Effects of Abattoir Effluents on Heavy Metal Tolerance, Bacteriological Quality and Physicochemical Parameters of Contaminated Soil in Yola, Adamawa State, Nigeria

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Abstract

This study was carried out to evaluate the effect of effluent produced from Yola abattoir on the heavy metals, the physicochemical parameters and the bacteriological quality of the contaminated soil. Stratified sampling technique was used to collect soil samples from the abattoir environment. Isolation of bacteria, cultural and biochemical characteristics were assessed using pour-plate and conventional techniques. Heavy metals presence was determined using atomic absorption spectrophotometer (AAS). Heavy metal tolerance by bacteria was done by agar plate method. The molecular identification was carried out using 16S rRNA gene of the bacteria. All data obtained were subjected to statistical analyses using analysis of variance (ANOVA) and t-test. Total bacterial count ranged from 6.19 x 10^5 and 8.50 x 10^5 CFU/mL. Bacterial species of *Pseudomonas*, *Klebsiella*, *Staphylococcus*, *Bacillus*, *Streptococcus*, *Staphylococcus* and *Escherichia coli* were isolated and identified. The highest mean value of the physicochemical parameters for pH, organic carbon, total nitrogen content, water holding capacity, total solid and total suspended solid of the effluent contaminated soil were 7.03, 7.97 %, 13.76 %, 2.48 %, 3346 g/cm, 1263 mg/L and 872 mg/L respectively. The minimum tolerance concentration of 50 ppm for copper, iron, zinc and cobalt was observed with a bacterium identified to be a strain of *Pseudomonas aeruginosa*. The isolated and identified *Pseudomonas aeruginosa* HBS_2 strain has the potential to be used in bioremediation.

Keywords: Abattoir Effluents, Soil Bacteria, Heavy Metals, Minimum Tolerance Concentration,

Introduction

Abattoir effluents have complex compositions that can be very harmful to the environment. The logging of abattoir effluent in the soil leads to the depletion of the available oxygen in the soil (Williams and Dimbu, 2015). Discharge of animal blood and improper disposal of animal dung lead to nutrient enrichment of the receiving system and increase the rate of toxic wastes accumulation in biological systems (Abubakar and Tukur, 2014).

Various organs of cattle such as muscle, blood, liver, kidney, viscera and hair have been found to contain heavy metals such as Lead (Pb), Copper (Cu), Iron (Fe), Arsenium (As) and Chromium (Cr) (Olarewaju and Olufayo, 2014). When untreated abattoir effluent or waste water are discharged into the environment and soil in particular, some elements such as iron, lead, phosphorus, calcium and zinc initially absent or present in insignificant quantities are introduced into the environment leading to the increase of these elements, thereby altering the pH and other physicochemical properties of the soil.

These elements when present in high concentration are lethal to microbial flora in the soil that are responsible for biodegradation or may lead to the exponential growth of pathogenic and resistant microorganisms (Odoemelan and Ajunwa, 2010). Higher concentrations of these metals above threshold levels have deleterious impact on the microbial communities and their functional activities in soils. Microorganisms exposed to higher concentrations of toxic heavy metals are likely to develop resistance to these metals (Olarewaju and Olufayo, 2014). There is evidence of a correlation between tolerance to heavy metals and antibiotic resistance, which has become a global problem currently threatening the treatment of infections in plants, animals and humans (Williams and Dimbu, 2015). Furthermore, the presence of heavy metals and organic compounds in the soil alter the quality of the soil and ground water (Azzam and Tawfik, 2015).

Microbial interactions with metals may have several implications for the environment. The microbes are known to be responsible for the biogeochemical cycling of toxic metals as well as remediating metal contaminated environments (Sterritt *et al*., 2008).

This study was carried out to evaluate the effects of abattoir effluents on heavy metals concentration in the receiving soil and their tolerance by bacteria as well as physicochemical nature of the receiving soil, as well as its impact on the bacteriological quality of the soil in Yola, Adamawa State, Nigeria.

Materials and Methods

Sample collections

Soil samples were collected using adapted method of NCR-13 standard soil scoop described by Brown (1998). The soil samples were collected within the abattoirs in Jimeta-Yola, Adamawa State, Nigeria (Latitude 9º13’ 9º17’N and Longitude 12º24’ and 12º 28’E). In brief, 10.0 g of well stirred soil was scooped at the center of the sample point. Excess was tapped off from the scoop. The soil samples were collected from effluent contaminated sites including blood bank site (BBS), Dung dumping site (DDS), bone dumping site (BDS), herds burning site (HBS), green grass site (GGS), pens site (PNS), selling point site (SPS) and waste water site (WWS). The control soil samples were collected 150 m away from the abattoir premises and were marked as CTS. Three (3) soil samples were collected from each of the points, packed in sterile labeled polythene bags and transported to the laboratory in the Department of Microbiology, Modibbo Adama University Technology, Yola for preparation and analyses.

Bacteriological analysis of the soil samples

The bacteriological analysis was carried out based on the method described by Rabah *et al. (*2013). Ten g of soil sample from abattoir was crushed and was diluted in 90 mL of sterile distilled water. This was serially diluted in ten fold up to 10^{-6} dilutions. Then 0.1 mL of aliquots from 10^{-3} to 10^{-6} dilutions were aseptically inoculated into already prepared plates of nutrient agar and MacConkey agar using pour plate technique and incubated at 37°C for 24 h. After the incubation period, plates with distinct colonies were counted and recorded as CFU/g. Distinct colonies were sub-cultured severally to obtain pure isolates. The formula below was used to calculate the microbial load in each case:

$CFU/g = 1/V \times N \times 1/D$

V = volume of inoculum

N = number of colonies counted

D = dilution factor

Morphological and biochemical characteristics of the bacterial isolates

The morphological and biochemical characteristics of the isolated bacteria were carried out using the methods described by Cheesbrough (2000). An 18-24 h pure culture of each bacterial isolate was used to carry out the following tests Gram reaction, oxidase, catalase, indole, methyl red, Voges Proskauer, motility, coagulase and urease. Citrate utilization was incubated at 37 °C for 48 h. Other biochemical tests were incubated at 37 °C for 24 h. The reaction to Gram staining and shape were observed under microscope with oil immersion (Cheesbrough, 2000).

Determination of heavy metal concentration in soil samples using atomic absorption spectrophotometer (AAS)

Sample preparation

Three soil samples each were prepared according to the description of Zhang *et al*., (2011). Samples were air-dried and thereafter ground in a mortar using pestle and sieved through a 2 mm sieve. The sieved soil samples were further subjected to Conning and Quartering method in order to obtain the required amount of soil for digestion. This process involves making a cone of the parent soil sample (the three soil samples from each of the nine sites), and then halving it. The half portion was thoroughly mixed, flattened and quartered, and then the two opposite portions were collected while the two alternate portions were discarded. From the opposite half, the procedures were repeated once to get the desired soil samples. After which the remaining two portions were finally mixed as one and they are stored in polythene bags under room temperature (Zhang *et al*., 2011).

Digestion procedure for Metal Determination (Cu, Co, Zn and Fe)

Various dilutions were prepared from 1000 ppm of stock solutions of copper, cobalt, zinc and iron (ppm). The dilutions were used for the preparation of standard calibration solutions. Aqua regia was used as the digestion reagent. This was prepared by mixing 20 mL of concentrated HCl and HNO_3 in the ratio of 3:1 (v/v). A 1.0 g portion of each of the soil sample was weighed into a beaker and 10 mL of freshly prepared aqua regia was added to the digest beaker containing the samples and were well shaken. They were placed into a fume cupboard and allowed to stand overnight. This was to allow for gaseous particles arising from any vigorous reaction in the beaker to be given off, which may have increased the pressure during digestion. The digestion beaker containing the sample that was mixed with aqua regia was digested on a hot plate (flame). Furthermore, at the end of the digestion, the beaker and the contents were allowed to cool, and filtered through a Whatman No 1 filter paper into 100 mL standard volumetric flasks. The sample residues were washed with deionized water up to 100 mL flask and transferred into plastic bottle. A blank solution was prepared by treating 10 mL of deionize water in the same manner. The metals copper, cobalt, zinc, and iron were determined by aspirating the standard solution and the blank. The digest (sample solution) were analyzed at various wavelength for copper (Cu), cobalt (Co), zinc (Zn) and iron (Fe) using Brick Model 210 VGP flame atomic absorption spectrophotometer with an air - acetylene flame (Obijiofor *et al.*, 2018).

Physicochemical analysis of the soil samples: The following physicochemical properties pH, organic carbon, organic matter, total nitrogen content, water holding capacity and particle size of the effluent contaminated soil were carried out using standard methods.

Determination of pH: This was determined using pH-meter 3015 as described by Pramod *et al*. (2014). Ten g of the soil sample was placed in a beaker, then 10 mL of distilled water was added and the mixture stirred. It was allowed to stand for 30 min. A buffered solution was used to calibrate the pH-meter. The electrode of the pH-meter was inserted into the mixture and the reading taken and recorded.

Determination of organic carbon: Organic carbon content of the soil was determined using the method described by (Pramod *et al*., 2014). One gram of soil sample was weighed into a 500 mL conical flask. 10.0 mL of 1 N potassium dichromate (1N $K_2Cr_2O_2$) and 20 mL of concentrated sulphuric (conc. H_2SO_4) was added in order to oxidize the organic carbon. The flask was swirled carefully and allowed to stand for 30 min. Two hundred milliliter of distilled water and 10 mL of concentrated Orthophosphoric acid

(Conc. H_3PO_4) were slowly added. One millilitre (1.0 mL) of diphenylamine indicator was added before titrated against 0.5 N ferrous ammonium sulphate solution until green color started appearing, indicating the end point. The blank without soil was run simultaneously for control sample.

Determination of Organic Matter: The organic matter of the soil was determined by multiplying the percentage of organic carbon by factor of 1.724 (Pramod *et al*., 2014).

Determination of Total Nitrogen (Kjeldhal Method): The total nitrogen was determined by Kjeldhal method, which was described by Bremner (1965) cited by Pramod *et al*. (2014). In this method, five gram of air dried soil samples were weighed into digestion tube and moistened with distilled water. Twenty (20) mL of concentrated sulphuric acid (Conc. H_2SO_4) and 5.0 g of catalyst (mixture of K_2SO_4 10.0 g and selenium 5.0 g) respectively were added. The tubes were then placed in the digestion unit. The heating equipment was adjusted to 400 $^{\circ}$C and the tubes were heated till the mixture became transparent. The tubes were then allowed to cool, 40 % sodium hydroxide was added to the digest till the color change blackish and the contents were distilled (liberated ammonia) and collected into 10 mL of 2 %3 boric acid solution (H_2SO_4) until pink color started appearing. A blank without the soil was run for control sample.

Determination of water holding capacity: Water holding capacity was determined using the method described by Saiki *et al.* (2015). The soil sample was spread out and thoroughly air-dried; ten g of air dried soil sample was placed into a funnel which was placed on a measuring cylinder with a cotton wool plugged at the neck of the funnel. Hundred ml of water was measured and gradually poured into the funnel covering the soil sample, and the amount of water added was recorded. The sample was gently stirred until it is saturated, and the water was allowed to drip and when it stopped, the amount of water in the graduated cylinder was then recorded as the final water content and also the blank without the soil sample was run simultaneously for control sample.

$WHC = W_o - \Delta W \times 100\ \%$

$$W_o = V_o \times \frac{100}{V_o + D_o} \quad \% \Delta = \Delta V_o \times \frac{100}{V_o + D_o}$$

Where V_o = initial water contents of the sample ΔV_o = difference in water contents of the sample before and after centrifugation D_o = initial dry mass of the samples, WHC= water holding capacity, W_o= final water content

Soil particle analysis: Soil particle analysis was done by Hydrometer method as described by (Bouyoucous *et al*., 1962 cited in Drummond *et al*., 2013). One hundred gram of air dried soil sample was weighed into a 1000 mL plastic beaker and treated with hydrogen peroxide (H_2O_2) to destroy the organic matter. Two hundred millilitre of distilled water and 100 mL of sodium hexa metaphosphate solution was added to the treated soil sample and stirred with glass rod. The plastic beaker was then covered and kept for 4 h. The volume of the content was made up to 500 mL and stirred for 10 minutes. The whole contents were transferred to a suspension cylinder and the volume was made up to 1000 mL with distilled water and covered with stopper and shakes several times to allow the soil particles to disperse completely. The stopper was removed and hydrometer was immediately placed in the suspension. The first reading was taken exactly 40 sec after placement of hydrometer. The cylinder was closed with the stopper and inverted several times again to ensure complete dispersal of particles. The hydrometer was then placed in the suspension exactly after 2 h and the second reading was noted. The same method was used for thermometer. The blank was run simultaneously for control sample without soil and the room temperature was recorded. The texture of the soil was determined by applying the particle size distribution to the Marshal's textural triangle, which includes:

$\text{Sand}\ (\%) = 100 - (H_1 \pm 0.2\,(T_1 - 20) - 2.0)^2$

$\text{Clay}\ (\%) = (H_2 \pm 0.2\,(T_2 - 20\,(-2.0)^2$

$\text{Silt}\ (\%) = 100 - (\text{Sand} + \text{Clay})\ \%$

Where H_1 = Hydrometer readings after 40 sec

H_2 = Hydrometer readings after 2 h

T_1 = Thermometer readings after 40 sec

T_2 = Thermometer readings after 2 h

Determination of Total Suspended Solids (TSS)

One hundred ml of effluent solution was filtered through 0.45 µm cellulose Whatman's filter membrane which was previously oven dried at 105 °C to constant weight and the filter paper plus retained solids were dried in a desiccator. Total suspended solid (TSS) was computed as the weight of filter paper and dried residue minus the weight of filter paper. The blank containing distilled water was run simultaneously for control sample. This method was earlier described by Ademoroti (1996) as cited by Obijiofor *et al.* (2018).

Determination of Total Solid (TS):

This was estimated by gravimetric method as described by Ademoroti (1996) as cited by Afshin and Farid (20310). One hundred millilitre of unfiltered portion of the effluents was evaporated in a porcelain dish to dryness. Porcelain dish was previously oven dried at temperature of 105 °C, cooled in a desiccator to room temperature to a constant weight. Total Solid (TS) was computed as weight difference between the weight of dish after evaporating the 100 mL of effluent sample to dryness and weight of the dish alone. The blank with distilled water was run simultaneously for control sample.

Determination of bacterial tolerance to heavy metals

Preparation of 0.5 mM stock:

Analytical grades of metal salts which include $CuSO_4$, $CoCl_2$, $ZnSO_4$ and $FeCl_2$ were used to prepare 0.5 mM Stock solutions. Each of the metal salts were measured out as follows $CuSO_4$ (5.4 g), $CoCl_2$ **(**4.4 g) ,$ZnSO_4$ (5.4 g) and $FeCl_2$ (4.3 g) and each was dissolved in 1000 mL of distilled water, then the metal salts solution were all filtered sterile using millipore membrane with a pore size of 0.22 µm. Mineral salts medium containing NH_4 SO_4 (1.2 g), $MgSO_4$ (0.5 g), KCl (0.5 g) and NaCl (1.0 g) were added to Tris-Buffered Low-Phosphate Agar medium (TBLPA) which is made up of Tris-buffered (5 mL) and Low-phosphate agar (K_2HPO_4 4.1 g), which are all dissolved in 270 mL of distilled water (Clausen, 2000). Each working test metal solutions were prepared by diluting the concentrated stock solution to the final concentrations of 50, 75, 100, 150, 200, 250 and 300 ppm for the determination of the Minimum Tolerance Concentrations (MTCs) of the metal ions for each isolate.

The agar plate method for testing bacterial tolerance was used in this study (Adamchuk *et al*., 2011). The bacteria used in the study were *Pseudomonas* sp., *Bacillus* sp., *Escherichia coli*, *Klebsiella* sp., *Staphylococcus* sp., *Streptococcus* sp. and *Staphylococcus* sp. Bacterial cultures were grown overnight in Tris minimal broth which contain $MgSO_4$ (0.1 mL), $CaCl_2$ (0.02 mL), NaCl (0.03 mL) and Tris-buffer (5mL). Then 0.01 mL of each of the cultures was spotted on duplicate plates for each metal and they were incubated at 30 °C. The plates were observed for growth at interval of 48 h for one week. Positive result was recorded by the appearance of colonies on the surface of the plate, TBLPA agar plates without heavy metal was used as controls. The lowest concentration that allowed growth was considered the MTCs.

Screening of heavy metal resistance genes in *Pseudomonas aeruginosa*

DNA Extraction

DNA was extracted using the protocol described by (Wawrik *et al*., 2010 and Mihdhir *et al.,* 2016). In brief, pure colonies grown on medium were transferred to 1.5 mL of liquid medium and incubated on a shaker at 28 ºC for 48 h. It was then centrifuged at 4600 rpm for 5 min. The resulting pellets were re-suspended in 520 µL of TE buffer (10 mM Tris-HCl, 1 Mm EDTA, pH 8.0). Fifteen microliters of 20 % SDS and 3 µL of Proteinase K (20 mg/mL) were then added. The mixture was incubated for 1 h at 37 °C after which 100 µL of 5 M NaCl and 80 µL of a 10 % CTAB solution in 0.7 M NaCl were added and homogenized. The suspension was incubated for 10 min at 65 °C and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200 rpm for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1: 0.6) was added and DNA precipitated at –20 °C for 16 h. DNA was collected by centrifugation at 13000 rpm for 10 min, washed with 500 µL of 70 % ethanol, air-dried at room temperature for approximately 3 h and finally dissolved in 50 µL of TE buffer.

Polymerase Chain Reaction (PCR)

PCR preparation cocktail consisted of 10 µL of 5x GoTaq colorless reaction, 3 µL of 25 mM $MgCl_2$, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each 27F 5'–CAGGTCGTTACCGCAGG–3' and – 1525R, 5′ CTCTGATCTCCAGGACATTC-3′ primers and 0.3 units of Tag DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water 8 µL DNA template. PCR was carried out in a Gene Amp 9700 PCR System Thermal cycler (Applied Bio system Inc., USA) with a PCR profile consisting of an initial denaturation at 94 °C for 5 min; followed by a 30 cycles consisting of 94 °C for 30 s, 50 °C for 60 s and 72 °C for 1 min 30 s; and final termination at 72 °C for 10 min. A storage temperature of 4 °C was used just after PCR reaction (Brown *et al*., 1992).

Integrity of the gene fragment

The integrity of the amplified about 1.5Mb gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1X TAE buffer) was prepared and subsequently used to prepare 1.5 % agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60 °C and stained with 3 µL of 0.5 g/mL ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1X TAE buffer was poured into the gel tank to barely submerge the gel. Two microlitres (2 µL) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4 µL of each PCR product and loaded into the wells after the 100 bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120 V for 45 min visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparing the bands with the mobility of a 100 bp molecular weight ladder that was ran alongside experimental samples in the gel (White *et al*., 1990).

Purification of amplified product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µL of Na acetate 3M and 240 µL of 95 % ethanol were added to each about 40 µL PCR amplified product in a new sterile 1.5 µL Eppendorf tube, mix thoroughly by vortexing and keep at – 20 °C for at least 30 min. Centrifugation for 10 min at 130 which the pellet were washed by adding 150 µL of 70 % ethanol and mix then centrifuge for 15 min at 7500 g and 4 °C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10 – 15 min. then re-suspended with 20 µL of sterile distilled water and kept in – 20 °C prior to sequencing. The purified fragment was checked on a 1.5 % agarose gel ran on a voltage of 110 V for about 1h as previous, to confirm the presence of the purified product and quantified using a Nano drop of model 2000 from thermo scientific.

Sequencing of the amplified DNA fragments from PCR and Bioinformatics-based sequence comparison

The amplified DNA fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Bio systems using manufacturers' manual while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used to edit the sequence while neighbor to neighbor joining was used to construct the phylogenetic tree (Tamura *et al*., 2007).

Data Analysis: The data derived from various determinations was subjected to statistical analysis including mean, Pearson correlation, t-test and ANOVA. The means for the levels in soil in the abattoir and control areas were determined. Using ANOVA and t- test, the means was compared to determine whether they are significantly different at 0.01 confidence level. Pearson correlation was used to relate the levels of heavy metals in soil to the control.

Results

Mean viable counts of bacteria isolated from samples

The mean viable bacterial counts obtained ranged between 6.19 x 10^5 CFU/g and 8.50 x 10^5 CFU/g recorded at green grass site (GGS) and waste water site (WWS) respectively. The range

was higher than value of 4.71 x 10^5 CFU/g recorded in control site (Table 1)

The morphological and biochemical characteristics presented in Table 2 shows that *Pseudomonas* sp., *Escherichia coli, Klebsiella* sp., *Staphylococcus* sp., *Bacillus* sp., *Streptococcus* sp. and *Staphylococcus* sp. were identified. Only three *Bacillus* sp., *Pseudomonas* sp. and *Staphylococcus* sp. were obtained from the control.

Mean Concentration of Heavy Metals in Abattoir Soils

Table 3 shows the mean concentration of heavy metals in Yola abattoir soil samples. Iron (Fe) was most concentrated in the soil with values that ranged from 227.48 mg/kg to 295.29 mg/kg while the least was recorded in Copper 3.36 mg/kg to 10.29 mg/kg. The values for the control in all the metals were generally low, 2.15 mg/kg for Copper and 216.24 mg/kg for Iron.

Bacterial Tolerance to Heavy Metal Ions

The results presented in Table 4 show twenty six (26) isolates growing under different concentrations 50 ppm, 75 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm of four heavy metals used. *Pseudomonas* sp. from HBS sample 2 (Table 2) was able to tolerate the minimum concentration of 50 ppm of all tested heavy metals without decline in growth after 72 h of incubation. Based on this, *Pseudomonas* sp. (HBS_2) was selected for further identification by molecular technique.

Table 5 shows the pH values of the sampled soils within the abattoir from 6.00 – 7.03 while the control was 7.18. The organic carbon (OC) for the sampled soils from abattoir ranged from 6.30 – 7.98 %, observed with samples BDS and WWS respectively. These values were higher than 1.10 % observed with the control sample.

The organic matter (OM) was found in the sampled soils from abattoir ranged between 10.84 and 13.76 % for samples WWS and DDS, BDS respectively. The organic matter content for the control sample was low, 1.88 % (Table 5). The total nitrogen (TN) content observed in the soils samples ranged from 1.46 to 2.48 % (Table 5). However, the value of total nitrogen content in the control sample was 0.50 %. The water holding capacity was between 20.49 and 33.46 g/cm, recorded in samples HBS and BBS. The control sample value was 20.13 g/cm, which is lower than the least value from the abattoir samples, 20.49 g/cm (Table 5).

The total solid (TOS) recorded in the soil samples from abattoir was between 775.5 and 1263.4 mg/L, with sample PNS had the least value while the highest was observed in sample BBS (Table 5). The total solid value recorded in the control sample was 979.59 mg/L (Table 5). The total suspended solid (TSS) presented in Table 5 shows that 872.70 mg/L was the highest while 688.40 mg/L was the least. They were recorded in samples WWS and HBS respectively. The total solid recorded in the control was 728.17 mg/L (Table 5).

Molecular Identification of 16S rRNA genes of Isolate *Pseudomonas aeruginosa* HBS_2

The phylogenetic relatedness of *Pseudomonas aeruginosa* HSB_2 using the 16S rRNA gene is presented in Figure 1. The isolate was found to share 99.93 % similarity with *Pseudomonas aeruginosa* PA01 at the GenBank. The molecular size of the 16S rRNA gene of the isolate was found to be around 1500 base pairs (Plate 1).

Table 1: Mean viable counts of bacteria isolated from soil

Total Mean	Counts of Bacteria (CFU/g)
BBS	6.99±0.01
DDS	7.28±0.02
BDS	6.98±0.03
HBS	7.19±0.02
GGS	6.19±0.03
PNS	6.40±0.02
SPS	6.30±0.01
WWS	8.50±0.02
CTS	4.71±0.02
P-value	0.001

Key: CFU/g =colony forming unit per gram, DDS = dung dumping site, BBS = blood bank site, BDS = bone dumping site, GGS = green grass site, PNS = pens site, SPS= Selling Point Site, WWS =waste water site, HBS= Herd burning site and CTS = Control Site.

Table 2: Morphological and biochemical characteristics of bacteria isolated from soil

Code	Gram Reaction	Ox	Cat	In	Cit	MR	Urease	Vp	Mot	NR	Co	Probable identity
BBS_1	- Rods	-	-	+	+	+	-	-	-	+	-	*Klebsiella* sp.
BBS_2	- Rods	-	+	+	-	+	-	-	+	+	-	*Escherichia coli*
BBS_3	+ Cocci	-	+	+	-	-	-	-	-	+	-	*Staphylococcus* sp.
DDS_1	- Rods	-	+	+	-	+	-	-	+	+	-	*Escherichia coli*
DDS_2	- Rods	-	+	+	-	+	-	-	+	+	-	*Escherichia coli*
DDS_3	- Rods	-	+	-	+	+	-	-	-	+	-	*Staphylococcus* sp.
DDS_4	- Rods	+	+	-	+	-	-	-	-	-	+	*Pseudomonas* sp.
BDS_1	+ Cocci	-	-	-	-	+	-	+	-	+	-	*Streptococcus* sp.
BDS_2	+ Cocci	-	+	+	-	-	-	-	-	+	-	*Staphylococcus* sp.
BDS_3	- Rods	-	-	+	+	+	-	-	-	+	-	*Klebsiella* sp.
HBS_1	+ Cocci	-	-	-	-	+	-	+	-	+	-	*Streptococcus* sp.
HBS_2	- Rods	+	+	-	+	-	-	-	-	-	+	*Pseudomonas* sp.
HBS_3	+ Rods	-	+	+	-	-	-	-	-	+	-	*Bacillus* sp.
GGS_1	+ Cocci	-	+	+	-	-	-	-	-	+	-	*Staphylococcus* sp.
GGS_2	+ Cocci	-	-	-	-	+	-	+	-	+	-	*Streptococcus* sp.
PNS_1	+ Rods	-	+	+	-	-	-	-	+	+	-	*Escherichia coli*
PNS_2	- Rods	-	+	-	+	+	-	-	+	+	-	*Pseudomonas* sp.
PNS_3	+ Cocci	-	-	-	-	+	-	+	-	+	-	*Streptococcus* sp.
SPS_1	+ Cocci	-	-	-	-	+	-	+	-	+	-	*Streptococcus* sp.
SPS_2	+ Cocci	-	-	-	-	+	-	+	-	+	-	*Streptococcus* sp.
SPS_3	- Rods	-	-	+	+	+	-	-	-	+	-	*Pseudomonas* sp.
WWS_1	+ Cocci	-	+	+	-	-	-	-	-	+	-	*Staphylococcus* sp.
WWS_2	- Rods	+	+	-	+	-	-	-	-	-	+	*Pseudomonas* sp.
WWS_3	+ Cocci	-	+	-	-	-	+	-	-	+	-	*Staphylococcus* sp.
WWS_4	+ Rods	-	+	+	-	-	-	-	+	+	-	*Escherichia coli*
WWS_5	+ Cocci	-	-	-	-	+	-	+	-	+	-	*Streptococcus* sp.
CTS_1	- Rods	+	+	-	+	-	-	-	-	-	+	*Pseudomonas* sp.
CTS_2	+ Rods	-	+	+	-	-	-	-	+	+	-	*Bacillus* sp.
CTS_3	+ Cocci	-	+	+	-	-	-	-	-	+	-	*Staphylococcus* sp.

KEY:- = negative, + =positive, Ox = Oxidase test, Cat = catalase test, Cit = citrate utilization test, MR = Methyl red test, In = indole test, NR = nitrate reduction test, VP = Voges Proskauer, Ur = urease test, Mot = motility test, Co = coagulase test,t DDS = dung dumping site, BBS = blood bank site, BDS = bone dumping site, GGS = green grass site, PNS = pens site, SPS= Selling Point Site, WWS = water waste site, HBS= Herd burning site and CTS = Control Site.

Table 3: Mean concentration of heavy metals in abattoir soil

Soil sampling Point	**Cu (mg/kg)**	**Fe (mg/kg)**	**Zn (mg/kg)**	**Co (mg/kg)**
BBS	5.93±0.12	227.48±0.80	17.68±0.64	11.33±0.12
DDS	10.29±0.53	264.12±0.63	13.75±0.44	8.97±0.26
BDS	8.25±0.47	295.29±0.68	20.80±0.09	11.47±0.11
HBS	6.11±0.12	286.24±0.08	18.79±0.34	14.93±0.17
GGS	7.68±0.64	247.19±0.80	19.09±0.90	12.33±0.27
PNS	3.36±0.87	274.81±0.56	16.90±0.51	15.12±0.71
SPS	7.22±0.79	237.48±0.85	18.83±0.76	12.45±0.38
WWS	6.87±0.67	260.65±0.15	19.00±0.21	13.61±0.40
CTS	2.15±0.64	216.24±0.95	10.50±0.50	6.87±0.09
p-value	0.001	0.001	0.001	0.001

KEY: BBS=blood bank site, DDS=dung dumping site, BDS=bone dumping site, GGS=green grass site, PNS=pens site, SPS= Selling Point Site, WWS=water waste site, HBS= Herd burning site and CTS= Control Site

Table 4: Bacteria tolerance to heavy metals at different concentrations

Code	Bacteria	Copper	Cobalt	Zinc	Iron
BBS_1	*Klebsiella* sp.	75,100,150	75, 100	No tolerance	100,150
BBS_2	*Escherichia coli*	75, 100	150, 200	100,150,200,250	75, 100
BBS_3	*Staphylococcus* sp.	No tolerance	100, 150	150, 200	50, 75
DDS_1	*Escherichia coli*	75, 100, 150	No tolerance	100, 150	150
DDS_2	*Escherichia coli*	75, 100,150,200	150, 200	50, 75	75, 100
DDS_3	*Staphylococcus* sp.	100, 150	100	75, 100	No tolerance
DDS_4	*Pseudomonas* sp.	100, 150, 200	75, 100, 150, 200	100, 150	75, 100
BDS_1	*Streptococcus* sp.	No tolerance	100, 150	150, 200	No tolerance
BDS_2	*Staphylococcus* sp.	75, 100, 200	100, 150	150	100, 75
BDS_3	*Klebsiella* sp.	100, 150	75, 100, 150	No tolerance	100, 150, 200
HBS_1	*Streptococcus* sp.	No tolerance	100, 150	150, 200	No tolerance
HBS_2	*Pseudomonas* sp.	50, 75, 100, 150	50,75,100, 150	50, 75, 100, 150, 200, 250, 300	50, 75, 100, 150, 200
HBS_3	*Bacillus* sp.	No tolerance	100, 150	150, 200	100, 150, 200
GGS_1	*Staphylococcus* sp.	75, 100, 150	100, 150	150	100, 150
GGS_2	*Streptococcus* sp.	100, 150, 200	100, 150	150, 200	No tolerance

Table 4: Bacteria tolerance to heavy metals at different concentrations (cont'd)

Code	Bacteria	Copper	Cobalt	Zinc	Iron
PNS_1	*Escherichia coli*	75, 100	150, 200	75	75, 100
PNS_2	*Pseudomonas* sp.	100, 150, 200	75, 100, 150, 200	100, 150	75, 100
PNS_3	*Streptococcus* sp.	100,150	No tolerance	150, 200	75,100
SPS_1	*Streptococcus* sp.	100,150	150,200	100,150,200	75
SPS_2	*Streptococcus* sp.	75, 100, 150	100, 150	No tolerance	100, 150
SPS_3	*Pseudomonas* sp.	100, 150, 200	75, 100, 150, 200	100, 150	75, 100
WWS_1	*Staphylococcus* sp.	75, 100, 150	100, 150	150	100, 150
WWS_2	*Pseudomonas* sp.	75, 100, 150, 200	100, 150, 200	75, 100, 150	150, 200
WWS_3	*Staphylococcus* sp.	100, 150	75	75, 100	No tolerance
WWS_4	*Escherichia coli*	75, 100	150, 200	100, 150,200	100,150
WWS_5	*Streptococcus* sp.	No tolerance	100, 150	150, 200	100

Heavy metals used: $CuSO_4$, $CoCl_2$, $ZnSO_4$ and $FeCl_2$

Heavy metals concentrations used: 50, 75, 100, 150, 200, 250 and 300 ppmKEY: BBS=blood bank site, DDS=dung dumping site, BDS=bone dumping site, GGS=green grass site, PNS=pens site, SPS= Selling Point Site, WWS=water waste site, HBS= Herd burning site and CTS= Control Site

Table 5: Physicochemical characteristics of the abattoir effluent contaminated soil

Sampling Point	pH	OC %	OM %	TN %	WHC(g/cm)	TOS(mgL^{-1})	TSS(mgl^{-1})
BBS	6.3±0.02	7.88±0.02	13.58±0.02	2.13±0.01	33.46±0.11	1263.4±0.01	804.99±0.21
DDS	7.00±0.02	7.97±0.24	13.76±0.01	1.46±0.02	24.52±0.20	1055.8±0.02	794.10±0.10
BDS	6.00±0.13	6.30±0.24	13.76±0.31	1.82±0.00	20.82±0.21	896.58±0.01	818.18±0.33
HBS	6.45±0.01	7.90±0.01	10.84± 0.03	2.48±0.01	20.49±0.50	1021.1±0.10	688.40±0.20
GGS	6.32±0.02	6.85±0.02	11.84±0.62	2.00±0.02	22.16±0.30	1183.2±0.12	738.30±0.22
PNS	6.16±0.02	6.83±0.31	11.82±0.27	2.14±0.02	23.11±0.01	775.50±0.02	689.19±0.02
SPS	7.03±0.40	6.79±0.01	11.68±0.23	1.65±0.01	23.23±0.02	819.31±0.30	765.49±0.03
WWS	6.07±0.01	7.98±0.22	10.84±0.03	2.11±0.03	26.33±0.51	1033.9±0.41	872.70±0.01
CTS	7.18±0.01	1.10±0.15	1.88±0.11	0.50±0.01	20.13±0.12	979.59±0.11	728.17±0.02
P value	0.00 1	0.001	0.001	0.001	0.001	0.001	0.001

KEY: BBS=blood bank site, DDS=dung dumping site, BDS=bone dumping site, GGS=green grass site, PNS=pens site, SPS= Selling Point Site, WWS=water waste site, HBS= Herd burning site and CTS= Control Site, OC = Organic carbon, OM = Organic matter, TN = Total nitrogen, WHC = Water holding capacity, TOS = Total Solids, TSS = Total Suspended Solid

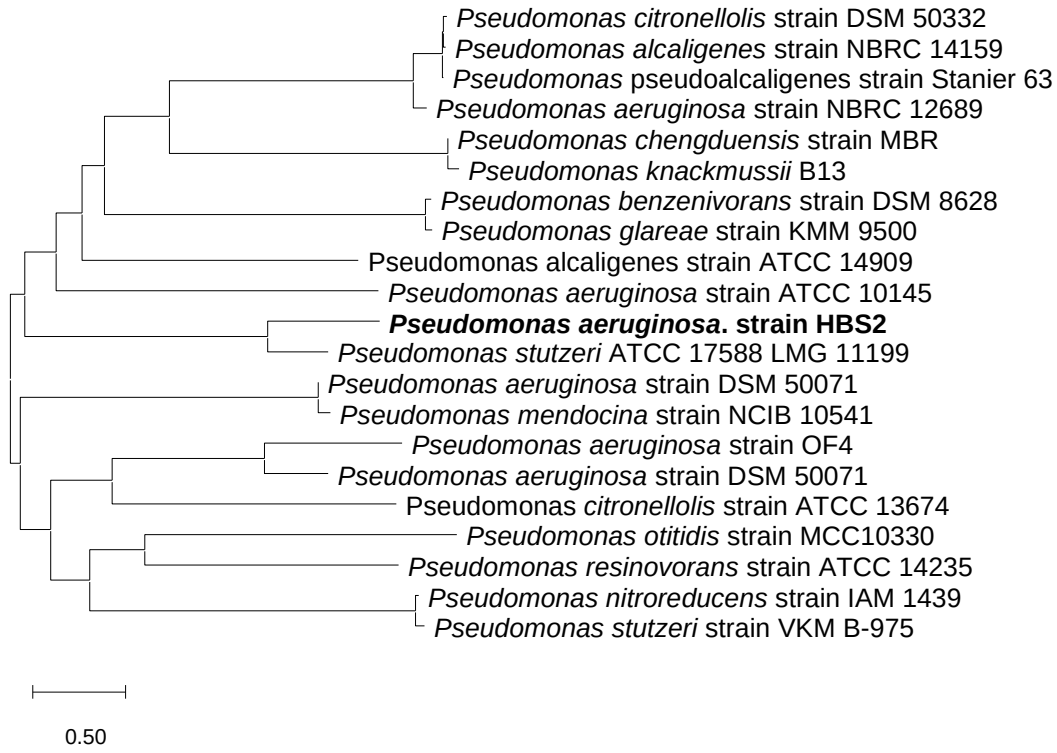

Figure 1 Phylogenetic tree based on 16S rRNA gene showing evolutionary relatedness of *Pseudomonas* sp. HBS_2 to other strains of *Pseudomonas* at GenBank

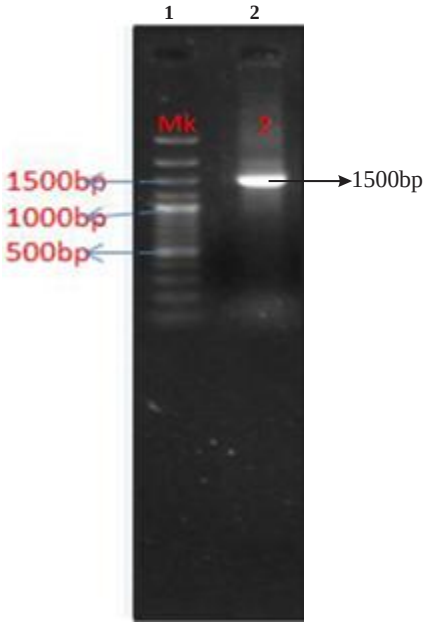

Plate 1. PCR amplified product of 16S rRNA gene of 1500 bp of *Pseudomonas aeruginosa* HBS_2 on agarose gel electrophoresis, 1 = molecular ladder (100 bp) and 2 = Sample

Discussion

Uncontrollable discharge of untreated abattoir effluents into the surrounding environment increases the concentration of heavy metals in the soil. Accumulation of heavy metal in the soil according to the report of Adekanmbi and Falodun (2015) could be toxic to the living organisms inhabiting the soil. These organisms could develop resistance to the metals and then antibiotics and these could pose danger to human health.

This study revealed that the total viable counts of bacteria isolated from the soil samples of the abattoir as presented in Table 1 ranged between 6.19 x 10^5 CFU/g and 8.50 x 10^5 CFU/g which were higher than the value recorded in the control, 4.71 x 10^5 CFU/g. Similar findings were reported in a study by Ezeoha (2010) who recorded a mean bacterial count of 3.32 x 10^7 CFU/mL in wastewater collected from Agege (Nigeria) abattoir. However, a lower total bacterial count of 3.6-6.4 x 10^1 CFU/mL was reported by Adekanmbi and Falodun (2015). The differences observed in the total viable bacterial counts in the contaminated soil sample could be attributed to the microbial community with the sampling points and other anthropogenic activities in the area (Adekanmbi and Falodun, 2015).

Different genera of bacteria were identified based on their morphological and biochemical characteristics in this study. These genera *Pseudomonas, Escherichia coli, Klebsiella, Staphylococcus, Bacillus, Streptococcus* and *Staphylococcus* were identified and presented in Table 2. The isolates with the highest occurrence in abattoir soil samples are

Streptococcus sp. followed by *Staphylococcus* sp., *Pseudomonas* sp., *Escherichia coli*, *Klebsiella* sp while the least was *Bacillus* sp. (Table 2). The results showed the diversity in the bacterial community in the soil contaminated with abattoir effluents. Some of these bacteria could be resident of the soils that were sampled while others are associated with the abattoir waste (Rabah *et al*., 2010). The reports being presented here are in tandem with the previous report of Yilmaz (2013). This could be attributed to the common microbial flora in the gut of animals.

High concentration of heavy metals in the soil is toxic to the growth and the development of both plants and animals (Gupta *et al*., 2012). They are non-biodegradable and they may remain as environmental contaminants for a long time during which they can be deposited or adsorbed into the plant tissues (Sharma *et al*., 2008). The mean concentration of heavy metals in recorded in this study presented in Table 3 showed that Iron (Fe) has highest concentrations values across the sites followed by Zinc (Zn), Cobalt (Co) and Copper (Cu) respectively. However, the concentrations of theses heavy metals in the control sample were quite low compared to the values obtained in the contaminated soil. In the study carried out by Ediene and Iren (2017), the authors also reported high concentrations of Iron, followed by Zinc and Copper. Our findings are similar to this report. Similarly, the results obtained in this study are in agreement with previous report of Singh *et al*. (2015) and Nasiru *et al*. (2016) who reported the presence of heavy metals such as lead, copper, cadmium, manganese, nickel chromium and zinc in their reports. Different concentration of organic materials in the affluent might be responsible for the differences observed in heavy metals concentration (Sharma *et al*., 2014).

Most of the bacterial isolates that obtained in this study had minimum tolerance concentration of the heavy metals used at 75 or 100 ppm as shown in Table 4. However, isolate HBS_2 that was identified to be *Pseudomonas aeruginosa*-HBS_2 was observed to tolerate minimum concentration of 50 ppm of all the four tested heavy metals at 72 h of incubation while the highest tolerance concentration was at 300 ppm by the same isolate. There was no decline in the growth of the strain as incubation progressed. Heavy metals resistance is common among Gram negative bacteria such as *Pseudomonas*, *Alcaligenes* and *Burkholderia* (Singh *et al*., 2010). The result obtained in this study is similar to the earlier findings of Bhojiya and Joshi (2016) who reported the isolation and identification of a strain of *Pseudomonas putida*. The reported strain possessed the potential to tolerate a wide range of heavy metals that included zinc, cadmium, cobalt, nickel, copper and lead. Similar results on the tolerance of heavy metals by *Pseudomonas* species have been mentioned by Naz *et al*., 2015 and Haroun *et al*., 2017). They all corroborate the findings of this study where an identified strain of *Pseudomonas*, *P. aeruginosa* HBS_2 was observed to tolerate heavy metals. The results of this work showed that the levels of tolerance exhibited by isolate *Pseudomonas aeruginosa*-HBS_2 were considerably higher than those reported by Liu *et al*. (2015) for a strain of *Pseudomonas aeruginosa* that they used in their study. Kang *et al*. (2014) had earlier reported that tolerance to heavy metals often occurs over a range of metals and not for a specific metal only. *Pseudomonas aeruginosa*-HBS_2 could be a good candidate for bioremediation of contaminated soil. Previous studies have shown that *Pseudomonas* species are tolerant to most heavy metal due to the presence of gene *PcoR* (Yilmaz, 2013). It has been previously mentioned that the resistance of bacteria to heavy metals may be due to different strategies used by these microorganisms during detoxification. These strategies include development of complexes using exopolysaccharides, reduction of metals and binding metals with bacterial cell envelope (Adekanmbi and Falodun, 2015).

One of the physicochemical properties of the abattoir effluent contaminated soil that was determined is the pH shown in Table 5. The pH of an environment influences the growth and biochemical activities of organisms including microorganisms such as bacteria. Also, the bio-accumulation of heavy metals by bacteria has been mentioned to be a function of the pH of the bacteria (Bhojiya and Joshi, 2016). It was observed in this study that the pH of the effluent contaminated soil ranged between 6.00 and 7.03 while that of the control was slightly higher, 7.18. The observed results are in agreement with earlier report of Ediene and Iren (2017) who mentioned that the pH of contaminated soil in their study was slightly acidic to alkaline. The report of Naz *et al*.

(2015) from their study shows that the pH values ranged between 7.19 and 7.70 while the control was 7.83. These pH values are quite higher than what we are reporting from this study. This could be as a result of types of wastes such as blood, fats, animal trimming and urine which could increase microbial activities in the soil, thereby altering the pH of the soil. The observed results are in agreement with the previous findings of Rabah *et al*. (2010) who reported pH that ranged within the same values. The value of pH observed in this work is within the acceptable WHO standard of 6.5 – 8.5. Such pH conditions as observed for the abattoir sites favor the proliferation of soil microbes as well as the growth of various crops. pH is also a major factor in all chemical reactions associated with formation, alteration and dissolution of minerals in the soil (Mejare and Bulow, 2011).

The remaining physicochemical properties of sampled soils from abattoir and control site are presented in Table 5. The organic carbon and organic matter recorded in this study were 7.98 % and 13.76 % respectively which are higher than those reported by Ediene and Iren (2017) but lower than previous report of Neboh *et al*. (2013) who reported a very low organic carbon that ranged between 1.10 % and 1.88 %. Similarly, the nitrogen content of the effluent contaminated soil was higher than that of the control. This may be attributed to the fecal wastes present in the effluent (Saiki *et al*., 2015). The total solids and total suspended solids obtained in this study were between 775.5 mg/L to 1263 mg/L and 688.4 mg/L to 872 mg/L respectively. Several authors in the past have reported different total solids and total suspended solids in their studies. For instances, Eze and Eze (2015) reported 152 mg/L as total solid and 97.3 mg/L as total suspended solids while 7726 mg/L - 47630 mg/L (total solids) and 1489 mg/L - 6803 mg/L (total suspended solids) were put forward by Osibanjo and Adie (2007). Our findings also follow the same pattern of lower total suspended solids than the total solids. This may be attributed to organic loads and dissolved solid matters from the abattoir wastes.

The amplification of the 16S rRNA of isolate HBS_2 shows that the extracted DNA revealed a 6.3 kilo base pair length of the total extracted genome on 1.5 % agarose gel electrophoresis which is similar to the report of Ramirez, et al. (2011). The extracted DNA subjected to PCR using the 16S rRNA bacterial universal primer and resulted in the PCR product of about 1500 bp fragment (Plate 1).

Conclusion

The study focused on the evaluation of the effects of Yola abattoir effluent waste on heavy metals, physicochemical parameters as well as bacterial viable count of the effluent contaminated soil. The pH and water holding capacity values are within the WHO/FEPA standards. All other physicochemical parameters measured such as organic carbon, total nitrogen content, total solid and total suspended solids exceed limits. One of the bacterial isolates HBS_2 was found to grow or tolerate minimal concentration of 50 ppm of the four metal used for an incubation period of 72 h. The isolate HSB_2 was identified to be a strain of *Pseudomonas aeruginosa* and could be used in the recovery of environment contaminated with heavy metals. However, proper treatment and disposal of Yola abattoir effluent is necessary to prevent development of heavy metal and antibiotic resistance strains in the environment.

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