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Effects of lead and mercury on crude oil utilization by *Pseudomonas aeruginosa*

Eze Chibuzor Nwadibe, Okobo Uchenna Jude and Odo Chuks Kenneth

Department of Microbiology, University of Nigeria Nsukka
Correspondence to: chibuzor.eze@unn.edu.ng ; +234 (0)8151510547

ABSTRACT

Heavy metal and hydrocarbon contaminations from the technosphere are critical issues assailing the biosphere today. Though microbes are known to play a major role in restoring petroleum polluted ecosystems, more often than not they are hampered by interferences from other contaminants in the environment. In this study, the effects of lead and mercury on crude oil degradation and utilization by a soil isolate identified as *Pseudomonas aeruginosa* were investigated. Four different concentrations (50mg, 100mg, 150mg, or 200mg w/v) of each metal salt were used and each administered per litre of medium containing 10% Bonny light crude oil and 2ml of a broth culture of cells of *Pseudomonas aeruginosa* suspended in normal saline to a level of 0.5% McFarland standard. Hydrocarbon degradation and utilization were assessed through time-course optical density (OD_{600}) and infrared spectroscopy. At low concentrations (50mg and 100mg) the heavy metals significantly ($p < 0.05$) enhanced the growth of the organism and its utilization of crude oil but at higher concentrations (150mg, 200mg) both growth and utilization declined. The lowest OD was obtained with mercury, suggesting that mercury exhibited higher toxicity to the organism than lead. The appearance in the infra red chart of new bands within the wave number ranges of 1630-1780cm^{-1}, 1710-1780cm^{-1} and 3590-3650cm^{-1} were interpreted from standard manuals to mean the emergence of the aldehyde, carboxyl and hydroxyl functional groups respectively which suggest an oxidative dissimilation of the oil. The effects of the heavy metals used in this study on the hydrocarbonoclastic efficacy of *Pseudomonas aeruginosa* were metal-dose and metal- species-dependent.

Key words: Technosphere, Pollution, Lead, Mercury, Restoration, ecosystem

INTRODUCTION

Crude oil is a very important source of energy globally and has remained the mainstay of many national economies since its discovery. Leaks and accidental spills occur regularly during its production, storage, refining and transportation. This has made petroleum hydrocarbons an important global environmental pollutant. Crude oil is a complex mixture of hydrocarbons and other organic compounds including some heavy metals and metallic compounds (Iyagba and Offor, 2014; Esin *et al*., 2012). In high concentrations the hydrocarbon molecules that make up crude oil and petroleum products are highly toxic to many organisms, including humans. Annually, about six million tons of crude oil enters the global environment causing damage both to living organisms and the physical environment.

Drilling wastes such as drilling mud and waste waters also constitute pollutants in the environment. Additionally, refined petroleum products are found to contaminate soils around fuel stations, power stations and mechanic workshops. Sometimes, when motor tankers are involved in road

accidents they spill petroleum products over a vast area. All these have a profound effect on the ecosystem of the affected areas.

Heavy metal contaminations of the soil also pose a serious threat to both man and animals in the environment. A heavy metal is any metallic element that has a relatively high density (at least five times that of water) and is toxic at low concentrations. They are present in the soil as natural components but of late their presence has accelerated due to human activities. Heavy metals released through anthropogenic activities are one of the major environmental problems in the world (Abioye 2011; Mona *et al*. 2014; Giwa et al. 2017; Igiri et al. 2018). This is a serious problem in areas where these metals occur in excessive amounts.

Many approaches have been used in an attempt to remove oil pollution from the environment. Among them is microbial remediation which has been shown to outshine other methods because it is ecofriendly and cost-effective. The major drawback of this technique is the slow rate at which biodegradation occurs, and this pace is reduced further by the presence of toxicants such as heavy metals at concentrations above trace amounts. Mustafa *et al*, (2013) stated that one factor affecting the bioremediation of crude oil is the presence of stressors such as heavy metals that halt the biodegradative potentials of the indigenous microbiota resulting in prolonged bioremediation and accumulation of toxic hydrocarbons in the environment. This work was designed to evaluate the impact of the presence of two heavy metals (lead and mercury) on crude oil utilization in liquid medium by *Pseudomonas aeruginosa.*

MATERIALS AND METHODS

Collection of Materials

- Bonny light crude oil and crude oil contaminated soil samples: These were obtained from Warri Refinery, Delta State, Nigeria.
- Stock culture of *Pseudomonas aeruginosa*: This was obtained from the author's collection. The organism was isolated from crude oil polluted soil sample obtained from Warri Refinery, Delta State, Nigeria.

Inoculum Preparation

The organism was subcultured into sterilized nutrient broth and allowed to grow for 48hours. Thereafter the cells were washed and suspended in normal saline to a level of 0.5 McFarland standard.

Experimental Setup

Determination of the effects of heavy metals on the hydrocarbonoclastic capabilities of the organism was carried out using four different concentrations (50mg/l, 100mg/l, 150mg/l and 200mg/l) of the metals in a liquid medium containing crude oil. The control contained crude oil in liquid medium inoculated with the organism but without heavy metals. The pH of the medium was adjusted to 7.0 before sterilization. The time course growth optical density measurement to determine the rates of crude oil utilization in the presence and absence of heavy metals was carried out over a period of eighteen days

using a visible spectrophotometer set at 600nm with samples collected every 48h. The effect of the heavy metals on bacterial degradation of crude oil was also evaluated using infrared spectrophotometric analysis of the residual crude oil after the degradation experiment.

RESULTS

Figure 1 depicts the optical density (OD) standard curve for the growth of *Pseudomonas aeruginosa* . The curve shows a positive correlation between OD_{600} and cell number. In figure 2, the effects of lead on the utilization of crude oil by *Pseudomonas aeruginosa* was reported and shows the growth of the organism in 50 mg/l lead-treated crude oil progressing at par with the control up to the 240^{th} hour. This shows that lead enhanced the growth of *Pseudomonas aeruginosa* at low levels (50mg/l and 100mg/l) for ten days (240 hours) whereas at high levels (150 mg, 200 mg) growth enhancement occurred for only four days (96 hours) after which there was stagnation. The same growth pattern was observed with mercury (Figure 3) except that the organism seemed to have had a higher tolerance for lead than mercury as shown by its slightly better growth performance with lead when compared with mercury (Figures 2 and 3). According to the figures (2 and 3) optimum growth of the microbe, indicated by the highest OD_{600} was obtained with 50mg/l (lowest concentration) of heavy metals which was confirmed by the bacterial cell number extrapolated from the OD standard curve.

Each of the flasks for the experiment contained an initial cell concentration of $2x10^{15}$ cfu/ml at 0 hour. At 240 hours the cell concentrations of *Pseudomonas aeruginosa* in crude oil control and in the test samples containing 50mg/l of lead and mercury were $21.4x10^{15}$, $21.2x10^{15}$ and $15.5x10^{15}$ cfu/ml respectively (Figures 2 and 3). Crude oil degradation at 150mg/l produced reduced cell concentrations (in cfu/ml) of $15x10^{15}$ and $13x10^{15}$ also for lead (Pb) and mercury (Hg) respectively. The growth rates declined further in 200mg/l of Pb and Hg giving cell concentrations of $10.8x10^{15}$ and $10.6x10^{15}$. Generally, there was a significant ($p < 0.05$) decrease in growth in all concentrations of heavy metals in comparison to their control.

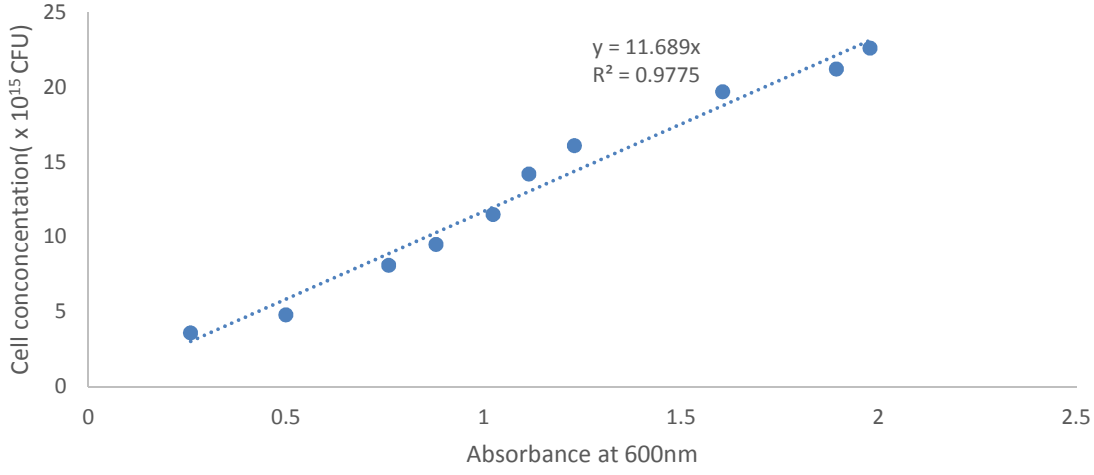


Figure 1: Optical density standard curve for *Pseudomonas aeruginosa*

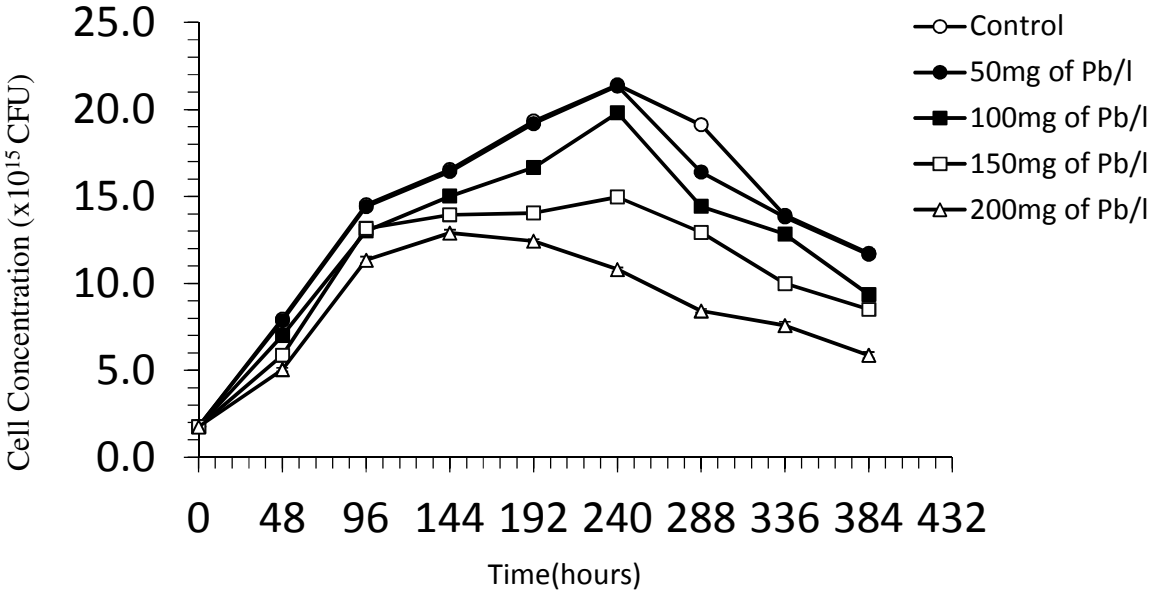


Figure 2: The effects of lead on the growth rate of *Pseudomonas aeruginosa* in crude oil

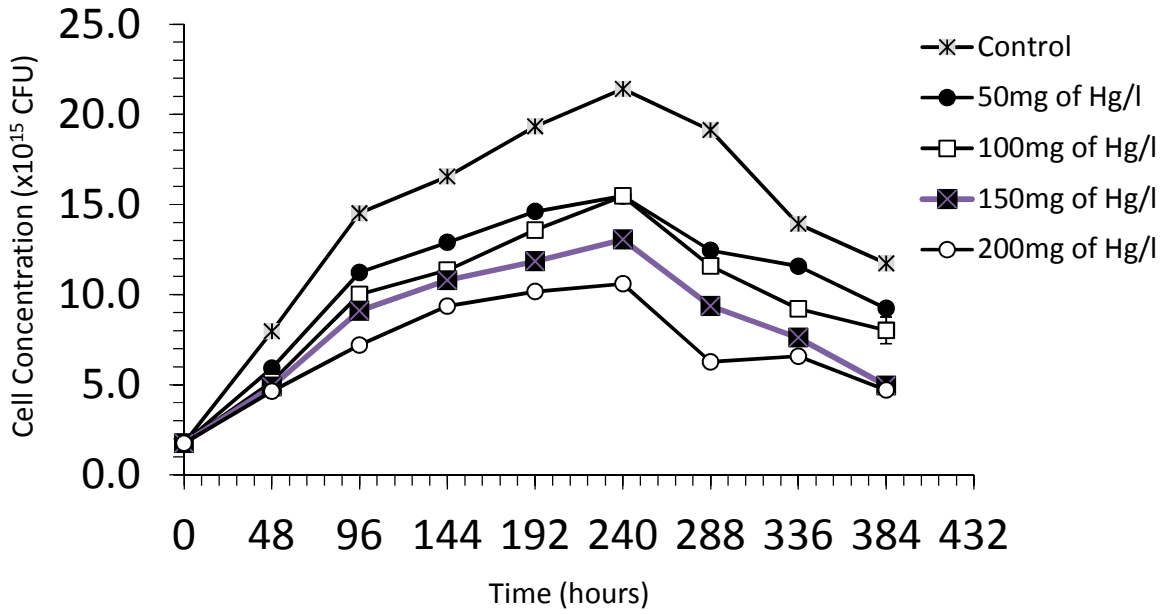


Figure 3: The effects of mercury on the growth rate of *Pseudomonas aeruginosa* in crude oil

Infra-red Spectrophotometric Analysis of Residual Oil

Comparing the Infrared (IR) profiles of the raw crude oil (control) in (Fig.4) with the degraded crude oil in the absence of metals (Fig.5) and crude oils degraded in the presence of metals (Figs.6, 7) one will see the emergence of new peaks and disappearance of old peaks in the infra red band. In figure 5 which represents crude oil degradation in the absence of heavy metals there was a disappearance of old peaks and emergence of new peaks within the wave number ranges of 2500-3500 cm^{-1} , 1690-1770cm^{-1} and 1060-1230 cm^{-1} . In crude oil samples containing heavy metals (Figs.6,7) the removal of peaks within 2500-3500 cm^{-1} , 1690-1770cm^{-1} and 1060-1230 cm^{-1} wave number ranges was not pronounced. There was a general reduction in peak size/intensity in the degraded crude oils, especially in the unpolluted sample (Fig.5) when compared with the raw crude oil sample (Fig.4).

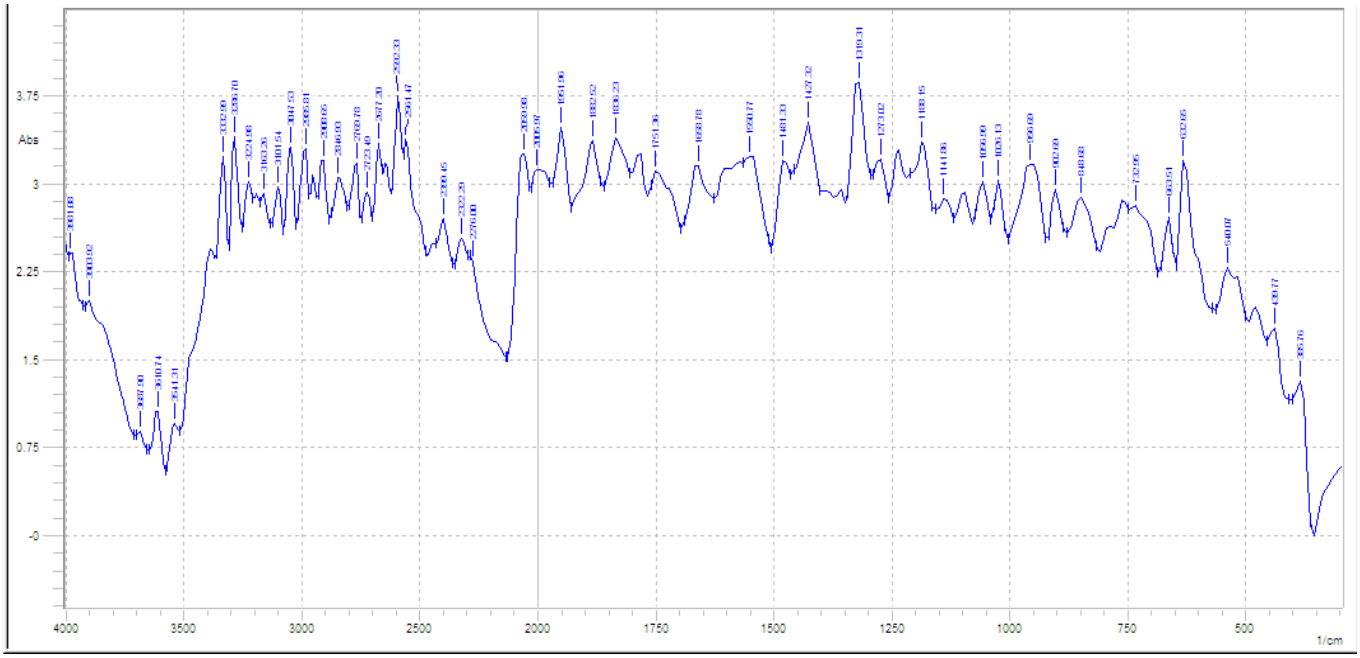


Figure 4: Infra-red Spectrophotometric profile of raw crude oil (control)

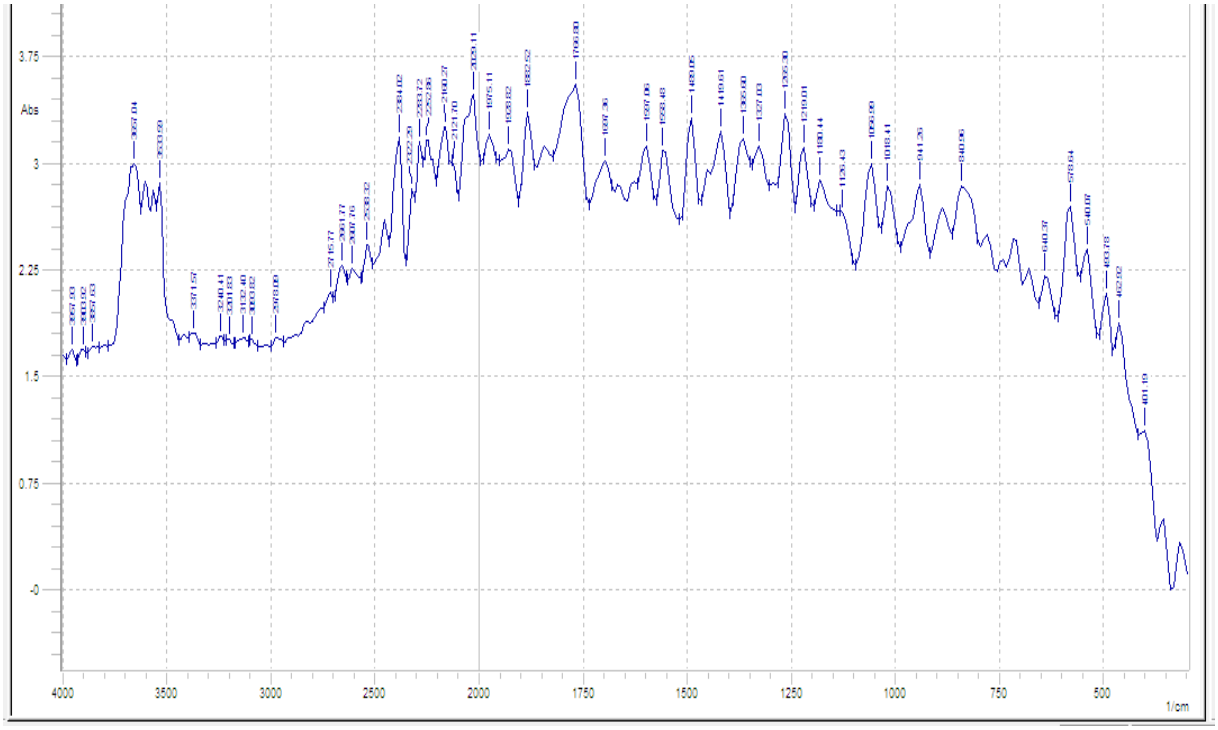


Figure 5: Infra-red spectrophotometric profile of crude oil after 16 days of degradation using *Pseudomonas aeruginosa*

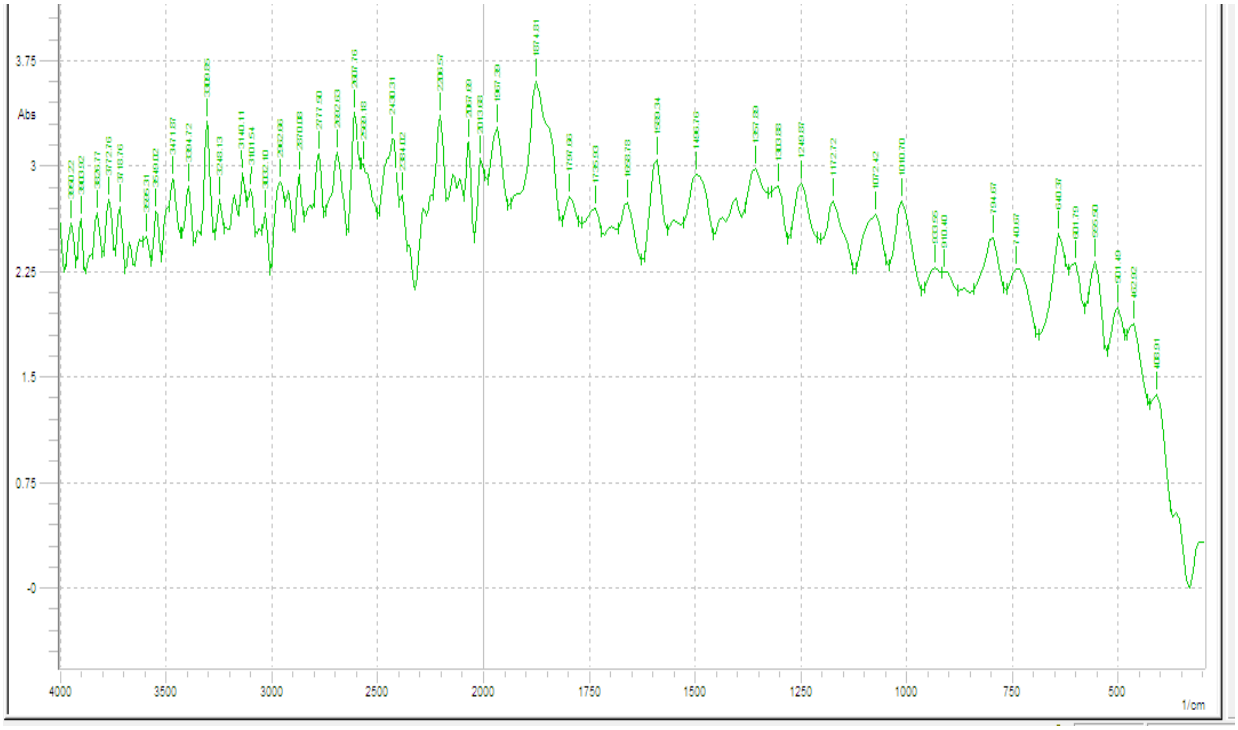
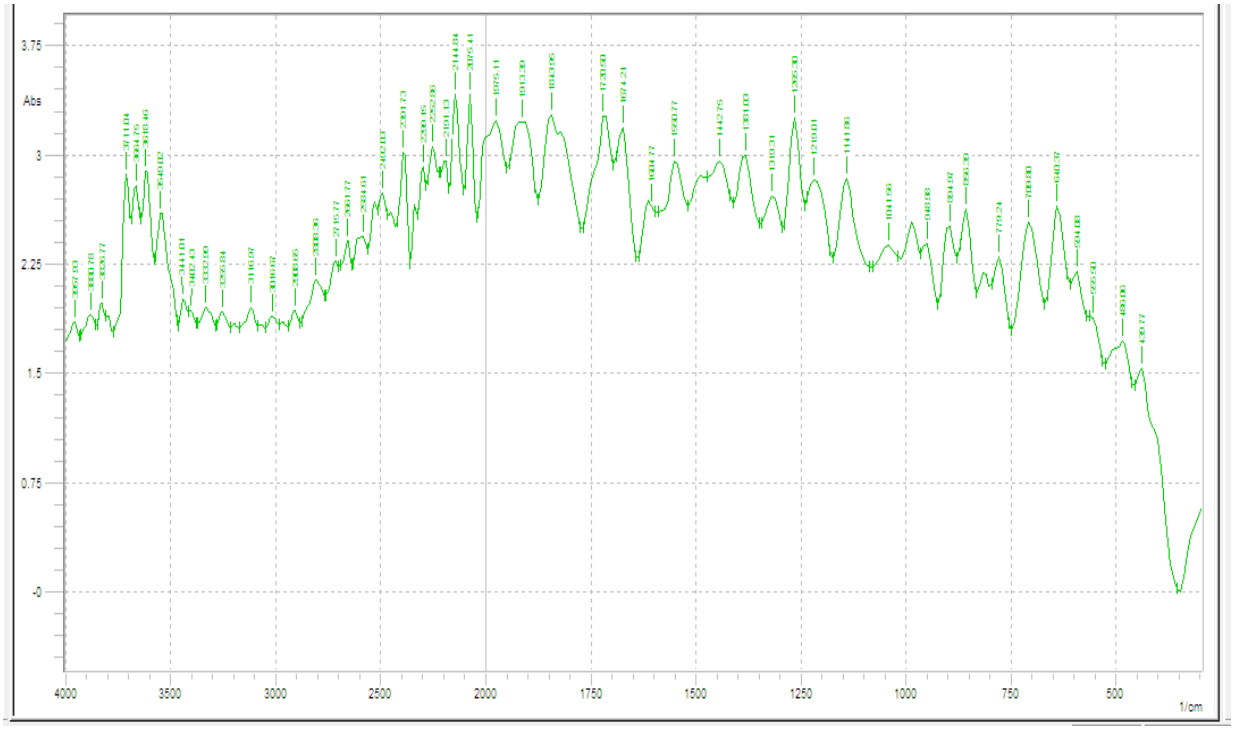


Figure 6: Infra-red Profile of Crude oil degraded for 16 days by *Pseudomonas aeruginosa* containing 200mg/l lead



Fiigure 7: IR profile of Crude oil degraded for 16 days by Pseudomonas aeruginosa containing 200mg/l mercury

DISCUSSION

Effects of heavy metals (lead and mercury) on the growth rate of *Pseudomonas aeruginosa* in crude oil

The optimum growth of the microbe observed with 50mg/l of lead (Fig. 2) which occurred on a par with the control during the 240th hour shows that lead at low levels could enhance crude oil biodegradation by *Pseudomonas aeruginosa*. Anirudda and Hemen (2010) reported in their work that heavy metals enhanced the biodegradation of oil products by some microbial isolates. Furthermore, Owabor et al. (2011) working on polycyclic aromatic hydrocarbon bioremediation observed a slightly enhanced enzyme production in *Bacillus* sp and *Aspergillus niger* at low concentrations of lead and mercury and subsequent reduction in hydrocarbon content. Our own findings eventhough with a different organism support the aforementioned reports.

Pseudomonas aeruginosa cells exhibited a steady decline in their growth from the 288th hour and above. This could be the result of accumulation of toxic metabolites which negatively affected enzyme activities causing growth inhibition. At heavy metal levels of 100mg/l and above there was a significant

(P<0.05) decrease in the growth of *Pseudomonas aeruginosa* in comparison to the control. The decrease was metal dose-dependent.

The findings associated with mercury in this study shows that mercury was more toxic to the microbe. This might be connected to the fact that mercury by virtue of its affinity for thiol-groups in protein acts as an inducer of oxidative stress. The result is the inactivation of enzymes and ultimately the death of the microbes (Owabor *et al*, 2011).

Infrared profiles of raw and degraded crude oils

Differences in absorption spectra between the raw (figure 4) and degraded crude oils (figures 5,6,7) are indicative of the biodegradation process. The metabolism by *Pseudomonas aeruginosa* of unpolluted crude oil (figure 5) and polluted crude oil (figures 6 and 7) produced new absorption bands characterized by the removal of old peaks and emergence of new peaks at the regions within the wave number ranges of 2500 - 3500 cm^{-1} , 1690-1780 cm^{-1} and 1060-1230 cm^{-1} which were interpreted from standard manuals to mean the emergence of the hydroxyl, aldehyde and carboxyl functional groups. These suggest an oxidative dissimilation of the oil (Guilhaumou *et al*, 2005; Puro *et al.,* 2011; Yasin *et al,* 2014). The removal of old peaks and emergence of new peaks were however reduced in the lead and mercury-treated oils (figures 6 and 7) when compared to the untreated/unpolluted oil (figure 5) implying inhibition of degradation in the treated oils.

CONCLUSIONS

Results of this work show that lead and mercury could either enhance or inhibit crude oil degradation by *Pseudomonas aeruginosa* depending on their concentration. Additionally the findings also show mercury demonstrating higher toxicity to the microbe than lead. While the results portray *Pseudomonas aeruginosa* as a good candidate for the bioremediation of crude oil polluted soils it also highlights the need for more research to engineer the organism for higher heavy metal tolerance for possible use in heavy metal remediation

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