Heavy Metal Levels, Physicochemical Properties and Microbial Diversity of Soil Matrix from University Solid Waste Collection sites in Benin City, Nigeria

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ABSTRACT: This study was carried out to assess the microbial, physicochemical and heavy metal characteristics of soil samples from five different waste collection sites within the University of Benin, Benin City and evaluated using standard analytical and classical microbiological methods. The heavy metal concentration includes Zn (4.45±0.00 - 5.76±0.02 mg g⁻¹), Cd (1.59±0.00 - 2.54±0.00 mg g⁻¹), and Fe (1.02±0.00 - 1.07±0.00 mg g⁻¹). The physicochemical properties include pH (4.91±0.01 - 5.82±0.04); TOC (6.49±0.01 - 7.65±0.02 %); NO₃⁻ (29.32±0.07 - 32.81±0.11 mg g⁻¹); and SO₄²⁻ (47.30±0.12 - 63.25±0.04 mg g⁻¹). The mean of culturable heterotrophic and coliform bacteria ranged from 4.03x10⁷ ± 0.35 - 4.51x10⁸ ± 0.12 CFU/g and 1.02x10⁷ ± 0.12 - 3.10x10⁷ ± 0.02 CFU/g respectively. At p-value < 0.01 level EC significantly correlates total heterotrophic bacteria (r=0.971); coliform bacteria significantly correlate NO₃⁻ (r=0.989); while clay significantly but negatively correlates coliform bacteria (r=-0.989). Some of the bacteria isolated and identified from the waste collection sites include Klebsiella oxytoca, Enterobacter aerogenes, Staphylococcus aureus, Pseudomonas putida, and Bacillus macerans. Findings from this study reveal that the municipal solid waste on the collection sites has impacts on the indicator variables of the resident soil as well as serving as breeding sites for pathogenic organisms.

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Most urban centres in Nigerian are the embodiment of urban decay and branded by poor housing plan, public health infrastructure and cleanliness (David and Oluyege, 2014). The continuous increase of housing units together with an upsurge in population has led to health hazards in the environment (Adefemi and Awokunmi, 2009). Wastes are generally produced from human activities and in most instances not appropriately managed in most urban centres or communities in developing countries (Gupta and Rajamani, 2015). This can result in reduced quality of the environment which is responsible for 25% of all ill health that can be preventable in the world (Majolagbe et al., 2017).

Wastes are usually collected and disposed of in uncontrolled dumpsite situated close to suburban communities. These wastes are accumulated and/or burnt, polluting the air and contaminating water bodies in close proximity (Uffia et al., 2013). Wastes usually result in the growth of disease-causing microorganisms and accumulation of heavy metals which can impact negatively on the environment. Leachates resulting from dumpsites constitute a significant source of pollution from heavy metal impacting on both aquatic and terrestrial environments (Sungur et al., 2014). This can be detrimental to human health, crop and soils (Bahnasawy et al., 2011; Balkhair and Ashraf, 2016).

The solid waste articulates elevated varied nature of biological and physicochemical perspectives which is significantly subjective by socioeconomic activities (Atalia et al., 2015). The microbial multiplicity studies are chiefly important so as to decipher the trend of microbial ecology in the environment (Igbinosa and Igiehon, 2015). The community of microorganisms remains as one of the most unexplored due to their immense classical microbial diversity (Igbinosa, 2015). Adequate inorganic nutrients, temperature, relative humidity and pH of the environment are significant factors that affect the proliferation of microbial consortia responsible for degradation (Ogunmwonyi et al., 2008; Dubey, 2009). These microbial populations obtain energy and nutrients for optimal multiplication from wastes by way of degrading them (Chikere and Ekwuabu, 2014). In the University community, there is no standard, specialized and organized waste collection system. In this study, an attempt was made to characterise the native microbial diversity, physicochemical and heavy metal concentrations from some waste collection sites within the University of Benin community.

MATERIALS AND METHODS
Study Area: This study was conducted within the University of Benin campus environment located at 6.3999° N, 5.6135° E Benin City, Nigeria. The
sampling collection sites are situated in the different halls of residence of the University community. The student population within the institution is about 40,000.

**Sample Collection:** Soil samples were obtained from collection sites from five major halls of residence (student’s hostels) within University of Benin. The sampling sites are designated A to E for the purpose of confidentiality. This was carried out every two weeks from January to March 2017. A garden rake was applied to remove waste at the top of the collection site, so as to expose the soil underneath. Soil samples were thereafter obtained with hand trowel into aluminium foil paper, labelled appropriately. Control samples were obtained from sites within the University of Benin with no history of waste collection or dumpsite. The samples were transferred to the Applied Microbial Processes & Environmental Health Research Laboratory, University of Benin, Benin City and analysed within 4 h after collection.

**Heavy metals and physicochemical analysis of Soil Samples:** Soil samples were pretreated following the methods described by Bassey et al. (2014). Heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) concentrations were carried out using Atomic Absorption Spectrophotometer (Perkin Elmer AA Analyst 800 series Graphite Furnace AA). Percentage organic matter was conducted through the process of the chronic acid titration technique (Walkley and Black, 1934), soil particle size was determined by the hydrometer method (Boyoucos, 1951); total nitrogen was determined using the Kjeldahl and steam digestion procedure (Boyoucos, 1951); cation exchange capacity (CEC) was determined by Bower method (Rowell, 1996); pH was determined using pH meter model: HI 2210 (Peech, 1965); temperature was measured using a mercury thermometer; total organic carbon content was characterised by the wet oxidation method (Rowell, 1996); electrical conductivity was carried out as previously described (Chopra and Kanzar, 1988); soil phosphate (PO₄³⁻), nitrate (NO₃⁻) and sulphate (SO₄²⁻) were determined using previously described protocol (Dewis and Freitas, 1984).

**Isolation and Enumeration of the Bacteria Isolates:** Ten (10) g of the soil sample was dissolved in 90 mL of sterilized distilled water which makes up the stock solution and serially diluted (10⁻¹⁻¹⁰). An aliquot of 0.1 mL of the 10⁻⁵⁻¹³ diluent was inoculated on Nutrient agar (Lab M, United Kingdom) using spread plate technique and incubated for 18-24 h at 37 °C. An aliquot of 0.1 mL from the 10⁻⁴⁻¹⁰ diluents was spread on m-FC agar plates (Merck, Germany) and incubated at 44.5 °C for 18-24 h. After incubation, plates were observed for growth. Distinct colonies from the Nutrient agar plates with different morphological characteristics were selected and purified using repeated streaking on a nutrient agar plate and stored on agar slants prior to identification procedure.

**Identification of the Bacterial Isolates:** The respective selected distinct bacterial isolates were subjected to Gram staining, motility, catalase, oxidase, urease, indole, Voges-Proskauer, methyl red, citrate utilization, hydrogen sulphide production and sugar fermentation test using standard protocol. The results were thereafter compared to identification guide on Bergey’s Manual of Systematic Bacteriology.

**Statistical Analysis:** All data were analysed using the statistical package SPSS version 21.0. Descriptive statistics were used to estimate the mean and standard deviation of variables. One Way ANOVA and correlation analysis were used to compare variables while Duncan Multiple Range Tests (DMRT) was used to show a significant difference between mean. The p-values <0.05 were considered statistically significant.

**RESULTS AND DISCUSSION**

**Heavy metal concentrations and physicochemical characterization:** The range of heavy metal indicators include Zn (4.45±0.00 - 5.76±0.02 mg g⁻¹) with control (1.76±0.01 mg g⁻¹); Pb (14.10±0.13 - 17.10±0.01 mg g⁻¹) with control (0.30±0.00 mg g⁻¹); Cr (1.15±0.00 - 2.54±0.00 mg g⁻¹) with control (1.01±0.01 mg g⁻¹); Mn (2.60±0.02 - 2.67±0.01 mg g⁻¹) with control (0.68±0.00 mg g⁻¹); Cu (2.12±0.00 - 4.10±0.01 mg g⁻¹) with control (1.05±0.00 mg g⁻¹); Cd (1.59±0.00 - 2.54±0.00 mg g⁻¹) with control (0.05±0.00 mg g⁻¹); Ni (38.09±0.11 - 42.01±0.14 mg g⁻¹) with control (3.42±0.00 mg g⁻¹) and Fe (1.02±0.02 - 1.07±0.00 mg g⁻¹) with control (0.81±0.01 mg g⁻¹). A significant difference was observed in all physicochemical variables, heavy metal and microbial density when compared to the control site (p<0.05). The physicochemical and heavy metal indicators from different waste collection sites are presented in Table 1.

The range of the physicochemical variables include pH (4.91±0.01 - 5.82±0.04) with control (6.81±0.02); temperature (28.0±0.01 - 30.0±0.03 °C) with control (29.0±0.09 °C); electrical conductivity (542.01±0.08 - 639.56±0.23 μS cm⁻¹) with control (83.20±0.05 μS cm⁻¹); cation exchange capacity (2.98±0.05 - 4.52±0.02 cmol kg⁻¹) with control (8.76±0.01 cmol kg⁻¹); total nitrogen (0.31±0.01 - 639.56±0.23 %) with control (0.63±0.00 %); total organic matter (3.57±0.01 - 5.15±0.01 %) with control (1.25±0.01 %); total organic carbon (6.49±0.01 - 7.65±0.02 %) with control (4.72±0.03 %); sand (87±0.13 to
The physicochemical variables such as pH, clay content, total organic carbon, total nitrogen; biological factors such as population densities, catalytic and inhibiting interactions between microorganisms; as well as pollutants such as heavy metals, heavy metal and the microbial cell density. The correlation of the heavy metal and microbial density from the waste collection sites are presented in Table 2. A p-value less than 0.05 level of significance, NO$_3^-$ negatively correlates SO$_4^{2-}$ ($r=-0.954$) but positively correlates Mn ($r=0.934$); while Mn negatively correlates SO$_4^{2-}$ ($r=-0.914$).
A p-value less than 0.01 level of significance, Cu significantly correlates Fe ($r = 0.969$); coliform bacteria significantly correlate NO$_3^-$ ($r = 0.989$). A soil which sand predominates is referred to as sand textured soil. They are coarse in texture and drain easily and quickly after the rain. It is lower in moisture-holding and nutrient-holding capacity than another type of soil (Dou et al., 2016). Sand negatively correlates silt and Ni in this study. This could be attributed to the dominant nature of sand (87.00±0.13 – 88.00±0.15 %) on the soil which also affects the formation and accumulation of nickel on the soil. Silt is usually found in soil along with other types of sediment, sand, clay and gravel (Xu et al., 2016). Clay soil can vary in different places but usually, act the same way. It tends to be slow to drain and quick to harden (Cai et al., 2016). Silt negatively correlates clay in this study. This could be ascribed to the dominant nature of clay (6.00±0.00 – 7.00±0.02 %) against silt. A study by Khan et al. (2014) on bioremediation of diesel-contaminated soil site revealed clay content (39.67%), sand content (34.34%) and silt content (25.98%) which differs from the findings in this study. Organic carbon in the soil is principally derived from residual plant and animal matter, synthesized by microbes and degraded via the influence of moisture, temperature and ambient soil conditions (Bowles et al., 2014). The availability of fixed nitrogen in a form that could be available to plants is of prime importance in revealing the fertility of soil (Mooshammer et al., 2014). Inorganic nitrogen compounds such as nitrates, nitrates and ammonia are converted to organic nitrogen such as protein and nucleic acids using nitrogen assimilation in the presence of bacteria (Hoffman et al., 2014). However, total organic carbon did not correlate with other variables in this study, total nitrogen positively correlates Zn. Iron (Fe) is the fourth most abundant element on earth. Plants require iron for the synthesis of chlorophyll and generally contain between 1 and 5 % iron in their content. There was no correlation of Fe on any of the variables. However, it has been reported that soil pH and aeration influence the availability of iron (Hermann et al., 2016). Though Pb did not correlate any of the parameters in this study, urban environments have generally received higher deposition of Pb from vehicular emission than rural areas or environment less of vehicular emission (Yang et al., 2016). Zn correlated total nitrogen in this study. Although Zn occurs naturally, most Zn finds its way into the environment as a result of human activities. In soil, most of the Zn stays bound to the soil particle. When high levels of Zn are present in the soil, the metal can steep into groundwater (Gua et al., 2016). Cd correlated pH and EC in this study. Cd is much less mobile in soils than in air and water (Mahar et al., 2016). The major factors governing Cd speciation, adsorption and distribution in soils include soluble organic matter content, pH, clay content and types, inorganic ligands and competition from other metal ions (Thakur et al., 2016). Cu significantly correlated Fe. The effect of FeO$_2$ on Cu from soil has previously been described (Diagboya et al., 2015). Cu is not mobile in soils, it is attached to soil organic matter and clay minerals. Studies on the assimilation and bioaccumulation of heavy metal which could be phytotoxic have been reported (Balkhair and Ashraf, 2016). Heavy metals in the likes of Zn, Pb, Cu, Co, and Hg from ground and surface water in close proximity to dump sites have previously been reported (David and Oluyege, 2014).

As soil temperature increases, more soil phosphates become available (Zhang et al., 2014). Soil pH also influences the availability of soil phosphate with an optimum pH (6.5-7.0). Soil high in cation levels such as Mn, Fe, Al and Ca immobilizes phosphate between pH 6.5-7.0; while Fe and Al can bind to phosphate below pH 6.0 (Liang et al., 2014). There was no correlation of phosphate on any of the parameters investigated which could be ascribed to the soil temperature (28.00±0.01 - 30.00±0.03 °C) and pH (4.91±0.01 - 5.82±0.04) observed in this study. A study by Atalia et al., (2015) shows that temperature, pH, moisture, total organic carbon and total nitrogen from municipal solid waste were in accordance with the findings of this study. In addition, Olukunle (2013) reported similar findings on moisture content, pH, organic matter, Mn, total nitrogen and total organic carbon from oil-polluted sites. Chihere and Ekwuabu (2014) reported similar total organic carbon, pH, Ni and Pb to this study but with higher levels of electrical conductivity, total nitrogen, phosphate, and Zn from crude oil-impacted sites. Gupta and Rajamani (2015) reported higher pH, electrical conductivity, Cr, and sulphate but with lower Pb from municipal solid waste landfill leachate. Eze et al., (2014) reported similar temperature, total organic carbon, sulphate, nitrate, nitrite, phosphate, Mn, and Zn but with significantly higher pH and Fe from soil contaminated site with used petroleum products.

Correlation matrix of the physicochemical and the microbial variables: The correlation of the physicochemical and microbial density from the waste collection sites are presented in Table 3. A p-value less than 0.05 level of significance, temperature significantly correlates total organic carbon ($r = 0.900$); pH significantly correlates cation exchange capacity ($r = 0.896$); cation exchange capacity negatively correlates moisture ($r = -0.921$); total organic matter negatively correlates sand ($r = -0.943$); Sand negatively correlates silt ($r = -0.881$); silt
negatively correlates clay ($r = -0.881$). A $p$-value less than 0.01 level of significance, electrical conductivity significantly correlates total heterotrophic bacteria count ($r = 0.971$); while clay significantly but negatively correlates coliform bacteria ($r = -0.989$).

| Parameters | pH | Temp | EC | CEC | TN | TOM | TOC | Sand | Silt | Clay | Moisture | HBC | TC |
|------------|----|------|----|-----|----|-----|-----|------|------|------|----------|-----|-----|
| pH         | 1  |      |    |     |    |     |     |      |      |      |          |     |     |
| Temp       | 0.79 | 1    |    |     |    |     |     |      |      |      |          |     |     |
| EC         | -0.47 | -0.71 | 1  |     |    |     |     |      |      |      |          |     |     |
| CEC        | 0.89* | 0.84 | -0.35 | 1  |    |     |     |      |      |      |          |     |     |
| TN         | 0.73 | 0.65 | -0.10 | 0.70 | 1  |     |     |      |      |      |          |     |     |
| TOM        | -0.23 | -0.60 | 0.31 | -0.29 | -0.67 | 1  |     |      |      |      |          |     |     |
| TOC        | 0.71 | 0.90* | -0.68 | 0.85 | 0.35 | -0.25 | 1  |      |      |      |          |     |     |
| Sand       | 0.06 | 0.44 | -0.03 | 0.23 | 0.62 | -0.94* | 0.14 | 1  |      |      |          |     |     |
| Silt       | 0.05 | -0.27 | 0.05 | 0.02 | -0.54 | 0.93* | 0.10 | -0.93* | 1  |      |          |     |     |
| Clay       | -0.19 | 0.00 | -0.06 | -0.35 | 0.32 | -0.72 | -0.40 | 0.64 | -0.88* | 1  |          |     |     |
| Moisture   | -0.81 | -0.58 | -0.01 | -0.92* | -0.69 | 0.09 | -0.61 | -0.12 | -0.13 | 0.44 | 1       |     |     |
| HBC        | -0.30 | -0.54 | 0.97*** | -0.12 | 0.04 | 0.25 | -0.49 | 0.04 | 0.05 | -0.16 | -0.23 | 1  |     |
| TC         | 0.26 | 0.13 | -0.01 | 0.46 | 0.24 | 0.61 | 0.52 | -0.54 | 0.81 | -0.01 | -0.12 | 0.12 | 1  |

Values are means of the overall readings of triplicates ± standard deviations (SD). EC: electrical conductivity, CEC: cation exchange capacity, HBC: heterotrophic bacteria count, TOC: total organic carbon, TOM: total organic matter, TN: total nitrogen, TC: coliform count. 
*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

The soil pH directly impacts the proliferation of plants and microorganisms as it affects the availability of nutrients. Between pH 6.0 and 6.5, most plant nutrients are in their most available state while between pH 6.5 and 7.5, most microorganisms proliferate effectively (Balkhair and Ashraf, 2016). The pH in this study ranged from 4.91±0.01 – 5.82±0.04 which could be detrimental to the proliferation of some group of microorganisms compared to soil pH (6.80 – 7.58) from Ogunmwonyi et al. (2008) which favours microbial growth. Soil pH in this study correlated cation exchange capacity (CEC) positively. Soil electrical conductivity is a measurement that correlates cation exchange capacity, soil texture, total organic matter level and subsoil characteristics (Pathak et al., 2011). Electrical conductivity consequently correlates strongly to soil particle size and texture. It varies depending on the concentration of moisture in the soil (Grisso et al., 2009). A significant difference was observed in all physicochemical variables, heavy metal and microbial density when compared to the control site ($p<0.05$). Soil contamination causes pressure on sensitive microbes and, consequently, changes in the diversity of the microflora representation of the soil trophic groups of microorganisms (Igbinosa, 2015).

Population densities of bacteria from the different waste collection sites: The population densities of bacteria from the different waste collection sites and the control site are presented in Table 4.

| Bacteria type          | Sites | Minimum CFU/g | Maximum CFU/g | Mean ± Standard Deviation CFU/g |
|------------------------|-------|---------------|---------------|--------------------------------|
| Culturable heterotrophic bacteria | A     | 1.56x10^6     | 2.68x10^7     | 5.50x10^6 ± 0.92*               |
|                        | B     | 3.25x10^6     | 5.16x10^7     | 4.23x10^6 ± 0.03*               |
|                        | C     | 4.18x10^6     | 6.67x10^7     | 7.05x10^6 ± 0.06                |
|                        | D     | 3.12x10^6     | 6.24x10^7     | 4.51x10^6 ± 0.12*               |
|                        | E     | 2.23x10^6     | 2.16x10^7     | 4.03x10^6 ± 0.35*               |
|                        | Control | 2.20x10^6   | 2.43x10^7     | 5.31x10^6 ± 0.02                |
| Total coliform bacteria | A     | 1.30x10^6     | 1.30x10^8     | 1.02x10^6 ± 0.12*               |
|                        | B     | 5.20x10^6     | 1.50x10^8     | 2.53x10^6 ± 0.03*               |
|                        | C     | 3.50x10^6     | 5.10x10^8     | 3.10x10^6 ± 0.02*               |
|                        | D     | 4.10x10^6     | 7.10x10^8     | 3.10x10^6 ± 0.04*               |
|                        | E     | 3.20x10^6     | 3.50x10^8     | 1.80x10^6 ± 0.06                |
|                        | Control | 4.10x10^6   | 2.20x10^8     | 4.10x10^6 ± 0.01*               |

Values are means of the overall readings of triplicates ± standard deviations (SD). Site A; B; C; D and E are different waste collection sites. Values which carry different alphabets across column show a significant difference ($p<0.05$).
The culturable heterotrophic bacteria density range between $4.18 \times 10^2$ and $6.24 \times 10^5$ CFU/g across the studied waste collection sites. For the control site, the culturable heterotrophic bacteria density range between $2.20 \times 10^4$ and $2.43 \times 10^6$ CFU/g. The coliform bacteria density ranged from $1.30 \times 10^5$ - $1.50 \times 10^6$ CFU/g across the studied waste collection sites. For the control site, the coliform bacteria density ranged from $4.10 \times 10^4$ - $2.20 \times 10^6$ CFU/g.

**Microbial consortium and identification of the bacterial isolates:** Inappropriate management of waste collection sites could result in significant adverse environmental consequence such as attraction of mice, wind blow litter, the culmination of pollutants such as leachate and toxic heavy metals which can contaminate underground aquifer/soil bed and also serve as breeding sites of pathogenic microorganisms (Antai et al., 2015). When waste is discarded on land, soil microorganisms readily inhabit the waste carrying out the cleavage and transformation of degradable organic matter in the waste (Antai et al., 2016). Microorganisms in waste collection sites use the waste materials as nutrients via digestive mineralization /transformation of complex organic matter into simpler/less toxic molecules (Verla et al., 2014; Balkhair and Ashraf, 2016). The microbial density of the different waste collection sites is as shown in Table 4. The range of heterotrophic count in the study range between $2.20 \times 10^4$ and $5.16 \times 10^9$. Similar heterotrophic bacteria count in agreement with the findings of this study have been reported (Ogunmwonyi et al., 2008; Chikere and Ekwuabu, 2014; Eze et al., 2014; Atalia et al., 2015). Microorganisms from organic waste have been applied in the bioremediation of palm oil mill effluent (Ojonomo and Udeme, 2014). The bacterial identified from this study are presented in Table 5.

**Table 5:** Distribution of the bacteria isolated from the different waste collection sites

| Sites   | Bacterial Isolates                                                                 |
|---------|-----------------------------------------------------------------------------------|
| A       | *Escherichia coli, Klebsiella oxytoca, Pseudomonas aeruginosa* and *Salmonella* sp. |
| B       | *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella oxytoca* |
| C       | *Bacillus subtilis, Escherichia coli,* Bacillus macerans, *Klebsiella oxytoca* and *Pseudomonas aeruginosa* |
| D       | *Enterobacter aerogenes, Micrococcus luteus,* *Escherichia coli* and *Staphylococcus aureus* |
| E       | *Proteus vulgaris, Micrococcus roseus,* *Enterococcus faecalis* and *Pseudomonas putida* |
| Control | *Bacillus macerans,* *Escherichia coli,* *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus* |

They include *Escherichia coli, Klebsiella oxytoca, Pseudomonas aeruginosa, Salmonella* sp., *Bacillus subtilis,* *Enterobacter aerogenes,* *Micrococcus luteus,* *Staphylococcus aureus,* *Proteus vulgaris,* *Micrococcus roseus,* *Enterococcus faecalis,* *Pseudomonas putida,* *Bacillus macerans,* and *Staphylococcus saprophyticus.* Studies on the contamination of ground and surface water in close proximity to dump site and the public health implication have been reported (David and Oluyearge, 2014). Eze et al., (2014) also reported bacterial isolates to be *Pseudomonas aeruginosa,* *Escherichia coli,* *Klebsiella* spp., *Staphylococcus aureus,* *Micrococcus spp. Bacillus* spp., *Citrobacter* spp. and *Streptococcus* spp. which were similar to the bacteria isolates in our study. The elevated number of CFU/g in case of all these soil samples from waste collection sites indicates the spontaneous composting and enrichments potentials of the catabolic/anabolic profile of the microbial diversity in concomitance to the physicochemical nature of the collection sites at the various stages of compost process.

**Conclusion:** Findings from this study reveal that the municipal solid waste on the collection sites has impacts on the microbial community of the resident soil. We, therefore, call on relevant authorities, government and environmental agencies to help introduce/provide well planned and closed waste collection sites, together with good waste management systems, so as to help decrease or stern further public health risk and environmental hazards that may emanate from the use of open and unplanned waste collection sites.

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