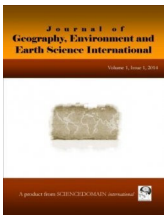

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Global Warming Potentials of Municipal Solid Waste Dumpsites in Calabar Metropolis, Cross River State

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Authors' contributions

This work was carried out in collaboration between all authors. Author IUB designed the study, wrote the protocol and wrote the first draft of the manuscript. Author LO managed the literature searches, analyses of the study and managed the experimental process while author IEA identified the greenhouse gases encountered in the studied dumpsites. All authors read and approved the final manuscript.

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ABSTRACT

Total anthropogenic greenhouse gases have continued to increase with municipal solid waste dumpsites contributing immensely to the high concentrations of greenhouse gases with high warming potentials. This study is aimed at determining the global warming potentials of municipal solid waste dumpsite in Calabar metropolis. Soil, leachate and solid wastes samples were collected from the dumpsite and subjected to standard microbiological analysis. The samples were examined for temperature, p^H, mean heterotrophic bacterial/ fungal counts and greenhouse gases emissions from the dumpsite using Combustible Gas Leak Detector. The mean temperature values for both soil and leachate samples ranged from 82°F-83°F while the mean p^H values ranged from 6.57-7.0. The proportion of Carbondioxide, Methane and Nitrous oxide in the studied dumpsite has significantly increased to 39%, 161% and 19% respectively. The mean total viable aerobic heterotrophic bacterial count in both leachate and soil samples ranged from 1.7×10^3 - 8.0×10^3 cfu/ml and 1.2×10^4 - 8.0×10^4 cfu/g, while the mean total viable fungal counts for both leachate and soil samples ranged from 1.0×10^5 – 5.0×10^5 cfu/ml and 2.1×10^3 – 6.0×10^3 cfu/g. The

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prevalent bacteria isolate from the dumpsite soil, leachate and decomposing solid waste include: *Escherichia coli*, *Bacillus sp, Pseudomonas aeruginosa, Enterobacter sp, Klebsiella sp, Proteus sp, Salmonella sp, Staphylococcus aureus, Micrococcus luteus, and Methanococcus sp.* The fungi isolated include: *Candida tropicalis, Aspergillus sp, Penicillium sp, Candida parapsilosis, Candida albicans, and Saccharomyces sp.* Statistical analysis of the bacterial and fungal counts showed significant difference ($p<0.05$) between the various sampling points. This study reveals the health, environmental, and climate hazard that could result from indiscriminate dumping of untreated wastes.

Keywords: Global warming potential; solid waste; Lemna dumpsite; greenhouse gases.

1. INTRODUCTION

The increasing awareness of global warming all over the world, and most recently its association with solid waste biodegradation is a cause for concern as temperature ranges around such waste dumpsites are of increase than normal. Warming of the climate system is unequivocal since the 1950s with observed changes [1]. Most researches on the causes of this high temperature have reported greenhouse gases (GHG) emitted from microbial activities of solid waste dumpsite [2]. Lemna solid waste dumpsite formerly known as Ikot-Effanga Mkpa dumpsite is an open dump that encourages the breeding of rodents and proliferation of most pathogenic bacterial and fungal species with no report of the dangers associated with these emissions [3,4].

Waste is any substance, solution, mixture for which no direct use is envisaged but which is transported for reprocessing, elimination by incineration or other methods of disposal [5].With urban industrialization, social development and population increase, solid waste production are growing rapidly making pollution a serious problem [6,7]. Solid waste disposal poses threat to humans, animals and the environment at large. Like chemical hazards, etiologic agents might be dispersed in the environment through water and wind.

Waste management in developing countries is usually equated with land disposal or discharge water [7,8]. This method of waste management is unscientific and causes nuisance to the public and most of all results in global warming as a result of GHG emitted into the atmosphere. This gases alter the climate and also deplete the ozone layer when waste is dumped on land, soil micro-organism including fungi and bacteria, readily colonize the waste carrying out the degradation and transformation of degradation (organic) materials in the waste [9]. Micro-organisms in the waste dump use the waste constituents as nutrients, thus detoxifying the materials as their digestive processes breakdown complex organic molecules into simple less toxic molecules [10].

Calabar city does not have a sanitary landfill. Improper disposal of untreated municipal solid waste is not only harmful to human's health but also constitute a threat to the ecological environment [7]. Temperatures around waste dumps seemed to be higher than normal, hence, the need to study keenly the global warming potentials associated with waste biodegradation in Lemna waste dumpsite.

2. MATERIALS AND METHODS

2.1 The Study Area and Sampling Site

The Lemna dumpsite in Calabar Municipality which is where this study was carried out, lies geographically along longitudes 08°18E and 08°26°E Greenwich meridian and latitudes 04°55°N and 04°58°N of the equator. In the North, Calabar Municipality is bounded by Odukpani and Akamkpa Local government Area, at the East by the Great Kwa River. At the south, it is bounded by the Calabar River and Calabar South Local government Area. It has an area of 331.551 square kilometres. Calabar municipality is characterized by a double maxima rainfall regime which occurs in June and September. The annual temperature is 28°c with a high evapotranspiration and an average humidity of 90%.

The vegetation of the study area is characterized by mangrove swamp and rainforest, but due to human activities like cutting down of tress, for roads, building of houses, schools, petrol stations, hotels and market it has resulted in the depletion of the rainforest.

It has an annual rainfall of 3000 mm and a harmattan wind blowing over the area in December and January respectively.

The sampling site was a dump located within Lemma road Calabar Municipality. It is a waste dump site used majorly by the Calabar urban development authority. The dump site was measured with tape and mater rule. The length of the landfill was 960 m, the width 430 m. Sampling station was established on the waste dumpsite and was represented as soil (S), leachate (L), solid waste (SW) and air sample (AS).

2.2 Sample Collection

2.2.1 Collection of soil sample

For soil sample collection, the wastes were first removed using a garden rake to expose the soil under the waste dump from where the soil samples were collected. The soil samples were taken at about 15 cm depth with the use of hand-driven auger and immediately transported to the laboratory in labeled polythene bags in ice-cold boxes at approximately 4℃ for microbiological analysis. A control sample was taken from a location of about 500 meters away from the dumpsite.

2.2.2 Collection of decomposing solid waste sample

A sterile large wooden spatula that had been properly rinsed with sterile distilled water was used to collect the decomposing wastes into sterile glass petri- dishes and sealed with masking tape. The samples were immediately transported to the laboratory for microbiological analysis or stored in a refrigerator at 4℃ until they were needed for analysis.

2.2.3 Collection of leachate sample

The method described by [3], was employed for leachates sampling. In this method, Pvc pipes were cut into four parts, each of 1 m and 0.5 m in length. The base end of each pipe was permanently sealed with a pipe cover and an adhesive while the top ends were just fitted with pipe covers. The pipes (both 1 m and 0.5 m length) were perforated evenly at considerable distances from their base ends to allow for water (leachates) percolation and collection. The perforated pipes were then buried at the studied dumpsite for a period of 3-4 weeks to allow percolation of leachates. After 3-4 weeks, sterile enema pumps or sterile syringes were used to collect the leachate into sterile bottles and transported to the laboratory for microbiological and physicochemical analysis.

2.3 Preparation of Diluent and Media

A ten-folds serial dilution of the sample was made by weighing one gram of sample into 9mls of sterile distilled water to obtain 10^{-1} dilution, further dilutions were made until 10^{-9} diluent was obtained.

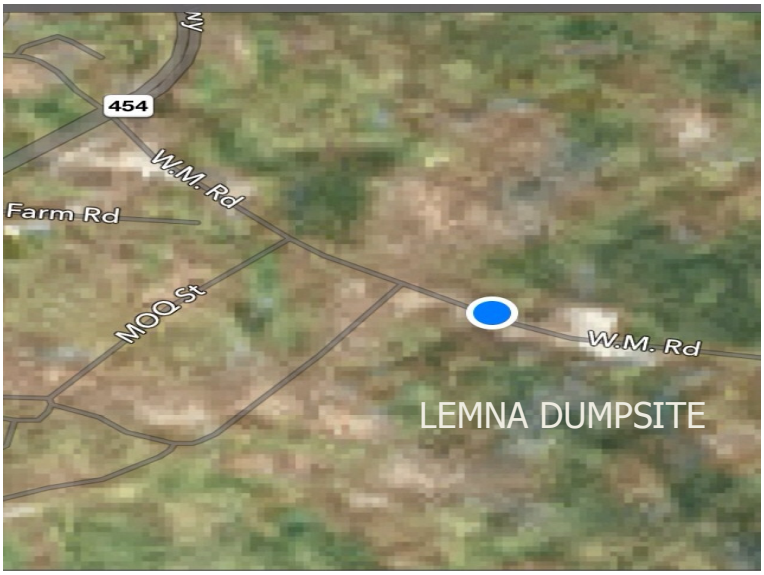

Fig. 1. Map of the study area

Fig. 2. Lemna road waste dumpsite

The media used to grow bacterial were nutrient Agar, Eosin methylene Blue Agar, *Salmonella-shigella* Agar, while the media used to grow fungi was potato Dextrose Agar. All were originally in a powdered form the media is usually prepared by weighing out a certain gram, depending on the number of plates to be prepared. The weighed quantity is then mixed with the appropriate amount of sterile distilled water in a conical flask. The conical flask is usually plugged with enough cotton wool and then placed in an autoclave for sterilization at 121℃ for 15 mins after which the media is removed from the autoclave and allowed to cool.

2.4 Determination of P^H and Temperature

The samples were made in suspension (solution) and $_P$H and temperature probes were inserted into it. The $_P$H and temperature was recorded accordingly.

2.5 Microbiological Analysis of Soil and Decomposing Waste Sample

One gram(1 g) of the sample was dissolved in 9ml sterile distilled water from the solution, ten-fold serial dilutions in the range of 10^{-1} – 10^{-9} were prepared. One milliliter (1 ml) aliquot from the dilution of 10^{-4} of each samples were aseptically transferred into freshly prepared nutrient agar plates then eosin methylene blue agar and salmonella-shigella agar as selective media for isolation and identification while 0.1 ml from the dilution of 10^{-3} was aseptically transferred into potato dextrose agar plates were incubated at 37℃ (24 hours) for bacterial isolates and 28±0.2℃ (3 to 5 days) for fungal isolates. Visible colonies of between 30-300 were multiplied by the reciprocal of the dilution factors and recorded as colony forming units per gram (cfu/g) of waste.

2.6 Microbiological Analysis of Air at Dumpsite

Settle plates method described by Bassey et al., 2015, was adopted for this analysis. According to this method, nutrient agar in triplicate was exposed at the dumpsite (10 m, 20 m, 30 m away from the dump) 1 hour between 10-12 noon. The plates were later covered and transported to the laboratory for incubation at 25-28℃ for 24 hours. The bacterial colonies that appeared on the plate were counted.

2.7 Microbiological Analysis of Leachate

Leachates from such a dumpsite are often turbid therefore, serial dilutions were made. One milliliter (1 ml) of the leachate was dissolved in 9ml of sterile distilled water to give 10^{-1} dilution from which further dilution up to 10^{-9} was made [11]. The dilutions were then further analyzed as those of decomposed waste and soil sample previously described above. The colony counts were expressed as colony forming units per milliliter of leachate (cfu/ml).

2.8 Isolation, Characterization and Identification of Bacteria in the Waste Dump Site

Pure cultures of bacteria were obtained by asceptically streaking representative colonies of different morphology types which appeared on the culture plates onto freshly prepared Nutrient agar plates which were incubated at 28℃ for 24 hours. Discrete bacteria colonies developed and were sub-cultured on nutrient agar slope, incubated at 28℃ for 24 hours. The nutrient agar slope served as pure culture stock for subsequent characterization tests. The pure cultures were identified based on their cultural, physiological and morphological characteristics. For tests conducted for characterization and identification, see appendix.

2.9 Isolation, Characterization, Identification of Fungi in the Waste Dumpsite

Pure cultures of fungi were obtained by sub-culturing discrete colonies into freshly prepared sabouraud dextrose agar plates and inoculated at 28℃ for 5-7 days. The fungal isolates which

developed were further sub-cultured onto agar slope and incubated at 28℃ for 5 to 7 days. The isolates which developed were pure cultures which were stored in the refrigerator as stock cultures for subsequent characteristic test. Macroscopic and microscopic examinations of fungal growth were carried out.

2.10 Determination of Greenhouse Gas Emissions from the Dumpsite

The emission of greenhouse gases from the studied dumpsite was done using combustible gas leak detector.

3. RESULTS

The frequency of occurrence of bacterial and fungal species isolated from samples obtained from Lemna waste dumpsites are presented in Tables 1 and 2. The table(s) identifies the bacteria and fungi with the highest and lowest frequency of occurrence. From the results obtained, *Escherichia coli* had the highest frequency of occurrence while *Micrococcus luteus* had the lowest frequency of occurrence Table 1. *Penicillium w*ere observed as the most occurring fungal species while *Candida tropicalis* had the lowest frequency of occurrence.

The mean bacterial and fungal counts of isolates from the different sources sampled are presented in Tables 3 and 4. The results revealed *Escherichia coli* with the highest mean counts of 27±15.60 cfu/g of decomposing solid wastes while *Pseudomonas aeruginosa* had the least mean counts of 5±3.00 cfu/g of decomposing solid wastes (Table 3). Table 4 also identified the fungus with the highest and lowest mean count for the different samples. *Candida albicans* had the highest mean count of 20±11.60 while *Candida tropicalis* had the lowest mean count of 5±3.00. Tables 5 and 6 showed the mean temperature and P^H values for both soil and leachate samples obtained from the dumpsite.

4. DISCUSSION

The gradual increase of temperature around municipal solid waste dumpsites within Calabar Metropolis were glaring over the years with serious impacts to the immediate environment and biodiversity. The increase is attributed to the continuous emissions of greenhouse gases (GHGs) from the municipal solid waste dumpsites [2,3]. The temperature values of soil and leachate samples from this study ranged from 82℉ to 83℉. While the mean temperature value for soil samples was 82.3℉ and 83℉ for leachates. The pH values ranged from 6.6 to 7.0. The mean pH value for soil sample was. 6.9 and 7.0 for leachates. The observed temperature ranges in this study are such that support the growth of mesophilic bacteria (mesophiles). From this study, mesophilic bacteria species are observed to be chiefly involved in biodegradation of the organic components of waste at the studied dumpsites (Table 1 and Table 3). This implies that, the digestion and decomposition of organic matter present in the waste is only achievable with the high presence of these organisms. In the decomposition process of the waste, it is observed that psychrophiles were more prevalent. This again is a clear indication that psychrophiles are the major organisms that

Table 1. Distribution and frequency of occurrence of bacteria species isolated from the dumpsite samples

Isolated bacteria species	Frequency of occurrence (%) of bacteria from different sources			
	S	**L**	**SW**	**AS**
Escherichia coli	80(16.50)	55 (10.83)	44 (17.0)	25 (7.50)
Bacillus sp	63(6.19)	48 (9.45)	39 (15.1)	30 (9.00)
Pseudomonas aeruginosa	52(10.72)	38 (7.48)	20 (7.72)	15 (4.50)
Enterobacter sp	37(7.63)	50 (9.84)	30 (11.50)	20 (6.00)
Klebsiella sp	49 (10.10)	33 (6.50)	27 (10.42)	40 (12.00)
Proteus sp	40(8.25)	44 (8.70)	15 (5.80)	38 (11.34)
Salmonella sp	39 (8.04)	30 (5.91)	18 (6.94)	40 (12.00)
Staphylococcus aureus	43 (8.87)	64 (12.50)	21 (8.11)	45(13.43)
Micrococcus luteus	29 (5.98)	74 (14.40)	- (-)	56 (16.71)
Methanococcus sp	53 (10.93)	73 (14.40)	45 (17.40)	26 (7.80)
Total	**485**	**508**	**259**	**335**

Key: S = soil, L = leachate, Sw = solid waste , AS = Air sample

Table 2. Distribution and frequency of occurrence of fungal species isolated from dumpsite samples

Isolated fungal species	Frequency of occurrence (%) of fungal from different sources			
	S	L	SW	AS
Candida albicans	60(22.00)	45(23.40)	35(16.00)	0(0)
Aspergillus sp	40(15.00)	30(15.62)	38(17.40)	25(45.50)
Saccharomyces sp	45(16.50)	32(16.70)	58(26.50)	30(45.60)
Candida tropicalis	20(7.33)	15(7.80)	18(8.23)	0(0)
Penicillium sp	58(21.23)	39(20.30)	50(22.83)	0(0)
Candida parapsilosis	50(18.30)	31(16.15)	20(9.13)	0(0)
Total	**273**	**192**	**219**	**55**

Table 3. Mean Count of bacteria species isolated from dumpsite samples

Isolated bacterial species	Mean count of bacterial spp from different sources			
	S	L	SW	AS
Escherichia coli	27±15.6	18.3±10.6	14.7±8.50	8.3±4.8
Bacillus sp	21±1.20	16±9.30	13±8.00	10±6.00
Pseudomonas aeruginosa	17±1.00	12.7±7.3	7±4.00	5±3.00
Enterobacter sp	12±7.00	17±9.60	10±6.00	7±4.00
Klebsiella sp	16.3±9.40	11±6.40	9±5.20	13.3±7.70
Proteus sp	13.3±7.70	15±8.50	5±3.00	12.7±7.30
Salmonella sp	14.3±8.30	21.3±12.3	7±4.00	15±8.70
Staphylococcus aureus	13±8.00	10±6.00	6±3.50	13.3±7.70
Micrococcus luteus	15.6±9.00	19.6±11.4	0(0)	22.0±13.00
Methanococcus sp	17.7±10	24.3±14.00	15±8.70	8.6±5.00

Table 4. Mean Count of fungal species isolated from dumpsite samples

Isolated fungal species	Mean count of fungal species from different sources			
	S	L	SW	AS
Candida albicans	20±11.60	15±8.70	11.7±6.70	0(0)
Aspergillus sp	13.3±7.70	10±5.80	12±7.30	8.3±4.80
Saccharomyces sp	15±8.70	10.7±6.20	19.3±11.20	10±6.00
Candida tropicalis	6.7±4.00	5±3.00	6±3.50	0(0)
Penicillium sp	19.3±11.20	13±7.30	16.7±9.60	0(0)
Candida parapsilosis	17±9.60	10.3±6.00	7±4.00	0(0)
Total	**273**	**192**	**219**	**55**

actually started the decomposition process as they are active at lower temperature up to 55℉ thus generating heat in the process. As the temperature of the decomposing waste reaches 50-100℉, the psychrophylic bacteria were seen displaced by the mesophilic bacteria which then continue the biodegradation. The microbial activities of the mesophilic bacteria often elevate the temperature of the compost (decomposing solid waste) which now gives way for the thermophilic flora such as *Bacillus*, *Aspergillus* as reported in this study. The degree of acidity (pH), reported in this study for all the stations of the waste dump site ranged from 6.5 to 7.0. [12], reported that, the initial pH of solid waste is between pH 5.0 and 7.0 for refuse which is about 3 days old. The pH of the waste drops to 5.0 or less in the parts 2-3 days of composing and then begins to rise to about 8.5 for the reminder of the aerobic process.

Table 5. Temperature of the samples

Temperature reading (Days)	Sample code			
	S_1	S_2	L_1	L_{2a}
1	82	82	83	82
2	82	83	83	82
Total	**164**	**165**	**166**	**164**
Mean	**82**	**82.5**	**83**	**82**

Key: *S_1 = soil sample I, S_2 = Soil sample II, L_1 = Leachate sample I, L_2 = Leachate sample II*

Note: *Temperature in ℉ to ℃ (T℉ – 32 x 5/9) Temperature in ℃ to ℉ (T℃ x 9/5 + 32)*

Table 6. Mean pH values of samples collected from the refuse dump site

pH reading (Days)	Sample code			
	S_1	S_2	L_1	L_2
1	7.0	7.0	7.0	7.0
2	7.0	6.57	6.9	7.0
Total	**14**	**13.6**	**13.9**	**14.0**
Mean	**7.0**	**6.98**	**6.95**	**7.0**

According to the soil classification by [13], the degree of acidity for sampled soil and leachate ranged from slightly acidic to neutral. The frequency of occurrence (%) of isolated bacterial for the different samples showed the organisms that occurred most. Among all the bacteria isolated from the different samples only *Micrococcus* was not isolated from solid waste (SW).

The frequency of occurrence of isolated fungi for the different samples showed the fungal that occurred most. Among all the fungi isolated from the different samples *Candida albicans, Candida tropicalis, Penicillium spp, Candida parapsilopsis was not isolated from the Air sample.*

The total aerobic bacterial count was highest in leachate samples and lowest in solid waste (SW) samples. The order of decrease in the bacterial counts for all the samples indicates that, solid waste (259) < Air < soil (485) <leachate (508). The total viable fungal count was highest in the soil samples and lowest in Air samples. The order of decrease in the fungal counts for all the samples however shows that, Air (552) < leachate (192) < solid waste (219) < soil (273). The bacterial counts were higher than the corresponding fungal counts. This is because the temperature range (82℉ to 83℉) supports the proliferation of the bacteria than fungi.

This study shows the frequency of occurrence of bacterial and fungal species isolated from Lemna waste dumpsite in connection with their biodegradation potentials of organic wastes that often results to the emission of greenhouse gases. The prevalent bacteria species isolated from the dumpsite include: *Escherichia coli, Micrococcus luteus, Bacillus* sp*, Proteus* sp*, Pseudomonas aeruginosa, enterobacter sp, Klebsiella sp, Salmonella sp, Methanococcus sp, Staphylococcus aureus.* Of all the different genera of bacteria isolated from the waste dumpsite, *Escherichia coli* had the highest frequency of occurrence while *pseudomonas aeruginosa* had the lowest frequency of occurrence.

Most of the bacterial isolates reported in this study have been reported [4,3,14] to be associated with waste and waste biodegradation. *Bacillus* and *Pseudomonas species* were reported by [15] to be associated with waste. *Bacillus, E. coli, Klebsiella and Pseudomonas* were also reported by [16]. Also, [17] reported *Bacillus, E. coli, Proteus, Pseudomonas, Staphylococcus, Streptococcus* among others. *Pseudomonas* has been widely reported to be associated with waste [18]. Fungal species isolated from the Lemna road dumpsite include *Candida albicans, Aspergillus spp, Saccharomyces sp, Candida tropicalis, Penicillium spp, Candida parapsilosis.* From the results obtained, *Candida tropicalis* recorded the lowest frequency of occurrence while *Saccharomyces sp* recorded the highest frequency of isolation. All fungi isolates reported in this study have been reported to be associated with waste and waste biodegradation [17,19] reported *Aspergillus* and *Saccharomyces.*

The study has revealed the presence of various bacteria and fungi known to be associated with waste biodegradation with subsequent release of greenhouse gases that has contributed immensely to the upscale of temperatures within the study area. Amongst these bacterial species, *Methanococcus* spp was the most prevent organism in sampling periods when methane gas emissions are relatively high. However, [2], reported that the upscale of temperatures around Lemna waste dumpsite was as a result of the high percentage emissions of greenhouse gases with methanogens been the most occurring organisms. The percentages of greenhouse gases (Carbondioxide CO_2, Methane CH_4 and Nitrous oxide N_2O) measured at the studied dumpsites greatly contributed to the potential warming of temperatures around the dumpsites. If the activities of the encountered bacteria, fungi and yeast species are properly harnessed, it can be used in future treatment plant, in Nigeria for improving the bioconversion of waste compost into organic fertilizer for optimum use in agriculture and horticulture. All the bacterial genera reported in this study with exception of Enterobacter sp are potential [3,20,16,14]. Also, all the fungal genera reported in this study with exception of *Penicilium sp* are potential pathogens. The presence of these potential pathogens (bacterial and fungal) reported in this study may be attributed to the disposal of raw human faecal discharges and other human wastes at the dumpsite that possibly encouraged their proliferation.

The indiscriminate dumping of waste around residential areas and other ecologically sensitive area plays an inevitable role in global warming and also pose serious health challenges. In a case where wastes are dumped indiscriminately, microbes set in to carry out decomposition. During the process of decomposition leachate are formed, if close to a river source the leachate formed flows in and contaminate the river which then becomes hazardous to the community nearby. Also, during the process of decomposition greenhouse gases are formed such as methane which contributes to the depletion of the ozone layer. All these consequences are as a result of the indiscriminate dumping of waste that has over the years been under estimated. Therefore, Cross river state waste management authorities must as a matter of urgency direct her efforts towards the treatment of waste before disposal as to minimize the health hazards associated with dumping of waste.

5. CONCLUSION

The present study reveals the potential hazards of indiscriminate dumping of solid waste on the environment at large, with regards to global warming contributions from waste biodegradation. Therefore, the results highlight the importance of proper collection, transportation, disposal and treatment of solid waste in an environmentally friendly manner that will not deface the aesthetics of the ecosystem. Calabar with a vast rising population of over 400,000 people has led to high generation of municipal solid waste with decreased space for disposal. The high concentrations of carbon dioxide, methane and nitrous oxides around the studied dumpsite is a wakeup call for more research tailored towards generating necessary measures that can arrest the risk associated with the continual emission of these greenhouse gases (GHGs) from the dumpsites.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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