

Toxic effect of kerosene contamination on the survival of bacterial and fungal species in soil from Niger Delta, Southern Nigeria

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ABSTRACT

Bacterial species isolated from kerosene contaminated and pristine soil samples which included species of *Bacillus*, *Pseudomonas*, *Serratia* and *Micrococcus* exhibited a percentage log-survival in the range of 2.52%-3.13% at a ten percent level of kerosene contamination. Other species isolated from pristine soil samples such as *Streptococcus*, *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* exhibited a percentage log-survival in the range of 29.47%-37.58% at the same level of kerosene contamination. Fungal species of *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor* and *Rhizopus* exhibited a percentage log-survival in the range of 4.41%-8.04% at a ten percent level of kerosene contamination. Toxic effect of kerosene in microbial composition gives useful information about their adaptability in pollution studies based on their ability to metabolize or resist the toxicant present.

Keywords: Percentage-log survival, kerosene contamination, exhibited adaptability, resistance.

INTRODUCTION

The drastic increase in the production, refining and distribution of crude oil also brought with it an ever increasing problem of environmental pollution. A great part of this problem results from the fact that massive movement of petroleum have to be made from area of high production to those of high consumption (Morris, 1971). In general, microbial communities from contaminated ecosystems can adapt to the presence of pollutants producing shifts in the metabolic and genetic diversity of the community (Wang and Bartha, 1994; Macnaughton et al., 1999). In some cases, the microbial biocenosis decreases in species richness in the presence of a selective pollutant (Jesen, 1975). Holloaway et al., (1980) detected a higher abundance of hydrocarbon utilizing microorganisms in an active oil field in the gulf of Mexico compared to an oil free control, though the diversity of bacterial population was shown to be unchanged. In contrast, drastic changes in diversity of the autochthonous bacteriocenosis were reported after oil

pollution of a semi-arid soil in Patagonia, Argentina (Pucci et al., 2002). Kerosene as well as other hydrocarbon spillage may result in damaging effect on associated microbial community due to its suffocation and toxic properties. Kerosene being a petroleum hydrocarbon can sometimes induce accumulation of high electro-dense inclusions inside a bacterium. The presence of these inclusions might be considered to indicate ecotoxicity in sediments (Dietstra et al., 2007). The objective of this study is to determine the toxicity of kerosene on isolated bacterial and fungal species in the soil.

MATERIAL AND METHODS

Bacterial and fungal counts

The soil samples were microbiologically analyzed using pour plate method (Song and Bartha, 1990). Serial dilutions of the soil samples were carried out in triplicates. One (1ml) each from 10^{-4} dilution tube was seeded into Nutrient agar plates and incubated at room temperature for 24 hours. Similarly, one (1ml) from the 10^{-3} dilution

tube was seeded into Sabouraud dextrose agar plates and incubated at room temperature for 72 hours.

Isolation and characterization of isolates

Pure bacterial isolates were obtained using streaking techniques and stored at 4°C in agar slants. Individual colonies were identified by morphological and biochemical techniques using the taxonomic scheme of Bergey's manual of Determinative Bacteriology (Holt et al., 1994). Fungal isolates were examined macroscopically for pigmentation, diameter, colonial characteristics and microscopic examination of sexual and asexual reproductive structures like sporangia, conidial head, arthrospore, vegetative mycelia, nature of hyphae (Alexopoulos and Sun, 1962; Bartha and Hunter, 1972).

Toxicants

Kerosene was employed as the toxicant and toxicant concentrations of 10%, 5%, 1% were employed. Twenty-four (24) hour old broth culture of bacterial species were serially diluted up to dilution 10^{-4} to tease out the bacterial population. One (1ml) from the dilution tube was introduced into 9 ml of four mineral salt medium (Zajic and Supplison, 1972) test-tubes pH 7.0. In each of the four MSM test-tubes was added 1ml, 0.5ml and 0.1ml to constitute 10%, 5% and 1% kerosene toxicant concentration. The tubes were incubated at room temperature for 24 hours. After 24 hours all bacterial tubes were pour plated on Nutrient Agar in triplicates and incubated at 28°C for 24 hours bacterial counts were recorded as colony forming unit per ml.

Similarly, broth cultures of fungal species were serially diluted up to 10^{-3} to tease out fungal population and 1ml from this dilution tube was introduced into 9 ml of four mineral salt medium test tube pH 5.8. In each of the four MSM tubes, was added 1ml, 0.5ml, 0.1ml of kerosene to constitute 10%, 5% and 1% kerosene toxicant concentration. The tubes were incubated at room temperature for 72 hours. After 72 hours, all fungal tubes were pour plated on Sabouraud dextrose agar plates in triplicate and incubated at room temperature for 72 hours. Fungal counts were recorded as colony forming unit per ml.

Percent-log survival test

Percent-log survival as adopted from Williamson and Johnson (1981) was calculated by obtaining the log of count in each toxicant concentration (Log C) dividing by the log of count in the zero toxicant concentration (log c)

and multiplying by 100 i.e (% log survival) = $\text{Log C} / \text{Log c} \times 100$

All results obtained were plotted against toxicant concentration.

RESULTS

Table 1 and 2 shows the different bacterial and fungal species isolated from chronic kerosene contaminated and pristine soil samples. Figure 1 and 2 shows the mean bacterial and fungal counts at 1%, 5% and 10% level of kerosene contamination. Figure 3 presents the percentage log-survival of Gram positive bacteria at 1%, 5% and 10% levels of contamination. One percent kerosene contamination results in 98.67 percentage log-survival of *Bacillus* species. Increased kerosene concentration to five (5) and ten (10) percent resulted in 98.46 and 97.26 percentage log-survival respectively. One (1) percent kerosene resulted also resulted in 99.70 percent log-survival of *Micrococcus* species. Increased kerosene concentration to five (5) and ten (10) percent resulted in 99.28 and 97.59 percentage log-survival respectively. One (1) percent kerosene contamination resulted in 90.86 percentage log-survival of *Staphylococcus aureus*. Increased kerosene concentration to five (5) and ten (10) percent resulted in 76.53 and 63.20 percentage log-survival respectively. Finally, one (1) percent kerosene contamination resulted in 90.32 percentage log-survival of *Streptococcus* species. Increased kerosene concentration to five (5) and ten (10) percent resulted in 79.69 and 64.47 percentage log-survival respectively.

Figure 4 shows the percentage log-survival of Gram negative bacteria at 1%, 5% and 10% level of kerosene contamination. One (1) percent of kerosene contamination resulted in 98.55 percentage log-survival of *Pseudomonas* species. Increased kerosene concentration to five (5) and ten (10) percent resulted in 98.34 and 97.53 percentage log-survival respectively. One (1) percent of kerosene contamination resulted in 98.67 percentage log-survival of *Serratia* species. Increased kerosene concentration to five (5) and ten (10) percent resulted in 98.21 and 96.91 percentage log-survival respectively. One (1) percent of kerosene contamination resulted in 83.40 percentage log-survival of *Salmonella* species. Increased kerosene concentration to five (5) and ten (10) percent resulted in 75.14 and 73.18 percentage log-survival respectively. One (1) percent kerosene contamination resulted in 83.93 percentage log-survival of *Escherichia coli*, increased kerosene contamination to five (5) and ten (10) percent resulted in 78.51 and 66.30 percentage log-survival respectively.

Figure 5 shows the percentage log-survival of fungal species at 1%, 5% and 10% levels of kerosene contamination.

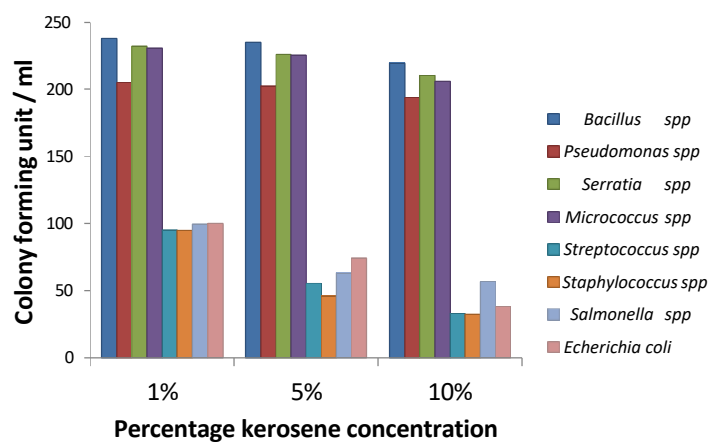


Figure 1. Mean count of isolated bacterial species at different level of kerosene contamination

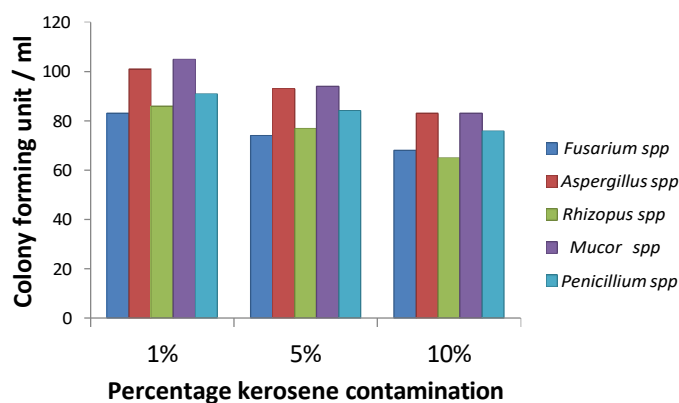


Figure 2. Mean count of isolated fungal species at different level of kerosene contamination

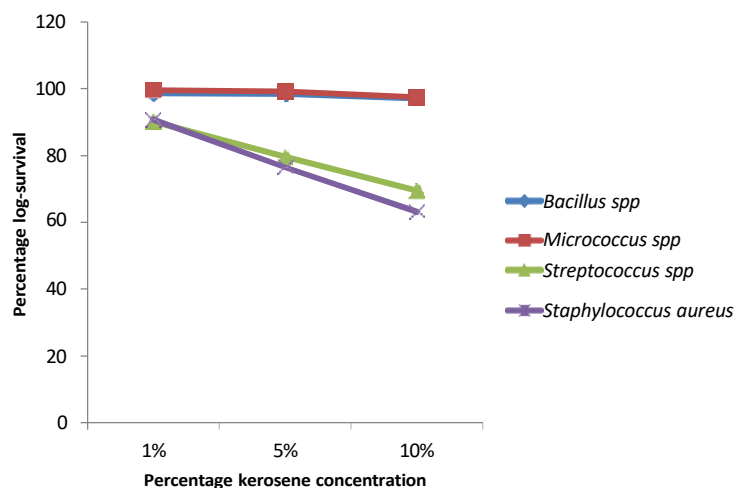


Figure 3. Effect of kerosene toxicity on isolated gram positive bacterial species

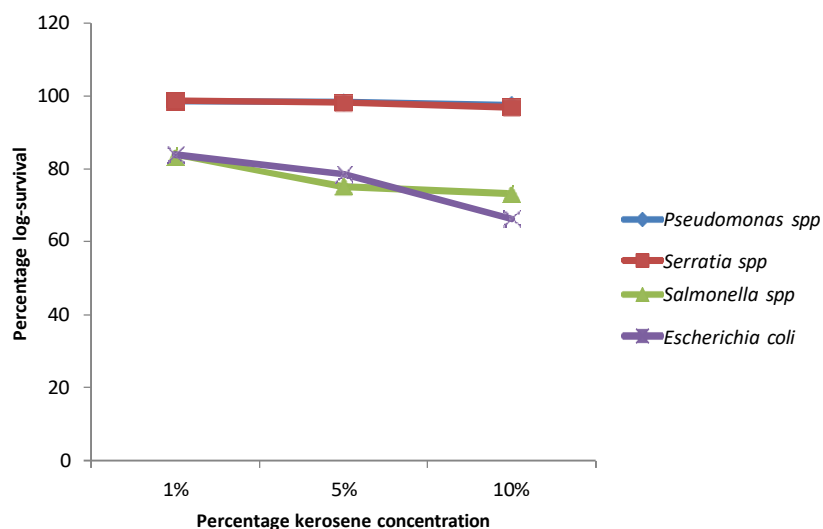


Figure 4. Effect of kerosene toxicity on isolated gram negative bacterial species

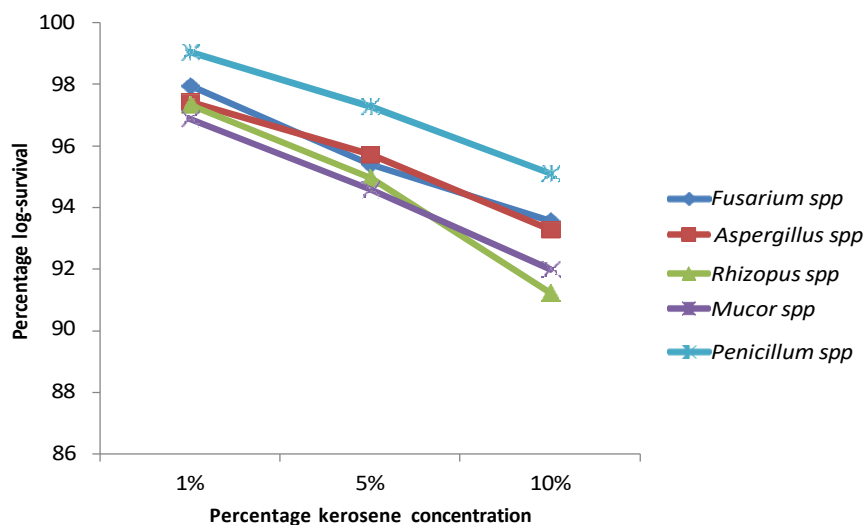


Figure 5. Effect of Kerosene toxicity on fungal species

nation. One (1) percent of kerosene contamination resulted in 97.33 percentage log-survival of *Rhizopus* species, increased kerosene concentration to five (5) and ten (10) percent resulted in 94.97 and 91.24 percentage log-survival respectively. One (1) percent kerosene contamination resulted in 97.42 percentage log-survival of *Aspergillus* species, increased kerosene concentration to five (5) and ten (10) resulted in 95.72 and 93.29 percentage log-survival respectively. One (1) percent of kerosene contamination resulted in 97.96 percentage log-survival of *Fusarium* species, increased kerosene

contamination to five (5) and ten (10) percent resulted in 95.41 and 93.57 percentage log-survival respectively. One (1) percent kerosene contamination resulted in 96.88 percentage log-survival of *Mucor* species; increased kerosene concentration to five and ten resulted in 94.58 and 91.99 percentage log-survival respectively. One (1) percent kerosene contamination resulted in 99.04 percentage log-survival of *Penicillium* species, increased kerosene concentration to five (5) and ten (10) percent resulted in 97.27 and 95.10 percentage log-survival respectively.

DISCUSSION

From table 1, it was observed that the microbial composition of chronic kerosene contaminated soil samples includes basically hydrocarbonoclastic bacterial species of *Bacillus*, *Pseudomonas*, *Micrococcus*, and *Serratia* this findings are similar to results of Ikpeme et al., (2007). The pristine soil samples included in addition to the above named species of *Streptococcus*, *Salmonella*, *Escherichia coli* and *Staphylococcus aureus*. The myco-flora between the chronic kerosene contaminated and pristine soil sample remained constant. With species of *Penicillium*, *Rhizopus*, *Aspergillus* and *Fusarium* as seen in table 2. Similar results were by Wemdo et al., 2002. From figure 1, the mean counts species of *Bacillus*, *Pseudomonas*, *Serratia* and *Micrococcus* was slightly reduced with increased concentration of the toxicant whereas, species of *Streptococcus*, *Staphylococcus*, *Salmonella* and *Escherichia coli* had a more pronounced reduction in mean bacterial count. From figure 2 it was observed that increased kerosene concentration had a minimal effect on the mean fungal count. Figure 3 presents the percentage log-survival of gram positive bacteria after contamination with 1%, 5% and 10% kerosene concentration for a period of twenty-four (24) hours. *Bacillus* species had a high percentage log-survival of 97.26% after ten percent level of contamination. Asitok and Antai (2006) reported a high optical density (OD) of 2.985 for *Bacillus* hence, its ability to degrade the kerosene contaminant and survive/thrive with it. Gram positive cocci *Micrococcus* sp, *Streptococcus* sp and *Staphylococcus aureus* had percentage log-survival of 97.59, 69.47 and 63.20 respectively. Boboye et al., 2010 reported that *Staphylococcus aureus*, *Streptococcus fecalis* and *Bacillus* sp had the highest degrading activities on the first day of exposure whereas, *Micrococcus luteus* had the highest on the third day of exposure to kerosene. Obire and Nwaebuta, 2002 isolated *Staphylococcus aureus* and *Micrococcus* sp from kerosene contaminated soil at twelve weeks of contamination. Figure 4 presents the percentage log-survival of gram negative bacteria after contamination with 1%, 5% and 10% concentration for a period of twenty-four (24) hours. *Pseudomonas* sp had the highest percentage log-survival of 97.53% after ten-percent level of contamination. Asitok and Antai (2006) reported a high optical density of 1.976 for *Pseudomonas* sp hence their survivability in kerosene concentration at this level of contamination. Species of *Serratia*, *Salmonella* and *Escherichia coli* had a percentage log-survival of 96.91, 73.18, and 66.30 respectively. These values indicated their susceptibility to kerosene toxicity at ten (10) percent level of contamination. Obire and Nwaebuta, 2002 reported that *Escherichia coli* and *Serratia marcesens* persisted in kerosene contaminated soil from two (2) weeks of contamination. From the above information, it is observed

that kerosene had a low toxic effect in the range (2.41%-3.09%) at a ten (10) percent level of contamination in a twenty four (24) hour interval period on the percentage log-survival of hydrocarbonoclastic *Bacillus* sp, *Pseudomonas* sp, *Serratia* sp and *Micrococcus* sp which were predominant in chronic kerosene contaminated soil samples. The survivability of these microorganisms is due to their ability to degrade the hydrocarbon in the kerosene contaminant and utilize it as a source of carbon and energy for metabolism. Whereas, the other bacterial genera which were only present in pristine soil sample *Streptococcus* sp, *Salmonella* sp, *Staphylococcus aureus* and *Escherichia coli*, a more pronounced response to the kerosene toxicant in the range (26.82%-36.8%) was observed at the same period of exposure and incubation. The ability of for these hydrocarbon degrader to survive kerosene toxicity may be due to a number of resistance mechanisms such as efflux pump, enzyme-linked mediated resistance, genetic adaptation, level of metabolic activity within the biofilm, outer membrane structure. Alekshun and Levy (2007); Anderson, (2006); Levy (1994); Prescott et al., (2005).

Figure 5 presents the percentage log-survival of fungi after contamination with 1%, 5% and 10% concentration for a period of twenty-four (24) hours. *Penicillium* sp, *Fusarium* sp, *Aspergillus* sp, *Rhizopus* sp, and *Mucor* sp had a percentage log-survival of 95.59, 94.02, 93.13, 91.96 and 92.06 respectively. This finding indicates that kerosene had a lower toxic effect in the range of (4.41%-8.04%). This lower toxicity may be responsible for the availability of similar species of fungi in both chronic kerosene contaminated and pristine soil samples. Oboh et al., 2006 reported that fungal species of *Penicillium*, *Aspergillus* and *Rhizopus* were implicated in hydrocarbon degradation.

The degree of degradation of kerosene and hence survivability of the microorganisms is largely dependent on the concentration of the kerosene contaminant in the medium with respect to the duration of exposure. Thus, it is expected that an increase in the concentration of the contaminant would result in further decrease in percentage log-survival of these bacterial genera and chronic contamination will result in their elimination. These would account for the absence of certain bacterial genera in chronic kerosene contaminated soil samples.

Statistical analysis on the percentage log-survival showed that there was significant difference ($p \leq 0.05$) in the effect of kerosene contamination on bacterial and fungal species at different levels of contamination i.e 1%, 5% and 10%. Bacterial species of *Bacillus*, *Pseudomonas*, *Serratia* and *Micrococcus* had higher counts than that of *Salmonella*, *Escherichia coli*, *Streptococcus* and *Staphylococcus aureus*. Effect of kerosene concentration on fungal counts was in the order *Mucor* sp > *Aspergillus* sp > *Penicillium* sp > *Rhizopus* sp > *Fusarium* sp. Hence, a decrease in microbial counts is indicative of susceptibility to kerosene toxicity.

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