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MICROBIAL DIVERSITY AND DEGRADATION OF PETROLEUM HYDROCARBON IN IMPACTED SOILS AND WATER BODIES IN NIGER DELTA AREA OF NIGERIA

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ABSTRACT

Microbial diversity of hydrocarbon in polluted soil and water bodies explains the variability that exists among various species of microorganisms inhabiting such environments. Sources of these pollutions include among others polycyclic hydrocarbons, heavy metals contamination, industrial wastes and the unregulated use of pesticides. No doubt, Niger Delta region is heavily polluted due to unregulated exploration activities of the petroleum prospecting and exploration industries, including the illegal activities of the locals. This has grossly affected the distribution and existence of some species of microorganisms. The survival of microbes in such polluted sites depends largely on their ability to adapt, tolerate and/or degrade these pollutants. The degradation of these hydrocarbons has resulted in the formation of metabolites some of which influences diversity among the indigenous microbial species. There is a significant relationship between the level of contamination and alterations in the microbial community. Decontamination of polluted sites can be used to increase biodiversity. Therefore, biodiversity is influenced by the concentration of the pollutants. The ecotoxicological effect of petroleum pollution is the need for oxygen during degradation of the hydrocarbon. In a reduced oxygen supply, serious ecological damage may take place. Also, the presence of heavy metals and organic compounds may enhance toxicity to microorganisms.

Contribution/ Originality: Previous works sourced have failed to link biodiversity to remediation of the polluted site and also, the analysis of the associated enzymes which are involved in the degradation of the pollutants. This work established that the application of hydrocarbon degraders can increase microbial diversity in petroleum hydrocarbon polluted sites.

1. INTRODUCTION

Microbial diversity explains the variations existing among different and/or within species of microorganisms in a given ecological system. Microorganisms are diverse in nature and play major role in the biogeochemical cycles. This role enhances the survival and sustenance of most micro- and macro-biota in the different ecological systems by recycling nutrients and making them available to the indigenous species (e.g. microbes, plants and animals). The diversity of microorganisms depends on their adaptability to varying environmental pollutants. No doubt, Niger Delta region is heavily polluted due to unregulated exploration activities of the petroleum prospecting and exploration industries, including the illegal activities of the local inhabitants through bunkering, blowing of pipelines and unauthorized refining of crude oil. The toxic effect of these petroleum products has grossly affected the distribution and existence of some species of microorganisms. The presence of crude oil and the associated intermediate products of its metabolism alter the physicochemical state of the affected soil and water bodies [1] and since these microorganisms have optimal conditions under which they thrive, their distribution in the environment are equally affected. However, some of these microorganisms facilitate energy recycling within the ecosystems through organic matter degradation that serve as energy sources to the indigenous flora. These processes of energy cycling ensure stability and balance in the essential nutrients required for growth and proliferation of the existing microbial species. To achieve this, microorganisms mineralizes organic matter to carbon dioxide, water and various inorganic salts [2]. For instance, in aerobic degradation, end products are usually water and carbon dioxide while in anaerobic degradation; it leads to biogas and suspended liquor (e.g. methane and digestate). Hydrocarbons are equally not left out in this process of mineralization. Biogeochemical cycles such as nitrogen, sulphur, phosphorus, iron and manganese cycles depend primarily on microbial activities [1, 2] most of which are enzymes mediated. The above listed cycles which are essential for the sustenance of soil and aquatic lives are disrupted by pollution. This inhibits proliferation and growth of most microorganisms that drive these processes. Bacteria are ubiquitous in nature and are capable of rapid growth when provided with nutrients and favourable environmental conditions for metabolism and cell division. Studies have shown that bacteria are involved in catalysis and synthesis of some organic matters in aquatic and terrestrial environments [2]. However, many substances mainly organics are degraded by microbial actions. Such substances include lignin, cellulose, chitin, pectin, agar, hydrocarbons, phenols and other organic chemicals which are in turn utilized by some microbes to sustain their growth and proliferation. It is imperative to note that both mobilization and transformation of pollutants are microbial-driven processes. Thus, allochthonous materials which are foreign to their present sites are left ultimately to the microorganisms for recycling [1]. Also, the toxic effects of pollutants on the autochthonous microbial populations is of major significance in ecotoxicological studies [3, 4]. The problem of petroleum input to aquatic and terrestrial environments has resulted to intense scientific investigation into the sources, quantity and biological impact of this major form of pollution. These hydrocarbons are mainly xenobiotic and are not easily degraded so can persist in the environment for a very long time. Their existence in the natural soil environment causes soil contamination as part of land degradation [5]. The concentration of these xenobiotics in unpolluted soil may be low enough that they do not pose threat to the environment as such can increase or sustain biodiversity. This is because toxicity is a function of the concentration of the pollutant. It implies, therefore, that toxicity effect is directly proportional to the concentration of the pollutants [2, 4]. Biodiversity is negatively affected when the environment is polluted. Pollution of soil or water bodies is said to occur when the concentration of the pollutants is high enough to cause damage to the environment [6-8]. Investigations into the effects of petroleum hydrocarbons on microbial communities have largely been focused on the role of microorganisms in hydrocarbon degradation. Few studies are focused on the effect of petroleum hydrocarbons on the activities of some heterotrophic microbial community [3]. Because the role of microorganisms is important to decomposition and mineral cycling in ecological systems, an understanding of the responses of the microbiota to petroleum spills is very key [1, 3].

Hydrocarbon degraders are mostly found in petroleum contaminated soils and water and have potential for the production of both toxic and non-toxic metabolites. Some of these metabolites facilitate competitiveness and survival of these microorganisms in such environments. According to Ahmed and Fakhruddin [9]; Varjani, et al. [10], the associated metabolites may have antimicrobial effect as it is with the case of pyocyanin and these have adverse effect on microbial biodiversity. Ekhaise and Nkwelle [11]; Barrett [7] believed that the wide range of the biological activity of pyocyanin is due to its ability to catalyze the formation of toxic radicals such as superoxides and hydrogen peroxide. Some researchers have proposed that those microorganisms capable of expressing high level production of enzymes catalase and superoxide dismutase (SOD) can tolerate pyocyanin more effectively. According to Ekhaise and Nkwelle [11]; Barrett [7] limited nutrients and carbon supply leads to coupling of pyocyanin production to intracellular ATP levels resulting in increased pyocyanin level. Such increase alters microbial community structures by inhibiting growth of microorganisms that are sensitive to pyocyanin.

There is a significant association between the level of contaminants and alterations in the microbial community. Reduction in the diversity of bacteria community is common after petroleum exposure, but an increase in bacterial diversity has also been reported [6, 7, 10]. A wide variety of pollutants contaminate the environment due to oil spills and various oil related industrial activities. This may lead to organic pool, impacting on microbial diversity and cause disruption of the natural degradation processes in aquatic and terrestrial microbial community [4, 5, 12]. Various sources of soil pollution are listed in Figure 1 and these include polycyclic aromatic hydrocarbons, domestic and industrial wastes, pesticides, and heavy metals discharge. In this study, the objective is to examine microbial community composition and activity after long term exposure to multiple pollutants such as heavy metals and petroleum hydrocarbons.

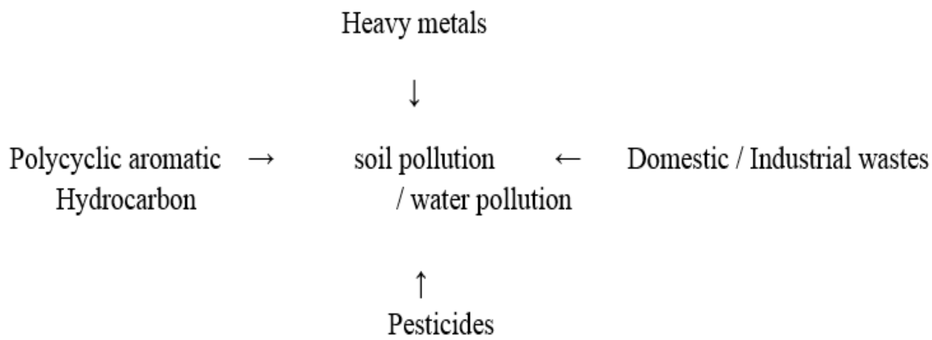

Figure 1. Sources of soil and water pollution.

2. EFFECT OF POLLUTANTS ON MICROBIAL DIVERSITY AND ITS DEGRADATION POTENTIAL

The effects of pollutants in soil and water environments are quite enormous and only decontamination can salvage the already damaged ecosystem. The decontamination of such polluted sites requires the service of microorganisms through the process of biotransformation or biodegradation of the pollutants [4]. Bacteria genera such as *Gordonia*, *Brevibacterium*, *Aeromicrobium*, *Dietzia*, *Burkholderia* and *Mycobacterium* isolated from petroleum contaminated soil proved to be the potential microorganisms for the degradation of hydrocarbons [3, 4]. Besides, microbial genera such as *Pseudomonas*, *Bacillus* and *Achromobacter* have also been incriminated. Other hydrocarbon degraders investigated and confirmed by Rhodes [4] include fungal such as *Aspergillus*, *Trichoderma*, *Penicillium*, *Candida*, *Sacharomyces*, *Rhizopus*, *Pichia*, *Lipomyces* and *Hansenospora*. Mycorestoration methods employ the use of fungi to repair and restore the negative impacts of hydrocarbon pollution. This method can also restore and repair habitat whether damaged by human activity or natural disaster. According to Rhodes [4] fungi are 'tools' in this restoration processes. The ability of these fungi to grow mycelia enhances easy penetration of its substrate. The filamentous fungi have advantages over bacteria due to their ability to breakdown a large number of persistent compounds. Also, the filamentous fungi are robust in their approach to biodegradation due to their high tolerance to high concentrations of recalcitrant organic molecules and are more tolerant to environmental stressors than bacteria [1, 5]. In addition, filamentous fungi have the potential of producing large amounts of extracellular enzymes during hyphal colonization of soil, resulting in enhanced rates of bioremediation [6]. However, fungi are known to produce oxidative enzymes such as peroxidases and laccase that catalyze polymerization of different organic pollutants [7]. According to Elisashvili, et al. [8] fungal enzymes such as oxidoreductases, laccase and peroxidases have relevant application in the elimination of polyaromatic hydrocarbons (PAHs) pollution of soil and aquatic water bodies. Some have shown great potential for xenobiotic transformation due to their ability to produce lignin peroxidase and manganese dependent peroxidases. These can degrade a wide range of pollutants including polychlorinated biphenyls (PCBs) and nitroaromatic explosives.

The processes in Figure 2 explains photo-degradation 0f hydrocarbon while Figure 3 and 4 explain both aerobic and anaerobic pathways for the degradation of hydrocarbon and are influenced by certain physical and chemical factors that render the pollutants susceptible to transformation and/or degradation. Both processes are explained within the context of oxidation and reduction and are mediated by oxidoreduction enzymes [10]. Besides, degradation can also be by hydro-degradation (i.e. hydrolysis followed by oxidation) and oxo-biodegradation.

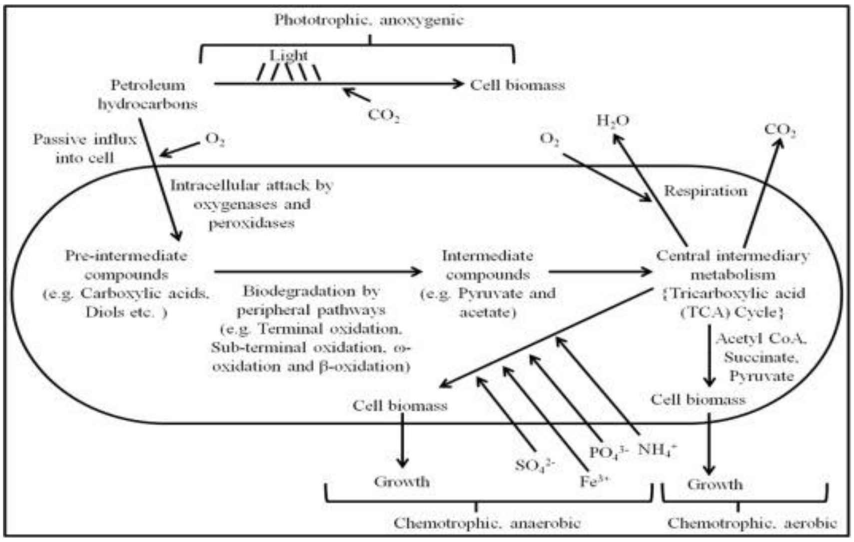

Figure 2. Photo degradation of hydrocarbon [10].

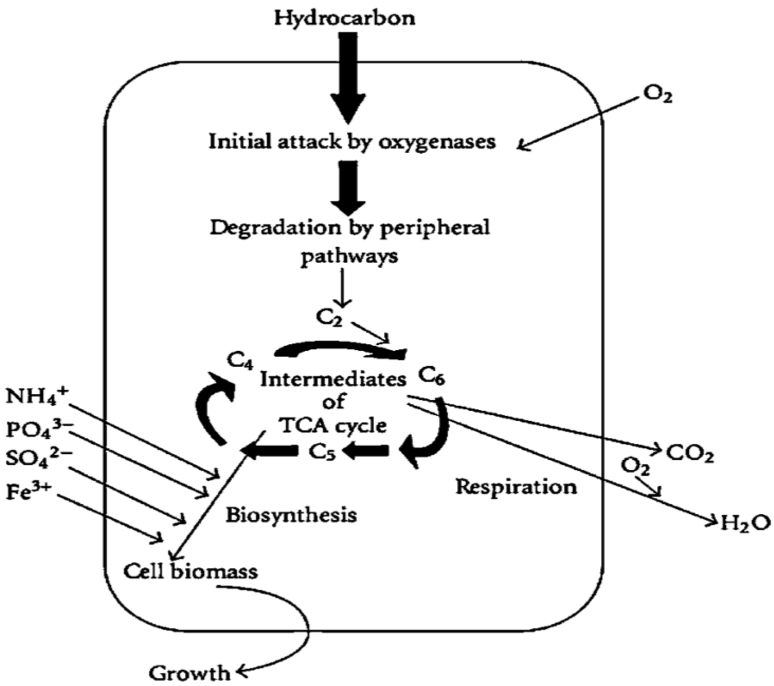

Figure 3. Aerobic degradation of hydrocarbon [10].

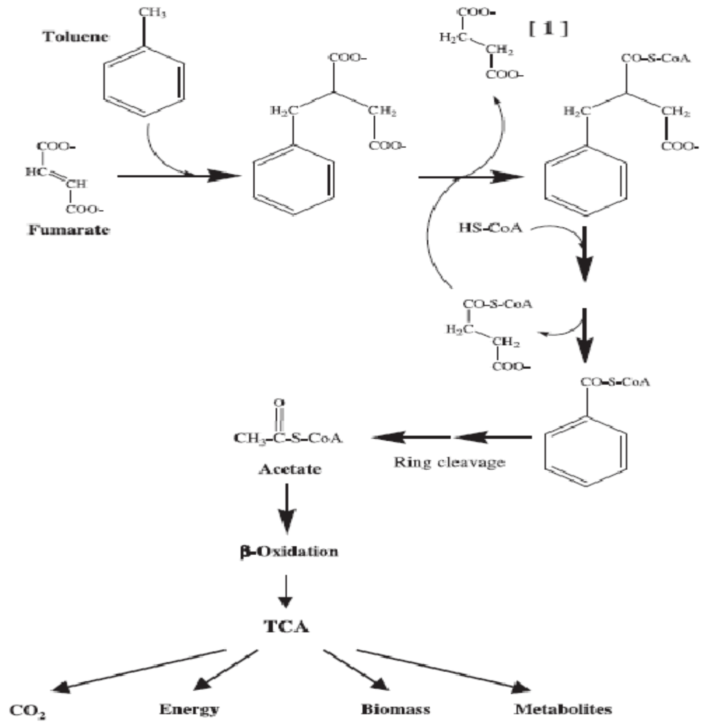

Figure 4. Some products of hydrocarbon degradation [10].

In aquatic environment, degradation is influenced when hydrophobic contaminants interact with dissolved organic substances and particulate organic matters in water column. Biotransformation and biodegradation can increase biodiversity in polluted environment since the aim is to remediate impacted sites. For biodegradation to take place, the susceptible substrates are brought into closer contact with those microorganisms that are capable of degrading the substrates [10]. However, increase in the production of toxic metabolic products reduces degradation processes and consequently reduces microbial diversity. This affects negatively the biological components of the ecosystem such that microorganisms that are susceptible to the metabolites are eliminated. In contrast, solubilization of pollutants into dissolved organic phases stimulates their breakdown through availability of co-metabolizable substrates [13]. The production of less toxic substances enhances the growth and proliferation of most microorganisms indigenous to the habitat thereby increasing population and microbial diversity [14]. Oil spills offer a good example of the effect of pollutants on microorganisms in aquatic and terrestrial microbial population in the Niger Delta area of Nigeria.

3. MICROBIAL ECOTOXICOLOGICAL EFFECT OF PETROLEUM

As explained previously and depending on the composition, petroleum products are recalcitrant and persist in the impacted environment. Certain environmental conditions favour the degradation of these recalcitrant molecules [7, 10]. For instance, the combination of temperature, water chemistry and initial microbial community are potential factors for the development of oil degrading microbial communities in oil polluted sites. Temperature is known to be the most critical factor. If water is present in oil, in the presence of oxygen and appropriate temperature condition, the microbial species multiplies, infesting the ecosystem. Thus, reducing the presence of water prevents microbial infestation. Under optimal conditions, selected fraction of these products can be removed in a matter of days or weeks [13] such that a certain percentage of the oil by weight disappears over a short period of time (e,g, n-alkane). It implies, therefore, that the remaining fractions maybe more resistant to microbial attack.

Petroleum hydrocarbons are highly toxic environmental pollutants comprising of mixtures of several hydrocarbons. The ecotoxicological effect of petroleum hydrocarbons is not limited to microorganism; plants and animals are also affected. In man, petroleum products toxicosis can be as a result of ingestion and inhalation of, and /or dermal contact with petroleum hydrocarbons in crude oil, diesel, naphtha, kerosene, gasoline or the hydrocarbon mixture [10, 15]. Exposure to these hydrocarbons has been incriminated in headaches, dizziness, nausea, vomiting, and so on. Exposure to high levels can cause coma and death [8]. Accordingly, no petroleum substance, including used oil can be hazardous substance except to the extent that it is listed as hazardous waste under Resource Conservation and Recovery Act, 1976 (RCRA) or under any other such status.

In nature, conditions are rarely favourable for complete biodegradation to take place. As such, when the rate of degradation is slow, oil may persist in the environment for a long time. Floodgate [16] opined that of millions of tons of oil from the oil spill of the Second World War that found its way into the sea, certain percentage is still in the ocean till date. The impact of this spill is still being felt on aquatic lives many years after it occurred. Therefore, in determining the ecotoxicological effect of petroleum, the amount and qualitative differences in hydrocarbon content of it must be considered since it influences its susceptibility to and availability for biodegradation.

Petroleum products are found to have relatively little effect on heterotrophic microorganisms except yeasts which tend to be slightly inhibited by fuel oil. Crude oil is also known to inhibit the growth of lipolytic, proteolytic and chitinolytic bacteria. Fuel oil, however, is observed to be more toxic than crude oil, though both can support the growth of certain individual species of heterotrophic and cellulolytic bacteria. The addition of petroleum hydrocarbon to water samples collected from oil-free environment has been shown to limit the growth of bacteria that are normally found in the oil-free water. In contrast, addition of petroleum hydrocarbon to water samples collected from an oil-polluted environment promotes the growth of bacteria already present in that oil-polluted water. This is because these bacteria species have been exposed and have the capacity to produce petroleum hydrocarbon degrading enzymes. So the pollutants at this point become source of nutrient to the microorganisms. The toxic effect of pollutants on microorganisms suggests that selection for oil-resistant species had already taken place in the oil-polluted environment [17, 18].

Addition of sub-lethal concentration of hydrocarbon can interfere with microbial chemotaxis by reversing its positive chemotactic responses. This is an important ecological process since microorganisms depend on chemotaxis for attachment to substrates required for growth. Researches done have shown that there is an anticipated pattern of activities in the breakdown of petroleum hydrocarbon with differences in time of occurrence, in the steps taken, in the degradation process and in organized changes in mixed microbial population [19].

Bacteria are selective in their choice of substrates and many different bacteria species in mixed cultures are required for significant degradation of pollutants. Bacterial oxidation of hydrocarbons may produce many intermediate metabolic products which may be more toxic than the original hydrocarbon compounds. Toxic aromatic hydrocarbons are degraded very slowly. According to Xie, et al. [19] an ecotoxicological effect of petroleum degradation is the essential need for oxygen during bacterial oil degradation. Xie, et al. [19] added that where oxygen content is lowered by other forms of pollutions, microbial degradation of oil may cause additional ecological damage due to oxygen limitation or degradation may not take place at all.

Another aspect of oil pollution problem is the presence of heavy metals and organic compounds in petroleum hydrocarbon pollutants. For instance, mercury can combine with oil to suppress degradation of oil and enhances toxicity to microorganisms due to separation of the mercury in the oil phase [18]. Under anaerobic conditions, bacteria in sediments will methylate mercury in contrast to the production of elemental mercury under aerobic condition. In this case, microorganisms act as mobilizing agents. In both cases, the mercury is transported from sediment to water through microbial activity [20, 21]. The metals can be mobilized through the food chain and are fed upon by higher forms (plants and animals). The methylation of metals such as mercury, cadmium and tin by microorganisms results in either primary or secondary ecotoxicological effects [21, 22]. Where there exist a significant heavy metal resistant bacteria population actively metabolizing, as well as accumulating heavy metals; their involvement in the bioaccumulation of these metals will be significant. Such bacterial population may not be adversely affected.

4. EFFECTS OF HYDROCARBON AND HEAVY METALS COMBINATION ON AQUATIC AND SOIL MICROBIAL COMMUNITY

Heavy metals at a very high concentration may have a reduction effect on soil and aquatic microbial community structure, biomass production and microbial activities [22, 23]. Low biomass and microbial activity may limit the decomposition of soil organic matter and lead to the accumulation of organic materials in metal contaminated soils and water bodies [21]. Scholars have opined that reduced microbial activity may originate from the change in microbial community structure over a long-term exposure to heavy metals [24, 25]. These researchers observed that the metal-resistant microorganisms have a restricted ability to degrade organic pollutants. Heavy metals are often mixed with organic pollutants in contaminated sites. The presence of multiple pollutants may present extreme challenges to the maintenance of a functionally diverse microbial community [26].

In sites contaminated with multiple pollutants, only microorganisms that tolerate those pollutants at toxic levels may survive. Besides, some microorganisms may also utilize hydrocarbons as their carbon and energy sources. The changes in community structure may not simply equal the microbial response to the individual pollutant. Moreover, the restricted ecological niche may simultaneously affect microbial function and activity.

5. IMPACT OF INDUSTRIAL WASTES AND PESTICIDES ON SOIL MICROORGANISMS

Industrialization has led to increase in soil and water pollution. The effluents discharge from the industries enters water bodies or percolates through the soil pore spaces down to the ground water. At low concentration, depending on the composition of the wastes, it may favour the growth of some species of algae, fungi and bacteria and cause increase in microbial population. It is believed that wastes with high phosphate concentration enhance algal bloom. These microorganisms in turn helps to modify the soil texture [27]. At high concentration of toxic industrial wastes, microbial profile of the receiving soil and water are adversely affected [28-30].

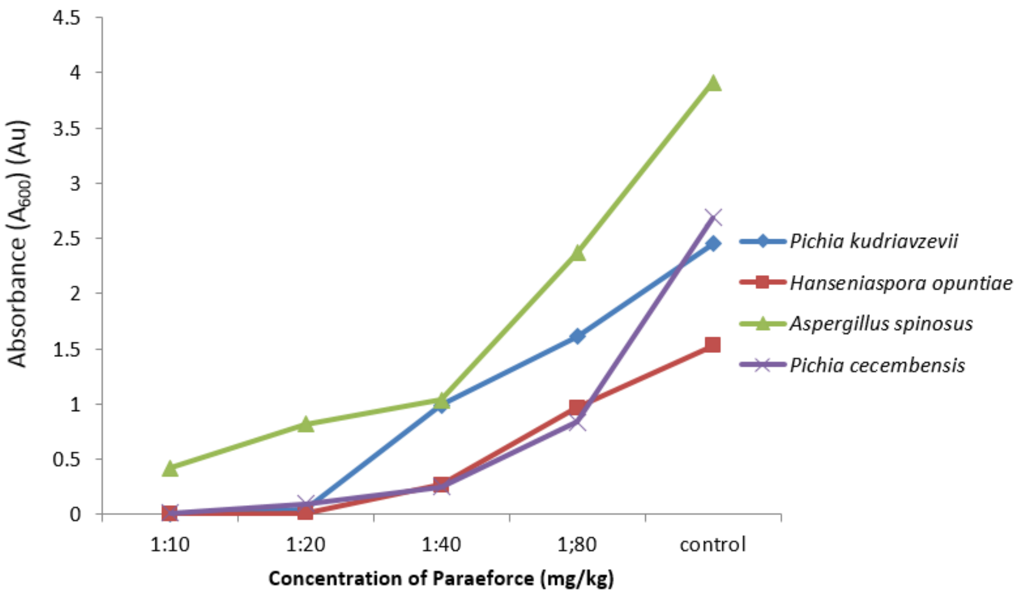

Figure 3. Effect of Paraeforce concentration on fungal growth [33].

Pesticides are toxic chemicals used by farmers to eliminate weeds and insect pests and in so doing, increases crop yield. They influence microbial diversity in soils by increasing or inhibiting the growth of some species depending on the concentration of the pollutants [31]. According to Meena, et al. [32] and Onianwah, et al. [31] the extent to which the pesticides affect microbial flora depends on the concentration applied, its bioavailability, adsorption, bioactivity, toxicity, bioaccessibility and biodegradability. At very high concentration, it tends to inhibit or completely eliminate certain species of microorganisms [33]. At relatively low concentration, the indigenous microbial flora may get adapted to these pollutants, synthesize the degradative enzymes and breakdown these compounds to non toxic products [33].

According to the work of Onianwah, et al. [33] the assessment of effect of different concentration of paraeforce (herbicide) in Figure 3 showed that higher growth rate was observed at a dilution of 1:80 (50mg/L) which is the concentration indicated by the manufacturers as requirement for the control of weeds in farms. At a higher concentration of 1:10, growth of some of the fungi was inhibited while at a concentration of 1:80, allows the growth of all the fungal species. This is supported by the work of Wibawa, et al. [34] which states that high concentration of herbicides inhibits the activities of most soil microorganisms. Similarly, Subhani, et al. [35] recorded that over application of herbicides in soil can inhibit some of the natural processes in the ecosystem and may decrease the performance of the non-target organisms. Onianwah, et al. [31] demonstrated in his work that the ability of most microorganisms especially fungi to degrade petroleum products is based on their ability to produced hydrocarbon degrading enzymes. Table 1, showed the potential of some fungal isolates to produce oxidative enzymes catalase, liccase and peroxidases. This work established that fungal enzymes are involved in detoxification of this herbicide (paraeforce). The preliminary screening test done identified three species of fungi capable of degrading paraeforce. Thus, *Trichoderma* sp, *Aspergillus* sp. and *Rhizopus* sp. were selected as the most potent microorganisms for the production of the enzymes as contained in Table 1. They argued that certain factors such as changes in pH, nutrient depletion and presence of inhibitory metabolites could impede the production of the oxidative enzymes consequently slowing down the degradation process. This also, affects biodiversity adversely.

Table 1. Qualitative assay of associated fungal enzymes in Paraeforce degradation.

Organism	Catalase	Mn peroxidase	lignin peroxidase	Laccase
Trichoderma sp.	+	+	+	++
Aspergillus sp.	++	++	++	++
Rhizopus sp.	++	++	+++	+++

Note: (+) = weak reaction, (++) = moderate reaction, (+++) = strong reaction.

Microbial utilization of these products leads to increase in the proliferation and growth of the soil microbial flora and of course, a consequent increase in microbial population [36-39] in a given community.

6. CONCLUSION

There is no doubt that the involvement of illegal oil refining merchants, oil theft, industrial wastes discharge and oil spills have grossly degraded the ecosystems in the Niger Delta region of Nigeria. The consequence is reduction in the population of soil and water biota. The direct effect on microbial population has reduced its activities leading to environmental degradation. Hydrocarbons no doubt are toxic and recalcitrant, and adversely pose serious health challenge to the ecosystem. The major effect of these pollutants on microorganisms is the impediment of their role in the earth's biogeochemical cycles. To ensure stability and sustenance of the ecological systems, hydrocarbon pollution or any other form of chemical pollution must be drastically reduced if not completely avoided.

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