

## Original Research Article

### Comparative effects of mashed mushroom and N.P.K Fertilizer on the bioremediation of crude oil polluted soil

U.Udochukwu<sup>1</sup>, O.C.Udinyiwe<sup>2</sup>, O.M.Adeghe<sup>3</sup> and F.I.Omeje<sup>4</sup>

<sup>1</sup>Department of Bioscience, College of Natural and Applied Sciences, Salem University-Lokoja, Kogi State, Nigeria

<sup>2</sup>Department of Science Laboratory Tech, Faculty of Life Science University of Benin, Edo State, Nigeria

<sup>3</sup>Department of microbiology, Faculty of Life Science University of Benin, Edo State, Nigeria

<sup>4</sup>Departments of Microbiology, Faculty of Science, Federal University Otuoke, Bayelsa State, Nigeria

\*Corresponding author

#### ABSTRACT

#### Keywords

Mushroom, N.P.K fertilizer, bioremediation and crude oil polluted soil.

The comparative effects of mushroom and N.P.K fertilizer on the bioremediation of crude oil polluted soil were examined. Mashed mushroom was used as a nutrient source for the bioremediation process which stimulated indigenous organisms. Before now, different types of fertilizers have been used in bioremediation process including N.P.K fertilizer. This research actually focused on the comparative advantage of using mushroom as a natural bio stimulant instead of synthesised fertilizers. A polluted soil sample was divided into two; half of it was amended with the mashed mushroom and the other with N.P.K fertilizer. The experiment was monitored at four intervals, the zero day, first day, 14th day and 28th day. The amended soil sample was analysed at the four different intervals., The pH value range from 6.49 to 7.30, total organic carbon (TOC) reduced from 5.00 to 1.20%, phosphate reduced from 10.10 to 5.54 (mg/kg), nitrate reduced from 9.50 to 4.48 (mg/kg) and the hydrocarbon content also reduced from 232.60 to 73.17 (ppm). The rate of crude oil degradation was examined in percentage also for both soil samples, N.P.K soil sample had a value ranging from 17.90 to 68.54% and that of the mushroom soil ranged from 28.75 to 84.46%. This showed that the soil amended with mushroom had higher bioremediation ability and will not cause secondary pollution in the soil as compared with chemically synthesized fertilizers.

#### Introduction

Bioremediation is a natural process which occurs as a result of the utilization of a crude oil contaminant as nutrient sources for bioactivities of the microorganism. This then leads to the breakdown of the long chain of

the chemical bond of the crude oil, but due to inhibition of microbial growth by petroleum products, crude oil products tend to persist during bioremediation. This makes bioremediation that occurs naturally to be

very slow but the introduction of an enhancer brought a great hope to crude oil bioremediation as the enhancers enhances the outgrowth of the microorganism so that bioremediation can take place within the shortest period of time. Crude oil pollution tends to persist in soils until remediation measures, involving the application of nutrients are taken, because oxygen and nitrogen are limiting factors in all types of petroleum degradation. The application of a mushroom and NPK 20:10:10 fertilizers were as effective as the use of bio-augmentation with indigenous hydrocarbon-utilizing bacteria (HUB) in the degradation of the hydrocarbon contaminant. This is because HUB is present in almost all types of soils and would multiply where the right types and concentrations of metabolic feedstock exist (Odokuma et al., 2003). A combination of treatments, consisting of the application of fertilizers and oxygen exposure on bioremediation of a crude oil-polluted agricultural soil was also evaluated by Ayotamuno et al., (2006). Enhanced Bioremediation technology exploits various naturally occurring mitigation processes: natural attenuation, bio-stimulation, and bio-augmentation. Bioremediation which occurs without human intervention other than monitoring is often called natural attenuation.

Bio stimulation also utilizes indigenous microbial populations to remediate contaminated soils. Bio-stimulation consists of adding nutrients and other substances to soil to catalyse natural attenuation processes. Bio-augmentation involves introduction of exogenous microorganisms capable of detoxifying a particular contaminant, sometimes employing genetically altered microorganisms (Agarry et al., 2012). During bioremediation, microbes utilize chemical contaminants in the soil as an energy source and through oxidation-reduction reactions, metabolize the target.

Petroleum hydrocarbons can be degraded by microorganisms in the presence of oxygen through aerobic respiration. When oxygen is limited in supply or absent, as in saturated or anaerobic soils or lake sediment, anaerobic respiration prevails. Generally, inorganic compounds such as nitrate, sulphate, ferric iron, manganese, or carbon dioxide serve as terminal electron acceptors to facilitate biodegradation (Department of Environmental Quality, 1998). Petroleum hydrocarbons are naturally occurring chemicals; therefore, microorganisms which are capable of attenuating or degrading hydrocarbons exist in the environment. Microorganisms have limits of tolerance for particular environmental conditions, as well as optimal conditions for pinnacle performance.

Pollutant degradation rate can be enhanced by the addition of nutrients, oxygen, and primary substrates into the contaminated systems. This could increase the populations of indigenous microorganisms and thus improve the efficiency of pollutant biodegradation. In bio-stimulation technology, nutrient supplementation for petroleum hydrocarbon degradation has traditionally focused on addition of nitrogen (N) and phosphorus (P), either organically or inorganically, (Agarry et al., 2012). Mushroom is more likely to be used as a soil conditioner; however, it may also have a potential application as a soil fertilizer (Vasudevan and Rajaram, 2001). Research and trials have been conducted regarding the value of mushroom as a nutrient amendment. The advantages of using mushroom as a soil fertilizer over chemical fertilizers is that it provides slow-release of nutrients which is enhances optimum growth of crops upon application. Furthermore, mushroom contains a wealth of micronutrients that usually are not present in standard NPK chemical fertilizers.

## **Materials and Methods**

The soil samples were collected from Rumudumanya village in Obiakpor Local Government Area, River state, Nigeria. The mushroom used for this study was collected from the University of Port-Harcourt Agricultural Demonstration Farm. The mushroom was grinded to fine mashed particle, dried for 60 minutes in an oven at a temperature of 125°C. The crude oil was collected from Port Harcourt Refinery. These samples were all transported to the laboratory aseptically. The media and the reagents were sterilized by autoclaving at 121°C at 15psi for 15minutes. The culturable hydrocarbon utilizing bacteria and fungi of the soil were enumerated in duplicate by the viable count method using the spread plate technique. Bushnell Haas agar media was used for the enumeration of hydrocarbon utilizing bacteria and fungi.

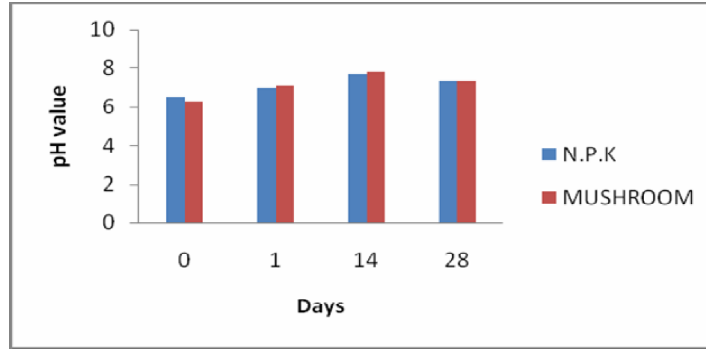
Bushnell Haas broth powder (0.33g) and Agar media (1.5gram) were aseptically prepared using standard method. After preparation of the media, 0.1ml of lactic acid was aseptically pipetted into the Petri dishes labeled for hydrocarbon utilizing fungi. Soil suspensions were prepared by 10 fold serial dilutions with 1g of soil and 0.1ml of  $10^{-5}$  and  $10^{-6}$  dilution was spread on the plates. After inoculation of the agar plates with the sample, a sterile filter paper (Whatman No.1), saturated with crude oil was aseptically placed onto the inside of the lid (cover) of the Petri dishes. The filter paper saturated with crude oil served as a sole carbon and energy source for growth of the organisms on the surface through vapour phase transfer. The plates were then incubated in an inverted position at room temperature for 7days, after which the average counts were recorded. Physico-

chemical analysis carried out were Soil pH, Total Organic Carbon (TOC), nitrate content, phosphate and total hydrocarbon content (Saari et al., 2007).

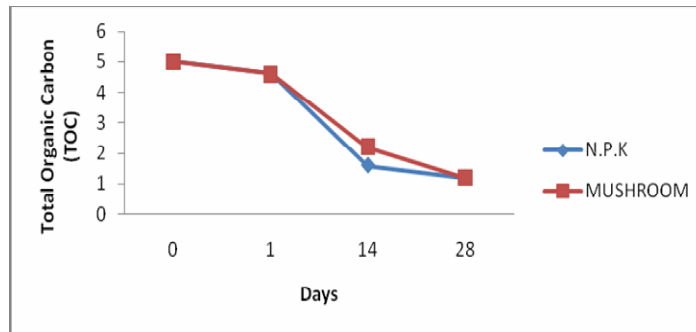
## **Results and Discussion**

It was observed that the rate of crude oil removal was 84(%) for treatment with mushroom and 68.54(%) for NPK, on the 28th day of the study. Though there was no much difference in the nitrate content, phosphate content and total organic carbon between the two soil samples. It can simply be seen that the indigenous microorganisms utilized the crude oil present in the soil for its growth and metabolic processes and this led to the removal of the contaminant from the soil. The bioremediation of the crude oil polluted soil to its normal and original status of the soil using mashed mushroom is highly effective as shown from (fig 6).

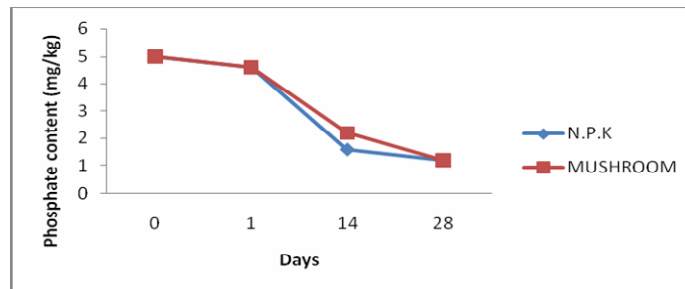
It was observed that the amount of crude oil in the soil were removed depending on the rate of enhancement of the growth of the indigenous microorganism which in turns led to the biodegradation of the crude oil contamination in the soil environment. From the dynamics of the growth, from the first day of treatment to the last day of the treatment, as the pollutant reduced which showed that the rate of bioremediation is directly proportional to the rate of microbial growth and metabolism. It can therefore be said that the rate of removal of total hydrocarbon content (THC) is directly proportional to the rate of growth of the microorganism in the sample analysed and the enhancement of these indigenous microorganism makes the remediation of the crude oil polluted soil samples faster and more efficient.



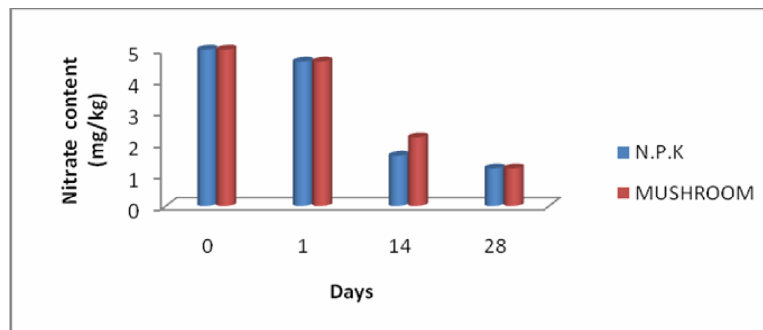
**Fig.1** pH value of the two polluted soil samples



**Fig.2** Total organic carbon of the two test systems



**Fig.3** The Phosphate content of the two soil samples within the 28 days



**Fig.4** The Nitrate content of the two soil samples within the 28 days

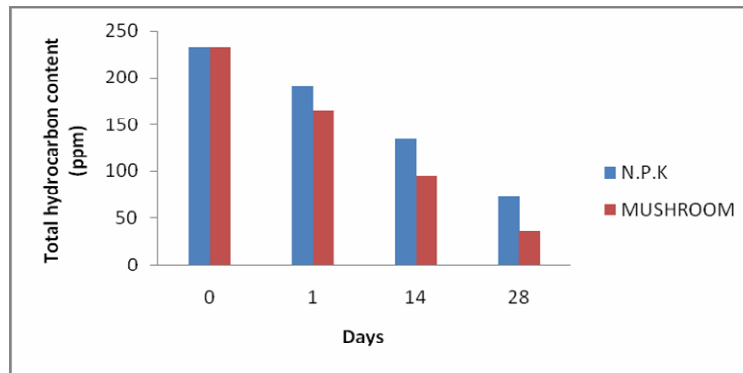


Fig.5 Total hydrocarbon content (ppm) of the two soil samples

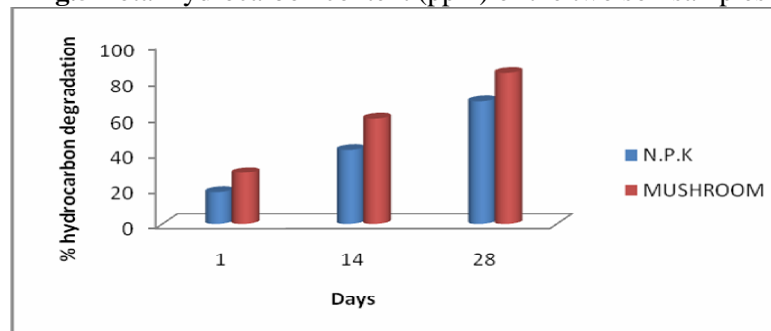


Fig.6 Percentage hydrocarbon degradation of mashed mushroom and NPK fertilizer

## References

- Agarry S.E., C.N.Owabor, and Yusuf R.O, (2012) Studies on biodegradation of kerosene in soil under different bioremediation strategies. *Bioremediation Journal*.14:135-141.
- Agnès, G., G.Vincent, and J. E.Graham, (2003). Assessment of Jessica oil spill impacts on intertidal invertebrate communities. *Marine Pollution Bulletin*: 46(11), 1377- 1384.
- Atlas R.M.(1991). Microbial hydrocarbon degradation of oil spills. *Journal of Technology and Biotechnology*. 52, 149 156
- Ayotamuno, M. J., R.B.Kogbara,, S.O.T.Ogaj, and S.D.Probert, (2006). Bioremediation of a crude oil polluted agricultural-soil at Port Harcourt, Nigeria. *Applied energy*, 83: 1249-257.
- Bossert, I.D. and.G.C.Compeau, (1995). Clean up of petroleum hydrocarbon contamination in soil. *Microbial transformation a degradation of toxic organic chemicals*.New York: Wiley; 77 -125.
- Bragg, J. R., , R. C.Prince, J. B.Wilkinson and, R.M.Atlas (1994). Effectiveness of bioremediation for the Exxon Valdez oil spill, *Nature*, 368, 413-418
- Chikere, C.B., G.C.Okpokwasili and B.O.Chikere (2009). Bacteria diversity in tropical crude oil polluted soil undergoing bioremediation. *African journal of biotechnology* 8(11)2535-2540
- Chorom, M., H.S.Sharili, andHotarnedi. (2010). Bioremediation of a crude oil polluted soil by application of fertilizers. *Iran journal of Environmental Health Science and Engineering*, 2010 (7) 319-326
- Chuma, C.O. (2010). Enhanced Bioremediation of hydrocarbon contaminated mangrove swamp in the Nigeria oil rich Niger Delta using seawater microbial inocula amended with crude biosurfactant and micronutrient. *Nature and science* 2010-8(8) Department of Environmental Quality.(1998).

- Fundamental Principles of Bioremediation.
- Hamme, J.D., A.Singh, and, O.P.Ward (2003). Recent advances in petroleum microbiology. *Microbiology of Molecular Biology Rev.* 67:503-549
- Ibiene, A.A., F.A.Orji, C.O.Ezidi and C.L.Ngwobia, (2011) Bioremediation of hydrocarbon contaminated soil in Niger Delta using spent mushroom compost and other organic fertilizers. *Nigeria journal of agriculture food & environment* 7(3):1-7
- Ihekweazu., N.S. (1998). Effects of Crude Oil Pollution on the Biochemical Composition of Soil in Izombe Area of Imo State, unpublished B.Sc. thesis, Department of Industrial Biochemistry, Federal University of Technology, Owerri, Nigeria.
- Leahy, J.G., and R.R.Colwell (1990). Microbial degradation of hydrocarbons in the environment. *Microbiol Rev.*;54:305-315.
- Mandal A. K, D., Manish, S.Abu, P.M.Sarma,, B.Lal, A.Agnihotri,, U. S.Hazra, S A.Fazal, A K. Rout and U.S. Prasad,(2007a). Remediation of oily sludge at various installations of ONGC: a biotechnological approach, Proceedings of Petrotech 007: International Oil & Gas Conference and Exhibition, New Delhi, India, 753.
- Moacir, A. T., M.P.Barros, S. C.G.Campos, E.Pinto,, S.Rajamani, R.T.Sayre,, P. Colepicolo, (2008). Biochemical biomarkers in algae and marine pollution: A review of Ecotoxicology and Environmental Safety, 71(1), 1-15.
- Mostafa C. and H.San Sharif, (2011) Bioremediation of crude oil polluted soil by sewage sludge published 2011. 204-301.
- Odokuma, L.O. and A.A.Dickson, (2003). Bioremediation of a crude-oil polluted tropical rain-forest soil. *Global Journal of Environmental Science.* 2 (1): 29-40.
- Prince, R. C., D. L.Elmendorf,, J. R.Lute,, C. S.Hsu,, C. E.Haith,, J. D.Senius, G.J.Deichert, G. S.Douglas, and E. L.Butler,(1994). Hopane as a conserved internal marker for estimating the biodegradation of crude oil. *Environmental Science and Technology*, 28, 142-145.
- Rosenberg, E., R.Legman,, A.Kushmaro,, R.Taube,, E. Adler, and E.Z. Ron, (1992). Petroleum bioremediation: a multi-phase problem. 3:337-350.
- Szaro Robert,C. P. D.Michael,, H.H.Gary and F. F.John (1978). Effects of chronic ingestion of south Louisiana crude oil on mallard ducklings. *Environmental Research*, 17(3), 426-436.
- Tanee, F.B.G. and P.D. S.Kinako, (2008). Comparative studies of bio-stimulation and phyto-remediation in the mitigation of crude oil toxicity in tropical soil *Applied and Science of Environmental manager.* USEPA (U.S. Environmental Protection Agency). (2006). A Draft Report on the incidence and Severity of Sediment Contamination Surface Waters in the United States, National Sediment Quality Survey: Second Edition. U.S. Environmental Protection Agency Office of Water, Washington D.C, EPA/823/F-01/031.
- Vasudevan, N. and Rajaram, P. (2001). Bioremediation of oil sludge-contaminated soil. *Environment International*: 26: 409-411.
- Wilson S. C. and Jones K C. (1993). Bioremediation of soil contaminated with polycyclic aromatic hydrocarbons: A review *Journal of Environmental pollution*, 81(3): 229-49.