: Map showing the locations in Edo State, Nigeria

### 2.2 Physicochemical Analysis

The parameters (pH, electrical conductivity, ammonium-nitrogen and total organic carbon) were analyzed using standard analytical methods [11].

### 2.3 Determination of heavy metals

The samples were analyzed for some heavy metals (iron (Fe), lead (Pb), manganese (Mn), zinc (Zn), chromium (Cr) and copper (Cu)) using an Atomic Absorbance Spectrophotometer (AAS) (Solar 969 Unicam series, UK) [11].



Plate 1: Pictorial view of a leriage location in Benin City

#### 2.4 Ecological Risk Assessment

Ecological risk assessment was conducted on the sampled soils to determine the level of risk associated with heavy metals detected in the soil samples. Contamination factor, degree of contamination, pollution load index and ecological risk index were used as risk analysis tools [12, 13].

#### 2.5 Contamination factor

The level of metal contamination in a particular matrix is expressed using contamination factor;

$$CF = C_{\text{metal}} / C_{\text{background}} \quad (1)$$

Where:  $C_{\text{metal}}$  is the concentration of a particular metal in the soil and  $C_{\text{Background}}$  is a metal concentration of a reference sample [13]. The CF values for describing the contamination levels are summarized in Table 2A.

**2.6 Degree of contamination:** Degree of contamination ( $C_d$ ) is used to express the extent of overall contamination of soil sample by all analyzed heavy metals. It is calculated as the summation of contamination factor of the individual metals.

$$C_d = \sum Cf. \quad (2)$$

According to [12], degree of contamination ( $C_d$ ) is classified into four groups as shown in Table 2B.

#### 2.7 Pollution load index (PLI)

Pollution load index is used to determine the degree of overall contamination at the sampled site. PLI is able to give an overview of the metal contamination status of a polluted site and suggest appropriate measure to be taken to mitigate the pollution. According to [13], PLI value of  $\geq 100$  is an indication that action should be taken immediately to curtail the pollution; value  $\geq 50$  means that further monitoring of the site should be done while PLI value  $< 50$  indicates that no measure is needed.

$$PLI = \sqrt{Cf_1 + Cf_2 + Cf_n} \quad (3)$$

where Cf is contamination factor of specific metal,  $C_n$  is contamination factor of nth metal [13].

## 2.8 Potential Ecological Risk Index (PERI)

According to [13] potential ecological risk index is used to assess the level of harm done by metals in the studied environment. PERI can be calculated for both single metal and comprehensive overall metals analyzed in a study.

Single factor PERI can be calculated as;

$$E_R^i = C_f^i \times T_R^i, \quad (4)$$

where  $C_f^i$  is the pollution index which is obtained by,  $C_f^i = C_s^i / C_n^i$  ( $C_s^i$  is the concentration of metal in the sample and  $C_n^i$  is concentration of metal in the control sample.  $T_R^i$  is the toxicity response coefficient of single metals,  $T_R^i$  for Zn, Cu, Cr and Pb is 1,5,20 and 5 respectively. The comprehensive PERI was calculated using;

$$RI = \sum E_R^i, \quad (5)$$

Where  $E_R$  is the ecological risk index of single metals. Based on the calculated Ecological risk index, the grading standards of PERI can be obtained (Table 2C).

## 2.9 Enumeration and isolation of heterotrophic bacteria and fungi

The enumeration and isolation of soil heterotrophic bacterial and fungal population was carried according to [14, 15, 16].

## 3.0 Results and Discussion

### 3.1 Physicochemical analysis

The result of the collected soil samples is shown in Table 1. There were relative variations between studied soils, which clearly indicated alteration of the physicochemical qualities of the soil, probably due to contamination. The pH values range from 6.04 – 7.73. Akinnibosun and Ayejuyoni [17] and Aighewi *et al.*[18] had earlier suggested that the pH of a soil from a lairage location can be greatly influence by the presence of dissolved potassium chloride and sodium chloride in animal manures. The electrical conductivity of the collected soil samples ranges from 931 – 3100 ( $\mu\text{S}/\text{cm}$ ). The percentage organic carbon mean values were 7.29 % -14.6 %. The ammonium nitrogen values ranged from 5.21 mg/kg – 24.5 mg/kg. Results of the heavy metals contents of the locations and control soils contain relative levels of toxic metals and variations in composition among the analysed samples. Iron had the highest concentration which range from 322.3 – 573.2 -573.2 mg/kg. Manganese levels ranged from 2.92 - 29.6 mg/kg; range concentrations for copper, chromium and lead were 0.28 -3.10 mg/kg, 0.19 – 1.50 mg/kg and 0.11-2.18 mg/kg respectively. The presence of high concentrations of toxic heavy metals in soils and water bodies must be checked and properly managed due to their public health implications to humans and other animals; and consequence on the food chain transfer [19, 20].

### 3.2 Ecological risk assessment

Result of contamination factor for the six heavy metals showed that manganese and copper showed moderate level of contamination while iron zinc and chromium showed considerable level of contamination in the studied locations (Table 3). Contamination degrees were of considerable levels, an indication that heavy metals analyzed in lairages are collectively of high levels. Pollution load index results of this studied soil samples were greater than 100, thus indicating that heavy metal pollution require immediate remediation action. This clearly indicates that the soils were highly polluted with the heavy metals as they have exceeded the acceptable background levels. This is likely to be as a result of the continuous contamination of the lairage soil with heavy metals from multiple sources during operation. The animal excreta may be one source depending on the composition of these metals in the feed. Another source maybe their drinking water. Potential ecological risk index for individual heavy metals showed slight degree of ecological risk as values were less <40, with lead having ecological risk value of 10.62. PERI

value for all metals collectively showed medium level of ecological risk (risk level B). The individual metal ecological risk result showed that heavy metals posed slight potential risk to the soil. The main metal that could cause risk to the soil was lead with Er value of 10.62. Soils in this study were in medium ecological risk as ecological risk indices were above 40 but less than 60.

### **3.3 Enumeration and isolation of heterotrophic bacteria and fungi**

The total heterotrophic bacterial and fungal counts range from 2.3 – 16.1 ( $\times 10^4$  cfu/g) and 0.6 – 3.6 ( $\times 10^4$  cfu/g) respectively. The predominant bacterial isolates (Table 4) were *Micrococcus luteus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* sp. and *Enterobacter aerogenes*. The predominant fungal isolates (table 4) were species of *Aspergillus*, *Mucor*, *Penicillium* and *Trichoderma*. These microbial isolates had been implicated as common spoilage organisms in the beef industry and are known soil inhabiting microorganisms [17, 21]. The observed high microbial counts and diversity may be due to favourable pH and high deposition of organic wastes in the soil [17, 20].

### **4.0 Conclusion**

In conclusion, this study hereby strengthens the concern that administrators and owners of lairages should monitor, manage heavy metals pollution.

**Table 1: Physicochemical qualities of soil samples from liirage locations in Benin City, Nigeria.**

Parameter	Month										Control
	November, 2016		December, 2016		January, 2017		February, 2017		March, 2017		
	BTCM	AHCM	BTCM	AHC M	BTCM	AHC M	BTCM	AHC M	BTCM	AHC M	
<b>pH</b>	6.72	7.53	6.46	7.10	6.30	6.27	7.41	7.73	6.04	6.30	5.72
<b>EC (<math>\mu\text{S}/\text{cm}</math>)</b>	1240	931	1946	1150	2050	1841	2740	3100	2230	2520	157.7
<b>Organic Carbon (%)</b>	9.38	7.46	12.4	10.9	17.3	14.6	11.7	8.94	9.54	7.29	1.11
<b>NH<sub>4</sub>N (mg/kg)</b>	9.17	7.40	15.8	11.0	24.5	21.7	8.14	6.39	6.63	5.21	0.73
<b>Fe (mg/kg)</b>	526.0	345.6	474.2	411.5	573.2	498.3	395.5	410.9	322.3	334.9	112.5
<b>Mn (mg/kg)</b>	6.48	2.92	19.5	21.2	15.4	10.7	29.6	21.4	24.1	17.4	6.30
<b>Zn (mg/kg)</b>	56.7	23.4	70.3	59.5	94.2	88.0	67.2	42.5	54.8	34.6	10.2
<b>Cu (mg/kg)</b>	0.51	0.28	0.68	0.71	1.47	1.13	1.47	3.10	1.20	2.53	0.41
<b>Cr (mg/kg)</b>	0.34	0.19	0.27	0.12	0.96	1.50	0.74	0.55	0.60	0.39	0.11
<b>Pb (mg/kg)</b>	0.16	0.11	0.36	0.47	0.75	1.33	0.82	2.18	0.67	0.78	0.35

Key: Benin Technical Cattle Market (BTCM), Asoro-Hill Cattle Market (AHCM)

**Table 2A: Contamination scale based on contamination factor**

Cf value	Classification
$Cf < 1$	Low degree of contamination
$1 \leq Cf < 3$	Moderate degree of contamination
$3 \leq Cf < 6$	Considerable degree of contamination
$Cf \geq 6$	Very high degree of contamination

**Table 2B: Levels of degrees of contamination**

$C_d$ class	Level of degree of contamination
$C_d < 8$	Low degree of contamination
$8 \leq C_d < 16$	Moderate degree of contamination
$16 \leq C_d < 32$	Considerable degree of contamination
$C_d \geq 32$	Very high degree of contamination

**Table 2C: Grading standard for ecological and potential ecological risk**

Ecological risk ( $E_R$ )	Pollution degree	PERI	Risk level	Risk degree
$E_R < 30$	Slight	$RI < 40$	A	Slight
$30 \leq E_R < 60$	Medium	$40 \leq RI < 80$	B	Medium
$60 \leq E_R < 120$	Strong	$80 \leq RI < 160$	C	Strong
$120 \leq E_R < 240$	Very strong	$160 \leq RI < 320$	D	Very strong
$E_R \geq 240$	Extremely strong	$RI \geq 320$	E	Extremely strong

**Table 3: Ecological risk assessment of studied soil**

Location and Metal	Contamination Factor (CF)	Degree of contamination (CD)	Pollution Load index (PLI)	Ecological risk (ER)	Potential ecological risk (PERI)
<b>BTCM</b>		20.57	187.3		33.16
<b>Fe (mg/kg)</b>	4.44			ND	
<b>Mn (mg/kg)</b>	2.60			ND	
<b>Zn (mg/kg)</b>	5.43			5.43	
<b>Cu (mg/kg)</b>	1.72			8.60	
<b>Cr (mg/kg)</b>	4.26			8.52	

<b>Pb (mg/kg)</b>	2.12			10.62
<b>ATCM</b>		20.09	203.92	43.21
<b>Fe (mg/kg)</b>	3.88			N.D
<b>Mn (mg/kg)</b>	2.01			N.D
<b>Zn (mg/kg)</b>	3.93			3.93
<b>Cu (mg/kg)</b>	2.50			12.50
<b>Cr (mg/kg)</b>	4.02			8.05
<b>Pb (mg/kg)</b>	3.75			18.73

**Table 4: Heterotrophic microbial counts for the respective soils sampled**

Parameter	Month									
	November, 2016		December, 2016		January, 2017		February, 2017		March, 2017	
	BTCM	AHCM	BTCM	AHCM	BTCM	AHCM	BTCM	AHCM	BTCM	AHCM
<b>THBC (10<sup>4</sup> x cfu/g)</b>	2.3	3.2	3.4	5.8	3.4	5.8	12.4	11.6	16.1	7.1
<b>THFC (10<sup>4</sup> x cfu/g)</b>	0.7	0.6	1.5	1.5	0.8	1.1	3.6	2.5	2.7	3.0



**Table 5: Distribution of microbial isolates in soil samples**

Isolates	November to December, 2016		January to March, 2017	
	A(BTCM)	B (AHCM)	A (BTCM)	B (AHCM)
<i>Streptococcus faecalis</i>	+	+	+	+
<i>Bacillus subtilis</i>	+	+	+	+
<i>Pseudomonas aeruginosa</i>	+	+	+	+
<i>Escherichia coli</i>	+	+	+	+
<i>Staphylococcus aureus</i>	-	+	+	+
<i>Klebsiella</i> sp.	+	-	+	-
<i>Enterobacter aerogenes</i>	-	-	+	+
<i>Micrococcus luteus</i>	-	-	-	+
<b>Fungi</b>				
<i>Aspergillus flacus</i>	+	-	+	+
<i>Aspergillus niger</i>	+	+	+	+
<i>Mucor</i> sp.	-	+	+	+
<i>Penicillium</i> sp.	+	+	-	+
<i>Trichoderma</i> sp.	-	-	+	+

KEY: A= Soil sample from Benin-Technical college road cattle market, B = Soil sample from Asoro Hill cattle market, + = Present, - = Absent, Benin Technical Cattle Market (BTCM), Asoro-Hill Cattle Market (AHCM)

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