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EFFECT OF PH AND TEMPERATURE ON THE REMOVAL OF HEAVY METALS BY AUTOCHTHONOUS BACTERIA FROM SOIL IN A GAS FLARING ENVIRONMENT

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ABSTRACT

The concentration of heavy-metals in soil is constantly being altered by activities of gas flaring in the environment. This study was carried out to assess the effect of pH and temperature on the removal of heavy-metals: Lead, Chromium, and Cadmium present in soils of gas-flaring communities using autochthonous heavy-metal resistant bacteria. Thirty soil samples were each collected from three communities and a total of 120 bacteria strains isolated amongst which two were found to withstand mixtures of lead, Chromium and Cadmium contamination up to 500ppm. The isolates were subjected to 16S rRNA gene sequencing for identification which ares *Salmonella enterica* and *Alcaligenes faecalis*. The Effect of pH and temperature was studied at pH 5, 7, and 9 at 37℃ and temperatures of 35, 55, and 65℃ at pH 7 for 48hours to determine the rate of metal removal. At the end of 48hours, *Salmonella enterica* recorded the highest lead removal (74%) at pH 5 with a concentration of 300ppm, Chromium(85%) and Cadmium(86%) removal at pH 7 with a concentration of 400ppm. At a temperature of 55℃ with a concentration of 400ppm, the highest lead removal (82%) was recorded with *Alcaligenes faecalis*, while Chromium (85%) and Cadmium (86%) removal with *Salmonella enterica*. Statistical analysis showed a significant difference in the concentration of heavy-metals before and after bioremediation. This study reveals that pH 5 and 7 and temperature of 55℃ at concentrations of 400ppm can thus be used for the removal of lead, Chromium and Cadmium in polluted soil in gas-flaring environments.

Keywords: heavy metals, gas flaring, bioremediation, pH, temperature, autochthonous bacteria

INTRODUCTION

The threat to human, animal, and plant life posed by pollution due to gas flaring cannot be overemphasized. Gas flaring is the unscientific burning of excess hydrocarbon gathered in an oil/gas production flow station. The most flaring sight in gas production flow stations is the ten-meter-high flame that burns continuously from vertical pipes at the many facilities owned by oil companies. Gas flaring in oil rigs and wells have been known to cause greenhouse gases in our atmosphere (Doran and Safly 2007). Nigeria tops the list of ten countries responsible for 75% of gas flaring emissions in the world. She flares 16% of the total associated gas, the highest amount by any country in the world after Russia (Dung *et al*., 2008). These gases are mostly emitted in the Niger Delta area of Nigeria.

Pollution with heavy metals is a consequence of anthropogenic activities which are released into the environmental media, especially water, soil, and sediments, and has currently become a significant threat to living organisms in the environment and they pose serious health problems throughout the world (Igiri *et al*., 2018; Deepa & Suresha, 2014; Hrynkiewicz & Baum, 2014; Okolo *et al*., 2016; Siddiquee *et al*., 2015; Su, 2014) With the growth of industry, there has been

a considerable increase in the discharge of industrial waste to the environment, chiefly soil, and water, which has led to the accumulation of heavy metals, especially in urban areas. Heavy metals are natural components of the earth's crust which are known as metallic elements. They are toxic in low concentrations and have relatively high densities e.g., arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, zinc, etc. (Elekofehinti *et al*., 2012). They are cytotoxic, carcinogenic and mutagenic in nature and have been defined with several criteria such as specific density greater than 5 g/ml, hard-soft acid and bases, cationic-hydroxide formation, complex formation, and environmental toxicity. They cannot be degraded, and unlike organic contaminants are able to build up and accumulate in plants and animals and can then be passed to humans in the food web as a consumer.

To make the environment healthier for human beings, contaminated water bodies and land need to be rectified to make them free from heavy metals. There are several techniques to remove these heavy metals, including chemical precipitation, oxidation or reduction, filtration, ion exchange, reverse osmosis, membrane technology, evaporation, and electrochemical treatment. However, most of these techniques become ineffective when the concentrations of heavy metals are less than 100 mg/L.

The use of microorganisms for remediation purposes is thus a possible solution for heavy metal pollution since it includes sustainable remediation technologies to rectify and re-establish the natural condition of soil which is also a natural process and its importance of biodiversity (above or below the ground) is increasingly considered for clean-up of metal contaminated and polluted ecosystem. Moreover, the response of microbial communities to heavy metals depends on several environmental factors such as the type of metal, the nature of the medium, and microbial species. Thus, this study is aimed at assessing the effect of pH and temperature in the removal of heavy metals from polluted soil in gas flaring environments using autochthonous heavy metal-resistant bacteria.

MATERIALS AND METHODS

Sampling area

Three different villages where gas flaring takes place were chosen for this work. These include Kwale/Okpai in Ndokwa East (5.7175° N, 6.4847° E), Uzere in Isoko South (5.3269° N, 6.2433° E), and Irri in Isoko South (5.4860° N, 6.2415° E) which are all remote villages situated in Delta state (Figure 1).

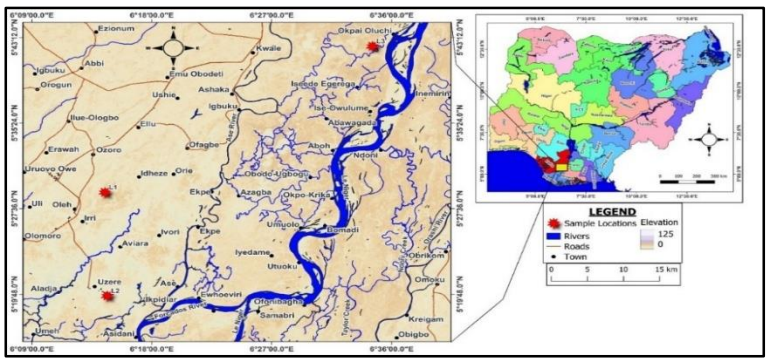


Figure 1: Map of Study Area and Sampling Location

Sample collection

A total of 30 Soil samples (10 samples per site) were obtained from three different sites; Uzere flow station, Obotoke (5°19'39.0"N 6°14'40.0"E), Irri flow station,1, Oleh (5°29'06.0"N 6°14'29.0"E) and Kwale-Okpai IPP (5°42'24.0"N 6°34'35.0"E) located in Delta state, Nigeria from a depth of 5cm using sterile trowel into sterile polythene bags. The samples were collected at distances 20m, 40m, 60m, 80m, 100m, 120m, 140m, 160m, 180m and 200m from the actual gas flaring point and transported immediately to the laboratory for analysis.

Digestion and Analysis of Soil Samples for Heavy Metal

Digestion was carried out by air drying 1.0 g of soil samples collected to eliminate moisture content and then placed in a 100 ml beaker in a fume cupboard, followed by the addition of 10 ml of nitric acid. This mixture was heated on a hot plate till the evolution of brown fume stopped. Distilled water was added when necessary. The digest was thereafter filtered using Whatman filter paper after cooling into a 50 ml volumetric flask and made up to mark with distilled water. The filtrates were then analyzed using Atomic Adsorption Spectrophotometry (AAS). Concentrations of heavy metals of Pb, Cr, and Cd were determined and expressed in ppm (Butler *et al*., 2009).

Isolation and Enumeration of Bacteria

Isolation and enumeration of bacterial was carried out using serial dilution, isolates with unique morphological characteristics were selected and sub-cultured on fresh nutrient agar plates using the streak plate method and incubated at 37℃ for 24 hours. These colonies were further sub-cultured into nutrient agar slants in bijou bottled, incubated for 24 hours at 37℃, and preserved in the refrigerator at 4℃ for further identification and characterization.

Characterization of Bacteria

This was carried out using appropriate methods of identification in accordance with the methods reported by Cheesbrough (2004). The bacterial isolates were subjected to morphological and various biochemical characterization such as gram staining, motility test, catalase test, citrate utilization test, indole production, oxidase test, and triple sugar ion (TSI) tests. Pure cultures of the isolates were identified according to Bergey's Manual of Determinative Bacteriology (Garrity *et al*., 2004).

Screening for Potential Heavy Metal-Resistant Bacteria

Pure cultures of bacterial isolates were screened for their ability to grow on increasing concentrations of a mixture of heavy metals; Pb, Cr, and Cd by agar plating method. Nutrient agar was sterilized at 121℃ for 15 minutes and allowed to cool at room temperature (37℃). This was followed by the addition of 100, 200, 300, 400, 500, and 600 ppm concentrations of a mixture of the heavy metals into the nutrient agar medium in separate conical flasks and transferred into petri dishes. Pure cultures of isolated bacteria were introduced into the Sterile Nutrient medium using the streak plate technique and then incubated at 37℃ for 24 hours (Rajkumar and Freitas, 2008, Jamaluddin *et al*., 2012). Isolates that were able to tolerate growth up to a concentration of 500ppm of the mixture of heavy metals (Pb, Cr and Cd) were selected as potential metal resistant isolates for further studies.

Molecular characterization

The heavy metal resistant isolates were grown overnight in a Nutrient broth medium for the isolation of genomic DNA using a method described by Hiney *et al*. (1992). Universal forward primers 27F- 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse 1429R- 5'-GGTTACCTTGTTACGACTT-3'were used for the amplification of the 16s rRNA gene from the genomic DNA using ethidium bromide-stained ion (Tamura *et al*., 2007). The BLAST search from NCBI was used in the identification of the heavy metal-resistant isolates.

Effect of pH

The effect of pH was studied at a pH range of 5, 7, and 9 to monitor the rate of metal removal. 0. 1M of NaOH and 0.5M of HCl were used as pH regulators. 5ml each of the freshly prepared inoculums of the heavy metal-resistant isolates was dispersed into 50 ml each of sterile nutrient broth contained in 250ml conical flasks containing 100, 200, 300, 400, and 500ppm of the metals Pb, Cr, and Cd separately. All flasks were incubated and maintained at different pH values at 37°C for 48 hours. This experiment was carried out in triplicate. 15 ml of the solution was withdrawn and centrifuged at 4000 rpm for five minutes. The supernatant was analyzed by AAS for residual metal ion concentration and the mean value was recorded.

Effect of Temperature

The effect of temperature on the growth of the heavy metal-resistant bacteria was studied at temperatures of, 35, 55, and 65°C to monitor the rate of metal removal at a pH of 7.0. Metal-resistant isolates were cultured in nutrient broth and incubated for 48 hours. 5 ml each of freshly prepared metal-resistant isolates was dispersed into 50 ml each of sterile nutrient broth contained in 250 ml conical flasks containing 100, 200, 300, 400, and 500 ppm of the heavy metals Pb, Cr, and Cd separately. This experiment was carried out in triplicate. 15 ml of the solution was withdrawn and centrifuged at 4000 rpm for Five minutes. The supernatant was analyzed by AAS for residual metal ion concentration and the mean value was recorded (Butler *et al*., 2009).

The biosorption percentage was determined by Beer Lambert's law.

$$\text{Percentage biosorption (\%)} = \frac{\text{Initial metal concentration} - \text{Finial metal concentration}}{\text{Initial metal concentration}} \times 100$$

Bioremediation of soil

Bioremediation of the soil was carried out in sterile plastic bowls. Metal-resistant isolates were cultured in 250ml of sterile nutrient broth and incubated at 37°C for 24hours. The polluted soil samples were air dried and autoclaved for 15minutes at 121°C to kill the indigenous microorganisms. The soil samples were divided into three parts prior to inoculation. Part A and B were each sprayed with 20ml of the liquid culture of the metal resistant isolates using a syringe, part C without the isolates was used as control. This experiment was carried out in triplicate. The samples were kept at room temperature (37°C). The polluted soil in each bowl was turned every 48hours to facilitate mixing of microbes and nutrients and to provide the necessary aeration (Ayotamuno et al., 2006). The water content was adjusted with distilled water by adding 45% of each soil's water holding capacity at three days' interval as described by Baldrian *et al.,* (2000). This remediation exercise lasted for 2weeks (14 days). At the end of this treatment, the residual metal concentration was determined using AAS to determine the extent of bioremediation by the selected metal resistant isolates.

Statistical Analysis

Graphs drawn from the data generated from this study were done using Microsoft Excel and data was subjected to statistical analysis using paired T-test to compare the concentration of heavy metal before treatment and after treatment using heavy metal-resistant organisms. All statistical analysis was performed using Statistical Package for Social Science (SPSS) version

RESULTS

Heavy metal concentrations in soil samples

Heavy metal concentrations of Pb, Cr, and Cd present in polluted soil samples collected from Gas flaring environments were determined to ascertain the concentrations present in these samples. It was observed that in all the samples collected from the three different sites, the concentration of the heavy metals was reduced meters away from the actual gas flaring site (Figure 2-4)

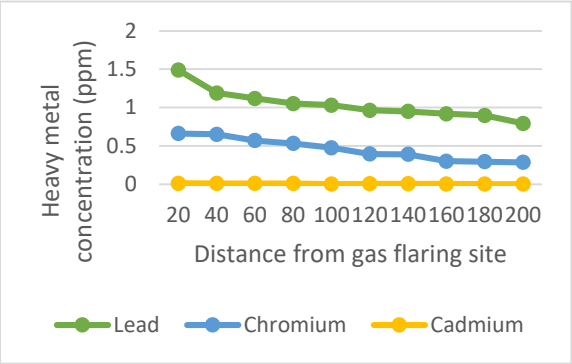


Figure 2: Heavy metal concentrations in Oleh

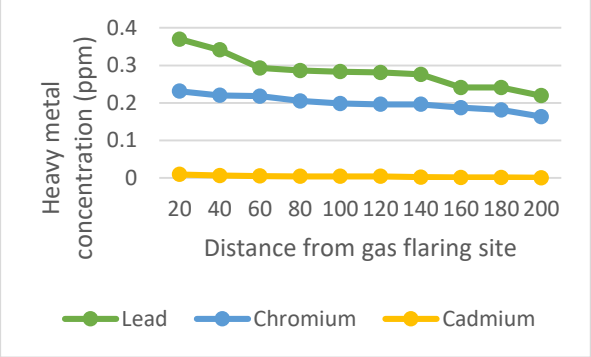


Figure 3: Heavy metal concentrations in Irri Uzere

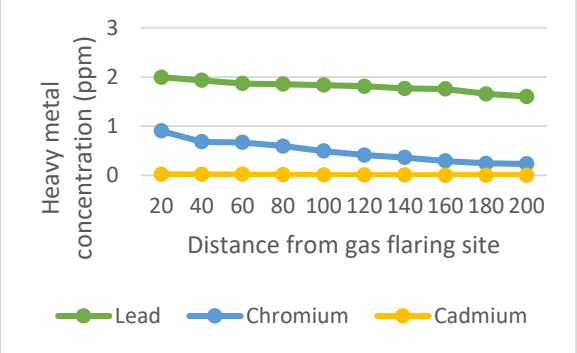


Figure 4: Heavy metal concentrations in Kwale

Microbiological Enumeration of Bacterial Loads in Soil Samples (cfu/g)

The microbiological enumerations of viable aerobic bacteria present in soil samples are presented in Table 1 which follows a similar trend observed in the concentration of heavy metal whereby samples closest to the flare point have the least number of bacteria counts. The enumeration shows that the highest bacteria count in Uzere, Irri, and Kwale was observed in sample 10 (200m from the flare site) with a bacteria count of 1.70×10^7, 2.36×10^7 and 1.85×10^7cfu/g respectively and the lowest was observed in sample 1 (20m from the flare site) with a bacteria count of 3.1×10^6, 3.3×10^6 and 4.5×10^6 cfu/g respectively.

Table 1: Microbiological Enumeration of Bacterial Loads in Soil Samples

Distance (m) from gas flaring site	Bacteria counts		
	Uzere	Irri	Kwale
1 (20m)	3.1×10^6	3.3×10^6	4.5×10^6
2 (40m)	3.8×10^6	3.5×10^6	7.9×10^6
3 (60m)	5.0×10^6	3.3×10^6	6.3×10^6
4 (80m)	4.9×10^6	7.9×10^6	4.8×10^6
5 (100m)	3.6×10^6	4.6×10^6	9.3×10^6
6 (120m)	8.1×10^6	7.2×10^6	7.0×10^6
7 (140m)	6.3×10^6	1.05×10^7	1.45×10^7
8 (160m)	9.2×10^6	2.26×10^7	1.02×10^7
9 (180m)	9.6×10^6	1.93×10^7	1.89×10^7
10 (200m)	1.70×10^7	2.36×10^7	1.29×10^7

Biochemical Characterization of Isolated Bacteria in Soil Samples

After a series of subcultures of the bacteria cultures isolated from soil samples, a total of 15 bacteria species were identified. The bacteria species identified include *Bacillus* sp (8%), *Pseudomonas* sp (10%), *Micrococcus* sp (6%), *Staphylococcus* sp (16%), *Proteus* sp (8%), *Clostridium* sp (4%), *Salmonella* sp (7%), *Klebsiella* sp (7%), *Shigella* sp (3%), *Enterobacter* sp (3%), *Enterococcus* sp (5%), *Streptococcus* sp (4%), *Escherichia. coli* (10%), *Alcaligenes* sp (4%), and *Aeromonas* sp (5%). At 16% frequency of occurrence, *Staphylococcus* sp was the predominant isolated while *Shigella* sp and *Enterobacter* sp with a frequency of 3% being the lowest (Figure 5).

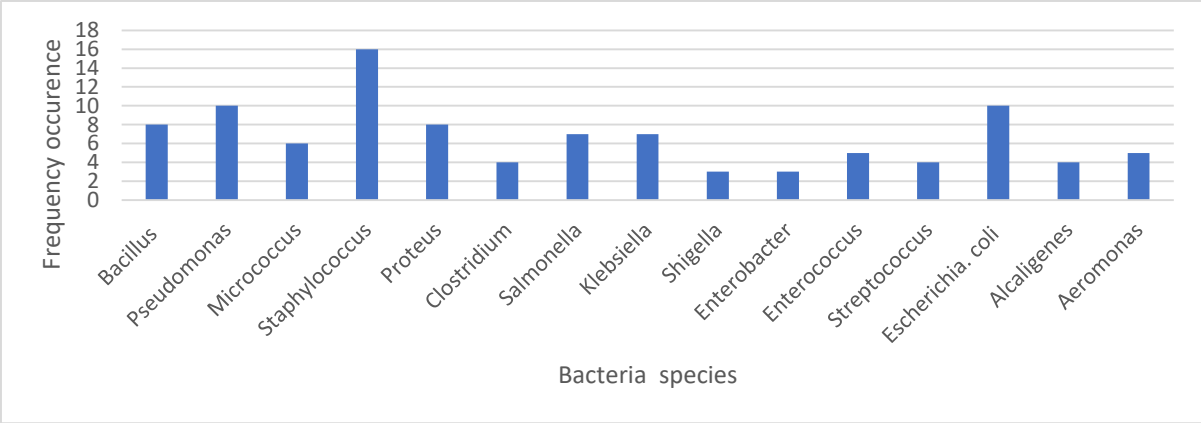


Figure 5: Bacterial species percentage occurrence from soil samples in gas flaring vicinities

Screening for Potential Heavy Metal-Resistant Bacteria

Since gas flaring soil contains a large number of heavy metals, the effect of Pb, Cr, and Cd on bacterial growth was an important part of this study. A total of 120 bacterial isolates isolated from soil samples were screened for heavy metal resistance. It was observed that as the metal concentration increased from 100 to 600ppm there was a decrease in the number of organisms that were able to grow on nutrient agar medium. Two (2) isolates (US2B and IS7C) that showed slight growth at 500ppm were selected for further studies as potential metal resistant isolates.

Molecular characterization

The genomic DNA was extracted from the two heavy metal-resistant bacteria isolates; US2B and IS7C using the universal primers 27F- 5'-

AGAGTTTGATCCTGGCTCAG-3' and reverse 1429R- 5'-GGTTACCTTGTTACGACTT-3'. The BLASTn search from the NCBI identified the organisms as *Salmonella enterica* and *Alcaligenes faecalis* (Table 6).

Table 6: 16S rDNA Gene Sequence-Based Identification of the Isolates and Their Accession numbers

Isolate code	Isolate name	Accession number	Percentage Identity (%)	E value
US2B	*Salmonella enterica* strain 16OCT84	OQ581800.1	100	0.0
IS7C	*Alcaligenes faecalis* Strain SbR-6	MH779820.1	100	0.0

Effect of pH

pH is an important factor to be considered in the study of heavy metal removal. The effect of pH was studied at pH 5, 7, and 9 for 48 hours. Figures 8 and 9 show the different percentage removal of heavy metals by *Alcaligenes faecalis* and *Salmonella enterica*. At the end of 48 hours, the highest Pb, Cr and Cd removal was observed in *Salmonella enterica* which recorded the highest Pb removal (74%) at pH 5 with a concentration of 300ppm while Cr and Cd removal (85% and 86%) at pH 7 with a concentration of 400ppm.

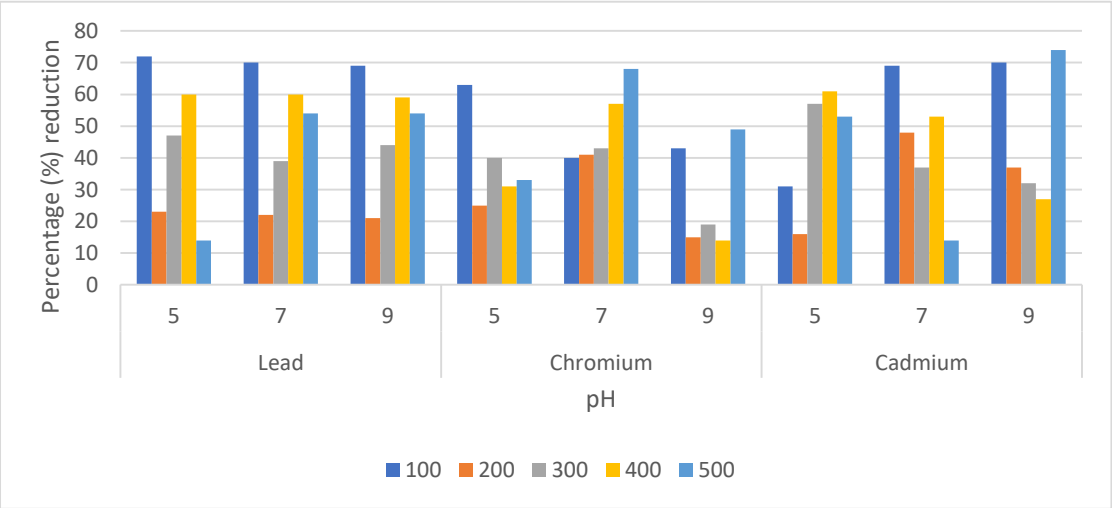


Figure 8: Effect of pH on Alcaligenes faecalis for 48 hours

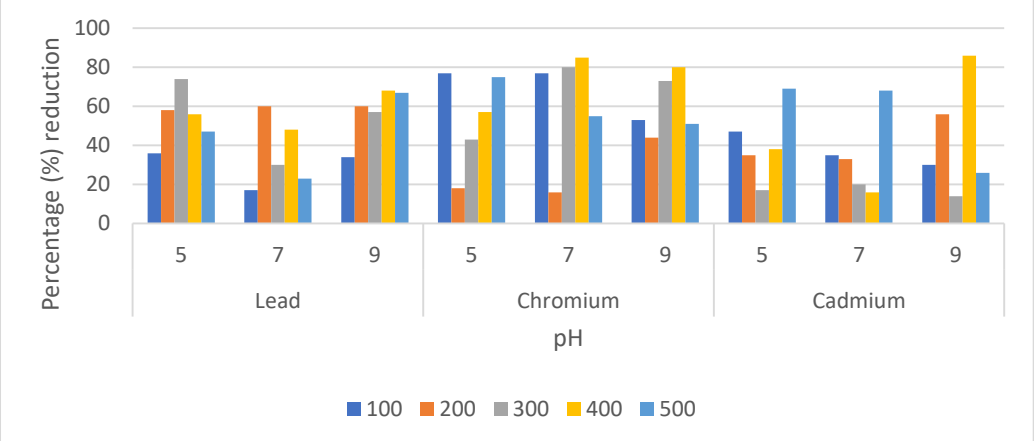


Figure 9: Effect of pH on Salmonella enterica for 48 hours

Effect of Temperature

The effect of temperature was studied at 35, 55, and 65 ℃ at a pH of 7 for 48 hours. Figure 10 and 11 shows the different percentage removal of heavy metals by *Alcaligenes faecalis* and *Salmonella enterica*. At the end of 48 hours, the highest Pb removal (82%) was recorded in *Alcaligenes faecalis* at 55℃ in 400ppm (Figure 4.11), while Cr and Cd recorded the highest removal (85 and 86%) in *Salmonella enterica* at 55℃ in 400ppm.

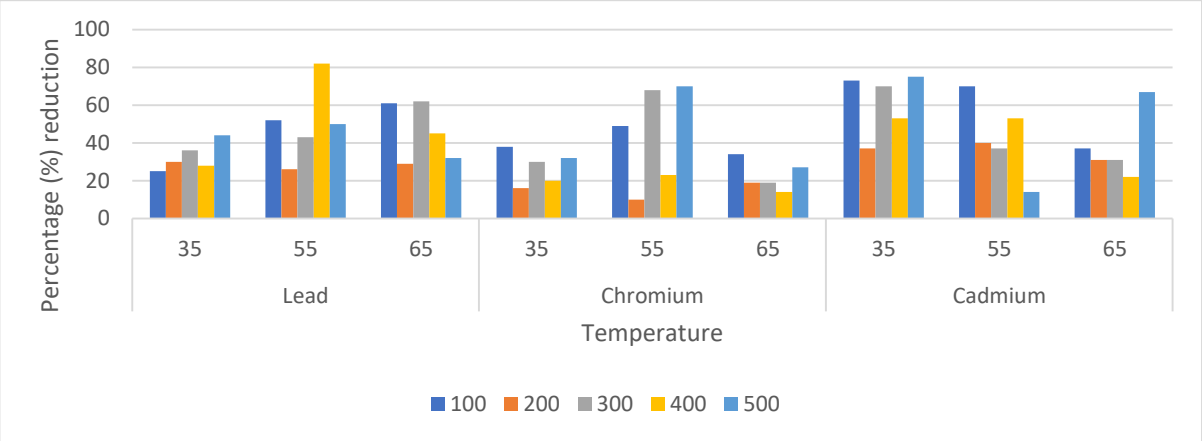


Figure 10: Effect of temperature on *Alcaligenes faecalis* for 48 hours

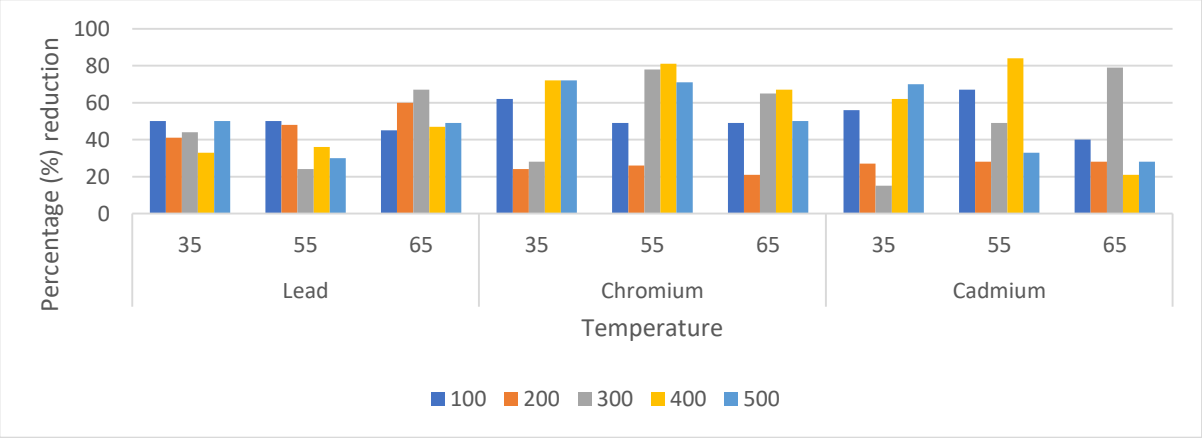
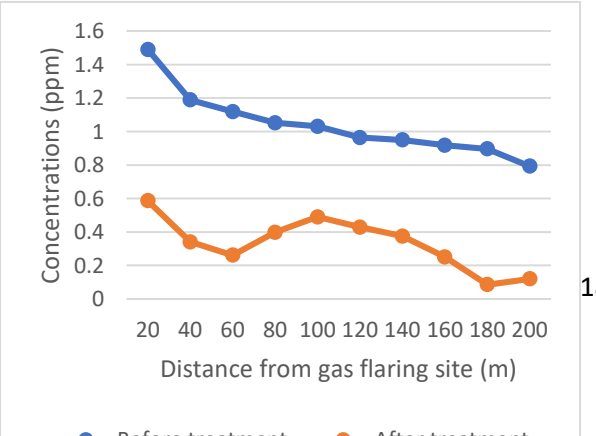


Figure 11: Effect of temperature on *Salmonella enterica* for 48 hours

Bioremediation of Soil samples

Figures 4.12- 4.35 show the results of heavy metal concentrations of Pb, Cr, and Cd in soil samples after 14days of treatment *Salmonella enterica* and *Alcaligens faecalis*. A drastical Pb and Cr reduction was seen in the samples and no cadmium was detected in the samples according to the AAS report.



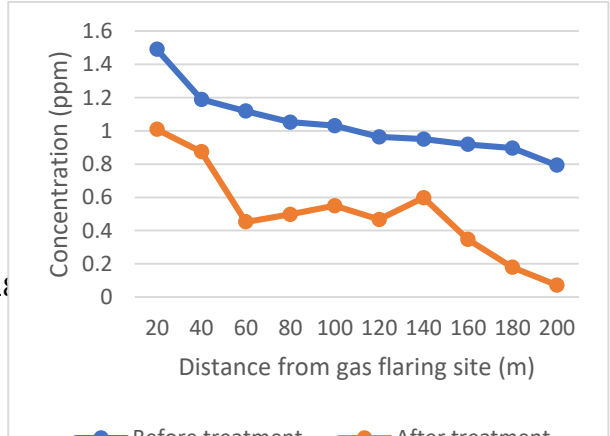


Figure 4.12: Trends in Pb concentration in Uzere at increasing distance from flaring site and effect of treatment with *A. faecalis*

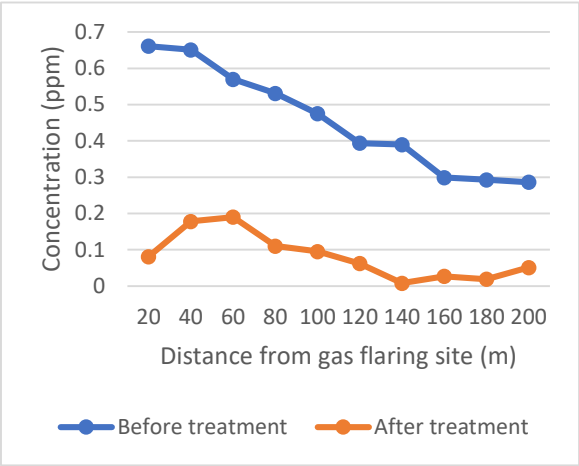


Figure 4.13: Trends in Pb concentration in Uzere at increasing distance from flaring site and effect of treatment with *S. enterica*

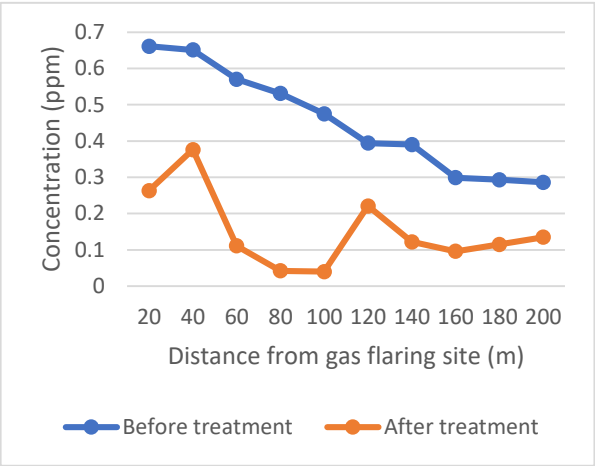


Figure 4.14: Trends in Cr concentration in Uzere at increasing distance from flaring site and effect of treatment with *A. faecalis*

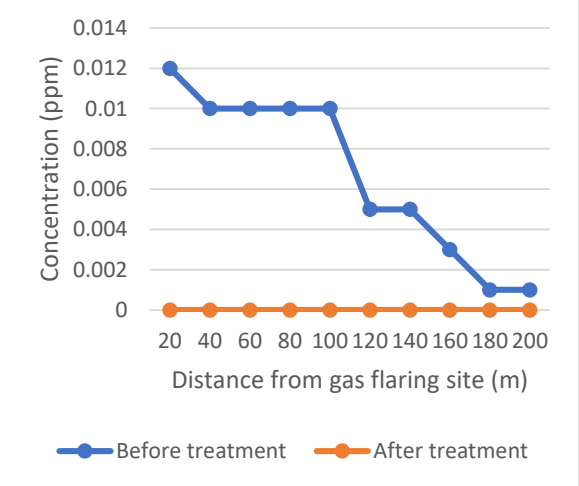


Figure 4.15: Trends in Cr concentration in Uzere at increasing distance from flaring site and effect of treatment with *S. enterica*

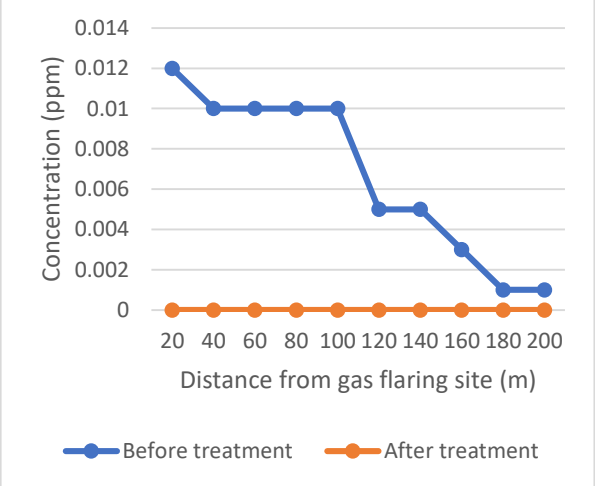


Figure 4.16: Trends in Cd concentration in Uzere at increasing distance from flaring site and effect of treatment with *A. faecalis*

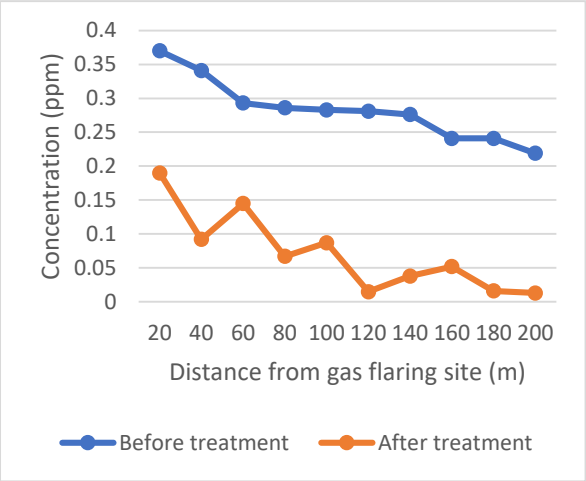


Figure 4.17: Trends in Cd concentration in Uzere at increasing distance from flaring site and effect of treatment with *S. enterica*

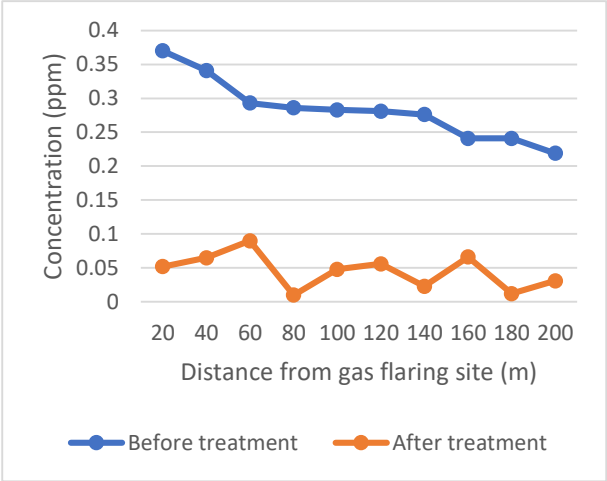


Figure 4.18: Trends in Pb concentration in Irri at increasing distance from flaring site and effect of treatment *A. faecalis*

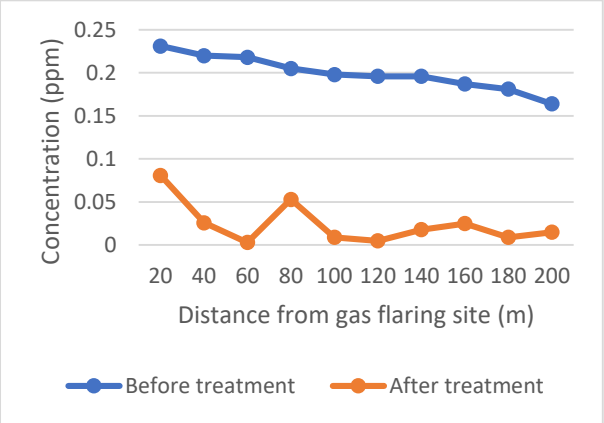


Figure 4.19: Trends in Pb concentration in Irri at increasing distance from flaring site and effect of treatment *S. enterica*

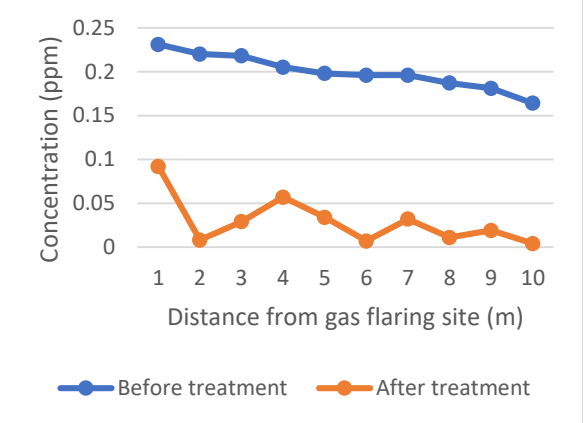


Figure 4.20: Trends in Cr concentration in Irri at increasing distance from flaring site and and effect of treatment *A. faecalis*

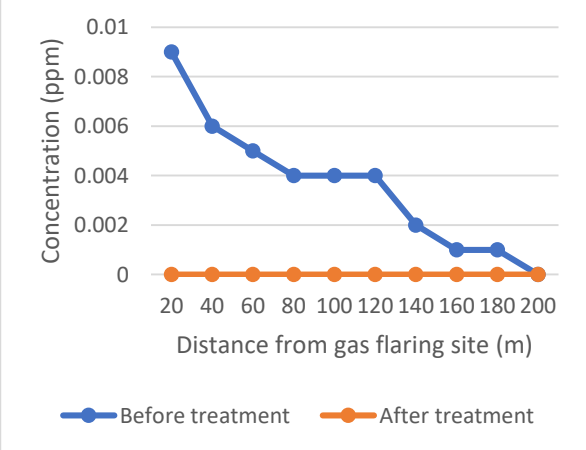


Figure 4.21: Trends in Cr concentration in Irri at increasing distance from flaring site and effect of treatment *S. enterica*

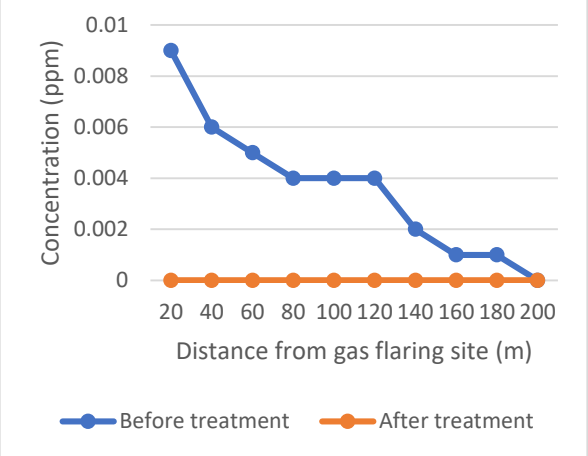


Figure 4.22: Trends in Cr concentration in Irri at increasing distance from flaring site and effect of treatment *A. faecalis*

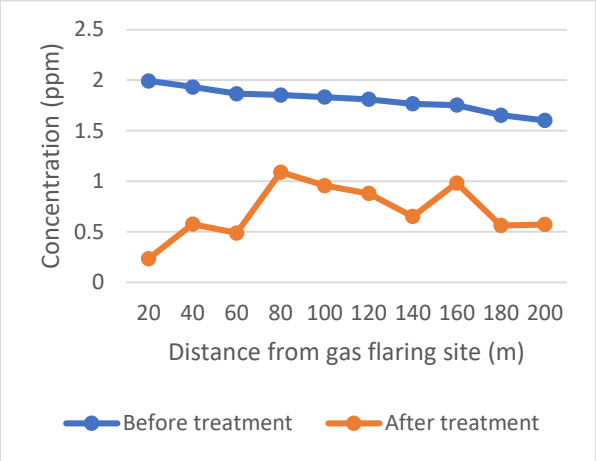


Figure 4.23: Trends in Cr concentration in Irri at increasing distance from flaring site and effect of treatment *S. enterica*

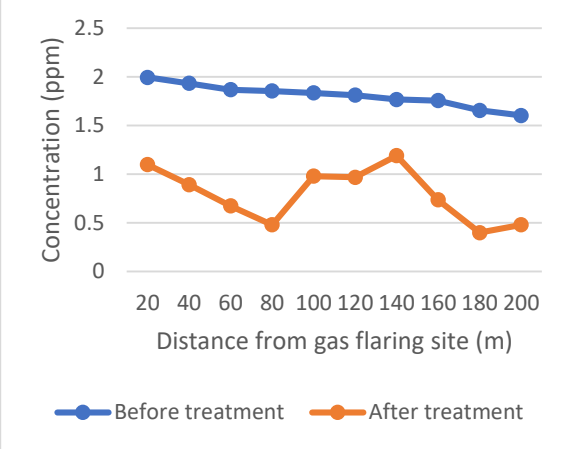


Figure 4.24: Trends in Pb concentration in Kwale at increasing distance from flaring site and effect of treatment *A. faecalis*

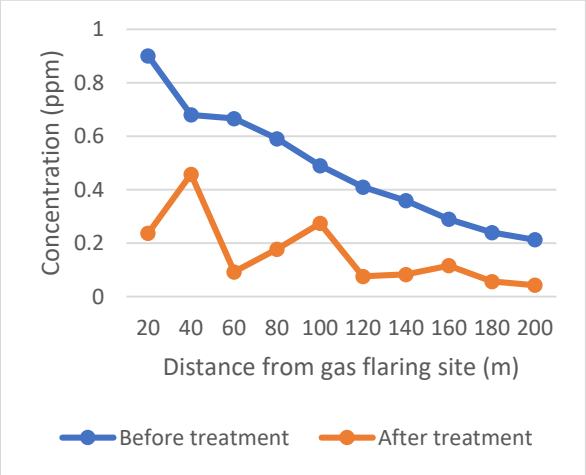


Figure 4.25: Trends in Pb concentration in Kwale at increasing distance from flaring site and effect of treatment *S. enterica*

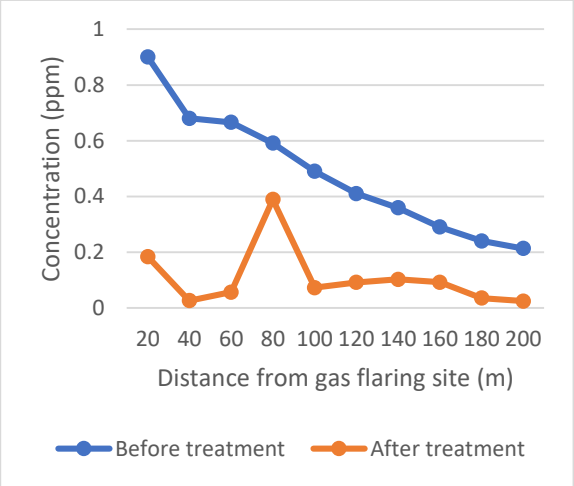


Figure 4.26: Trends in Cr concentration in Kwale at increasing distance from flaring site and effect of treatment *A. faecalis*

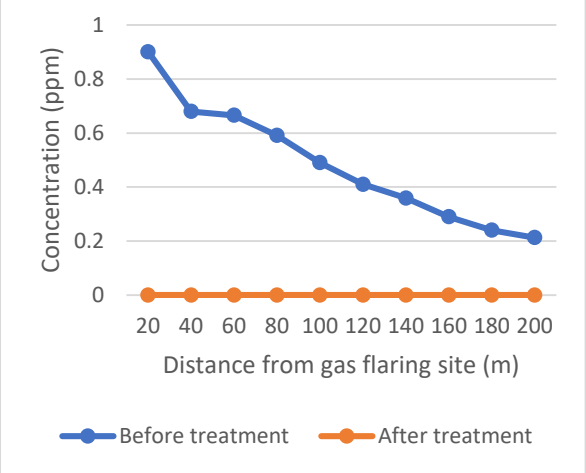


Figure 4.27: Trends in Cr concentration in Kwale at increasing distance from flaring site and effect of treatment *S. enterica*

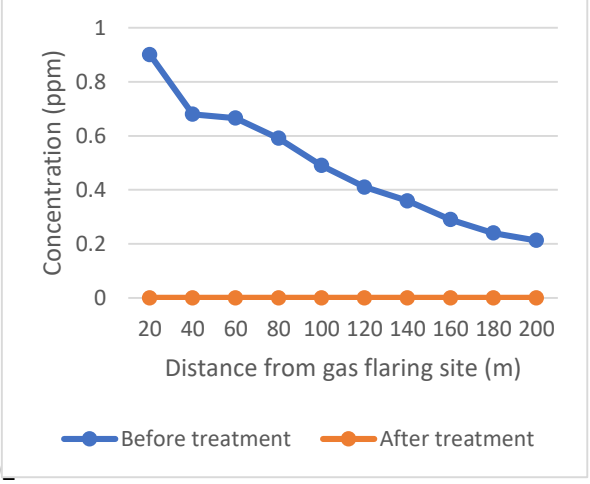


Figure 4.28: Trends in Cd concentration in Kwale at increasing distance from flaring site and effect of treatment *A. faecalis*

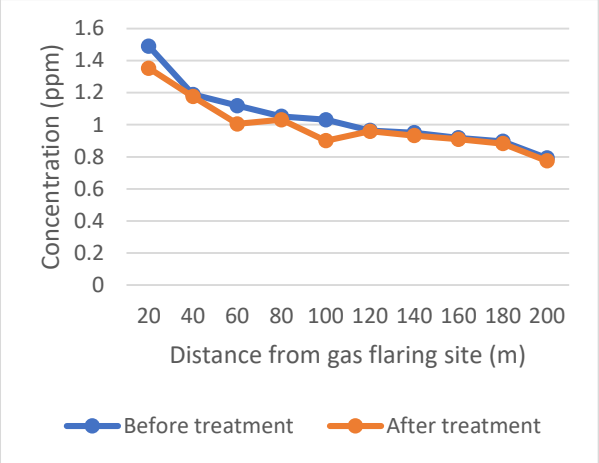


Figure 4.29: Trends in Cd concentration in Kwale at increasing distance from flaring site and effect of treatment *S. enterica*

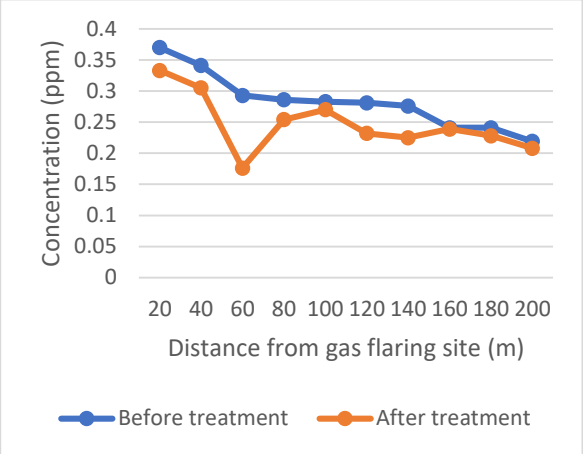


Figure 4.30: Trends in Pb concentration in Uzere at increasing distance from flaring Site and effect of treatment without isolates

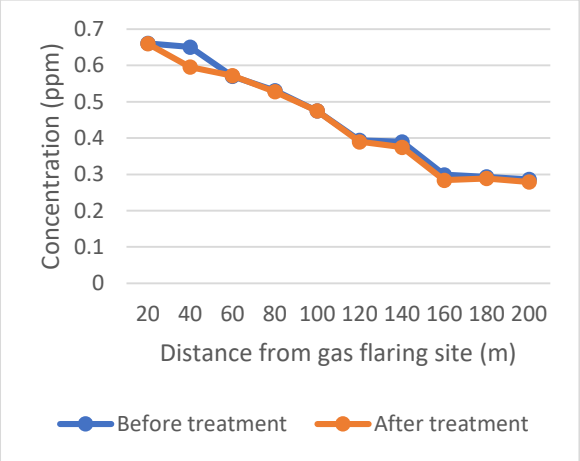


Figure 4.31: Trends in Pb concentration in Irri at increasing distance from flaring site and effect of treatment without isolates

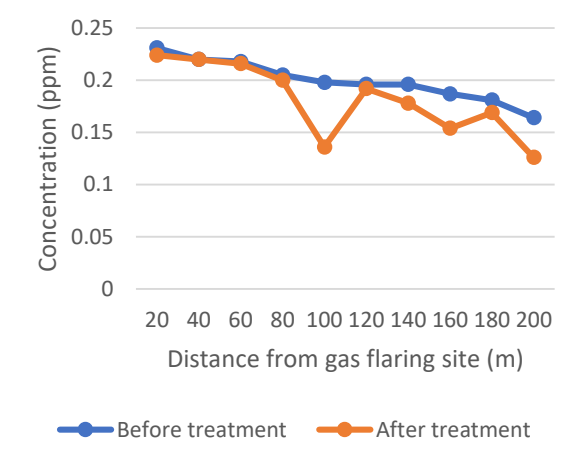


Figure 4.32: Trends in Cr concentration in Uzere at increasing distance from flaring site and effect of treatment without isolates

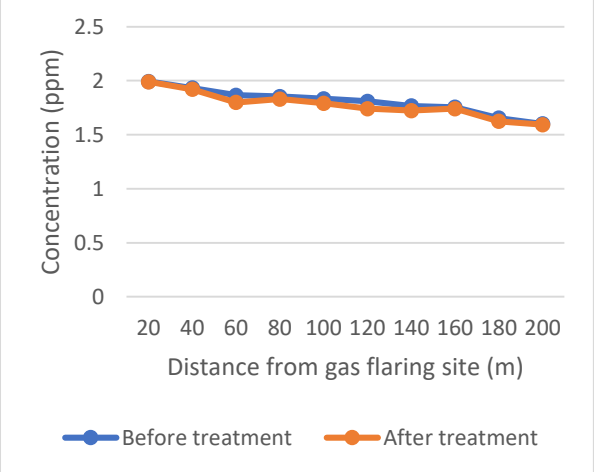


Figure 4.33: Trends in Cr concentration in Irri at increasing distance from flaring site and effect of treatment without isolates

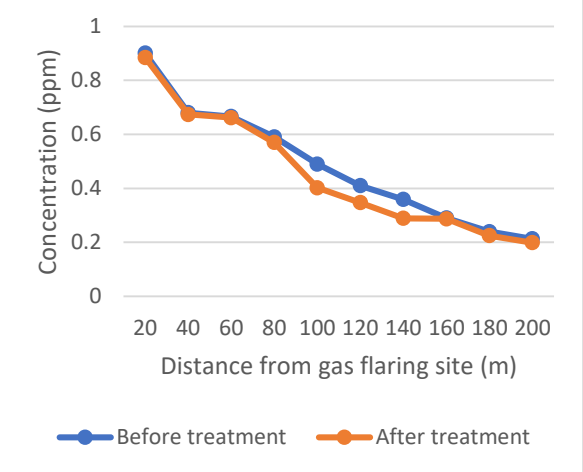


Figure 4.34: Trends in Pb concentration in Kwale at increasing distance from flaring Site and effect of treatment without isolates

Figure 4.35: Trends in Cr concentration in Kwale at increasing distance from flaring site and effect of treatment control

The statistical comparation between the concentration of heavy metals before bioremediation and after bioremediation using the student t-test statistics at 0.05 level of confidence is presented in Tables 7 – 8. The result indicates that was a significant difference in the concentration of heavy metals before and after bioremediation using heavy metal resistant organisms. Therefore, we are able to reject the null hypothesis which asserts that there is no difference between means and conclude that there is an effect in the bioremediation of heavy metals using heavy metal resistant organisms.

Table 7: The overall effect of treatment with *Alcaligene faecalis* on the concentrations of heavy metals in soil in the vicinity of gas flaring.

Location	Metal	Mean concentration of soil samples (ppm) ±SD		(*P*)
		Before treatment	After treatment	
Uzere	Pb	1.040±0.195	0.334±0.157	0.001
	Cr	0.455±0.144	0.082±0.063	0.001
	Cd	0.007±0.004	0.000±0.000	0.001
Irri	Pb	0.283±0.046	0.071±0.059	0.001
	Cr	0.199±0.019	0.024±0.025	0.001
	Cd	0.004±0.003	0.000±0.000	0.002
Kwale	Pb	1.806±0.119	0.699±0.267	0.001
	Cr	0.484±0.223	0.161±0.129	0.001
	Cd	0.006±0.007	0.000±0.000	0.025

Key: (*P*)= Significant difference, SD= Standard deviation

Table 8: The overall effect of treatment with *Salmonella enterica* on the concentrations of heavy metals in soil in the vicinity of gas flaring.

Location	Metal	Mean concentration of soil samples (ppm) ±SD		(*P*)
		Before treatment	After treatment	
Uzere	Pb	1.040±0.195	0.505±0.284	0.001
	Cr	0.455±0.144	0.152±0.105	0.001
	Cd	0.007±0.004	0.000±0.000	0.001
Irri	Pb	0.283±0.046	0.045±0.026	0.001
	Cr	0.199±0.019	0.029±0.027	0.001
	Cd	0.004±0.003	0.000±0.000	0.002
Kwale	Pb	1.806±0.119	0.788±0.278	0.001
	Cr	0.484±0.223	0.107±0.109	0.001
	Cd	0.006±0.007	0.000±0.000	0.025

Key: (*P*)= Significant difference, SD= Standard deviation

DISCUSSION

Gas flaring for decades has been a major source of heavy metal pollution in the Niger Delta which has been reported to be hazardous as well as a threat to the biotic component of the ecosystem. The soil around gas flaring sites are rich sources of potential bacterial populations that are resistant to heavy metals which can be as a result of metals in their environment, their cell wall composition that binds and interacts with the metal ions, and their genetic make-up (Ndeddy Aka and Babalola, 2017; Zampieri *et al*., 2016; Choudhary and Sar, 2009; Çolak *et al*., 2011; Abou-Shanab *et al*., 2012). Gas flaring water and soils contaminated with metals are sources of metal-resistant isolates belonging to *Alcaligenes, Staphylococcus, Enterobacteriaceae, Bacillus, and Pseudomonas* species (Ndeddy Aka and Babalola, 2017). In this study, water and soil samples from gas-flaring environments, with the natural occurrence of heavy metals, were used for the isolation of metal-resistant isolates and for the remediation of these contaminated samples.

Assessment of the heavy metals found in soil samples collected from Uzere, Irri, and Kwale as shown in Figures 2- 4 revealed a significant variation in the concentration. The values recorded showed that Kwale samples had the highest concentration of Pb, Cr, and Cd followed by Uzere samples, and the lowest concentration was recorded in Irri samples. However, it was observed that the concentration of the heavy metals in all the samples decreased meters away from the flaring site. Samples collected 20m away from the flaring point contained more concentration of heavy metals than those collected 200m away.

Similar trends were observed in the enumeration of bacteria contained in each sample. The bacteria counts were low for samples collected from 20m compared to the samples taken 200meters away from the flare site (Table 1) ranging from 3.1×10^6 to 1.70×10^7 in Uzere soil samples, 3.3×10^6 to 2.36×10^7 in Irri soil samples, 4.5×10^6 to 1.85×10^7 in Kwale soil samples. The low bacteria count in samples closest to the flare point could be because of growth inhibition in the presence of heavy metals which suppresses the diversity and population of bacteria in the growth medium (Chihomvu *et al*., 2014; Zampieri *et al*., 2016). In agreement with these findings, a study by Ibrahim et al (2021) reported a scarce count of bacteria from heavy metal-contaminated environments.

A total of one hundred and twenty bacteria isolates were isolated from soil samples. Based on biochemical identification the prevalence of bacteria isolated were *Bacillus* sp (8%), *Pseudomonas* sp (10%), *Micrococcus* sp (6%), *Staphylococcus* sp (16%), *Proteus* sp (8%), *Clostridium* sp (4%), *Salmonella* sp (7%), *Klebsiella* sp (7%), *Shigella* sp (3%), *Enterobacter* sp (3%), *Enterococcus* sp (5%), *Streptococcus* sp (4%), *Escherichia. coli* (10%), *Alcaligenes* sp (4%), and *Aeromonas* sp (5%). At 16% frequency of occurrence, *Staphylococcus* sp was the predominant isolated while *Shigella* sp and *Enterobacter* sp with a frequency of 3% being the lowest (Figure 5).

The bacteria encountered in this study have previously been associated with gas-flaring environments by previous researchers (Abo-Amer *et al*., 2015) which have been known to cause several diseases. For example, *Escherichia coli* has been known to cause intestinal diseases characterized by diarrhea, urinary tract infection, nosocomial pneumonia, etc. *Klebsiella* causes several diseases such as meningitis, pyogenic liver abscess, urinary tract infection pneumonia, and intra-abdominal infection which are characterized by flu-like symptoms, rash, fevers and chills, light-headedness, etc. *Alcaligenes* have been associated with soft tissue infection, bacteremia, meningitis,

urinary tract infection, etc. *Salmonella* species are enteric pathogens that cause enteric fever, and intestinal diseases with symptoms like vomiting, fever, nausea, and even death. Also, *Shigella* species causes shigellosis characterized by symptoms like stomach cramps, fever, and diarrhea. Some species of Staphylococcus such as *Staphylococcus aureus* produce toxins that may increase the severity of diseases such as toxic and septic shock syndrome, food poisoning, etc. Furthermore, some species of *Pseudomonas* could cause septicemia and bacteremia.

Screening of metal-resistant bacterial isolates is a vital process in the search for isolates that have the potential to remediate contaminated environments. Hence there is a need to screen for resistant isolates from gas-polluted soil to harness the potential benefits of these organisms. The different responses of each isolate to different concentrations of heavy metals suggests different degree of toxicity of each metal ion, unique chemistry, and different mechanism of action (Oladipo and Ifebajo, 2018; Edwards and Kjellerup, 2013). Resistance to multi-metal by microbial strains gives mutual benefits to the single component and is suitable for metal removal (Alisi et al., 2009). Multi-metal resistance could be attributed to the existence of concomitants regulating factors for the co-selection of metals that is widespread in the environment (Rahman and Singh, 2020) and to the presence of various genetic determinants that encode for specific metal transport proteins involved in the regulation of the active efflux and the sequestration of metal ions for resistance to metals which could have evolved in the natural environment. The result of the screening for reduction potential disclosed that out of the ninety bacteria isolated, only two of the isolates were able to tolerate a maximum of 500ppm of a mixture of Pb, Cr, and Cd. The resistant isolates recorded were gram-negative which studies (Neethu *et al*., 2015) showed to be more tolerant to heavy metals than Gram-positive. This trait could be due to the interaction between the metal ion on the surface and the interface of the bacteria and the bacteria cell wall. Bacterial tolerance to higher metal concentration influenced by complexation properties of the surface molecule, sorption, and chelating can be responsible for these characteristics. The observations were in accordance with Abo-Amer et al (Abo-Amer *et al*., 2015) who reported the isolation of heavy metal tolerant *Alcaligenes faecalis* from contaminated soil containing heavy metals. Molecular Characterization and phylogenetic analysis based on 16S rRNA gene sequencing however identified the two isolates in this study to be *Salmonella enterica* (OQ581800.1) and *Alcaligenes faecalis* (MH779820.1).

Growth condition respective to pH and temperature was optimized for the heavy metal-resistant isolates. For the effect of pH, *Salmonella enterica* was observed as the most suitable isolate in the removal of heavy metals at a pH of 5 for the removal of Pb at 300ppm, while a pH of 7 for the removal of Cd and Cr at 400ppm (Figures 8 and 9). This findings mimics that of Shridhar *et al.,* (2017) who reported maximum removal of Pb at pH5. Similarly, for the effect of temperature, the highest Pb removal of 82% was observed with *Alcaligenes faecalis* at 55℃ at a concentration of 400ppm while *Salmonella enterica* recorded the highest metal removal at 55℃ at 400ppm for both Cr and Cd (Figures 10 and 11). This findings disagree with the report of AL- Homaidan *et al.,* (2016) where the highest Pb and Cr removal was at 26℃. Optimization of growth parameters such as pH and temperature has a keen effect on metal resistant capacity of bacterial strains.

The study of bioremediation of the removal of individual heavy metal in the polluted soil samples around gas flaring sites for 14 days are shown in Figure 4.12-4.35. Microorganisms, through different metal complexation, efflux and influx

methods are able to contain and reduce heavy metals in soil samples. The study showed that Cd was completely removed in all soil samples by each of the resistant metal isolates. *Alcaligenes Faecalis* reduced Pb concentration by 68.6% and Cr concentration by 80% while *Salmonella enterica* reduced Pb concentration by 65% and Cr concentration by 75.8%. Control without the isolates however had the lowest percentage removal of Pb and Cd concentration by 5.9% and 6.7%. The removal activities for Pb, Cd and Cd in soil were high when compared to similar study by Fauziah *et al.,* (2017) who investigated bioremediation of heavy metal contaminated soil using indigenous microorganisms isolated from a close dump site. The discrepancy in the removal of heavy metals by the individual treatment groups may be ascribed to the fact that certain microorganisms have higher affinity and sensitivity to a particular heavy metal than another. Microbiological processes in the soil can either immobilize metals (reduce the bioavailability of metals) or solubilize them (increase their bioavailability and potential toxicity). The findings from this study on removal rate for heavy metals are similar to other studies on the bioremediation of heavy metal contaminated area (Fauziah *et al.,* (2017), Alvarez *et al.,* (2017), Guarino *et al.,* (2017)

According to Kuddus *et al*., (2013) bioremediation is successful when 65% or more of the heavy metals are removed from the original molecule. From this study, it signifies that the remediation of Pb, Cr and Cd in water and soil samples in this study was very successful.

CONCLUSION

It can be concluded from this study that pH and temperature contribute to a positive reduction of heavy metal concentrations in gas-flaring environments using heavy metal-resistant bacteria *Salmonella enterica* and *Alcaligenes faecalis* from polluted soil samples. These findings are highly relevant to the gas flaring environments from the perspective of bioremediation as this can also be practiced in the metal recovery techniques in the gas flaring industries. Therefore, the application of this method in a continuous system is among the future research plans.

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