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Comparative Roles of HBB5 Biosurfactant and Poultry Wastes in Polyaromatic Hydrocarbon Biodegradiation of Crude Oil-contaminated Sediment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The comparative study of poultry wastes- and HBB5 biosurfactant-mediated polyaromatic hydrocarbon biodegradation in sediment polluted with crude oil were investigated. The experiments were carried out for a period of 28 days by monitoring pH, nitrate, phosphate, polycyclic aromatic hydrocarbon and microbiological parameters using standard procedures. The pH values obtained ranged between 6.21 and 6.93 in days 1 and 28 for the most effective treatment recipes. Generally, there was depletion in the concentrations of nitrate and phosphate for all set ups, but the most effective recipe witnessed highest reduction. For the polycyclic aromatic hydrocarbons, the recipe with highest limiting nutrients depletion also recorded the most hydrocarbon loss, and yet highest increase in density of hydrocarbonoclastic bacteria and fungi. The sample containing polluted

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sediment + poultry wastes + HBB5 biosurfactant recorded PAH values of 1932.6472ppm on day 1 and 481.2272ppm on day 28. Total hydrocarbon-utilizing bacterial counts ranged from 1.48×10^4 cfu/g to 9.70×10^6 cfu/g, while hydrocarbon-utilizing fungal counts ranged between 2.30×10^3 cfu/g and 3.90×10^5 cfu/g. From the results obtained, poultry wastes combined with HBB5 biosurfactant recorded the highest efficiency in the biodegradation of polycyclic aromatic hydrocarbons in sediments, and HBB5 biosurfactant in isolation recorded higher degradation efficiency for polyaromatic hydrocarbons than the degradation effect mediated by poultry wastes alone. It is therefore recommended that a combination of surface-active agent, nutrient amendment source and viable microbial biomass be adopted and employed as potent recipe for the degradation of polyaromatic hydrocarbons in crude oil-contaminated sediments.

Keywords: Biosurfactant; poultry wastes; polyaromatic hydrocarbon; biodegradiation; crude oil-contaminated sediment.

1. INTRODUCTION

A wide variety of organic and inorganic contaminants enter the marine environment through sources which include; inputs from industrial or municipal effluents, ocean dumping of wastes, terrestrial runoff, and atmospheric deposition. In the marine environment, hydrocarbons are among the most widespread pollutants [1]. The nature, composition, chemical and physical characteristics of hydrocarbons after having spilled will always change due to weathering or aging. Weathering implies a complex series of processes including evaporation, photo-oxidation, emulsification, drafting, photolysis, spreading, absorption of particles and sedimentation [2]. The concentration of hydrocarbon contaminants in marine sediment can have negative effects on marine ecosystems and human health [3,4]. Generally, Polyaromatic hydrocarbons (PAHs) are organic pollutants that are widely distributed in the environment. They are toxic and very persistent [5,6,7]. Polyaromatic hydrocarbon compounds tend to accumulate in sediments rather than water [8,9]. Concentration of PAH compounds, in particular sediments, ranges from µg kg^{-1} to g kg^{-1} levels depending on the proximity of the area to PAHs sources such as industries, municipalities, and on water currents. Sediment core studies have shown an increase in PAH concentrations in the past 100-150 years with concentrations peaking in 1950 [8]. Bacteria and fungi are the main degraders of organic pollutants in marine environments. Many marine bacteria have degradability potentials, either they are enhanced by organic nutrients or augmented [10]. Polyaromatic hydrocarbons (PAHs) degradation has been examined in marine sediments, and the rates of degradation have been related to pre-exposure to related compounds [11,12]. Although many organic contaminants that associate with sediments have proven to be highly resistant to aerobic biodegradation, new approaches and understandings are promising increased potential for the biodegradation of many of these contaminants [13,14]. The importance of acclimatization of bacteria to contaminants in both aerobic and anaerobic environments is receiving increasing attention [14,15]. Properly applied surfactants have been shown to improve desorption, apparent aqueous mobility and bioavailability of hydrophobic organic compounds such as PAHs [16,17]. Surfactants that are produced by microorganisms tend to have lower toxicities and are effective at wider temperature, pH, and electrical conductivity ranges [18]. Ite et al. [19] observed that petroleum exploration and production has direct consequences on the various aspects of the environment including; the atmosphere, soils and sediments, surface and groundwater, marine environment to mention but a few in the Niger Delta. This study therefore compares the roles of poultry wastes and a bacterial biosurfactant (HBB5) in the PAH degradation of crude oil-contaminated sediment.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The polluted sediment samples were collected from Umuoka swamp in Obagi, while the control sediments were collected from Idu River, in Idu all in Ogba/Egbema/Ndoni Local Government area of Rivers State, Nigeria. Sediment samples were collected using Eckman Grab into sterile polythene bags and amber-colored glass bottles. Poultry wastes were collected from a poultry farm in Port Harcourt, Rivers State, Nigeria. Standard methods were observed during collection of samples.

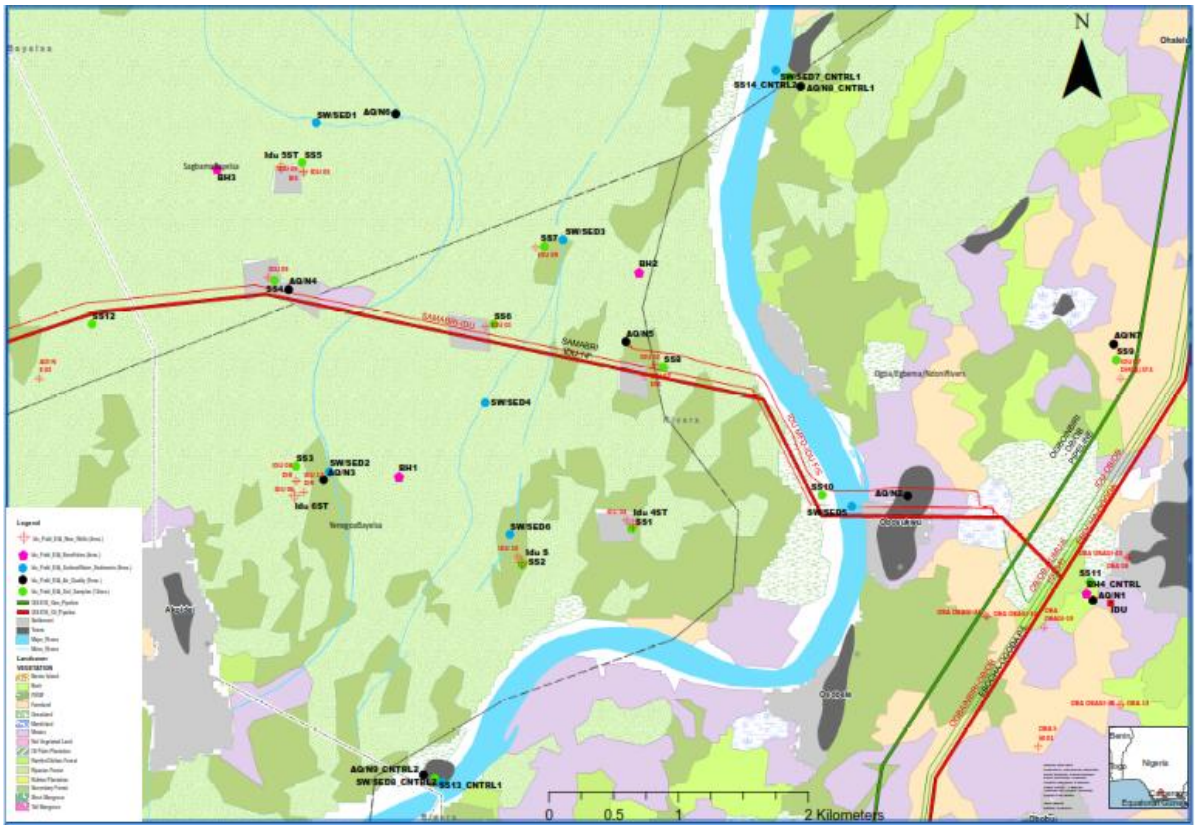

Fig. 1. Map showing study Area where samples were collected

2.2 Biosurfactant

The biosurfactant used was HBB5 biosurfactant produced from *Pseudomonas xiamenesis* that was isolated from the brackish water of Amadi-Ama creek in Port Harcourt, Rivers state.

2.3 Physicochemical Analysis

The pH was determined using the Hanna pH meter (HI 9829), which photograph is displayed in Plate 1. The ascorbic acid method as described in APHA [20] was employed in the determination of available phosphate while the nitrate content of samples was determined using the Brucine method [20]. The concentrations for polyaromatic hydrocarbons (PAHs) were determined by solvent extraction using dichloromethane, and analysis using gas chromatograph-mass spectrometer.

2.4 Determination of Total heterotrophic bacterial (THB) and Hydrocarbon-utilizing Bacterial (HUB) Counts

Total heterotrophic bacterial (THB) count was determined using the nutrient agar and spread plate technique as described by Prescott et al. [21]. An aliquot (0.1ml) of each serially diluted sample using dilution factors of 10^{-4} for all the treatment sediment samples were separately inoculated onto different freshly prepared sterile nutrient agar plates in triplicates. The plates were incubated at 37°C in an inverted position for 24 hours. After incubation, colonies that developed on the plates were counted using a colony counter, and only counts of between 30 and 300 were recorded. The average values of replicate plates were calculated and expressed as colony forming units per gram (CFU/g). The populations of the hydrocarbon-utilizing bacteria were determined by inoculating 0.1ml aliquot of the serially diluted (10^{-1} and 10^{-2}) samples of sediment onto mineral salt agar media using the spread plate technique described by Odokuma and Dickson [22] and modified by Nkwocha and Odokuma [23]. The vapour phase transfer method was adopted by the use of sterile filter paper discs saturated with crude oil, which served as the only carbon source in the mineral salt agar. The sterile crude oil-soaked filter papers were aseptically transferred to the inside covers of the inoculated Petri dishes and incubated for 5 days at room temperature [22]. Colonies that developed were counted, average

of triplicate colonies calculated colony forming units per gram of sediment measured.

Plate 1. Section of Hanna HI 9829 pH Meter

2.5 Determination of Total Fungi and Hydrocarbon Utilizing Fungal Count

The total count of fungi in the samples was also determined by the spread plate technique. An aliquot (0.1ml) of serial dilution (10^{-2}) of each of the various samples was plated onto separate Potato dextrose agar plates to which 0.1 ml of streptomycin solution was incorporated to suppress bacterial growth. The plates were incubated at 28°C for 5-7 days and the discrete colonies that developed were enumerated as the viable counts (CFU) of fungi in the sediment samples [24]. Hydrocarbon-utilizing fungal count of sediment samples was determined by inoculating 0.1ml of the serially diluted samples on mineral salt agar. The mineral salt medium was supplemented with streptomycin (0.1ml) to suppress bacterial growth [24]. The vapour phase transfer method described by Odokuma and Dickson [22] and modified by Nkwocha and Odokuma [23] was also adopted.

2.6 Biodegradation Experiment

2.6.1 Composition of biodegradation set up

Five glass troughs were used for the degradation experiment and were properly labeled. Each of the glass troughs contained 2kg of sediments (polluted and unpolluted), 200 ml of the poultry waste, and 100ml of HBB5 biosurfactant. (1:10) as shown in Table 1.

2.7 Bioremediation Experiment

The bioremediation experimental set-ups were incubated at room temperature. The set-ups containing the sediment samples and treatments were thoroughly mixed. Content of the experimental set ups was analyzed for pH, nitrate, phosphate, polycyclic aromatic hydrocarbons (PAHs), total heterotrophic bacterial count, hydrocarbon utilizing bacterial count, total fungal count and hydrocarbon utilizing fungal count at weekly intervals for 28 days.

Table 1. Experimental Set ups

Set up	Content
A	Unpolluted sediment only
B	Polluted sediment alone
C	Polluted sediment + poultry wastes
D	Polluted sediment + HBB5 Biosurfactant
E	Polluted sediment + poultry wastes + HBB5 Biosurfactant

3. RESULTS

Table 2 and Fig. 2 showed the pH profiles of the various sediment treated options. All the treated options increased with time except the unpolluted sediment (control) which decreased on day 7 and later increased on day 14, then decreased thereafter. Polluted sediment option decreased slightly on day 28. Generally the pH values ranged between 6.21- 6.93.

The results of the nitrate are shown in Table 3 and Fig. 3. Unpolluted sediment recorded nitrate range of 2.147 ppm – 2.214 ppm, polluted sediment ranged between 3.152 ppm – 3.163 ppm, polluted sediment + poultry wastes showed ranged from 1.5421 ppm to 2.110 ppm, polluted sediments + HBB5 biosurfactant recorded range of 2.253 ppm – 2.413 ppm. Polluted sediment + poultry wastes + HBB5 biosurfactant ranged between 0.866 ppm and 7.283 ppm. The nitrate values in all the treated options recorded lowest value on day 28.

Phosphate concentrations as shown in Table 4 and Fig. 4 recorded range of 0.533 ppm – 0.581 ppm for the unpolluted sediment. Values in the polluted sediment ranged between 0.283 ppm

and 0.296 ppm. The values of phosphate in the polluted sediment + poultry wastes ranged between 0.298 ppm and 0.398 ppm. Polluted sediment + HBB5 biosurfactant treatment option recorded values of phosphate which ranged between 0.168 ppm and 0.193 ppm. The treatment containing polluted sediment + poultry wastes + HBB5 recorded values ranging from 0.316 ppm to 3.418 ppm.

Table 2. pH in various Sediment treated recipes

Sample	Day 1	Day 7	Day 14	Day 21	Day 28
Unpolluted sediment only (control)	6.27	6.23	6.30	6.25	6.22
Polluted sediment	6.26	6.28	6.31	6.33	6.32
Polluted sediment + poultry wastes	6.26	6.39	6.43	6.47	6.53
Polluted sediment + HBB5 Biosurfactant	6.21	6.36	6.51	6.77	6.93
Polluted sediment + poultry wastes + HBB5 Biosurfactant	6.21	6.36	6.51	6.77	6.93

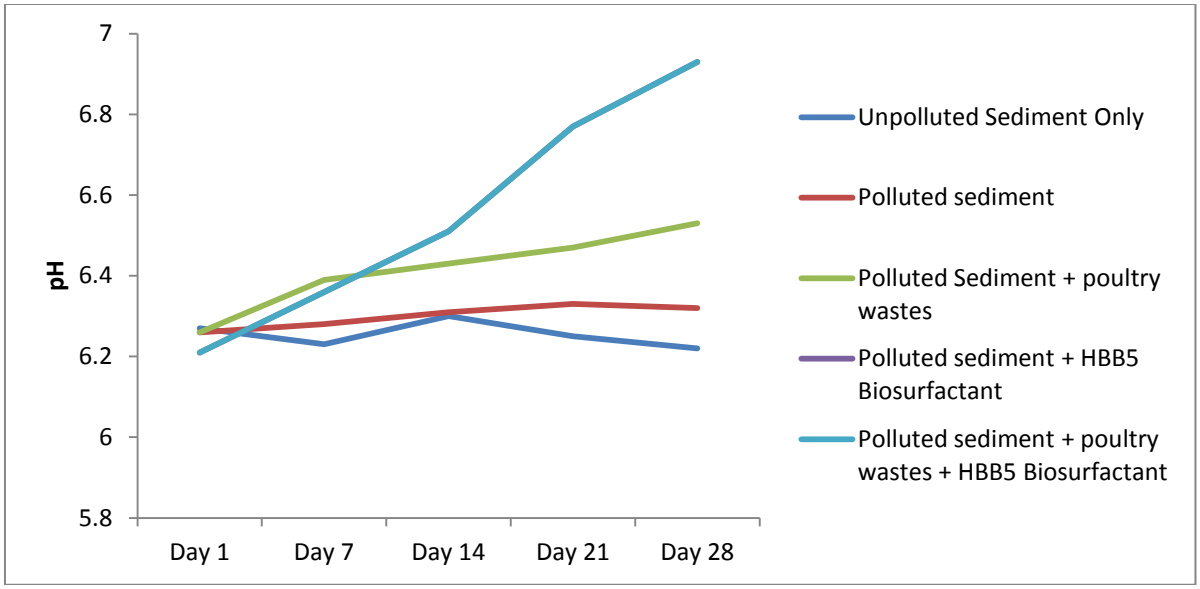

Fig. 2. pH perturbations in treatment recipes

Table 3. Nitrate concentrations (ppm) in various Sediment treatment options

Sample	Day 1	Day 7	Day 14	Day 21	Day 28
Unpolluted sediment only (control)	2.214	2.211	2.193	2.162	2.147
Polluted sediment	3.162	3.163	3.160	3.157	3.152
Polluted sediment + poultry wastes	2.110	2.018	1.993	1.875	1.542
Polluted sediment + HBB5 Biosurfactant	2.413	2.383	2.311	2.279	2.253
Polluted sediment + poultry wastes + HBB5 Biosurfactant	7.283	6.042	5.317	3.328	0.866

Table 4. Phosphate concentrations (ppm) in various Sediment treatment options

Sample	Day 1	Day 7	Day 14	Day 21	Day 28
Unpolluted sediment only (control)	0.572	0.533	0.581	0.575	0.563
Polluted sediment	0.296	0.293	0.290	0.286	0.283
Polluted sediment + poultry wastes	0.398	0.363	0.319	0.305	0.298
Polluted sediment + HBB5 Biosurfactant	0.193	0.188	0.184	0.180	0.168
Polluted sediment + poultry wastes + HBB5 Biosurfactant	3.418	3.114	2.318	1.146	0.316

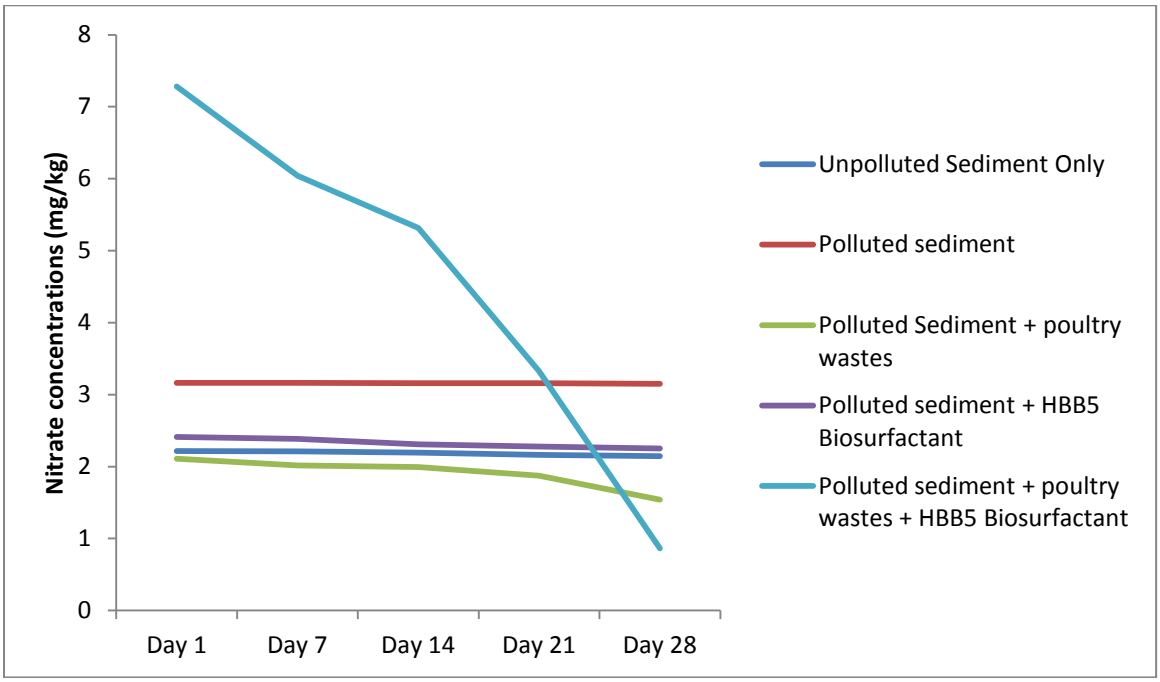

Fig. 3. Nitrate concentrations perturbations in treatment recipes

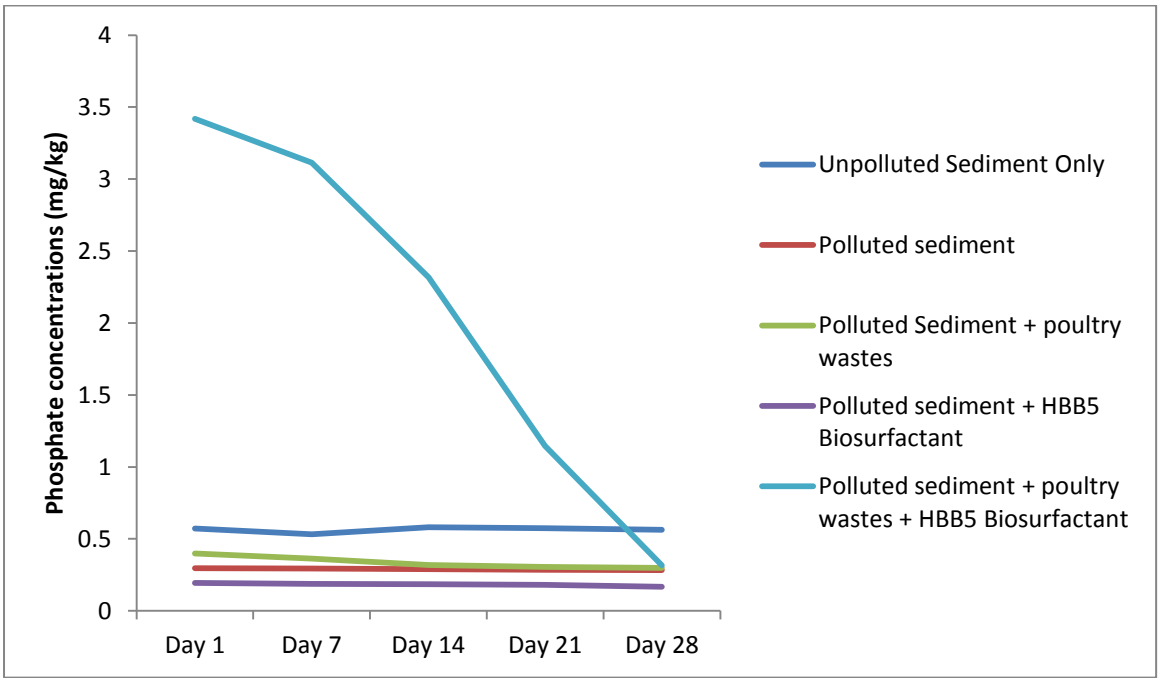

Fig. 4. Phosphate concentration perturbations in treatment recipes

The concentrations of polycyclic aromatic hydrocarbon in the various treatment options are shown in Table 5 and Fig. 5. The unpolluted sediment recorded 0.0093 ppm and 0.0068 ppm on days 1 and 28, respectively. The polluted sediment on day 1 was 1938.1446 ppm and 1921.1442 ppm on day 28. Polluted sediment + poultry wastes recorded values of 1946.3847 and 1336.8249 ppm on days 1 and 28, respectively. The values of 1932.64742 and 1014.7421 ppm were obtained on days 1 and 28, respectively for polluted sediment + HBB5 biosurfactant. The sample containing polluted sediment + poultry wastes + HBB5 biosurfactant recorded values of 1932.6472 ppm on day 1 and 481.2272 ppm on day 28.

Table 5. Concentrations (ppm) of polycyclic aromatic hydrocarbon in various Sediment treatment options

Sample	Day 1	Day 7	Day 14	Day 21	Day 28
Unpolluted sediment only (control)	0.0093	0.0084	0.0079	0.0074	0.0068
Polluted sediment	1938.1446	1933.6518	1929.4588	1926.4177	1921.1442
Polluted sediment + poultry wastes	1946.3847	1933.3898	1948.2390	1618.0384	1336.8249
Polluted sediment + HBB5 Biosurfactant	1932.6472	1822.1024	1517.2172	1321.2431	1014.7421
Polluted sediment + poultry wastes + HBB5 Biosurfactant	1932.6472	1779.1024	1283.2172	771.7021	481.2272

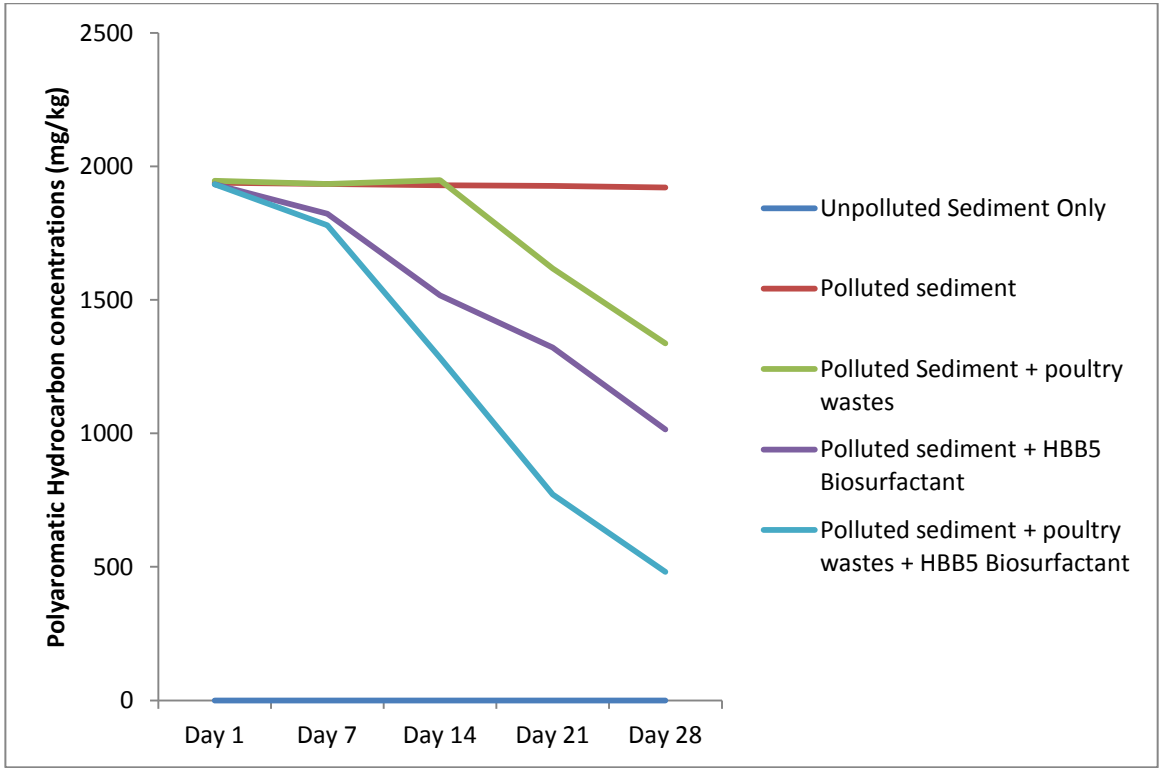

Fig. 5. Polyaromatic hydrocarbon perturbations in treatment recipes

Table 6. Total heterotrophic bacterial counts (cfu/g) in various Sediment treatment options

Sample	Day 1	Day 7	Day 14	Day 21	Day 28
Unpolluted sediment only (control)	1.56×10^7	1.55×10^7	1.49×10^7	1.52×10^7	1.61×10^7
Polluted sediment	3.00×10^4	3.30×10^4	4.30×10^4	6.50×10^4	8.80×10^4
Polluted sediment + poultry wastes	3.60×10^4	5.20×10^4	9.10×10^4	1.50×10^5	6.30×10^5
Polluted sediment + HBB5 Biosurfactant	3.30×10^4	8.20×10^4	1.27×10^5	4.20×10^5	8.90×10^5
Polluted sediment + poultry wastes + HBB5 Biosurfactant	3.00×10^4	1.52×10^5	1.38×10^6	8.40×10^6	1.93×10^7

Table 7. Hydrocarbon-utilizing bacterial counts (cfu/g) in various Sediment treatment options

Sample	Day 1	Day 7	Day 14	Day 21	Day 28
Unpolluted sediment only (control)	1.66×10^4	1.49×10^4	1.52×10^4	1.48×10^4	1.51×10^4
Polluted sediment	1.91×10^4	1.76×10^4	3.00×10^4	4.10×10^4	5.00×10^4
Polluted sediment + poultry wastes	1.62×10^4	2.74×10^4	4.50×10^4	8.80×10^4	2.93×10^4
Polluted sediment + HBB5 Biosurfactant	1.91×10^4	3.10×10^4	5.50×10^4	6.90×10^4	9.40×10^4
Polluted sediment + poultry wastes + HBB5 Biosurfactant	1.89×10^4	7.60×10^4	8.00×10^5	5.30×10^6	9.70×10^6

Table 8. Total fungal counts (cfu/g) in various Sediment treatment options

Sample	Day 1	Day 7	Day 14	Day 21	Day 28
Unpolluted sediment only (control)	3.30×10^5	3.50×10^5	3.70×10^5	3.60×10^5	3.80×10^5
Polluted sediment	2.10×10^4	2.37×10^3	3.60×10^4	4.20×10^4	5.30×10^4
Polluted sediment + poultry wastes	2.10×10^4	3.20×10^4	5.70×10^4	9.10×10^4	2.14×10^5
Polluted sediment + HBB5 Biosurfactant	2.10×10^4	3.00×10^4	5.10×10^4	8.60×10^4	1.73×10^5
Polluted sediment + poultry wastes + HBB5 Biosurfactant	2.10×10^4	8.80×10^4	6.20×10^5	1.40×10^6	8.70×10^6

Table 9. Hydrocarbon-utilizing fungal counts (cfu/g) in various Sediment treatement options

Sample	Day 1	Day 7	Day 14	Day 21	Day 28
Unpolluted sediment only (control)	2.30×10^3	3.00×10^3	2.40×10^3	2.20×10^3	2.70×10^3
Polluted sediment	2.30×10^3	2.40×10^3	4.00×10^3	4.40×10^3	6.40×10^3
Polluted sediment + poultry wastes	2.30×10^3	3.30×10^3	6.00×10^3	1.00×10^4	2.30×10^4
Polluted sediment + HBB5 Biosurfactant	2.30×10^3	3.60×10^3	5.50×10^3	1.10×10^4	2.70×10^4
Polluted sediment + poultry wastes + HBB5 Biosurfactant	2.30×10^3	9.30×10^3	7.50×10^4	2.80×10^5	3.90×10^5

Table 10. Biodegradation Kinetics for Polyaromatic hydrocarbon

Experimental setup	Model Equation	Prediction constant	Degradation constant	Half Life (days)	Degradation Efficiency (%)
Polluted sediment Alone (PSA)	y = -0.000x + 7.569	0.992	0.0001	6931	0.9
Polluted sediment + Poultry wastes (PS + PW)	y = -0.013x + 7.656	0.794	0.013	53	31.3
Polluted sediment + HBB5 Biosurfactant (PS + HBB5)	y = -0.023x + 7.639	0.967	0.023	30	47.5
Polluted sediment + Poultry wastes + HBB5 Biosurfactant (PS + PW + HBB5)	y = -0.053x + 7.765	0.958	0.053	13	75.1

$$Half\ Life = \frac{\mathrm{Ln}\ 2}{Slope}$$

The total heterotrophic bacterial counts obtained in various sediment treatment options are as shown in Table 6. On day 1, the unpolluted sediment recorded counts of 1.56 x 10^7 cfu/g, which decreased on days 7 and 14, then later increased on days 21 and 28. Other treatment options recorded progressive increase in the counts from day 1 through day 28. Polluted sediment recorded least count of 3.00 x 10^4 cfu/g on day 1 and highest count of 8.80 x 10^4 cfu/g on day 28. Lowest count of 3.60 x 10^4cfu/g and highest count of 6.30 x 10^5cfu/g on days 1 and 28, respectively was obtained in the polluted sediment + poultry wastes. Polluted sediment + HBB5 biosurfactant recorded lowest count of 3.30 x 10^4 and highest count of 8.90 x 10^5cfu/g on days 1 and 28, respectively. The option with polluted sediment + poultry wastes + HBB5 biosurfactant recorded least count of 3.00 x 10^4 cfu/g on day 1 and highest count of 1.93 x 10^7cfu/g on day 28.

The hydrocarbon-utilizing bacterial counts obtained in various sediment treatment options are as shown in Table 7. On day 1, the unpolluted sediment recorded counts of 1.66 x 10^4cfu/g, which decreased on day 7 and later increased on days 14 and 28. Polluted sediment recorded least count of 1.76 x 10^4 on day 7 and highest count 5.00 x 10^4cfu/g on day 28. Lowest count of 1.62 x 10^4cfu/g and highest count of 8.80 x 10^4cfu/g were obtained in the polluted sediment + poultry wastes on days 1 and 21, respectively. Polluted sediment + HBB5 biosurfactant recorded lowest count of 1.91 x 10^4 and highest count of 9.40 x 10^4cfu/g on days 1 and 28, respectively. The option with polluted sediment + poultry wastes + HBB5 biosurfactant recorded least count of 1.89 x 10^4cfu/g on day 1 and highest count of 9.70 x 10^6cfu/g on day 28.

Table 8 shows the total fungal counts in the various sediment treatment options. Unpolluted sediment recorded least counts of 3.30 x 10^5cfu/g on day 1 and highest count 3.80 x 10^5cfu/g on day 28. Polluted sediment recorded least count of 2.10 x 10^4 cfu/g on day 1 and highest count of 5.30 x 10^4 cfu/g on day 28. Lowest count of 2.10 x 10^4cfu/g and highest count of 2.14 x 10^5cfu/g were obtained in the polluted sediment + poultry wastes on days 1 and 28, respectively. Polluted sediment + HBB5 biosurfactant recorded lowest count of 2.10 x 10^4 cfu/g and highest count of 1.73 x 10^5cfu/g on days 1 and 28, respectively. The option with polluted sediment + poultry wastes + HBB5 biosurfactant recorded least count of 2.10 x 10^4cfu/g on day 1 and highest count of 8.70 x 10^6cfu/g on day 28.

The hydrocarbon-utilizing fungal counts in treated options are as presented in Table 9. Unpolluted sediment recorded least count of 2.20 x 10^3cfu/g on day 21 and highest count 3.00 x 10^3cfu/g on day 7. Polluted sediment recorded least count of 2.30 x 10^3 cfu/g on day 1 and highest count of 6.40 x 10^3 cfu/g on day 28. Lowest count of 2.30 x 10^3cfu/g and highest count of 2.30 x 10^4cfu/g were obtained in the polluted sediment + poultry wastes on days 1 and 28, respectively. Polluted sediment + HBB5 biosurfactant recorded lowest count of 2.30 x 10^3cfu/g and highest count of 2.70 x 10^4cfu/g on days 1 and 28, respectively. The option with polluted sediment + poultry wastes + HBB5 biosurfactant recorded least count of 2.30 x 10^3cfu/g on day 1 and highest count of 3.90 x 10^5cfu/g on day 28.

The polluted sediment recorded degradation efficiency of 0.9%, polluted sediment + poultry waste obtained 31.3% degradation efficiency. 47.5% was recorded in the treatment with polluted soil + HBB5 biosurfactant, while 75%.1 degradation efficiency was recorded for polluted soil + poultry wastes + HBB5 biosurfactant.

4. DISCUSSION

Biodegradation of polluted sediments was investigated using poultry wastes and HBB5 biosurfactant. The control (unpolluted sediment) and polluted sediment alone were compared with the other treatments. The pH value in the polluted sediment + HBB5 biosurfactant and polluted sediment + poultry wastes +HBB5 biosurfactant recorded the least values while the unpolluted sediment only recorded the highest. The pH values generally were slightly acidic. The pH values obtained in this study favoured microbial growth and in turn increased the biodegradation. This supports the observation of Pawer (2005), Singh et al. [25] that alkaline or slightly acid pH improves biodegradation of contaminated environment. Highest value of nitrate on day 1 was recorded in polluted sediment + poultry wastes + HBB5 biosurfactant. This was followed by polluted sediment, polluted sediment + HBB5 biosurfactant, unpolluted sediment. The least value of nitrate on day 1 was recorded in the polluted sediment + polluted wastes. There was reduction of nitrate values in all the treatment options at day 28, with the least value recorded on polluted sediment + poultry wastes + HBB5 biosurfactant. The phosphate

values on day 1 was more in the polluted sediment + poultry wastes + HBB5 biosurfactant treated option and the least was recorded in the polluted sediment + HBB5 biosurfactant. On day 28, there was decrease in all the treatment option. The least remaining value of phosphate was observed in the polluted sediment + HBB5 biosurfactant. Reduction in the nitrate and phosphate values in the treated options could be that they were utilized by microorganisms present in the samples. The high amount of nitrate and phosphate recorded in polluted sediment + poultry wastes + HBB5 biosurfactant could be attributed to the poultry waste and HBB5 biosurfactant added to the polluted sediment, suggesting there use in biodegradation of polluted sediment were nutrient level is limited. Leahy and Colwell [26] reported that although oil hydrocarbons are rich source of carbon and energy, they do not contain significant concentrations of other nutrients (such as nitrogen and phosphorus) necessary for the growth of microorganisms. The carbon/nitrogen/phosphorus/potassium ratio can be adjusted by adding fertilizers or organic nutrients which accelerate the biodegradation of oil hydrocarbons [27,28]. Results from the present study confirm the level of contamination of the sediment by polycyclic hydrocarbons. The contamination could be as a result of industrial activities, discharge of waste containing hydrocarbon and its contents. High level of contamination of polycyclic aromatic hydrocarbon in marine coastal area caused by industrial, petrochemical plants effluent, agricultural runoffs and navigation waste discharges were also reported by researchers [29,30,31]. The polycyclic aromatic hydrocarbon in the treated options reduced progressively in all the days. Polluted sediment + poultry wastes + HBB5 biosurfactant had the least remaining amount of polycyclic hydrocarbon with 75.1% degradation efficiency, this was followed by the polluted sediment + HBB5 biosurfactant (47.5% degradation efficiency), polluted sediment + poultry wastes (31.3% degradation efficiency). The highest amount remaining was observed in the polluted sediment (0.9% removal). The results obtained showed that biodegradation of polluted sediment using combination of poultry wastes + HBB5 biosurfactant was a better combination compared to their use individually. The polluted sediment only also degraded the PAHs although the processes were relatively slow. Bioremediation treatments have been increasing by emerging in recent years, for the low environmental impact, low costs, and ability to degrade organic contaminants and for the possibility to use post treatment sediments [32]. Bioaugmentation strategy is one of the most important issues in bioremediation [33]. The ubiquitous distribution of oil degrading microorganisms has already been reported [26]. The total heterotrophic bacterial counts in the treated options on day 1 showed that the polluted sediment and polluted sediment + poultry wastes recorded the least counts. The highest count was obtained in the unpolluted sediment. The heterotrophic bacterial counts increased as the experiment progressed. The highest count on day 28 was observed in polluted sediment + poultry wastes + HBB5 biosurfactant while the least count was obtained in polluted sediment. The least count recorded on day 1 could be attributed to the impact of hydrocarbon pollution on the microbial community. Hydrocarbon utilizing bacteria on day 1 were higher in the polluted sediments and polluted sediment + HBB5 biosurfactant. The polluted sediment + poultry wastes recorded the least count. On day 28, highest hydrocarbon utilizing bacterial count was observed in the polluted sediment + poultry wastes + HBB5 biosurfactants. The high counts recorded could be as a result of the addition of poultry wastes + HBB5 biosurfactant. The total fungal counts were higher in the unpolluted sediment compared to other treatment options. On day 28 the highest count was observed in the polluted sediment + poultry wastes + HBB5 biosurfactant. The hydrocarbon utilizing fungi recorded same values on day 1.on day 28 the highest count was observed in polluted sediment + poultry wastes + HBB5 biosurfactant. Researchers have reported that culturable hydrocarbon degrading and PAHs degrading populations are widely distributed and can be enriched from sites of contamination in marine environments [34,35]. However other studies indicate that high levels of PAHs can be toxic to marine bacteria which might inhibit PAHs degrading bacteria and other microorganisms [36,37].

5. CONCLUSION AND RECOMMENDATION

This study showed that the polluted sediment demonstrated impact with polycyclic aromatic hydrocarbons. The environmental and physiological factors were observed to favour the growth of microorganisms, especially those of the hydrocarbon degraders. Treatment options used suggest that poultry wastes and HBB5 biosurfactant has great efficiency in the

biodegradation of PAHs in sediments. For bioremediation of PAH, the HBB5 biosurfactant should be considered for use in combination with poultry wastes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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