Using Compost to Reduce Oil Contamination in Soils

Chindo Anulika Nwankwo

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The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

The use of mineral fertilizers as amendment for bioremediation of crude oil contaminated soils has been investigated in many situations; however, the average farmer in Niger Delta, Nigeria is unable to access mineral fertilizer for routine farming purposes due to cost. The waste stream in the Niger Delta has a high percentage of biodegradable materials that are suitable for composting. Given the abundance of biodegradable materials in the region, it should be possible to use the product as a bioremediation material. This project investigated the possibility of using waste-derived compost to treat soils contaminated with crude oil.

The level of oil at 5,000 mg oil/kg soil (0.5% w/w) is the limit used in Nigerian legislation. For this study, contamination values much above this level were used ranging from 5, 7.5 and 10% (w/w) as this was found to better reflect the actual situation in the Niger Delta. The total petroleum hydrocarbons were determined using spectrophotometre.

The ability of compost to improve the fertility of the soil was determined using seedling germination, chosen because of its relatively rapid response. The results from this study showed that germination of seeds without the addition of compost was adversely affected by the oil pollution. There was total inhibition to growth at initial 10% oil level suggesting that 10% oil concentration is above the trigger level for plant growth. The addition of compost diluted the contamination levels producing approximately 50% increase in overall germination observed within 5 weeks. Plants grew in soil with the least diluted content of 7.5% oil level. Soils treated with compost recorded higher biomass yields compared to those not treated with compost. This suggests that compost improved the quality of contaminated soils and sustained the yield of tomatoes seeds. The factors used for evaluating the effectiveness of treatment on soil quality included soil pH, electrical conductivity and seedling test as demonstrated by germination and growth of tomato seeds. The results were used to develop equations and charts for determining the most suitable treatment regime to be used in the field. Coming from this treatment regime, a relatively simple protocol was developed for use by local farmers to enable them to make the most effective use of compost on their own contaminated soils.

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Map of Nigeria within the red dotted line

Source: Infoplease Atlas (2000–2007, p184-85)

1 Introduction

1.1 The role of petroleum products in environmental pollution

Oil spillage continues to be a global concern not only due to the environmental impact but also due to the public health hazards associated with any spill. The rise in energy consumption worldwide is not without a price and the activities associated with crude oil production, exploration, transportation and marketing have led to increased number of oil spills both on land and into water bodies. The trend shows continuous increases in oil pollution which can be attributed to the increasing dependence on oil based technology such as fuels for aircrafts, automobiles and heating systems, although there have been recent advances in alternative sources of energy such as production of biofuels (Wokocha *et al.*, 2011). It is difficult to draw a line between effects of oil pollution on man, soil and plants as these three are interwoven.

In general, it is acceptable that the responsibility of the polluter and part of any contingency programme for oil polluters is clean up of any spill whether on land or water to restore the area back to its former state. In some cases, the oil polluters are bound to pay compensations (largely determined by the legislation) both to the state and to individuals affected by such spills. However, in many cases financial compensation is not an end to the problem, the patent question to ask is the loss in terms of the environment, livelihoods and health of both man, animals and plants. For example, the recent explosion (April, 2010) of one of the deep water rigs in the Gulf of Mexico, saw a United States (US) court rule that the oil company in question pay a fine of $4.5 billion which is the largest penalty to be paid by any polluting company. In this case, the corporation as well as individuals that are liable was charged. The aim was to make this a deterrent to the oil company and the threat of prison to deter the employees. Despite this huge financial compensation, environmental campaigners are agitating that the payoff cannot be commensurate to the losses.

Despite the legislation, the situation in developing countries such as Nigeria does not always hold most oil polluters accountable for the many oil spills across the country. It may be the case that public officials do not have the will to implement the law to the letter or that the leaders are not responsive. This attitude has contributed largely to how oil spillages and clean-ups are managed in such countries. As recently pointed out by a Dutch court in their ruling against Shell petroleum Development Company in a case brought against them by four farmers from the Niger Delta in Nigeria, that under Nigerian law, there is liability for the consequences of oil spillage but none for failure of the offenders to respond adequately (Gaughram, 2013). However as alluded to earlier, in many cases the payment of fines cannot be compared to the environmental damages caused by oil spillage, sometimes hydrocarbons do persist in the environment even after a clean-up programme thereby affecting agricultural activities over the longer term (Osuji and Adesiyan, 2005).

Various authors (Odu, 1978, Anoliefo and Vwioko, 1995, Ekundayo and Obuekwe, 2000, OuÃ©draogo *et al.*, 2001, Bernal *et al.*, 2009) have reported that the effect of oil spillage on flora and fauna varies depending on the amount and type of hydrocarbon involved, the sensitivity of the species and the topology of the land. Oil spillage could also introduce metals such as copper, zinc and lead into the environment which might be toxic to plants and damage soil ecosystems (Ekundayo *et al.*, 2001). The first major oil spill that received a great deal of public attention was in 1967 and is popularly referred to as the Torrey Canyon oil spill in the English Channel. After the tanker ran aground, it released approximately 30 000 tonnes of oil and was left for six weeks before any organised response by the authorities concerned, by which time she had discharged all the oil into the water (Burrows *et al.*, 1974). A spill of such magnitude was unprecedented and so there was not enough time to respond. The first fear about the oil release had to do with the aesthetics of the beaches even though it affected marine life as well.

Recently, the Gulf of Mexico oil spill has shown the potential impact of a major oil spill, which in this case caused the loss of human lives, flora and fauna. Many family businesses suffered as they lost their source of livelihood to the oil spillage; beaches were ruined as oil coated the white sands even wildlife was affected as they became smothered in oil (Plate 1.1).

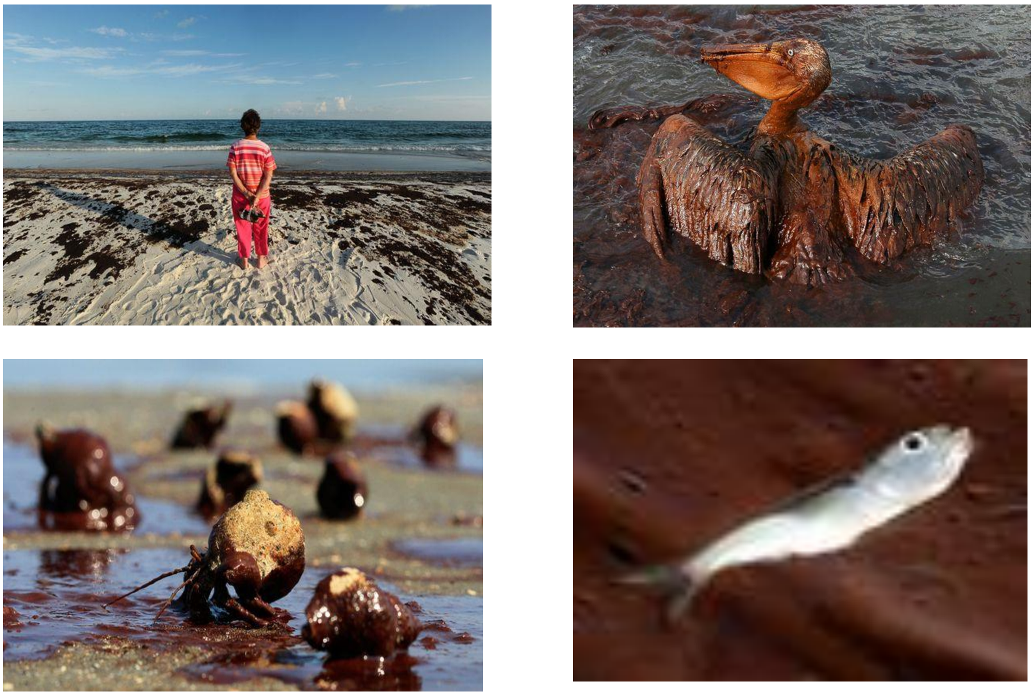

Plate 1.1: Some potential impact of oil spillage such as oil coated beaches, oil laden wildlife, sticky slog and dead fish; adapted from National Geographic, 2012

In the Niger Delta region of Nigeria, oil pollution and gas flaring have affected both land and water bodies. Oil spills in the Niger Delta, have devastated farmlands and other agricultural settlements. Many studies have investigated the environmental pollution in the Niger Delta yet the pollution problems continue to occur and very little clean up takes place (Antai, 1990, Adeniyi and Afolabi, 2002, Ijah and Antai, 2003, Sojinu *et al.*, 2010). The result is that more and more farmland is being lost to oil pollution incidents. It is not an overstatement to say that delays to and inadequate cleanup has aggravated environmental damages and peoples' human rights especially their right to health, water, food and livelihood (Gaughram, 2013). Anifowose, (2008) in his paper described exploration and transport of oil and gas in the Niger Delta as being under reactive rather than proactive environmental regulations.

According to the Energy Information Administration (EIA) an energy department in the USA, many oilfields in Nigeria lack the infrastructure to produce and market the associated natural gas and as a result it is often flared thereby releasing greenhouse gases to the environment. Nigeria ranks second only to Russia in relation to the quantity of gas which is flared off (Nwilo and Badejo, 2007).

1.2 Background to study

With a growing global population of approximately six billion (Lai, 2001) and growth of 1.3% per year, there is increasing concern over food security due to a rapid increase in world population and soil degradation due to various factors including soil contamination by crude oil. The importance of soil cannot be overemphasized; it supports terrestrial life such as recycling of major elements (carbon, nitrogen phosphorus and water), plants' growth and biomass production. Recently Amnesty International (05/02/2013) reported the case of four farmers from the Niger Delta, Nigeria who took SHELL Petroleum Development Company to court for damages to their farmland and environment. Even though a ruling by the Dutch court gave victory to only one of the farmers, it has served to show that both human rights and environmental abuses by multinational companies have gained international recognition (Gaughram, 2013).

When enormous oil spillage occurs, apart from affecting the aesthetic values of the affected environment, it also leads to reduced soil fertility which provides a major challenge in the reuse of crude oil impacted soils for agricultural purposes and could result in very poor crop yields (Nwachukwu, 2001). This challenge has necessitated the treatment of crude oil contaminated soils to regain the soil's fertility and allow reuse in agriculture.

As highlighted earlier, there is renewed concern regarding the implications of crude oil contamination on both land and water towards food security as aquatic lives are affected and more lands abandoned due to contamination both from municipal and industrial wastes. For example, China with a population of approximately 1.3 billion people has a record of increasing land degradation due to heavy metal contamination (Chen, 2007). Poor land management due to human impacts, activities of oil industries, indiscriminate discharge of industrial waste especially oil-based waste, inappropriate use of agrochemicals and urban wastes are among the major causes of soil pollution (Chen, 2007, Orji *et al.*, 2012).

There are a number of contaminated sites around the World without any form of treatment. In Europe alone, approximately three million (3 000 000) sites are considered to be at the risk of contamination due to anthropogenic activities, with over eight percent actually contaminated and in need of clean-up. A projection based on five years of study estimates an increase of fifty percent in 2025 of contaminated sites due for remediation. Therefore, lack of knowledge among the policy makers on implication of land damages and lost to soil services means that such losses will not be included in legislation (Guimaraes *et al.*, 2012). The economic importance of oil spillage can be summarised as (a) environmental degradation (b) soil contamination and land shortages (c) poor agricultural yields i.e. food insecurity and loss of livelihoods and (d) fire incidents.

From the available literature, one can draw the conclusion that the majority of the oil spillages affecting the West are limited to the marine environment since majority of activities relating to oil exploration including transporting and marketing are offshore. However the situation in Africa and the Middle East (e.g. Nigeria and Kuwait) is very different with most of the oil spillage affecting the land (Atlas and Bartha, 1972, Odu, 1972, Atlas and Bartha, 1973, Leahy and Colwell, 1990, Ekundayo and Obuekwe, 2000, Namkoong *et al.*, 2002, Ahiamadu and Suripno, 2009). The consequences are that local communities living around the contaminated sites are prone to both ecological and economic damages.

1.3 The study area

The Nigerian coast has been described as approximately facing the Atlantic ocean and lies between latitude 4° 10' to 6° 20'N and longitude 2° 45' to 8° 35'E. It has been described as comprising four distinct units from West to East (a) the Barrier Island coast (220km) (b) the Mihin Transgressive Mud coast (known as Mud coast approx. 450km) (c) the Niger Delta coast (450km) and (d) the Strand coast (100km) (French *et al.*, 1995, Nwilo and Badejo, 2007, Kadafa, 2012). Figure 1.1 shows the Nigerian coastal region. The focus of this research is the Niger Delta region located in the Atlantic coast of Southern Nigeria.

According to Osuji *et al.* (2006) and Anifowose (2008) the Niger Delta is one of the world's largest deltas and makes up approximately 7% of the land area of Nigeria.

There is no universal agreement on the size of the Niger Delta; however Sojinu et al., (2010) reported that it covers an area of approximately 75,000km^2 (7.5 % of Nigeria) and Akpokodje, (1987) reported that it covers an approximately 36,260Km^2 (about 4% of Nigeria). Anifowose, (2008) described the Niger Delta as containing the bulk of Nigerian's proven oil and gas reserves. The region has about 5284 oil wells and 527 flow stations of crude oil processing with more than 7000km of oil and gas pipeline traversing across the whole land area (Figure 1.2). The Niger Delta region in Nigeria comprises nine states out of the thirty-six states in Nigeria namely – Abia, Akwa Ibom, Cross Rivers, Rivers, Bayelsa, Delta, Imo, Ondo and Edo (Figure 1.3).

The contribution of the Niger Delta to the foreign exchange earnings of the Nigerian government makes it the treasure base of the nation. The statistics of oil wells in Nigeria reveals that there are 606 oil fields in the Niger Delta of which 360 are onshore and 246 offshore (Kadafa, 2012). According to NOSDRA (Nigeria National Oil Spill Detection & Response Agency), approximately 2,400 oil spills have occurred in the Niger Delta since 2006. Now, the Niger Delta refers to a geographical name that has been associated with the ugly menace of oil pollution in Nigeria for many years.

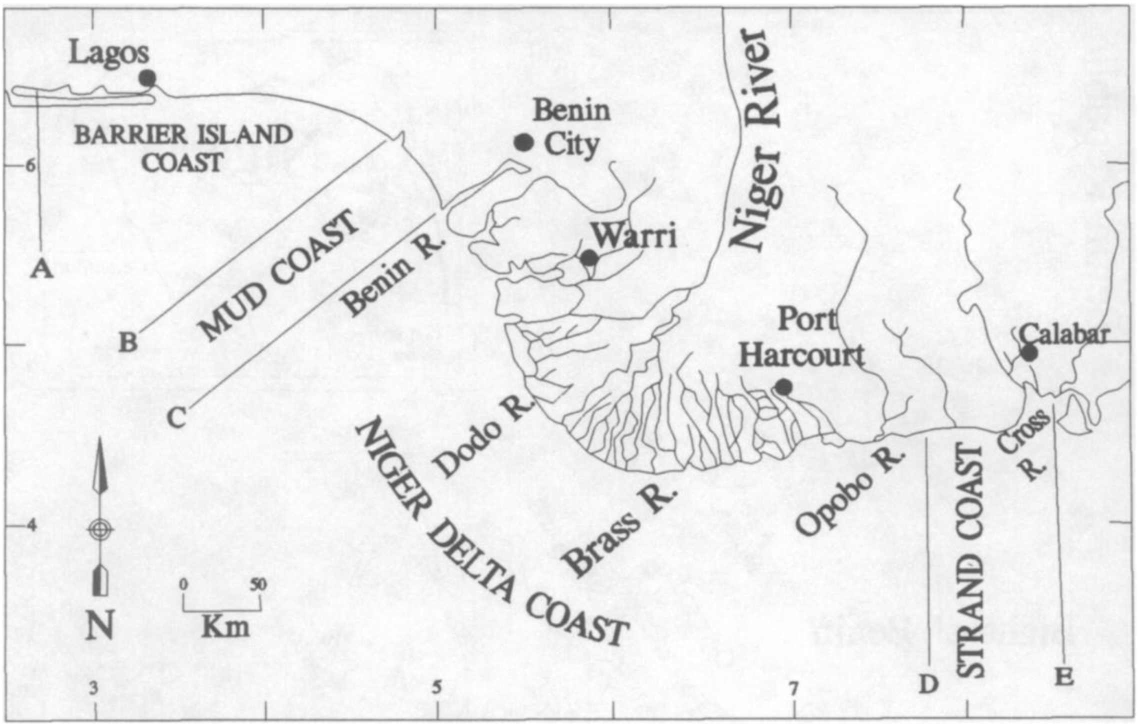

Figure 1.1: The geomorphic classification of the Nigerian coast, (adapted from (French et al., 1995).

Figure 1.2: Map showing wide distribution of SPDC oil fields in Niger Delta region, adapted from Nwilo and Badejo,(2007)

Figure 1.3: Map of Nigeria with a black ring around the Niger Delta states. The two states with red stars are not grouped as the Niger Delta states. Adapted from: (AllWestAfrica.com., 2009)

1.3.1 Context of Study: the Niger Delta, Nigeria

Oil spillage in the Niger Delta has a link to the historical background of the region for us to appreciate the current situation and complex drivers of the conflicts. The impact of politics and trade in the Niger Delta has sharpened the magnitude and effects of oil spillage in the region. Oil spillage has its complexities as discussed next and without an understanding of these complexities; it might be difficult to appreciate the effect of oil spillage on the public and human development of the people concerned.

1.3.1.1 Impact of transatlantic trade on the region

Before the advent of the oil boom, the region was known for other forms of trade especially oil palm trading such that it contributed significantly to the national treasury (Obi and Rustad, 2011). Like other parts of the country during the 20th century, the Niger Delta region experienced transformation economically especially with the coming of the European traders who became the principal merchants in the import –export trade after conquering the local merchant-princes and kings (Obi and Rustad, 2011, Ebenso, 2012).

1.3.1.2 Socio-cultural development

The Niger Delta people are primarily farmers and fishers. The land ownership belongs to the extended family, and is shared among the number of householders (usually the men) that make up the extended family. During the planting season, a householder can borrow more land from a different family, which sometimes attracts payment of token and other times no fees depending on the affluence of the owner. There are instances where land is leased for a given period. This does not necessarily involve transfer of ownership. The swampy terrain in the region has posed some challenges including scarcity of land and this has helped in defining the livelihood of the people of the Niger Delta as mainly subsistent farmers, fishers and traders.

1.3.1.3 Economic development

In 1954, Nigeria became a federation and the principle of governance based on revenue sharing, political representation and power distribution. These factors contributed in weakening the ethnic minorities in terms of the power equation at the regional and national levels (Obi and Rustad, 2011). This feeling of marginalisation became obvious after independence in 1960, with the struggle for resource control among the political elites. However, in the mid-1960’s the regional economy grew weaker and by the 1970’s petroleum became the nation’s main export earning approximately 85% of the government’s revenue (Nwilo and Badejo, 2007).

The campaign for resource control came out of the feeling among the oil producing minorities that the non-producing ethnic minority have more seats at the federal level. This means they also control the oil wealth whereas they who produce the oil suffer from neglect, exploitation and pollution. The Niger Delta case shows how natural resources within a region could enrich the multinationals, their partners and even local elites and on the contrary contribute to disempowerment and impoverishment of the local people (Obi and Rustad, 2011). A historical point of view, may help us understand how the struggle for local autonomy and resource control had developed from non-violet protest to militant resistance and attacks on oil installations, which unfortunately contributed its share of oil spillage.

However, with the change in land ownership, the states took control of the land and resources, therefore for the locals their title to their land was only customary and not legal (Obi and Rustad, 2011). Therefore, it was possible for the multinational companies (MNC) to directly get oil and land leases from the government without recourse to the local communities. This triggered local grievances as the people felt alienated from their resource and in turn this gave rise to different local groups such as the Movement for the Survival of Ogoni people (MOSOP), the Movement for the Emancipation of the Niger Delta (MEND), Niger Delta Peoples' Volunteer Force (NDPVF) and the Ijaw Youth council (IYC) to mention but a few.

Some of the local groups who want their own share of the oil wealth have taken to undertaking illegal oil businesses. They will drill into the oil delivery lines and tap from the crude oil which they then load into tankers and sell at the parallel market. For many this has actually turned into a commercial venture, however not without a cost as it has also led to severe pipeline damage leading to serious oil spills and widespread pollution. Osuji and Adesiyan (2005) reported a particular case of an oil spill from a pipeline leakage which occurred on the 21 August,1997 at Ogbodo-Isiokpo in Ikwerre, Rivers State, Nigeria releasing Twelve Thousand barrels of oil (a barrel is equivalent to 158 litres) and affecting an area of 15,900m^2.

1.3.1.4 Socio-economic and political context

There is a big divide between the political elites who want control of the oil rents derived from oil production in the Niger Delta and the ethnic minorities who are marginalised in the distribution of the wealth (Obi and Rustad, 2011). As a result, there is a collective desire by the people of the region to control their resources especially oil and land. Unfortunately, this struggle for resource control has metamorphosed into a vicious cycle of exploitation, protest, violence and even criminality. Combination of both domestic and international factors rather than a single cause have led to the violence in the Niger Delta. The rise in violence emanated from a context of long years of oppression of communities non-violet protests by prolonged military rule in the state of Nigeria. Either the Nigerian forces were pacifying the protesting communities or fighting the local groups (mentioned earlier) that were resisting oil exploitation by the oil multinationals and Nigerian state. This even led to the death of human rights environmental activist Ken Saro-wiwa and eight other Ogonis executed by the then military government on November 10, 1995. The militias felt that non-violet protests were not yielding the desired results so they resorted to violence.

In 1999, when Nigeria returned to democracy after very long years of military rule, the peoples' expectation included demilitarization of the region and that the elected leaders would address their grievances. In contrast, the return of democracy had increased the number of human rights and pro-democracy groups. Some unscrupulous politicians conscripted the unemployed youth into their camp and used them as tools against the opposition thereby increasing the wave of violence especially in the 1999 and 2003 elections (Obi and Rustad, 2011). This led to a full insurgency in 2006 with a combination of both lethal attacks, kidnapping of oil workers and families (released after ransoms are paid) and sabotage of oil installations leading to more oil spillages in the region, which meant that the little arable land available kept on reducing while the local farmers bear the pains.

In summary, the conflict in the Niger Delta region started because of peoples' alienation from the oil wealth produced in the region, however there is a shift from the initial goal of resource control to all sorts of goals embedded in greed and dishonesty. This is not to say that the oil multinational do not share some of the blame since some of the oil installations are very old, spanning twenty – twenty-five years of age without any replacement. Other causative factors associated with oil spillage are human error and equipment failure. However, the number of crude oil spilled sites left without any clean-up is of concern since the local community end up bearing the burden. The government has failed to take care of its responsibilities in respect of the Niger Delta crisis and as Obi and Rustad, (2011) rightly concluded; the economic capability of any nation rich in resources is dependent largely on the quality of institutions.

The involvement of the Nigerian state, the military, different ethnic movements, oil multinationals and the International communities complicated the conflict and its resolution. Therefore, the communities' feeling of neglect and deprivation will persist and are drivers of conflict in the region. In addition, the inability of government to implement the regulations has left the oil companies at their own wills and caprices in matters relating to standards of operation. This lapse has encouraged the oil multinationals to break both environmental and civic laws without consequences.

In Nigeria, as in some other developing countries, one of the issues with oil spills is not that of legislations but the fact that there is no commitment on the part of government and public officials for full implementation of the law. This has resulted in most of the Trans-national oil corporations (TNCs) operating at a double standard which is below the International Standard when compared to what is implemented in the West.

Given the quantity of oil spilled whenever there is oil spillage on land, the question is should this be left at the mercies of the TNCs until they come to rescue of the people. It invariably means that the locals whose mainstay is subsistence farming and fishing will continue to pay the price. Secondly, the current approach by local contractors in the Niger Delta region of excavating the contaminated land and back filling with fresh soil from a different location is unacceptable and not sustainable. This has a massive impact on people living in affected areas. One of the drivers for this study is the belief that if the local people are empowered through technology-knowhow, they can actually get involved in the cleanup of their own land and be able to recover soil to be used for agriculture once again.

The events in the Niger Delta, could be summed up under political and economic context of petroleum violence (a) ethnic minority agitation for local autonomy and resource control (b) non-violent protest before discovery of crude oil (c) militant resistance after oil became a national factor and (d) ecological devastation (especially oil spillages on land) as a result of petroleum violence (Obi and Rustad, 2011).

Table 1.1: Major oil spills recorded in Nigeria

Year	Location	Quantity(barrels)
1970	Bomu 11 oil well blowout	> 200,000
1978	GOCON's Escravos spill	300,000
1980	SPDC's Forcados Terminal	580,000
Jan,1980	Offshore well blow out	200,000
1980	Texaco Funiwa-5	400,000
1982	Abudu pipeline	18,818
Jan, 1998	Idoho oil spill	40,000
Aug, 2008 &Feb, 2009	Bodo oil spill	> 200,000
April 2009	K. Dere spill	> 300,000

Source :(Nwilo and Badejo, 2007)

Plate 1.2 shows the devastation caused by oil and gas exploration and exploitation in the Niger Delta.

a

b

c

d

Plate 1.2: Photographs showing some of the devastating effects of oil and gas exploration and exploitation. (a) farmland devastated by oil contamination, (b) oil installation cutting across a footpath (c) oil spillage site at Ogoni (d) Elume oil spill fire. Source: Energia.gr (2011)

1.4 Problems associated with waste management in the Niger Delta

Every society has its own social problems, these problems are identifiable and labelled by the society itself as a problem, and so waste management is a big challenge in Nigeria and the Niger Delta region in particular. Up until the late 1970s, backyard composting was a traditional practice in rural areas of the Niger Delta. The usual practice in the region is that people sort out biodegradable materials from their household wastes and animal house (from animal husbandry) and bury the waste in pits until the pit is almost full. They then cover it with red earth and dig different pits if need be. After a period of about three months, the humus- like material is ready for use during the planting season.

In the urban and semi-urban centres, there are government provided skips for refuse collection. Although sorting of waste was not popular, people tend to retain cans and bottles, which are then sold to the refuse scavengers. The refuse scavengers in turn recycle these wastes through sale to metal and manufacturing companies. In this way, most of the biodegradables and packaging are disposed of in the skips. Sometimes the ministry of Environment gets rid of the waste.

In recent times (from the 80's), with the explosion in population growth in Nigeria coupled with the mass exodus of people to urban centres, utility services such as waste collection have been non-existent and this has given rise to indiscriminate dumping of refuse (Plate 1.3). The inability of different authorities to curb this social vice at the beginning gave rise to the appearance of dumpsites all over the country. The incessant changing of different government from civilian to military and back to civilian government has not helped matters as each government comes with different policies regarding the environment. With time, the attitude of the locals towards waste management changed and gradually the practice of backyard composting started fading out.

Plate 1.3: A typical dumpsite in the Niger Delta region

Nigeria is an agrarian nation with many food (organic) wastes produced particularly during the harvesting period. This arises mainly because the local farmers do not have a means of preserving their produce for long periods. Consequently, during harvest, fruits and vegetables rot away and are disposed of in open dumps. In addition to this, many of the public abattoirs have no proper disposal routes and as a result, animal waste ends up in open dumps. The wastes are left to decompose in the open or are burnt in an uncontrolled manner releasing noxious gases into the atmosphere.

The use of composting is seen as a viable option in Nigeria because the raw materials are available locally. It is seen as being cost effective, relatively easy to manage and does not use sophisticated technology regardless that one of the key challenges is source separation of waste in Nigeria (Adekunle, 2011). In the hierarchy of waste management, waste recycling is preferred to other methods even if though it is not possible to recycle 100% of the recyclable material from municipal solid waste (Burnley *et al.*, 2011).The extent to which waste is managed can impact on the environment. This impact has been acknowledged both internationally and nationally as can be seen in directives for waste management to move from landfill-based to resource recovery-based (Burnley *et al.*, 2011).

To date, the government of Nigeria does not see it as a viable option spending money on what is already termed waste hence the poor and inadequate condition of waste management in most areas in Nigeria. The percentage of total municipal solid waste (MSW) thought to be decomposable is high in Rivers State (one of the states that make up the Niger Delta) an average of 81% (Igoni *et al.*, 2007). Adekunle (2011) reported that the volume of organic waste in Nigeria differs according to location but in general 70 – 80% of the total waste are organic in nature. Despite this, no effective large-scale composting plants are available and no proper recycling systems exist in the area. Therefore, these wastes are left to decompose in an open dump generally accompanied by open burning of waste with the resultant effect being emission of noxious gases into the atmosphere.

In Nigeria, if sorting of the organic waste is encouraged for example starting with government residential areas, this might lead to a change in the public's attitude towards waste management. As rightly observed by Adekunle (2011), waste are not source-separated in Nigeria otherwise it would have created an opportunity for generating compost as end products which could be readily used as well as providing employment opportunity. The refuse scavengers who ordinarily engage in sorting of waste in dumpsites can be gainfully employed as unskilled labour. This inability to separate waste has also limited the use of the organic materials in compost technology and subsequent application of the product as bioremediation material.

The use of waste derived compost is seen as a possible bioremediation option in Nigeria given the availability of the raw materials. By converting these wastes into compost, it has the potential to help to preserve the environment and reduce the amount of chemical treatment to the contaminated soil in the form of mineral fertilizers.

1.5 Current research and problems

Environmental degradation can be defined as any process (physical, chemical or biological) which can bring about a negative change to the quality of any component (air, water or land). Generally, it is accepted that natural resources such as land and water are infinite. This misconception arises due to lack of awareness about the risks associated with the life cycle of hazardous substances. Sources of contamination to the environment comes largely from anthropogenic activities thereby placing increasingly higher stress on the natural environment (Izonfuo and Bariweni, 2001, Otokunefor and Obiukwu, 2005, Adoki and Orugbani, 2007b, Rezaei *et al.*, 2010).

Nowadays environmental pollution is a global concern (Udo and Fayemi, 1975, Atlas, 1981, Leahy and Colwell, 1990, Bragg *et al.*, 1994a, Atlas, 1995a) and efforts are increasingly moving towards cleaning up of contaminated sites due to the public health hazards associated with hydrocarbons in the environment(Vorhees *et al.*, 1999). Various methods available for remediation of soils contaminated with hazardous compounds comprise traditional, chemical and biological approaches. The traditional approach mainly involves excavating the contaminated soil and disposing of it into landfill. The limitations to this approach includes restrictions due to regulations and logistics of handling and transporting the waste (Vidali, 2001). Presently in Europe, as part of the measures to manage what goes into the landfill, the legislation has set targets to reduce the amount of biodegradable municipal waste sent into landfill (Defra, 2007b). Approximately 40% (by weight) of biowaste still goes into landfill while in some member states up to 100%. Consequently, EU legislation demands that member states manage their waste in a way that will protect both the environment and human health, and so members are required to reduce biodegradable municipal waste going into the landfill to 35% by 2016 (Commission, 2010).

Other physical and chemical methods of treating contaminated soils are incineration of pollutants (which could release noxious gases into the atmosphere adding to the level of greenhouse gases emitted into the atmosphere global warming), containment to prevent migration of oil pollutants and soil washing. Despite the possibility of these methods in reducing levels of petroleum contaminants, the drawbacks associated with them include the fact that they involve rather complex technology and are expensive to run. In addition the public has a negative perception with respect to incineration for fear of contamination of site receptors (Vidali, 2001).

Bioremediation by land farming involves spreading the contaminated soils in layers or prepared beds and mixing with fresh soil, then tilling at intervals to allow oxygen for microbial degradation. The method requires a lot of land, and a failure of this bioremediation technique, results in previously uncontaminated soil becoming contaminated. Land farming practice is restricted to topsoil within the range of 10 – 35 cm (0.1 to 0.35 m).

In bio augmentation, the process of biodegradation is enhanced by addition of pre-selected microorganisms, with the aim of reducing the time taken to achieve an acceptable outcome. This process ensures the presence of sufficient microorganisms for the contaminant cleanup. Seeding with microorganisms has not proved very effective in the marine environment in contrast to adding fertilizer and adequate aeration (Atlas, 1995b). Prevailing factors against the use of seed organisms include strain selection, procedure for culture introduction, type of contaminant, and environmental constraints (Tyagi *et al.*, 2011).

Bio stimulation involves the addition of nutrients or other supplements to indigenous microbial populations to enhance growth. There have been contradictory results on the effect of addition of nutrients in the form of fertilizers to contaminated soil. Fertilizers in the form of urea-phosphate, N-P-K fertilizers, ammonia have been used. Some workers observed accelerated biodegradation (Ijah and Antai, 2003, Ahiamadu and Suripno, 2009), while in other cases there was no increase in biodegradation rates or rather, an increase after a delay of several months (Leahy and Colwell, 1990).

The use of chemical dispersants in cleaning up of contaminated land has resulted in greater ecological impact than the actual spill especially when a toxic dispersant is used (Cowell, 1971). This is in agreement with the report by Sullivan, (2010) who described the effect of dispersants on water as more harmful due to the fact that more dissolved oxygen was needed to degrade both the oil and dispersant and this can cause dangerously low oxygen concentrations in the water column. In the absence of toxicity, some dispersants can help in petroleum degradation as found with n-alkanes (Mulkins-Phillips and Stewart, 1974).

Considering the methods mentioned above, the main disadvantage is the impact on the environment and so clean up of petroleum pollutants have not been a complete success. These drawbacks have indeed opened up a gap between laboratory trials and field application (i.e. real life application). Therefore, one of the identified gaps, this research wants to address is how to assist the end user (in this case a local farmer) apply the current approach to his contaminated soils. In other words, without going through the complicated process of using seed or engineered organisms, nutrient supplements or analysing the different hydrocarbon components of the crude oil before he will be able to reuse the contaminated soils. This need arises because he does not have the skills, expertise and financial resources to do that.

Bioremediation simply defined is the use of microorganisms to remove or breakdown pollutants under enhanced conditions. The products are carbon dioxide, water and biomass under complete mineralisation (Hoff, 1993). Applying a more technical definition, bioremediation is the process which accelerates biodegradation by manipulating the contaminated matrix either through addition of nutrients, other supplements or exogenous microorganisms to stimulate indigenous microbial population (Atlas, 1995b). Bioremediation of crude oil contaminated sites takes place due to co-metabolism between microbial groups. Frutos *et al.* (2012) described bioremediation as a ‘green’ alternative for treating environmental pollutants and identified the advantages of bioremediation as follows; simple technology, cost effectiveness, treatment can be done on site saving cost on handling and transporting and can be applied over large areas.

However, the limitations of bioremediation include the degree of effectiveness, the process could be pollutant specific, it takes longer time and the need to do a pilot test to be sure that the design is effective at achieving a satisfactory result and the fact that the site owners and regulators have limited knowledge about the principles of operation (Vidali, 2001).

One of the central problems in the early 80’s was finding environmentally friendly options for enhancing petroleum hydrocarbon degradation. Earlier workers on bioremediation applied nutrient supplements in the form of nitrogenous fertilizers and mineral salts, as well as seeding with oil degraders (Bragg *et al.*, 1994b, Atlas, 1995b, Atlas, 1995a). Initial bioremediation of polluted sites through fertilizer application was done in marine environment Atlas (1995b), though it was more effective on oiled shoreline rather than open water oil pollution. The 1989 oil spill in Prince Williams, Alaska popularly known as Exxon Valdez which spilled 200 000 barrels (≃32 million litres) into the coast of Alaska was the ground-breaking event that opened new doors towards research on bioremediation through fertilizer application. It is the largest bioremediation project to date because of the amount of nitrogen and phosphorus that was used, about 50 000kg of nitrogen and 5 000kg of phosphorus was applied to the shorelines(Atlas, 1995b).

However, concerns about the accessibility, cost of inorganic fertilizers and its implication in environmental pollution have been identified as a major challenge especially in regions with high rainfall intensity such as Nigeria. Nitrogen can easily be washed away from the soil and pollute the water body. The introduction of compost as an organic fertilizer is a cleaner option because compost slowly releases its nutrients and acts as a soil conditioner.

Use of compost for bioremediation will be suitable in places such as the Niger Delta with high intensity of rainfall. In addition, it is a more practical way of reducing cost in contrast to chemical fertilizers and is more accessible. Compost has higher microbial diversity when compared to fertile soil and this feature has been implicated as contributing to higher degradation rate of organic contaminants(USEPA, 1998). As rightly put by Sullivan (2010), compost is the secret ingredient that allows the bacteria to do work.

The main microbial biomasses responsible for degradation of organic wastes are bacteria, fungi and actinomycetes. Treatment with compost and the use of composting can be applied as a bioremediation technique. In case of the latter bioremediation is achieved by mixing contaminated soil with traditional composting substrate such as garden and yard waste. This mixture may be treated in a windrow or in an enclosed reactor. Microorganisms consume the supplied substrates as well as the contaminants possibly by co-metabolism. Many hazardous wastes have been converted to innocuous products in this manner. The bacteria, actinomyctes and fungi present in composting break down pollutants into less harmful substances.

Consequently, the success of bioremediation depends on the bioavailability and biodegradability of the pollutants (Semple *et al.* 2001). This type of biological treatment is seen as a viable option because of the ability of these organisms to breakdown pollutants when compared to methods such as incineration, landfilling and land farming which merely transfers these pollutants from one medium to the other.

1.6 Scope and limitations of the current study

The scope of this research is limited to the use of compost for bioremediation of soils contaminated with Nigerian crude oil. Different soils from United Kingdom and Nigeria were used for the research. The work is laboratory based. Monitoring the reduction of hydrocarbons has been a traditional method to evaluate the impact of any protocol for agricultural purposes. However, this method is not enough from the point of view of public health and human development. There is need for a simpler and rapid test that will help to check the restoration of contaminated land for agricultural purposes. One of the promising features to monitor in terms of effectiveness of any protocol is seedling germination and biomass yield relative to the unpolluted soil. This approach of using biological indicators is relevant because the impact of oil has caused many distortions in the soil, flora and fauna and the traditional economies of the local people such as farming and fishing; the major sources of livelihood of local farmers living in regions where oil contamination is very frequent. Such a protocol should be user focused to allow the local people the opportunity to participate in clean-up of their contaminated soils.

1.7 Aim and Objectives of the current study

The overall aim of this research project is to determine the feasibility of using compost for the restoration of soils contaminated with Nigerian crude oil. Successful restoration will be assessed by the ability of the soil to sustain plant growth and improve yields. In order to achieve this overall aim, a number of study objectives are recognised:

1. To determine the relationship between carbon dioxide production and microbial activities in soils contaminated with Nigerian Bonny light crude oil.
2. To determine whether degradation of hydrocarbons is enhanced by treatment of the contaminated soil with compost at elevated temperature levels.
3. To use indicators such as seed germination, plant height and biomass yield to determine the degree to which successful restoration of contaminated soils has been achieved.
4. To determine the impact of different compost feedstock’s on the compost’s ability to achieve improvements in the germination and growth of plants.

5. To use the results from this study to develop treatment protocols for contaminated soils that can be used by the oil companies or local farmers.
6. To determine the potential financial implications of using compost as an alternative to inorganic fertiliser for the restoration of soils contaminated with Nigerian Bonny light crude oil.

1.8 Significance and impact of this research

The application of this study will be of high value in countries as diverse as Nigeria and other countries in the sub-Sahara area with a history of oil spillage. More especially in the Niger Delta region where there is (a) high cost and irregular supply of inorganic fertilizers to the local farmers (b) availability of raw material for composting and (c) cheaper labour cost. The abundance of compost and its accessibility by the local farmers make this approach a sustainable method of treating contaminated soils. The knowledge gained from this research will build on existing collaborations especially in the Niger Delta, between oil companies and communities, which aim to increase the amount of arable land.

The use of waste derived compost might improve the quality of contaminated soil and boost agricultural production under the conditions investigated because the organic matter content of the waste makes it ideal for use as composting raw materials. This approach might enhance the means of livelihood of the local farmers in the oil prone communities of developing countries by improving crop yield on recovered contaminated soils. In terms of the socio economics, this will recover the value of land for both immediate livelihood and future generations.

Therefore, the justification for this project can be summarised in the following:

I. The current approach for clean-up of contaminated soils in the Niger Delta is unacceptable and not sustainable. The method has an impact on the people living in the region. This new approach is more sustainable depending on the availability of organic material for composting.
II. The abundance, availability and accessibility to compost in the Niger Delta and similar areas will reduce cost.

III. The new approach is simple, cost effective and will hopefully work i.e. will produce soil to be used for agriculture once again

IV. The approach has double impact; local application in the Niger Delta and also in similar context with access to organic material.

An estimate of the daily volume of waste generated in Port Harcourt, the hub of the Niger Delta region is 8781.25 m^3. On the average, 1.11 kg per capita per day of refuse are generated in the metropolis (Igoni *et al.*, 2007). Koledzi *et al.* (2011) observed that waste generation ratio in developing countries ranges between 0.4 – 0.7 kg/cap/day. Similar characterisation of waste generated from some Nigerian cities have been reported by Babanyara *et al.* (2010). They pointed out an increasing trend in the generation of solid waste over the past decade, with Port Harcourt producing 265,129 tonnes in 1990 and further 352,853 tonnes in year 2000. Lagos and Kano states produced the highest volume of waste per year perhaps due to the population size of the two states. Of this volume, approximately 60% is organic fraction and suitable as composting material. Assume volume reduction during composting to be 50% where the density of compost is 600 kg/m^3 (approximately 0.6 $tonne/m^3$), then the amount of daily waste that is compostable is 1,580 tonnes.

2 A Review of the Academic Literature on Bioremediation

2.1 Journey of bioremediation

Bioremediation simply put is the use of microorganisms to remove or breakdown organic pollutants (a process of biodegradation). Under complete mineralisation, the products are carbon dioxide and water (Hoff, 1993). Perhaps bioremediation of oil contamination is due to co-metabolism between microbial species. More technically, bioremediation is the process, which accelerates biodegradation by manipulating the contaminated matrix through either addition of nutrients, other supplements or exogenous microorganisms to stimulate indigenous microbial populations.

Early work on bioremediation was to understand the underlying chemistry behind petroleum hydrocarbon degradation by indigenous microorganisms as well as the role of metabolic pathway and genetic composition of microorganisms in microbial degradation (Atlas, 1981, Leahy and Colwell, 1990).

Looking at the journey of bioremediation in the past four decades, one could say that the use of bioremediation as a clean-up protocol for contaminated soils came into the published literature after the 1980's. Given records from database – 'Web of Science', thirty-three Thousand, three Hundred and sixty-three (33,363) journal articles on bioremediation were published from 1980 – 2010, in contrast to Eight Thousand, three Hundred and thirteen (8,313) articles published from 2010 – 2012, which is approximately 25% increase in 3 years. A different database 'Engineering village' accessed on the same day (17 October 2012) showed similar results. Six Thousand, nine Hundred and twenty-three (6,923) journal articles were published between 1980 -2010, while from 2010 -2012, 2238 were published which is 32% increase (Figure 2.1). The search phrases included; bioremediation, types of bioremediation, crude oil contaminated soils, compost, bonny light crude oil, Niger Delta, contaminated soils in Niger Delta, Nigeria, crude oil and plants, effects of oil on tomatoes and biodegradation of n-alkanes.

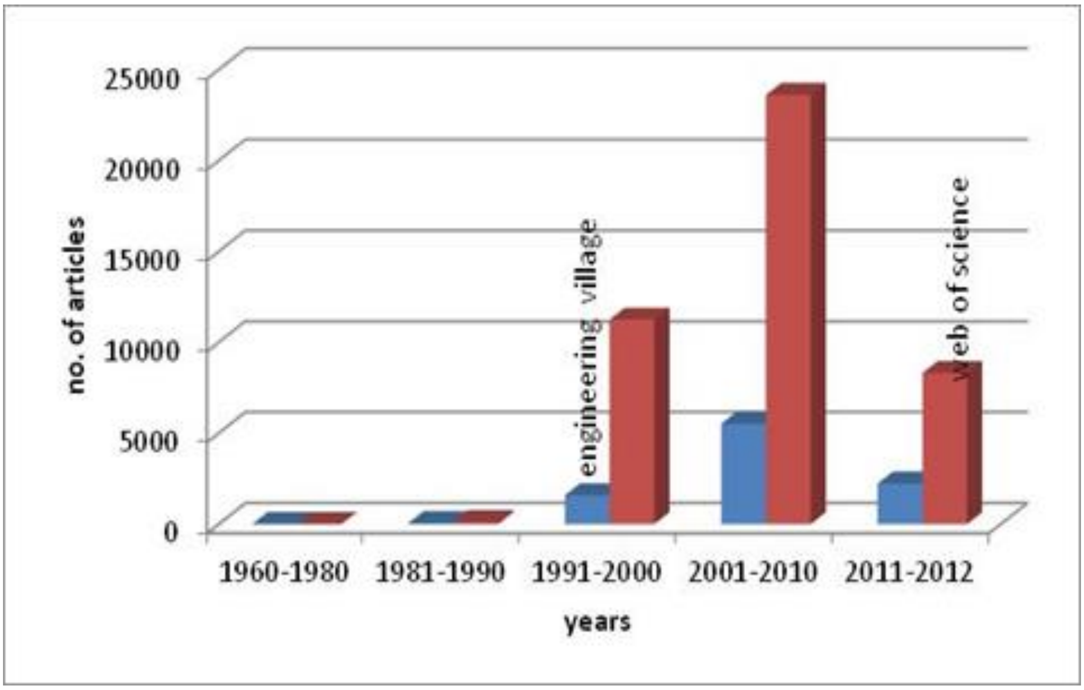

Figure 2.1: Publications on bioremediation from 1960 to 2012 (generated from online database accessed on 17 October 2012)

The 1989 oil spill from tanker Exxon Valdez which introduced over 200,000 barrels (a barrel ~ 159 litres) into the seacoast of Alaska, U.S.A. was the largest bioremediation project to date making use of 50 000kg of nitrogen and 5 000kg of phosphorus (Bragg *et al.* 1994a). The Exxon Valdez oil spill incident played a major role in the development of bioremediation as an oil spill response. The initial response to the oil spillage included soil washing, but while that was going on, a joint of United States Environmental Protection Agency (USEPA), Alaska Department of Environmental Conservative and Exxon investigated the feasibility of using bioremediation option to clean up the residual oil. Prior to the 1980s, various products to enhance bioremediation were commercially available and either nutrient based or cultured microbes.

From 1980’s -1990’s bioremediation received wide attention (Figure 2.1). However, different protocols in United States were developed both locally and internationally to evaluate various products. These protocols gave rise to the establishment of national testing standards by the United States Environmental Protection Agency (U.S.E.P.A.). Some of the oil spill incidents in which bioremediation technology was applied as clean up options included Exxon Valdez (Alaska), Apex barges and Mega Borg Spills (Gulf of Mexico), Prall's Island (New Jersey), and Seal Beach (California).

After several studies, (Atlas, 1981, Leahy and Colwell, 1990, Bragg *et al.* 1994a, Atlas, 1995b) it was evident that bioremediation technology had its own limitations which cuts across duration of treatment, monitoring of oil residual concentrations and poor experimental design. The years after the 1990s saw bioremediation receiving wide acceptance even though there were concerns about the toxicity of different fertilizers and cultured microorganisms if they were to be used in the marine environment (Hoff, 1993).

The first workers on bioremediation of contaminated matrices (Atlas and Bartha, 1973, Atlas, 1975, Leahy and Colwell, 1990, Bragg *et al.* 1994a, Atlas, 1995b, Atlas, 1995a) exploited the activities of indigenous microorganisms within the marine environment even though microbiologists have been using microorganisms in the treatment of wastewater for over two centuries.

2.2 Biology of biodegradation

Different methods have been used to speed up oil spill clean-up. Biodegradation of hydrocarbons by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants are eliminated from the environment (Leahy and Colwell, 1990). According to Riser-Roberts (1998), during biodegradation, anaerobic bacteria can act on organic compounds to produce short chain organic acids. However, the activities of aerobic microorganisms further convert them to innocuous compounds such as carbon dioxide, and water. *Pseudomonas Acinetobacter*, *Flavobacterium* are some of the natural soil microorganisms that breakdown hydrocarbons.

Petroleum hydrocarbons are organic compounds with mostly hydrogen and carbon atoms. They are divided into alkanes (paraffins), alkenes(olefins), aromatics (mono and poly aromatics), resins and asphaltene. The last two comprise polar molecules containing nitrogen, sulphur and oxygen (Balba *et al.* 1998). Paraffins are one of the major constituents of hydrocarbons and further divided into - normal alkanes, branched alkanes and cycloalkanes.

The bonding between the carbon atoms strongly influences the chemical characteristics of each group. Aromatics are polar but aliphatics are non-polar or slightly polar. Aromatics are more water soluble and less volatile when compared to alkanes (Potter and Simmons, 1998). In terms of chemical properties, the persistence of a hydrocarbon in the environment is related to its molecular structure – (Atlas and Bartha (1972); Leahy and Colwell (1990).

Among the class of aliphatics, the simplest is alkanes and they have single carbon-to-carbon (C-C) bonds. The prefix n-alkanes denote they are straight chain. In order of degradation, branched alkanes are more resistant to biodegradation than normal alkanes. Aromatics hydrocarbons have one/more benzene rings as structural components. The electrons are delocalised and this gives it its polar feature. Polar hydrocarbons are more resistant to biodegradation (Bragg *et al.* 1994b). The alkyl substitutes such as sulphur, nitrogen and oxygen may be present in large amounts in resins (Potter and Simmons, 1998).

For bacterial cells to survive and multiply, they need a carbon, energy source and nutrients. Carbon comes from organic carbon and carbon dioxide while energy sources are from substrate and sunlight. Microbes acquire energy from chemicals and substrates through oxidation –reduction processes, thereby extracting the energy available in the molecule through the transfer of electrons (Wong *et al.* 2003). The energy thus produced is available for synthesis of new cell mass and maintenance of old cells. The hydrocarbon degrading microorganisms abound in nature for example Pseudomonas, *Acinetobacter*, and *Flavobacterium* and reports have shown their ability to degrade alkanes and other aromatic hydrocarbons (Leahy and Colwell, 1990, Guimaraes *et al.* 2012). Various alkane degraders have the ability to use other substrates as carbon source in addition to alkanes, and in most cases, they prefer these other compounds before turning to alkanes; however there are hydrocarbonoclastic bacteria that are highly specialised in degrading hydrocarbons such as *Alcanivorax borkumensis* (Rojo, 2009).

Some organisms in the marine environment, for example *Arthrobacter* strain, *Pseudomonas* and *Corynebacterium*, have produced emulsifying agents. Though this attribute should have increased microbial degradation of hydrocarbons in oil, on the other hand, it is not yet clear why production of emulsifying agent has no correlation to hydrocarbon degradation by these organisms. Probably due to formation of ‘mousse’, this has unfavourably low surface to volume ratios (i.e. low surface area) and as a result the rate of biodegradation, is restricted (Atlas, 1981, Leahy and Colwell, 1990).

More complex ring structured compounds are more resistant to biodegradation, as the molecular weight and complexity increases. For those compounds that are not readily biodegraded, the greater are the chances of having partially oxidized intermediary metabolites, which can persist in the environment (Atlas, 1975). According to Atlas (1975), as biodegradation is taking place, there is production of residual materials but as long as the residual mixture is not bio available, it is of no implication to the environment.

Atlas (1975) stated that when there is contamination by petroleum, this stimulates the growth of hydrocarbon-degrading microorganisms, however, Sharabi and Bartha,(1993) and Kim *et al.* (2005) by contrast observed that when a spill occurs, the carbon to nitrogen ratio (C/N) of the soil becomes high. This high ratio is unfavourable to microbial activity and so they proposed that a standard test in the initial assessment of biodegradation of any contaminant is the measurement of the amount of CO2 evolved.

The metabolic pathway of hydrocarbon biodegradation by bacteria and fungi is that the substrate undergoes oxidation by oxygenase and carboxylic acid is formed which undergoes other stages and finally enters the Tricarboxylic cycle (Atlas, 1981). Despite having different fungi breaking down aromatic hydrocarbon rings in the environment to form trans-diols (which are carcinogenic), the bacteria always form cis - diols as intermediates and they are biologically inert. Given bacteria are predominant in the environment, the biodegradation of aromatic hydrocarbon results in detoxification and do not produce potential carcinogens (Atlas, 1995). Complete biodegradation (mineralisation) produces carbon dioxide and water as well as biomass (largely protein) useful in the food web.

Atlas (1975), came to these conclusions that; (a) bioremediation still remains the most viable and cost effective way of cleaning up oil spill (b) microbes need nutrients, carbon and energy to survive and multiply (c) biodegradation rates are therefore highly dependent on environmental factors such as temperature and moisture content. Bioremediation has its own limitations, which includes type of pollutant and available time for the treatment. On-site treatment reduces the risks associated with removal and transport of hazardous organic pollutants to sites of treatment (in terms of cost, operation etc.) .

2.3 Overview of bioremediation techniques

One of the remarkable events that attracted global attention to oil pollution was the 1967 oil spill by Torrey Canyon in the English Channel. After the Torrey Canyon oil spill, most of the studies focused on the marine environment. One of the key questions was finding answer to the fate of petroleum hydrocarbons in the environment relative to microbial degradation. Microorganisms are able to break down hydrocarbons and use them as sole source of carbon and energy (Atlas, 1981, Leahy and Colwell, 1990).

The following authors (Atlas, 1981, Bossert and Bartha, 1984, Leahy and Colwell, 1990, Hoff, 1993, Bragg *et al.* 1994b, Atlas, 1995b, Atlas, 1995a, Semple *et al.* 2001) have done extensive review on the process of bioremediation. Literature accounts for four categories of bioremediation whether on terrestrial or aquatic environment. These are bioaugumentation, biostimulation, land farming, compost and composting technology.

2.3.1 Bioremediation in marine environment

The most studied and referenced bioremediation application was the 1989 Alaskan oil spill in Prince William sound Alaska, United States of America, popularly known as the Exxon Valdez oil spill (Atlas, 1995b). The Exxon Valdez spill released approximately four million (4,000,000) litres of oil onto the shores of Alaska. Bioremediation through fertilizer application was used consuming fifty thousand (50,000) kilograms of nitrogen and five thousand (5,000) kilograms of phosphorus. Atlas examined the experimental approach used in the investigation of petroleum degradation at Prince William Sound, which was mainly seeding with microorganisms, and application of chemical fertilizers.

In two different scientific publications by the same author (Atlas, 1995b, Atlas, 1995a), with respect to the Exxon Valdez oil spill, he reported that seeding with oil degraders in marine environment was not effective, but the addition of nitrogenous fertilizers increased rates of petroleum biodegradation. In a bid to finding microbial cultures that would enhance biodegradation, different companies went into production of hydrocarbon seed cultures. About ten of such products were studied under laboratory investigation; degradation was observed after five days and reached a maximum after twenty to thirty days. Finally, two of the products were selected and was applied in Prince William Sound, however the results obtained from the field trials did not show any improved biodegradation by these products in comparison with the result from the laboratory investigations.

In his review, Atlas made reference to the Mega Borg spill in Texas, USA, a different spillage site where seeding was applied. Even though a report by the Texas General Land Office had claimed that, the use of Alpha culture on the Mega spill was effective at removing the oil, the claim lacked independent investigation. A different claim was made on the effectiveness of seeding during spill of the Apex barge that affected at Marrow Marsh along the Texas shoreline but in this case, unlike the Mega Borg spill, an independent investigation was carried out. The investigation concluded that the physical change observed in the oil might have resulted from emulsification by the Alpha product. Chemical analyses did not show any significant difference in pistine/phytane ratios (chemical compounds used as internal markers) between the impacted and control zone which would have indicated bioremediation.

There was no scientific evidence to determine the effectiveness of using seed organisms on open water or coastal pills. This led to the use of fertilizers for enhancing bioremediation of petroleum hydrocarbon. Three types of fertilizer were used as nutrient supplements namely (a) water soluble i.e. garden fertilizer formulation (b) slow-release and (c) oleophilic i.e. Inipol EAP22(Atlas, 1995b). Of the three, oleophilic fertilizer proved more effective. Within ten days of application, the rocks on the shoreline were covered with black oil turned white, though the result could be said to be visual, however, the result contributed to the conclusion that Prince Williams Sound trial was nutrient limiting and this was a boost to the use of fertilizers for bioremediation. The nutrients believed to have sustained higher numbers of oil degraders on oiled shorelines. The result from the joint monitoring action by USEPA-Exxon team showed that bioremediation of oil-contaminated beaches was a safe technology because no toxicity on the shoreline treated with fertilizer; more so, there was no evidence of eutrophication in near shore waters. The measurement of chlorophyll concentrations supported the claim that there was no eutrophication, and no algal growth in the near shore waters, and fertilizer treated zone. In addition, absence of non-degraded oil residues from the beaches means no toxicity.

Even though laboratory investigations yielded favourable result on application of Alpha culture, which was one of the two selected products that were used as trial for the bioremediation of Prince Williams Sound oil spillage, however the culture was not effective during the field trial. Therefore, it can be concluded that for bioremediation to be effective, it needs to be demonstrated both in the laboratory and field experiments. Although laboratory studies are easier than in-situ application it was observed that bioremediation using fertilizer application was more effective in oiled shorelines than open water oil pollution (Atlas, 1995b, Atlas, 1995a). In this current research, the work is laboratory based, though we identified the need for field studies to assess the feasibility of the cleanup protocol in real life application.

Atlas was able to highlight the limitations of bioremediation through application of seed organisms to marine environment and the effectiveness of bioremediation through fertilizer application in marine environment. Atlas, (1995b) noted that the type and quantity of hydrocarbon as well as the properties of the affected ecosystem influences the persistence of petroleum hydrocarbons in the environment.

In summary, Atlas's investigation contributed towards a better way of cleaning oil spill on beaches. Though it is possible to achieve greater stimulation by the addition of more nutrients, the threat posed by his approach is the fear that this might result in ecological side effects due to eutrophication and toxicity to marine life. His approach did not include details for analysis or protocol for monitoring hydrocarbons, which seems a gap in the study. For evidence that is more experimental it would have been useful if Atlas had used gas chromatography or spectrophotometry to confirm the removal of the hydrocarbons rather than just visual observations.

The success of his approach made bioremediation a possible option (though not the only option) in future clean-up protocol. In his review, Atlas, (1995a) compared the cost of bioremediation to other treatment options such as soil washing to show the cost effectiveness of the bioremediation technology. He reported that one million dollars ($1,000,000) a day was spent on physical washing of shorelines in other to clean up the oiled rocks of Prince Williams Sound, Alaska despite the low success recorded in contrast to the bioremediation of hundreds of miles of the shorelines which cost less than one million dollars.

Bragg *et al.*(1994b), in their review of the bioremediation application applied to the Exxon Valdez incident made a central claim that statistical and chemical analysis provided enough clues to understand the microbial breakdown of hydrocarbon components of oil. Such analysis used the ratio of the mass of the degraded oil components to that of hopane (a chemical compound and an internal marker) to measure bioremediation. Hopanes have low water solubility and low volatility and so are not easily lost. The study described by Bragg *et al.*(1994b) was a continuation of the work of other authors in this area. Microbiological data suggested that the addition of fertilizer stimulated the growth of oil degraders. However, this did not provide any statistical evidence about the breakdown of oil. Other studies (Atlas, 1995b, Atlas, 1995a) used only visual observations of the shoreline treated with fertilizer which was assessed to be cleaner than the reference zone to confirm the effectiveness of bioremediation. Atlas used visual assessment, as it was difficult to use analytical confirmation because crude oil is a complex mixture and its distribution is non-uniform when a spill occurs in a marine environment.

In other to show statistical evidence for the effectiveness of bioremediation, Bragg *et al.*(1994b) measured: (a) changes in oil composition, (b) microbial population and (c) physical measurements. Three sites were chosen from Prince William Sound which have not previously undergone bioremediation in 1989. The sites were subdivided into treated and untreated controls with the treated zone receiving fertilizer treatment.

Bragg *et al.*(1994b) used first order models and regression analysis to compare the statistical difference in results obtained from the fertilised and control plots. Their argument was that using hopane (a chemically stable compound) it was easier to quantify the rate and extent of oil biodegradation with statistical confidence, which was lacking when only visual evidence was used as reported by Atlas, 1995b. Hydrocarbon losses were monitored relative to hopane and it was observed that the addition of fertilizer enhanced biodegradation about five times or more. Using a compound from the crude oil as internal marker means its composition will remain stable even as the other hydrocarbon components are degraded.

Although, previously pristine and phytane (chemical compounds) have been used as non-degraded internal markers in other spills and Prince William Sound, they were found to biodegrade with time and could only serve as an internal marker for a short timescale (Atlas, 1981, Atlas, 1995b). They concluded that fertilizer application would be better when oil had reached the shoreline; and that the situation at the time of the spill should be assessed before fertilizer application rather than relying on a predetermined dosage.

In summary the results from the works of (Atlas, 1981, Atlas, 1995b) served as guideline for bioremediation strategy for other spills. The data presented were convincing as they reported compatible experimental evidence. Even though seeding was not effective in the Exxon Valdez spill, Atlas acknowledged that there was a possibility that seeding might be useful in other spilled sites.

Shortly after the Exxon Valdez oil spill, Leahy and Colwell, (1990) in their investigation acknowledged that contamination of the environment by petroleum hydrocarbon was a concern. The authors explored the use of molecular biology techniques (e.g. study of plasmid DNA in oil degrading bacteria) study of plasmid DNA) in understanding the principle of bioremediation and the use of genetically engineered or natural microorganisms as seed organisms. Through the process of conjugation or transformation, plasmid DNA is able to transfer hydrocarbon degrading ability to other organisms and can impart novel phenotypes. This might be the basis of for the appeal for use of genetically modified microorganisms as seed organisms in bioremediation.

Even though the advantages of using seed organisms include increasing the rate and extent of bioremediation, Atlas reported that fear of safety and ecological damage has a restriction on the use. Jones,(1988) concluded that until regulations are in place on the use of genetically engineered microorganisms, the benefits which were expected from broad applications of such organisms' in in-situ bioremediation could not be explored.

Leahy and Colwell (1990) agreed to earlier observation that variation in composition of crude oil and its refined products affects the overall degradation of crude oil and its components. They observed that the more acceptable pathway for degradation of petroleum hydrocarbon by oxygenase is under aerobic condition requiring molecular oxygen; however, microbial degradation of oxidized aromatic compounds such as benzoate and halogenated aromatic compounds e.g. halobenzoates have been reported to occur under anaerobic conditions. However, aromatic compounds such as toluene and benzene have been reported to degrade under methanogenic conditions to produce carbon dioxide (CO_2) and methane (CH_4). The amount of substrate removed under anaerobic conditions can be significant (Leahy and Colwell, 1990). Under methanogenic conditions, they reported conversion of fifty percent of toluene and benzene to CH_4 and CO_2 in sixty days. It is yet not defined the importance of anaerobic degradation of aromatic hydrocarbons in the environment; as a result, there is a research need for proper understanding of anaerobic metabolic pathway and the possibility of other classes of hydrocarbon such as alkanes undergoing degradation under methanogenic conditions.

There has been use of chemical dispersants in the marine environment. They are generally a mixture of surfactants (surface active chemicals) and solvents (Scholz *et al.*, 1999). Surfactants are molecules with two ends, i.e. they possess both hydrophilic and hydrophobic (oleophilic) groups. The hydrophobic group consists typically of a long-chain or halogenated hydrocarbons that have affinity for oil, whereas the hydrophilic group is ionic or highly polar. As the oleophilic end gets attached to the oil droplet, the hydrophilic end remains outside the oil surface and sticks to water while at the same time repelling other oil droplets which get suspended in water (Scholz *et al.*, 1999). This unique chemical structure results in the tendency for surfactants to adsorb at the interface between two phases and markedly alter the interfacial properties.

The case of Torrey canyon oil spill recorded treatment failure because of the application of degreasing agents rather than dispersants. The composition of the degreasing agents was made of over 50% aromatic solvents, which were toxic to the aquatic life. Secondly, the manner of application of the chemicals was a flaw because they were poured close to inland waters. The beaches were indiscriminately sprayed consequently the use of degreasing agents in Torrey canyon oil spill was not successful (Scholz *et al.*, 1999). Current formulations of dispersants are environmentally friendly and are for application in oil spill situations.

The application of bioremediation in marine environment can be summarised as follows; even though bioremediation succeeded in treating some of the marine oil spills, localised environmental factors, such as type of petroleum hydrocarbons, ambient temperature, waves and tides, and indigenous microbial population all influenced the success or failure of any bioremediation attempted in the marine environment.

One can say that the succession of bioremediation application in larger spill was in the following order; application of chemical dispersant to the Torrey Canyon oil spill, which resulted to toxicity of flora and fauna, and then came the development of seed organisms, which was used in the Exxon Valdez oil spill even though seeding was not very effective. At the same time, the use of chemical fertilizer was suitable in the Exxon Valdez spill after seeding failed and this had a positive impact on bioremediation. Bioremediation through fertilizer application serves as a way of providing nutrient supplement to the microorganisms.

2.3.2 Bioremediation in soil ecosystems

Semple *et al.*(2001) in their review recognised that different microbial species have been employed in the degradation of petroleum pollutants in the environment. In some cases, the microorganisms mineralised these pollutants to carbon dioxide (CO_2) and water while in others, there was transformation of pollutants to less harmful substances or had their bioavailability reduced by locking the pollutants up within an organic matrix. Semple *et al.* (2001) concluded that bioavailability and biodegradability of pollutants are the two key factors which determine the success of any remediation approach.

Semple *et al.*(2001)suggested that the mechanism for microbial degradation lies in their ability to interact with different compounds and in the process alter the structural makeup of the molecules. There is a large amount of literature on use of composting techniques for bioremediation of different classes of pollutant, such as aliphatic, aromatics, and pesticides, (Fogarty and Tuovinen, 1991, Jørgensen *et al.*, 2000, Namkoong *et al.*, 2002, Gogoi *et al.*, 2003, okoh, 2006, Onibon and Fagbola, 2008, Golodyaev *et al.*, 2009) with poly aromatic hydrocarbons (PAH) being the most popular.

Namkoong *et al.*(2002) investigated the feasibility of using composting for bioremediation of diesel contaminated soil in Korea using composting technology. The aim was to investigate the appropriate mix ratio that would enhance the degradation of diesel contaminated soil. Topsoil with no history of petroleum contamination was spiked with diesel and treated using compost and sludge at different ratios. The ratios of contaminated soil to organic amendment (compost and sewage sludge) were 1:0.1, 1:0.3, 1:0.5 and 1.1 on a wet weight basis. In their report, on addition of sewage sludge to the experiment, the degradation at mix ratios 1:0.1, 1:0.3, 1:0.5 and 1.1 was 86, 98.1, 98.1, and 94% respectively. Addition of higher mix ratio did not produce a corresponding increase in the degradation of TPH. On addition of compost, the degradation at mix ratios 1:0.1, 1:0.3, 1:0.5 and 1.1 was 67.1, 93.1, 98.4 and 97.1%. For the compost, highest Percent degradation was observed at mix ratio 1:0.5. This led them to conclude that even though addition of organic amendment could increase degradation of the target contaminant, but addition of excess amount of organic matter might actually inhibit the degradation rate.

It is possible that microorganisms preferentially degrade the carbon source from the organic amendment rather than that the target contaminant in which case the degradation of the target contaminant is inhibited. According to Namkoong *et al.* (2002), this phenomenon was not observed in their experiment as the addition of organic amendment produced high degradation rates of total petroleum hydrocarbons (TPH) in comparison with the soil only experiment. A mix ratio of 1: 0.5 (by wet weight) which is equivalent to 1: 0.3 (dw) diesel contaminated soil to compost showed the highest Percent degradation of TPH (98.4%). Even though the value is smaller, it can be compared to the work of Stegmann *et al.* (2001) whose highest percent degradation was achieved at 1: 0.5 on dry weight basis. This difference may have arisen due to variations in the composition and age of compost. The following conclusions were drawn from the work of Namkoong *et al.* (2002);

- That it will be better to do a feasibility study to determine optimum mix ratio suitable for bioremediation of a particular contaminated soil.
- Given the effectiveness of small amount of compost in the preferred mix ratio (0.5), it will allow more contaminated soil to be treated.

- The degradation of n-alkanes did not follow the paradigm, which believes that higher molecular weight petroleum compounds show reduced degradation.

Following on from the third conclusion the authors came up with two classes of n-alkanes with respect to degradation rate namely $C_{10} – C_{15}$ and $C_{16} – C_{20}$, and irrespective of the amount of organic amendment added their first order degradation rates were 0.37/day – 0.54/day and 0.18/day – 0.24/day respectively. Loss of hydrocarbons due to volatilisation was only about 2%. Also the study provided evidence to support carbon dioxide evolution as a tool for measuring microbial activity even though there was concern expressed by some authors that the addition of compost and sewage sludge could introduce metals which would inhibit soil enzymes (Martens *et al.*, 1992). However, the authors (Namkoong *et al.*, 2002) did not mention any public health hazards associated with the use of compost and sewage sludge.

Baptista *et al.*(2005) working on a contaminated site in Brazil investigated the production of carbon dioxide (CO_2) during a bioremediation process. Due to nutritional shortage, the soil was supplemented with $(NH_4)_2SO_4$ (2.5% w/w) and KH_2PO_4 (0.035% w/w) in other to stimulate the indigenous microorganisms to breakdown the petroleum hydrocarbons. Each bioreactor contained three hundred grams (300g) of contaminated soil. The soil in question was rich in nitrogen and low in phosphorus. In addition, they investigated CO_2 evolution as a parametre to measure microbial activity. CO_2 production was monitored for 45 days as well as oil and grease and organic matter content of the soil samples. In this experiment, care was taken to avoid loss of CO_2 gas to the environment, even though no standard method exists to estimate CO_2 gas leakages from such experiment. The parameters used to determine crude oil biodegradation after 45 days were organic matter (OM), oil and grease (OG) and total petroleum hydrocarbon (TPH). The results of the findings showed that highest degradation corresponded to 50% OM and 35% oil and grease) removal and 4,000 mg CO_2 production per kg of soil. For the control, OM and OG removal was below 5% of the soil mass. Their finding is in agreement with other works (Jørgensen *et al.*, 2000, Namkoong *et al.*, 2002, Ahiamadu and Suripno, 2009). These workers used CO_2 evolution as an indication of bacterial respiration and a measure of bioremediation process.

Previous studies in Nigeria involving soils contaminated with crude oil have used a combination of organic and inorganic sources for nutrients. Some studies used microbial seeding to enhance the rate of biodegradation (Odu, 1978, Antai, 1990, Ayotamuno *et al.*, 2006, Ahiamadu and Suripno, 2009) . The advantage of using different organic wastes, especially food waste, is that any deficiency in the soil in terms of nutrients such as nitrogen, phosphorus and potassium (N, P, K) can be supplemented from organic sources.

Different workers have investigated bioremediation of crude oil contaminated soils in Nigeria, in some cases; it involved study of real contaminated and other times investigation involved spiking soils with crude oil. The results from some of the investigations are presented in Table 2.1. The studies used microorganisms and treatment amendments such as nutrients, and enhanced aeration. Despite these studies, no protocol has been drawn that could enable a local farmer to clean up his contaminated land and reuse the soil for agriculture.

Table 2.1: Bioremediation methods applied under different studies in Nigeria

TREATMENT	FINDINGS	REFERENCE
Soil type: soil from 25 year old teak plantation. Contamination: spiked with light Nigerian crude at 1 or 2ml/20g moistened soil. Amendments: nutrient (mineral salt medium fertilizer), nitrogen as 2ml of 10mg-N/ml $(NH_4)_2SO_4$ & 1ml of mineral salt solution. Conditions: 28^0C Experimental time: 12 weeks	More oxygen consumption in polluted soil than unpolluted soil, however oxygen consumption was depressed by the addition of $(NH_4)_2SO_4$. Microbial degradation of oil was not limited to indigenous hydrocarbon lovers rather other microorganisms found in soils remote from area of contamination were involved. Rehabilitation of oil contaminated soil needed aeration and nutrients especially nitrogen.	(Odu, 1978)
Soil type: non-contaminated agricultural topsoil Contamination: spiked with 0.5ml of BLCO. Amendments: microbial inoculations- *Bacillus* & *Psuedomonas sp*, nutrients (mineral salts medium). Conditions: 20 – 44^0C, pH 5.8 -8.2 Experimental time: 14 days	4g/L of nitrogen and 3g/L of phosphorus concentration supported the highest level of crude oil degradation. Maximum degradation was at 37^0C and pH 7.0 -7.4. The two seasons (dry and wet) must be considered during bioremediation as they vary remarkably in temperature, pH and soil nutrients in order to know which of the seasons is preferable.	(Antai, 1990)
soil type: non-contaminated agricultural topsoil Contamination: spiked with Nigerian light crude, (Transniger pipeline TNP) at 10,20,30, 40% v/w	No extensive biodegradation recorded until after 3 months. The microorganisms identified as being effective in oil spill cleanup included *Bacillus, Micrococcus, Pseudomonas, Vibrio and Alcaligens* with presence of *Bacillus* more in soils treated with 30 & 40% crude oil due to its resistant spores.	(Ijah and Antai, 2003)

Amendments: Conditions: experimental microcosms, pH 5.6, 3% moisture & 2.1% organic matter. Experimental time: 12 months	Length of incubation plays a role in the degradation of hydrocarbons especially C_{12}-C_{32}. Fates of polluting oil in soils depend on the amount of oil spilled and the total exposure time.	
Soil type: non-contaminated agricultural topsoil Contamination: spiked with Bonny light crude oil (BLCO), 0.8L applied per $0.16m^2$ of soil. Amendments: nutrients (50-200g of 20-10-10 NPK fertilizer) Conditions: soil bed (40cm x 40cm x 30cm), tilling – twice a week. Experimental time: 6 weeks	50 -90% reduction in hydrocarbon levels. Increased supply of nutrients led to greater rates of biodegradation; however, inhibition was recorded after 2 weeks at 200g NPK/$0.16m^2$ of soil. For accelerated biodegradation, clean up should be carried out during dry season.	(Ayotamuno *et al.*, 2006)
soil type: soil from crude oil spilled site (OML 58 in Nigeria) contamination: crude oil (Total petroleum hydrocarbon concentration 3830 mg/kg ds) Amendments: bulking agent(woodchips), commercial microbial inoculums and nutrient (NPK), soil and woodchips (3:1) Conditions: soil cell (1.2m x 3m x 20m), mixed weekly for 6 weeks, and then mixed every 2 weeks. Experimental time :22 weeks	Decrease of total petroleum hydrocarbon by 93% to 268.1mg/kg ds. Highest reduction rate was in the first 5 weeks.	(Ahiamadu and Suripno, 2009)

Soil type: surface soil (0 – 20cm depth) from communities very close to oil installations in the Delta. contamination: PAHS(polyaromatic hydrocarbons) Amendments: Conditions: freeze-dried samples were Homogenised and lyophilized	Different levels of PAHs in sediments and soils were observed due to their different characteristics. The concentration of the 28 target PAHs ranged from 65-331ng/g in the sediment and from24 -120ng/g in the soils. The level of PAH in the sediments from river was lower which could be due to the action of tides from the Atlantic ocean. The most carcinogenic PAH found in the study was benzo(a)pyrene ranging from 0.17 -10.21ng/g (in sediment) & 0.09 – 2.05ng/g in soils.	(Sojinu *et al.*, 2010)

Agarry et al; (2010) investigated the efficacy of biostimulation with animal manure versus chemical fertilizers in the developing country using Nigeria as study area. His argument was that most farmers in developing countries struggle to get a supply of chemical fertilizer which they use for routine farming. Therefore given the cost component of using the chemical fertilizer, a cheaper and environmentally friendly option of enhancing petroleum hydrocarbon degradation was needed, possibly using organic fertilizers such as animal manures.

In their experiment, 190g of soil was spiked with 10% $_{w/w}$ of petroleum hydrocarbon mixture (kerosene, gasoline and diesel oil). In each experimental set up, the soil was amended with 20g each of poultry, pig, and goat manure and 17.5g of NPK chemical fertilizer. After 4 weeks of remediation, they found that poultry manure showed the highest total petroleum hydrocarbon degradation of 75% followed by piggery manure (65%), goat manure (50%) and NPK fertilizer (39%).

Antai, (1990) in her work added microbial inoculations in form of *Bacillus* and *Pseudomonas spp* and nutrients (mineral salts medium). She found out that 4g/L of nitrogen and 3g/L of phosphorus concentration supported the highest level of crude oil degradation rather than addition of inoculum. Her observation agrees with those of Loick *et al.* (2009), who noted that environmental conditions rather than the addition of microbial products had greater effect on oil degradation.

There have been contradictory results on the effect of the addition of nutrients in form of fertilizers to oil contaminated soil. Fertilizers in form of urea-phosphate, N-P-K fertilizers, and ammonia have been used. In some cases accelerated biodegradation was observed (Ijah and Antai, 2003, Ahiamadu and Suripno, 2009), while in other cases there was no increase in biodegradation rates or there was an increase after a delay of several months (Leahy and Colwell, 1990).

The above studies highlighted the rate and extent of biodegradation in addition to quantifying the residual hydrocarbons after each treatment. However, from the studies it is evident that the investigations succeeded in monitoring the reduction of hydrocarbons to evaluate the impact of the treatment on contaminated sites. This approach will be most welcoming as it marches the engineering and industry’s view on oil spillage, but lacks judgement for assessment of public health implication of oil spillage and the effects on human development. These two elements will have a large impact on the primary sources of livelihood activities of the affected communities' especially local farmers whose farmlands are contaminated.

2.3.3 Compost and composting for bioremediation

The use of compost in bioremediation has not been widely applied even though reports show that it has enormous potential for bioremediation (Semple *et al.* 2001, Trejo-HernÃ¡ndez *et al.* 2007, Agarry *et al.* 2010, Adekunle, 2011). Compost has a wide microbial population, which includes bacteria, fungi and actinomycetes that are able to degrade variety of pollutants; it can also slowly release its nutrients thereby potentially improving the quality of contaminated soil. A number of studies have reported use of composting for bioremediation of different classes of pollutant (Jørgensen *et al.* 2000, Namkoong *et al.* 2002, Van Gestel *et al.* 2003, Okolo *et al.* 2005, Godoy-Faúndez *et al.* 2008, Onibon and Fagbola, 2008, Niqui-Arroyo *et al.* 2009). Semple *et al.* (2001) in their review raised a concern about addition of compost to contaminated matrix. Their argument is that addition of compost increases the volume or weight of the original contaminated soil and if for any reason the bioremediation process fails, it means a total increase in the volume of contaminated material. Therefore, the needs for pilot scale trials before full field trials.

It is important to differentiate between 'composting' and 'compost'. Composting in a simple term is a stage-by-stage decomposition of organic matter by microorganisms first into intermediate compounds and then mineralised to carbon dioxide and water under aerobic condition. The final product is compost. Composting is the process that produces compost (manure). Therefore, a composting strategy would be to mix composting raw materials with the contaminated soil. The active microbial flora then degrades the pollutant as the composting process continues in the soil. In the case of using compost, it is added to the contaminated soil after its maturation and in some cases, the level of microbial activity is reduced.

Semple *et al.* (2001) in their review assessed the effectiveness of composting strategy as bioremediation option. They identified 'soil-pollutant interactions' as the most important factor that determines the effectiveness of any composting strategy. The limitations might be that processes such as volatilisation can lead to loss of pollutant and the possibility of the pollutants being immobilised through other intra soil processes, even though debates are on-going about the stability of such non-bioavailable residues over time. 'Ageing' as a factor has influenced soil-pollutant interaction. Simply put it means that bioavailability of the pollutant to microbial action decreases with time. There are a number of factors governing 'ageing' of pollutants such as the soil organic matter (SOM), pore size and structure, the binding forces (dipole-dipole, hydrogen bonding) between soil and organic pollutant, and lastly, sorption of pollutants either to soil organic or inorganic pollutants.

Semple *et al.* (2001)concluded that the primary factor that affects pollutant interaction with soil is soil organic matter. Although higher concentration of pollutants enhances both sorption and ageing, the dilemma is that high concentration of SOM affects the pollutants' bioavailability and biodegradation. In their review, two extremes for bioavailability of organic pollutants were recognised. At one extreme, the substance is completely available which favours bioremediation, while at the other extreme the reverse is the case. They concluded by saying that ageing makes compounds available in' less accessible compartments'.

The advantages of treating pollutants and polluted matrices with compost includes but not limited to the following:

- It has potential for bioremediation
- Compost is a rich source of microorganisms such as bacteria, actinomycetes and these organisms can degrade a wide range of petroleum pollutants because they are equipped with complex metabolic systems.
- It can act as a soil conditioner – because it can change the soil pH, structure, moisture content and act as nutrient source, which will enhance the native or seeded microbial population.
- Other losses

Hydrocarbons losses could take place in three ways namely through vaporization, dissolution and biodegradation showing that all the losses were not as a result of biodegradation (Bragg *et al.*, 1994b). The first two are physical weathering, however, it has been pointed out the difficulty in separating losses due to volatility and that as a result of removal through microbial metabolism (Zhou and Crawford, 1995). In their review of the effectiveness of bioremediation in the Exxon Valdez oil spill, Bragg *et al.* (1994b) reported an estimate of a thirty Percent (30%) loss of the Alaskan oil by physical weathering (though the total extent of weathering was not certain). The physical losses occurred within two weeks of oil spill. One year after the spill, the other losses occurred due to biodegradation. Because hopane was used as non-degraded internal marker (a C30 pentacyclic triterpane biomarker), they related the quantitative measure of mass loss of other hydrocarbons to hopane concentration in degraded oil and was able to calculate the amount of hydrocarbon remaining one year after the oil spill.

Leahy and Colwell (1990), in their review of microbial degradation of hydrocarbons talked about two different types of emulsion namely (1) oil in water emulsion and water in oil emulsion (mousse). Oil in water emulsion increases surface area of the oil and its availability for microbial breakdown but mousse builds unfavourable low surface area to volume ratio thereby inhibiting biodegradation. They pointed out that there could be losses from bio emulsification rather than biodegradation in aquatic ecosystem. Some bacteria produce emulsifying agents and so dispersion and emulsification (a form of physical weathering) of oil slick aids rapid biodegradation.

Atlas (1981) cited a number of oil spillages where losses other than biodegradation occurred. For example, a spill in the Mississippi River Delta in 1970 could only account for one Percent (1%) of the total sixty-five thousand (65,000) barrels of oil that had spilled, much of the oil have dissipated. A study on the experimental spiking of an artic ecosystem with Prudhoe crude oil showed fifty Percent (50%) loss due to combined abiotic and biodegradation losses.

Namkoong *et al.*(2002) reported volatilisation loss in the range of 0.9 -2.6% which was small compared to a much higher amount of degradation at 67.1 to 98.4%. In their study, they used activated carbon, made from coconuts, to adsorb the volatile organic compounds that were lost during bioremediation of diesel-contaminated soil using composting technique. The experimental design involved monitoring the amount of volatile organic compound, measured in percentage to determine when the absorption capacity of the charcoal reached breakthrough. The hydrocarbon loss was mainly from the n-alkane group (C_{10} to C_{16}).The method they used provided strong evidence to support loss of volatile organic compounds during bioremediation process. Namkoong *et al.* (2002) also monitored abiotic loss by adding a biocide – mercury chloride ($HgCl_2$) to one of the test conditions so as to distinguish it from total degradation. Only a negligible loss of total petroleum hydrocarbon resulted from the biocide treatment.

Leahy and Colwell, (1990) pointed out the role of soil composition in hydrocarbon losses. Reduced loss of volatile hydrocarbon by evaporation is possible due to soil infiltration. The volatile component is associated with microbial toxicity. Oil adsorption onto soil particulate matter also reduces the amount of losses. However, the oil adsorbed on humic substances could result in the formation of persistent residues. Odu, (1972) reported on the oil spillage in the Ogoni area of Nigeria in which approximately six million square metres (6,070,000 m^2) of land was affected. Two weeks after the spill, the affected area had reduced to 3,120,000 m^2 (approximately 51% of the original area). His assumption was that the reduction in the area affected by the original spill was a result of dispersal due to rainfall or decomposition by soil micro flora.

Even though bioremediation might be cost effective when compared to the other clean up options such as physical removal of pollutants, in real situation it is very important to do site characterisation before applying a particular bioremediation technique to minimise the chances of failure. Factors such as the characteristics of polluted site, the nature of pollutant, and the indigenous microorganisms are just a few determining factors for the best option. In addition, public opinion about bioremediation might influence decisions by top management as to use bioremediation and patiently wait for the benefits or to take a different option with more obvious results to please the public who would want immediate action on oil spillage.

2.4 Different mechanisms by which oil affects plant growth

Many workers have investigated environmental pollution as a result of oil spillages, for example in July of 1970, in the Ogoni area of Nigeria a spill from an oil well managed by the Shell-BP Petroleum Development Company of Nigeria Limited affected a total of 607 hectares (6070000 m^2) of land, though the oil was not uniformly distributed on the land. In August, 1997 a different site in Ogbodo, Rivers State Nigeria measuring

15900 m^2 was contaminated by a high-pressure pipeline leakage releasing 1900000 litres of crude oil (Osuji and Adesiyan, 2005). Such oil spillages had significant changes in soil properties which have been attributed to the presence of oil (Udo and Fayemi, 1975, Antai, 1990, Adeniyi and Afolabi, 2002, Ijah and Antai, 2003, Osuji and Adesiyan, 2005, Sojinu *et al.*, 2010). Odu, (1972) reported that early research on oil spillage on land was limited to laboratory investigations and greenhouses. The results from the above-mentioned spillages could be summarised as follows that the alteration of soil properties following exposure to crude oil contamination could influence agricultural productivity depending to what extent plant growth is inhibited.

Crude oil can affect plants in several ways but primarily by affecting soil qualities:

2.4.1.1 Physical effect on soil

The importance of soil as natural reservoir for plant nutrients cannot be overemphasized and so any external influence on the physicochemical qualities of soil will definitely affect the availability of nutrients to plants. Ekundayo et al; (2001) investigating the response of maize crop grown on crude oil contaminated soil having initial pH as 5.4, carbon content 2.62% and nitrogen contents as 0.16% claimed that maize crops could easily be affected if planted on soil immediately after contamination. The reason being that the roots are still at tender stage in development; however, when contamination occurs in advanced growth stage, the effect on the crop is lower. At advanced stage, the crops can exhibit higher inherent genetic resistance to heavy metals.

Odu (1972), reported that plants could respond differently to oil pollution due to environmental influences on their genetic makeup. Some plants could grow on freshly contaminated soils due to their tolerance, though it could pose a risk to human consumption due to high level of heavy metals (Ekundayo *et al.*, 2001). Ekundayo *et al.* (2001) went on to show how exposure of maize to crude oil at different stages of growth could affect yield. The most affected of the pot experiment was plant yield (1.41%) from seeds planted on soil one week after exposure to crude oil concentration of 4.98% (w/w). In a different pot experiment, on application of same oil concentration five weeks after planting, the yield was 3.47% whereas oil application seven weeks after planting was 41.67%. The unpolluted soil produced 100% yield. Application of oil above 3% could interfere with the physiological functions of the seeds (Udo and Fayemi, 1975).

Across literature, there seems not to be a remarkable change in pH after oil pollution, which suggests that the effect of petroleum products on soil flora and fauna might be more of the toxic action of the hydrocarbons rather than from change in pH. Ekundayo *et al.*(2001) after spiking topsoil with 4.98% oil concentration (w/w), the pH of the polluted soil (5.4) was found to be in same range as that of unpolluted soil (5.6), in contrast Essien and John, (2010) claims that the presence of oil increased the soil pH. In their experiment, it involved spiking loamy agricultural topsoil with crude oil; the pH values of both unpolluted and polluted soils were 4.5 and 5.8 respectively. Even though the change in pH is slight and still leaves the polluted soil in the acidic region, they argued that such a change was in response of the original soil to oil pollution and concluded that such a change indicates that contamination of agricultural topsoil with crude oil tends to buffer the soil pH towards alkalinity.

Table 2.2 shows some soil properties used in previous investigations in Nigeria. The physicochemical characteristics of soil samples taken from various locations in Niger Delta show that their chemical properties are poor with high soil acidity.

Table 2.2: Different soil properties used in previous investigations in Nigeria

Authors	Sample ID	pH	E/C (µS/cm)	Moisture content %	Total Bacterial Count	TPH mg/kg	N%	C/N ratio	Organic matter content %
Adekunle, 2011	Compost	8.43	1325±68		4 x 10^7		0.85	23	
	Soil (Abeokuta Southwest Nigeria)	7.45							5.46
Ebuhi et al;2005	Soil from Rumuekpe, Rivers State, South south Nigeria				1.22 x 10^8	1.10 x 10^4	24.6mg/kg		
Abu & Ogiji (1996)	Sediment from Kpene River (Niger Delta, Nigeria)	pH in the acidic zone							
Ijah & Antai (2003)	Soil (Calabar Southsouth Nigeria)	5.6		3	2.5 x 10^8CFU/g - 6.0 x 10^8CFU/g				2.1
Osuji & Nwoye (2007)	Soil from Owaza in Abia State (Niger Delta basin)	4.9 – 5.1(contaminated soil (up to 150mm surface) control – 5.6 i.e. unpolluted soil	430 (contaminated) & control – 1890 ±2328	30 (very high for contaminated soil) but for control is 9.5		3.4 x 10^3 – 6.8 x 10^3mgkg (control - 0.6)			
Leton, (in press)	Soil from Onne, Port Harcourt(Niger Delta)	4.3					1.1	12	SOC – 1.23%

2.4.1.2 Physiological effect

This can influence availability of nutrients and oxygen. In oil-contaminated soil, as microbial degradation proceeds, there is more demand on nutrient consumption resulting in nutrient competition between the plants and microbes. Plants mostly use carbon substrate as source of energy and as decomposition progresses the C/N ratio will be reducing if nitrogen is not lost as gas or leached out. The availability of nitrogen to plants is a complex process with the inorganic nitrogen (nitrate nitrogen and ammonium nitrogen) being the form that is readily available, there is need for the presence of microorganisms that are able to convert nitrogen to different forms. The deficiency in nutrients also causes stress to the plants (Merkl *et al.* 2005).

In a study reported by Osuji and Nwoye (2007), on the impact of petroleum hydrocarbons on a crude oil spillage site at Owaza, Niger Delta Nigeria, they identified that the oil spillage adversely affected soil physicochemical conditions. This they suggested had implications of low soil fertility. Clean up measures could still leave residual hydrocarbons, which would affect the soil flora and fauna (key participants in decomposition of organic matter). It is acceptable that in a hydrocarbon-rich environment of crude oil contaminated soil; the amount of oxygen available to plants for breaking down carbonaceous soil is reduced, leading to simultaneous increase in the utilisation of other available electron acceptors such as nitrate, which adds to the reducing environment. This direct use of nitrates as electron acceptor might partly explain the reduction in nitrogen levels observed in crude oil contaminated soil (Odu, 1972). As nitrogen is one of the major building blocks used by plants for cell synthesis, any reduction in available nitrogen poses stress on the plants and this in turn affects their yield.

In line with the works of other authors in this area, Odu, (1972) acknowledged that though rehabilitation of nitrogen deficiency in a crude oil contaminated soil could be enhanced by addition of nitrogenous fertilizers, he pointed out that it is the microorganisms that are equipped to incorporate these nutrients into the organic fraction of the soil and subsequently breakdown the hydrocarbons. This entire mechanism of action will actually depend on the number of viable microorganisms still present in the contaminated soil. Essien and John, (2012) investigating the effect of crude oil on agricultural properties of soil after exposing the soil to chemical remediation (use of surfactant). They observed an increase in nitrogen, phosphorous and carbon content in polluted soil. They attributed the increase to atmospheric nitrogen fixing and microbial degradation of the oil resulting to more carbon. In addition, Essien and John, (2012) reported as part of their findings that the presence of oil increased the soil pH in contrast to the unpolluted soil and that increased PH in the soil contributed to higher nitrogen content in the polluted soil.

2.4.1.3 Toxicity

This effect can occur on either the seeds or plants. In the case of the spillage investigated by Odu,(1972) mixed cropping was practiced on the affected farmland prior to the spill and both economic trees and vegetables were affected. Some trees died of suffocation such as oil palm trees (Elaeis *guineensis*), some showed leaf decolouration (coconut- Coco *nucifera* & raffia palms –Raffia *pedunculata*) and some were completely defoliated (mango trees Mangifera *indica*). Within two months of the spillage, some of the crops showed signs of regrowth however, yam (Dioscorea) a starchy annual crop was seriously affected as well as cassava (Manihot *utilisima*) which is a stable food in the region. For these two crops, regrowth was not noticeable until after six months even though they varied in their degree of recovery. From the data presented by him, it is evident that crude oil contamination resulted in noticeable changes in the characteristics of the soils, which affected the crops negatively.

2.4.1.4 Effect on soil microorganisms

Different arguments have been raised on the effect of crude oil contamination on soil microorganisms (Atlas, 1981, Antai, 1990, Leahy and Colwell, 1990, Atlas, 1995a, Ijah and Antai, 2003). Odu (1972) reported an increase in the microbial population after the initial drop following a major spill. His report was in agreement with the experimental evidence on viable counts of total microorganisms in the contaminated areas, which led him to conclude that a large spill initially depresses the microbial activity, but later the excess carbon source turns out to stimulate growth and multiplication.

Odu,(1972) did not adopt the usual approach of quantifying the residual hydrocarbon from the contaminated sites as shown by a number of authors (Al-Daher *et al.*, 1998, Jørgensen *et al.*, 2000, Namkoong *et al.*, 2002, Ahiamadu and Suripno, 2009, Golodyaev *et al.*, 2009), and this can be justified given the goal of the investigation was to contribute to a wider understanding of the effects of crude oil contamination on crops and indigenous microorganisms. Consequently, he explored different experimental evidence using plant trials and microbiological methods to support his approach.

2.4.1.5 Effects on different vegetable crops

Other workers have investigated the effect of petroleum products as well as heavy metals on tomato seeds. Poulik, (1999) observed that the development of tomatoes was adversely affected by nickel more than lettuce. In addition, the nickel levels were below the expected toxic level in lettuce and higher in tomatoes. Anoliefo and Vwioko, (1995) investigating the effect of lubricating oil on two crops- hot pepper (Capsicum *annum L.*) and tomatoes (Lycopersicon *esculentum Miller*) reported that growth was not inhibited on hot pepper, on the other hand the effect of the lubricating oil was more pronounced on tomatoes. Tomatoes are a popular vegetable used for preparing different sauces due to their high fibre content (approximately 35%) and the presence of unsaturated fatty acids such as oleic and linoleic acid. Tomatoes also contain minerals such as potassium, magnesium and phosphorus, plus about 30% protein and amino acids. These high nutrient contents are useful in fortifying foods.

Nwachukwu,(2001) in his investigation used sterile agricultural soil that was artificially contaminated with crude petroleum in the laboratory to represent a worst case scenario of the effects of oil spillage on vegetable crops using cress seeds. The experimental set ups were inoculated with *Pseudomonas putida* as well as supplemented with inorganic nutrients. The control set-ups contained inorganic nutrients as supplemented but no addition of *Pseudomonas putida*. At nine weeks after spiking with oil, oil was degraded in the experimental set ups and none in the control set-ups. As a test of recovery of the oil-impacted soil, cress seeds were planted on both soils. Growth on control set up (not seeded with *Pseudomonas putida*) was poor (27.5%) when compared to results from experimental set up which yielded 98.8% growth. He suggested that such negative effects of crude oil on vegetable crops are amenable by addition of inorganic nutrients and inoculation with *Pseudomonas putida*.

Udo and Fayemi (1975) and Gill *et al.* (1992) recognised the effect of crude oil on the morphology of plants. Morphological aberrations could influence the proper functioning of plants. Gill *et al.* (1992) investigated the effects of crude oil on growth and features of a plant (Chromolaena *odorata*) and observed that the effect of crude oil on the cells of the plant was caused by the osmotic effect of the crude oil. The oil created unfavourable conditions in the soil causing water stress and restricted the availability of nutrients; they concluded that the effect was not due to toxic hydrocarbons in the crude oil. On the other hand, Udo and Fayemi, (1975) after studying the effect of crude oil on germination and growth of corn came to a different conclusion that reduced growth observed in polluted soils was as a result of non-availability of nutrients and toxic action of crude oil on the seed embryo which disrupted the physiological functions within the seed.

2.5 Tomatoes and nutrients

The importance of nitrogen to tomatoes amongst others includes growth of leaves. The leaves are the site for making sugar, which the plants use. Nitrogen availability to plants is important because the form of nitrogen not only influences uptake of other macronutrients but more importantly the pathway for its assimilation which determines the amount of energy to be used by the plant (Passam et al;2007). Studies have shown that when ammonium is the sole source of nitrogen, tomato plants are susceptible to it. This could result to impaired growth and reduction in yield. There has been conflicting reports on the actual value of ammonium nitrogen needed by plants. When value is higher than 0.1%, there is restriction in vegetative growth and fruit yield (Siddiqi et al;2002 :In Passam et al;2007). In contrast (Claussen,(2002) and Dong et al;(2004):In Passam et al;2007) reported improved yield when ammonium fraction was 0.25%. However, it is noted that the lowest range of NH3-N/total N that affects plants growth is actually controlled by the pH at the root zone, other form of nitrogen and other environmental factors. 10% of the total nitrogen (i.e. 0.1% of NH4-N/total N) is able to enhance fruit flowers (Siddiqi et al; 2002:In (Passam *et al.*, 2007).

Phosphorus as a macronutrient encourages flowering and fruiting. Studies have shown that growth rate of tomatoes increases with higher concentration of plant phosphorus especially when the residual level is below the minimum requirement (de Groot et al; 2002:In (Passam *et al.*, 2007). These workers observed that the effect of mild phosphorus limitation is not as harmful as severe phosphorus limitation, which is noticeable in photosynthesis because of reduced starch accumulation. It can also affect nitrogen concentration due to decrease in leaf cytokinin levels (de Groot et al; 2002:In (Passam *et al.*, 2007). Phosphorus concentration of 1%dry basis is recommended as the critical level between sufficiency and toxicity for tomato plants (Jones, 1988).

Depending on the key crops identified to respond better to a particular oil concentration, nutrient in form of animal manure or chemical fertilizer could be added to improve yield.

2.6 Researcher’s summary

The biggest limitation to bioaugumentation is that the approach seems complicated, because it requires the selection of appropriate microbial species and possibly genetically modified microorganisms. Use of genetically modified microorganisms has not received wide acceptance in research. It is also an expensive option. Biostimulation seems more practicable provided the nutrient requirements are available in the right proportions. However, the disadvantage is the difficulty of determining when the supplements become limiting to the process.

There is a general agreement about the low cost of using compost and composting techniques for bioremediation of petroleum pollutants and the preference of the method over methods such as incineration and disposal to landfill. However, it is evident that opinions differ on the effectiveness of such a process, with the following factors as concerns:

- The presence or absence of bioavailable pollutants, soil organic matter (SOM), and pollutant concentration;
- The biodegradation pathway which is determined by the chemical structure of the pollutants and the microbial species present;
- The long term stability of ‘less bioavailable’ form of pollutants; and
- The complete mineralization of the pollutant is not achievable, but they are often transformed into less toxic compounds

In conclusion, given the discrepancies between laboratory and field experiments for which some of the factors responsible had a mentioned in Chapter 3, the use of particular bioremediation technique will depend mostly on site-specific goals that a user wants to achieve. For the oil companies, the management may give preference to the length of time required for any bioremediation approach rather than the benefits, especially when using compost, which means there are less chances of considering the use of compost.

There have been many studies on recovering land contaminated with oil but the results are in many cases conflicting. The intention of this study was not just to look at the impact of the oil on the soil but also to focus on the reclamation of the contaminated land from the point of view of the farmer. The intention being to come up with a methodology to allow the farmers local to a spill to be able to return to producing food from the land as quickly as possible but without needing massive financial resources to do it.

The present research applied bioremediation by use of compost on two different soils from England and Niger Delta to come up with a more practical protocol, applicable on field. This diversified approach included not just plant trials but different compost mix ratios and possible ways to help sustain germination and growth of plants on treated soils.

The local community might view use of compost as a viable option due to accessibility to and availability of compost and the low technology involved in using the technique even though source separation of waste in Nigeria is still at infancy. A better understanding of the effects of crude oil on tomatoes is relevant to the Niger Delta region where incessant oil spills have devastated farmlands and vegetable crops. In general, the findings of the study could help a local farmer reclaim his contaminated soil for agriculture.

2.7 Biodegradation of petroleum hydrocarbons

It is thought that there is an initial lag phase (several days after oil spill) before indigenous microorganisms are able to degrade oil (Hoff, 1993). This lag phase has been thought to occur due to the toxicity of the volatile fractions of the oil which are the first components to evaporate, after which any measurable degradation can be recorded (§2.3.3). Hoff, (1993) concluded that this period of lag phase must be built in when considering bioremediation as a first response.

2.7.1 Microorganisms and chemistry of biodegradation

Most readily degraded petroleum hydrocarbon groups are of normal alkanes (n-alkanes) in the range C_{10} –C_{26}, benzene, toluene, and xylene (BTX), though the BTX group are toxic. Compounds with higher molecular weights are more difficult to biodegrade (Atlas, 1995a).

Although degradation of different classes of petroleum hydrocarbons suggests this order of decreasing susceptibility; n alkanes >branched alkanes > low molecular weight aromatics >cyclic alkanes, there is no universal agreement on this matter (Leahy and Colwell, 1990). Leahy and Colwell pointed out that the variation in the composition of crude oil and its products has influenced the overall degradation of crude oil components.

Atlas,(1981) recognised the contribution of different classes of microorganisms in degrading petroleum hydrocarbon. There are basic metabolic pathways utilised by microorganisms depending on the class of hydrocarbon involved, for example, the biodegradation of n-alkanes (a branch of saturated hydrocarbons) by n-alkane utilizing microorganisms produces primary alcohol followed by an aldehyde and a monocarboxylic acid. Beta (β) oxidation of the carboxylic acid eventually produces carbon dioxide. Isoprenoids (organic compounds with two or more units of hydrocarbons) that are highly branched e.g. pristine, undergo omega (Ω) oxidation with formation of dicarboxylic acids. As the amount of branching increases so does the resistance of the petroleum hydrocarbon to biodegradation). This phenomenon could be the reason why cycloalkanes (type of alkanes with one or more rings of carbon atoms in their chemical structure) are resistant to microbial attack. It is believed that during microbial degradation of hydrocarbons, some new compounds are formed, though the biochemical mechanisms for the formation is unknown (Atlas, 1981). Atlas confirmed the presence of these new compounds by glass capillary gas chromatographic analysis showing the new compounds as having unresolved peaks. Atlas, in his recommendation, called for research on the role of microorganisms in formation of complex products, which could persist in the environment.

2.7.2 Environmental modification

As listed in section 2.8, environmental factors influence the activities of the indigenous microbial population. The type and amount of hydrocarbon as well as the abiotic factors of the ecosystem affects its biodegradation. Therefore, it is possible that a particular hydrocarbon could be highly biodegraded in one environment and persist in another.

In the marine environment, the rate limiting factors are molecular oxygen, phosphate and nitrogen concentration even though oxygen might not be limiting in a well-aerated marine environment (Atlas, 1995b). The factors that affect biodegradation are environmental and nutritional factors.

2.8 Environmental factors and biodegradation

2.8.1 Physical state

Though some hydrocarbons are soluble in water, this might not be the case at very high concentrations, which is usually the case in most oil spills. In the marine environment, the degree of spreading partly determines the surface area available for microbial attack, in contrast to land where spreading is limited by plant matter and soil particles, which could absorb the oil because of their high organic matter content. There is numerous literature available on the effects of physical state of hydrocarbons with respect to microbial degradation (Atlas, 1981, Leahy and Colwell, 1990).

Usually when oil spills in water, it forms a slick which is either oil in water (emulsion) or water in oil ('mousse') Mousse limits the access of microorganisms to oil for degradation as it has limited surface area and as such the hydrocarbon is not available for microbial degradation. Water helps in the movement of nutrients and oxygen as well as creating an oil-water interface for hydrocarbon degrading microorganisms to act. Oil forms an emulsion in water and it is believed that ability of microorganisms to produce emulsifying agents would enhance degradation of hydrocarbons. Some organisms that have produced emulsifying agents are *Arthrobacter* strain, *Psuedomonas* and *Corynebacterium*. Though this attribute should increase microbial degradation of hydrocarbon in oil, on the other hand, it is not yet clear why production of emulsifying agent has no correlation to hydrocarbon degradation by these organisms. Probably this is due to formation of large ‘mousse’ which have unfavourably low surface to volume ratios and as a result inhibit biodegradation (Leahy and Colwell, 1990).

2.8.2 Temperature

There is a correlation between degradation and temperature. Petroleum hydrocarbon degradation takes place over a range of temperature. Mulkins-Phillips and Stewart, (1974) observed that degradation was more favourable at 10°C than at 5°C in a cold region (Arrhenius equation that reactions doubles for every ten degrees Celsius rise might be more applicable to chemical reactions even though it is not ruled out in biological reactions). Atlas and Bartha,(1973) observed higher microbial growth at 5°C during winter than any other seasons. This effect is attributable to seasonal shift in microbial population, which was reflected in the rate of hydrocarbon degradation at a particular temperature. Horel and Schiewer, (2009) did their investigation within a temperature range of 6-20°C and observed that microbial activities was higher for a few days at 20°C whereas at 6°C, it took an average of three (3) weeks for the microorganisms to adapt to the colder environment and peak in their respiration rate.

Atlas, (1981) also noted that the effect of temperature depends on the hydrocarbon composition of the petroleum mixture. This is in line with the report by Leahy and Colwell, (1990) which says that temperature influences petroleum biodegradation by its effect on the physical nature and chemical composition of the oil, the rate of hydrocarbon metabolism by microorganisms and the microbial population. At low temperatures, the viscosity of oil increases and the volatilisation rate of low molecular weight hydrocarbons is slowed down and some of them are toxic to microorganisms (§2.3.2). The presence of such components can actually delay the onset of biodegradation. In addition, the decrease in rate could be due to the decreased rates of enzymatic activity especially for temperature-dependent processes. Leahy and Colwell, (1990) reported that higher temperature increase the rate of hydrocarbon metabolism to an optimum within the temperature range of 30 – 40^0C. Petroleum hydrocarbon degradation takes place over a range of temperature. This is possible because psychrophilic, mesophilic and thermophilic microorganisms have been isolated during microbial degradation of petroleum.

2.9 Nutritional factors

2.9.1 Carbon to Nitrogen (C/N) ratio

Microorganisms use carbon for building up protoplasm and the carbon oxidized to yield energy and carbon dioxide. Secondly, because carbon is used for cellular metabolism and also released as carbon dioxide, it is invariably required in abundance in bacteria and also in their nutrition (Leton, 1984). Nitrogen is one of the components needed for protein formation.

Namkoong *et al.* (2002), agreed that crude oil spillage increases the organic carbon content of the contaminated site and reduces the soil nitrates and phosphorus however this carbon might be unavailable to the microorganisms and this unavailability might affect the way the organisms use oil as substrate. This could cause a delay in the natural process of rehabilitating the contaminated soils. They suggested the need for an alternate carbon substrate.

Odu (1972) did acknowledge the occurrence of nitrogen deficiency at crude oil spillage sites and proposed that nitrogenous fertilizers be used to improve the availability of nitrogen. John *et al.*(2011) proposed that in the absence of an external nitrogen source, it was natural for the microorganisms to resort to soil nitrogen after which they would explore nitrogen fixation cycle in which case the nitrogen fixing bacteria will dominate. Soil microorganisms such as nitrifying bacteria (i.e. nitrogen fixers) can make nitrogen available to plants through the process of nitrogen fixation especially in leguminous plants due to the symbiotic relationship that exits between them. When legumes grow on crude oil contaminated soil, the nitrifying bacteria are retarded. The oil inhibits the action of enzyme ‘nitrogenase’, which is essential in protein synthesis. This invariably affects the growth and survival of such plants in the affected ecosystem.

However, given the limits of their experimental evidence, (John *et al.*, 2011) called for future research into the isolation of nitrogen fixing bacteria from recovered soil. Even though C/N ratio is an important measure of nutrient balance in microbial growth, however, some authors have given more preference to the effects of nitrogen and phosphorus as limiting with respect to hydrocarbon degradation (Atlas, 1981). The difference in results has to do with whether the study aims at assessing the biodegradability of hydrocarbon within an oil slick or the biodegradation of soluble hydrocarbons. Atlas, (1981) found that though temperature might be the major factor to consider most of the year; when temperature and nutrient supply were optimal, temperature rather than nutrient yielded a higher rate of hydrocarbon degradation especially for marine environment.

He observed that without the addition of nutrients, soil and marine microbes more readily degrade aromatic hydrocarbons than the saturated hydrocarbons. Also on application of fertilizer (containing nitrogen and phosphorus) only the bacterial population was increased and not the fungi. This observation questions synergistic relationship between classes of microorganisms implicated in crude oil degradation. Some workers have reported that seeding with microorganisms does not have much effect on degradation (Jørgensen *et al.*, 2000, Namkoong *et al.*, 2002). However Kim et al;(2005) reported that there was an increase in degradation of Arabian crude oil 3% (v/v) after incubation at 25°C for a week following the addition of microbial inocula – Corynebacterium sp.IC-10, Sphingomonas sp. KH3-2 and Yarrowia sp.

2.9.2 pH and Organic matter contents

Zuofa et a;l (1988) working with soil from Niger Delta observed that there was an increase in soil pH with higher oil level exposure. Generally, oil tends to buffer soil's pH towards neutral though the soil's buffering capacity plays a key role (Essien and John, 2010). In contrast, another investigator Adekunle, (2011) working on a different soil from Nigeria did not notice any change in pH as a result of the treatment. Even though it is possible for different soils to have different buffering capacities as suggested by Zuofa et al; (1988). Soils with low organic matter content tend to have low buffering capacity and so changes in pH are noticeable unlike soil with high organic matter content. Soils' organic matter tends to increase with the application of oil. As the oil, which is a hydrocarbon, is broken down, it serves as source of carbon and energy for microbial growth, which invariably increases the organic matter content. Any residual hydrocarbon also adds to the organic matter pool over time and this in turn improves the soil fertility. Zuofa et al; (1988) also reported an increase in total nitrogen due to activities of nitrogen fixing organisms.

By contrast, Jørgensen *et al.* (2000) observed a decrease in soil organic matter following oil contamination. In the same manner Osuji and Adesiyan, (2005) investigating the effect of Bonny light crude oil on the total organic carbon (TOC) and organic matter content (OMC) of affected soils at Isiokpo Niger Delta, reported low OMC in the polluted site contrary to the high OMC expected after oil spillage. Osuji and Adesiyan, (2005) concluded that the spilled oil adversely affected metabolic activities of the microorganisms which could have resulted in the addition of organic carbon from the organisms utilizing the petroleum hydrocarbons.

The variation suggests that microbial load, organic matter content and soil physical characteristics differ from soil to soil depending on location and soil type as well as the geological formation and these factors play an important role in deciding how a given contaminated soil behaves on treatment with organic amendment such as compost.

2.10 Parameters of importance in composting

As mentioned earlier (§1.4), there is an availability of raw materials in the region which can be converted into compost and the product could then be used for bioremediation of crude oil contaminated soils. We have also suggested the need for a local composting plant as this means availability of finished product within the reach of the local farmer. Therefore, it is important to discuss a number of factors that could affect effect composting.

2.10.1.1 *Aeration*

Composting is an aerobic process, such that presence of oxygen (air) influences microbial activity. The chemical and physical composition of waste also determines the rate of aeration required in the process, hence the use of bulking agent during composting to facilitate the free passage of air. A bulking agent is an organic/inorganic material of sufficient size to provide support and maintain air spaces within the composting matrix (Haug, 1993). Examples include woodchips (25 - 40 mm size), tyres and peanut shells. An amendment is a material added to the other substrate to condition the feed mixture (Haug, 1993). Examples include refuse straw, peat, rice hulls, yard waste and other waste materials. An ideal amendment is dry, has low bulk weight and is relatively degradable. Aeration is important because; (a) It supplies oxygen to support aerobic metabolism**;** (b) controls temperature and (c) removes moisture, carbon dioxide and other gases.

When aeration is insufficient, it can lead to formation of anaerobic zones and create bad odors whereas excessive aeration can lead to reduced moisture both of which affect microbial activities. Turning the composting materials with a front-end loader or a turning machine in the case of windrow systems improves aeration.

2.10.1.2 Moisture

Moisture affects bacterial activity. It is a function of physical structure of waste and ratio of air to water in the soil. Moisture, free air space, aeration, temperature and combination of other physical factors influence composting. The higher the moisture, the less the available air pores and the oxygen content. Moisture content can be defined as the weight loss after the sample has been dried to constant weight at 100-110°C (Fogarty and Tuovinen, 1991). Another factor that affects moisture is the ability of the material to withstand compaction, which changes the physical characteristics of compost layers, ultimately resulting in decreased aeration and microbial activity.

Excessive moisture limits oxygen diffusion and invariably affects the aeration metabolism, while insufficient moisture or water disrupts composting. The optimum range is 50-60% (for bacteria) with bacteria inhibition occurring when it falls below 40% but for fungi, their threshold is lower. Use of bulking agent is recommended due to the fact that they enhance porosity (Trejo-Hernández *et al.*, 2007). Composting is a drying process because microbial activity is an exothermic reaction and this buildup of heat results in a temperature rise, which evaporates more water.

2.10.1.3 pH

pH is a measure of hydrogen ion concentration. It is divided into three ranges (a) from 1-6.9 is referred to as acidic range (b) 7 is neutral and (c) 7.1-14 is referred to as basic range. The normal pH range for composting is between 5.5 and 8.5. Extreme pH has effect on microbial activity and enzymes. A typical composting process for food waste would start in the acidic range and as composting progresses work towards alkaline conditions, with most finished compost ending up with a pH in the 7.5 to 8.5 range. Different soils have different pH which could range from 2.5 – 11.0. Extremes of pH as found in some soils may be unfavorable for microbial degradation.

2.10.1.4 Temperature

Temperature affects microbial activity because microorganisms have optimal temperatures above which their growth rate is affected. Numerous studies have identified temperature as an important factor during composting (Ryckeboer *et al.*, 2003, Van Gestel *et al.*, 2003). Heat is a byproduct of composting process. This heat remains in the composting mass because of good insulating properties of the composting matrix. According to Ryckeboer *et al.* (2003), composting has four stages based on temperature rise. First the mesophilic stage, then thermophilic stage, with the temperature going down during cooling and maturation stages.

Fogarty and Tuovinen, (1991) noted that these variations in temperature also influences the organisms found within the compost matrix. The thermophilic organisms (optimum at 45 -65°C) replace the mesophilic population (which grows best at temperature range of 15 – 45°C). Above this thermophilic temperature, the fungi and yeasts become inactive; leaving the work of decomposition to mostly the thermophilic actinomycetes and spore forming bacteria (Lasardi, 1998). During this phase, the readily degradable substances are consumed and most human and plant pathogens destroyed. This suggests there is microbial succession during composting process. We can characterize microbial activities within these temperature ranges as shown in Table 2.3.

Table 2.3: Microbial activities during composting

25 – 45°C	highest biodiversity
45 – 55°C	highest rate of biodegradation
>55°C	Highest pathogen inactivation

Source: (Stentiford, 2010)

Due to the fact that temperature plays a role in microbial succession, the efficiency of the composting process drops at higher thermophilic levels (Golueke, 1986, Epstein, 1997). Epstein suggested that temperature should not be more than 50 - 60°C. If not controlled, temperature could exceed 70°C or 80°C in a space of few days and only a few thermophilic sporogeneous bacteria can survive and the rate of biological decomposition becomes very low (Lasardi, 1998).

Degradation is highest at the thermophilic phase as seen by the measurement of CO_2 evolution rates. In a compost pile, it shows temperature gradient with the center having highest temperature, which decreases, towards the outer zones as shown in Figure 2.2. In the third phase of composting i.e. cooling phase, the amount of readily available organic carbon becomes rate-limiting factor resulting in decrease in microbial activity as well as heat produced.

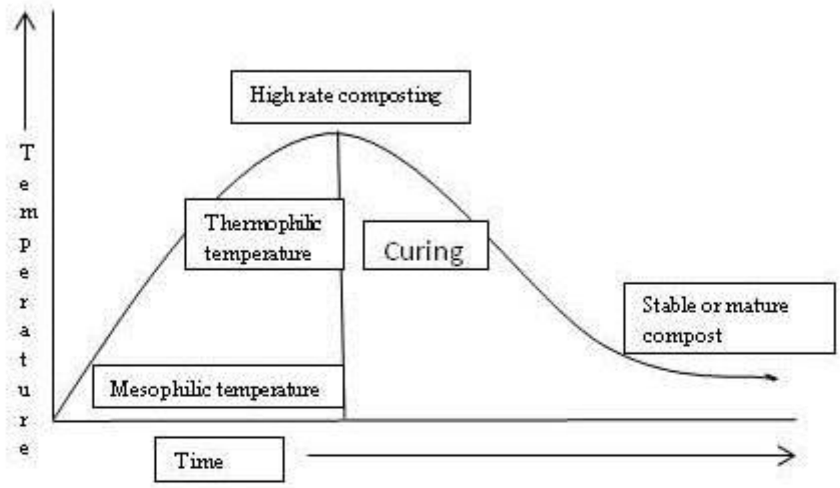

Figure 2.2: Temperature curve during composting adapted from Epstein, 1997

Fogarty and Tuovinen,(1991) supported the argument that there is microbial succession within the composting matrix. However, they reported that it is actually difficult to evaluate the relationship between microbial activity and temperature at any given point in time during composting but it is generally accepted that temperature greater than 60°C could result in rapid thermal inactivation of the desired microbial community.

Temperature also affects moisture content and aeration during composting. The type of waste used as compost feedstock must reach a certain temperature to be free of pathogens. In summary, mesophilic and thermophilic microorganisms are involved in the composting process and their succession is important in its effective management Goyal et al; (2005). Table 2.4 shows the recommended values for composting.

Table 2.4: Composting process operating guidelines

Condition	**Reasonable range***	**Preferred Range**
carbon to nitrogen (C/N) ratio	20:1 – 40:1	25:1 – 30:1
Moisture content	40 -65% **	50 -65%
Oxygen concentration	Greater than 5%	Much greater than 5%
Particle size (diametre in mm)	3 – 12 mm	Varies **
pH	5.5 -9.0	6.5 -8.0
Temperature (^{0}C)	43 - 65.	54 - 60

*The recommendations are for rapid composting. Conditions outside this range can also yield successful results.

**Depends on the specific material, pile size and / or weather conditions.

Source: adapted from Michael *et al.* (1995).

This review has shown there is gap in knowledge with respect to use of compost in bioremediation of soils contaminated with Bonny light crude oil. Thus, the gap that this research addresses can be summarised as follows; there is no adequate mix ratio of compost to soil for the restoration of soils contaminated with Bonny light crude oil in the Niger Delta region of Nigeria and areas of similar context.

3 Materials and Methods

The flow chart in Figure 3.1 is an overview of the experiments carried out during the research.

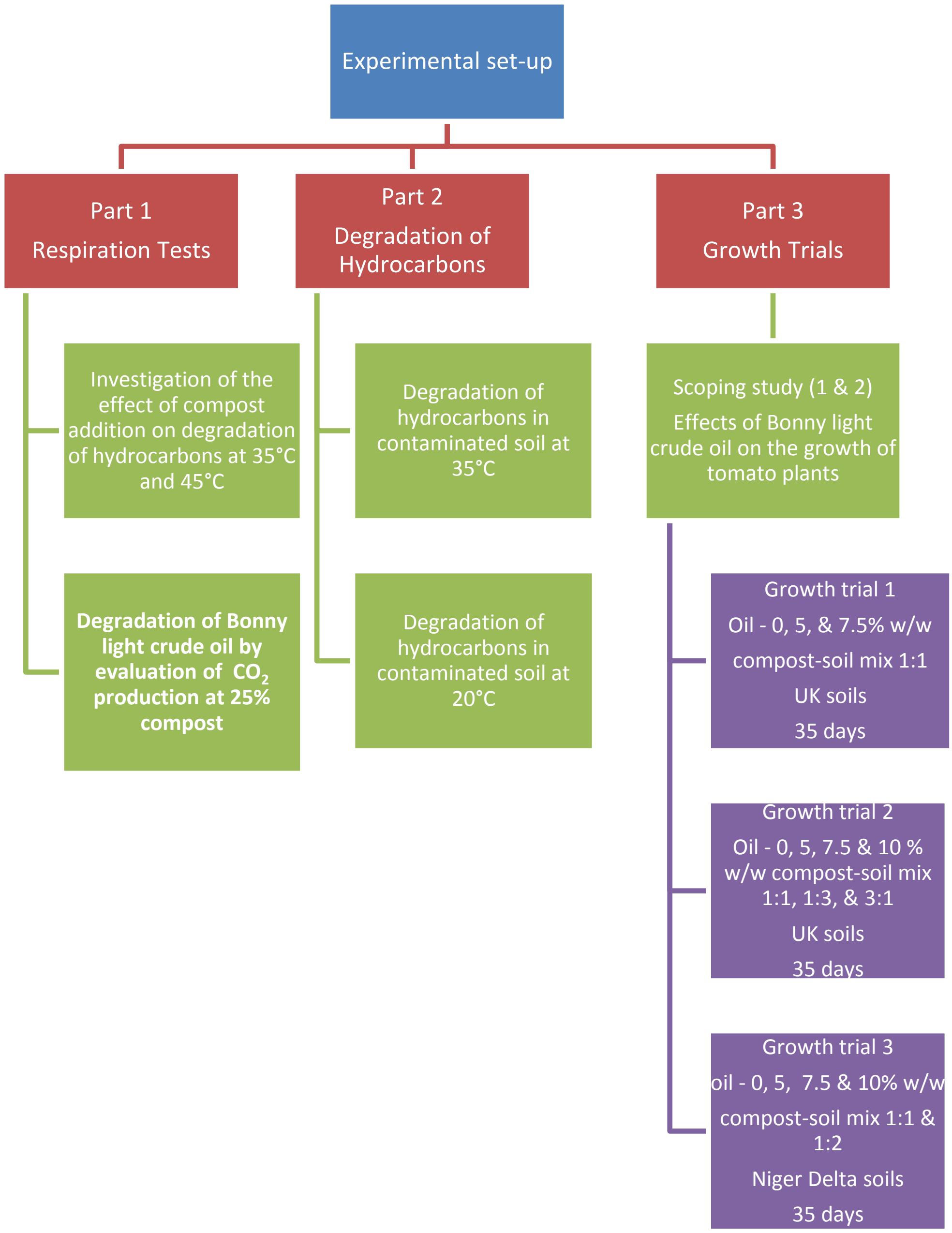

Figure 3.1: An overview of the different experiments carried out during the research

3.1 Introduction

The aim of this research was not just to look at the impact of the oil on the soil but also to focus on the reclamation of the land from the point of view of the farmer. The intention being to come up with a methodology to allow the farmers local to a spill to be able to return to producing food from the land as quickly as possible but without needing massive financial resources to do it. A wide range of compost and soils examined represents a diversity of conditions. This section comprises three parts namely: (a) respiration tests (carbon dioxide measurement), (b) degradation of crude oil contaminated soil (hydrocarbon degradation) and (c) growth trials.

From the literature, several tests have been standardised for the measurement of biodegradability of organic compounds however, they are based on indirect measurement of summary parameters such as oxygen uptake using respirometer and carbon dioxide (CO_2) production which reflects microbial activities (Sharabi and Bartha, 1993, Pagga, 1997, Namkoong *et al.*, 2002, Okolo *et al.*, 2005, Ahiamadu and Suripno, 2009). During aerobic metabolism in a living system, as food and oxygen are consumed to create energy and produce new cells, CO_2 is released (Lasardi, 1998). Sharabi and Bartha (1993) in their investigation agreed that conversion to CO_2 under aerobic condition is a standard method for assessment of biodegradability, however, they pointed out that majority of the CO_2 produced come from mineralization of biomass and soil organic matter and some may be unrelated to the test compound.

Different investigators have pointed out the advantages and disadvantages of measuring carbon dioxide evolved during the biodegradation process especially when using manual techniques (Odu, 1972, Pagga, 1997). Despite the low points of the method, it is widely used due to the low cost apparatus involved. The manual technique has its limitations in comparison to the automated techniques such as high time consumption and the likelihood of under estimating the CO_2 evolved (Száraz and Beczner, 2003).

In the present study due to cost, a manual set up was built in our laboratory using a DR4 (4-day Dynamic Respiration) rig to test the biodegradation of oil under controlled reactor conditions. In this case, the experiment lasted for more than 4-days. The measurement of CO_2 evolution served as an indicator of crude oil degradation because at the preliminary stages, it was not possible to quantify the hydrocarbon using gas chromatography since none was available in the school at the time of the experiment. This also made it difficult to confirm the validity of CO_2 evolution as a useful tool for monitoring hydrocarbon degradation.

Some studies have agreed on the difficulty of measuring CO_2 produced from hydrocarbon degradation mostly because of the challenges of using different types of closed systems to avoid leakage of carbon dioxide to the environment. Also, there is no universal acceptance as to whether the CO_2 is produced only from the organic contaminant since it has been suggested that other soil biomass have been involved in producing CO_2 at the same time (Alexander, 1994, Kim *et al.*, 2005). However, in the present study we monitored baseline CO_2 by using a control without oil. Another disadvantage of using CO_2 production is the conversion of the test material into biomass, not accounted for, and this has necessitated the use of empirical values in making comparisons with the values from the experiment.

During the study, we monitored changes in physicochemical and biological parameters as well as total petroleum hydrocarbon (TPH). We obtained the raw materials used in this study both from United Kingdom and from Nigeria. There were modifications on experimental procedures and some procedures dropped entirely in later trials due to inconclusive results from preliminary experiments.

3.2 Raw materials

3.2.1 Soil

Agricultural topsoil was collected from the top 150 mm of soil from different locations in UK (Bardsey Leeds, West Yorkshire and Selby, North Yorkshire) as well as the Niger Delta region of Nigeria (Rumuodara- a village in Port Harcourt, Rivers State). Port Harcourt is the capital of Rivers State, centre for crude oil and natural gas harnessing in Nigeria. It is located approximately latitude 05° 21'N and longitude 06° 57'E with average annual minimum and maximum temperature of 25°C and 31°C respectively (Igoni *et al.*, 2007). The region is characterised by two season’s namely dry and wet seasons.

3.2.2 Compost

Compost was obtainable from different sources. One of these was coarsely shredded compost, from an in-vessel compositing site of TEG Environmental located in Todmorden, West Yorkshire, UK. The compost consists of feedstock mixture of food and green waste. Some of the food waste came from refuse treatment plant operated by the Greater Manchester Council. Different compost (horse manure and straw) collected from Bardsey; Leeds and a third batch (green waste feedstock) obtained from Rufforth, in the county of York, West Yorkshire, all formed the feedstock.

The waste stream in the Niger Delta contains a significant proportion of organic waste good as raw material for composting. Table 3.1 represents the composition of a typical municipal solid waste (MSW) from the Niger Delta. For an estimate of organic waste produced per day in the region (see section 1.8).

Table 3.1: Physical composition of MSW in the Port Harcourt Metropolis

Waste component	Mass (kg)	Mass (%)	Volume as initially collected (cm^3)
Organic			
Food waste	43.5	29.2	0.14
Wood/leaves	12.7	8.4	0.05
Paper	18.5	12.4	0.21
Plastics	14.7	9.9	0.24
Textiles/rubber/leather	11.4	7.6	0.08
Miscellaneous organics	2.7	1.8	0.02
Inorganic			
Glass	20.1	13.5	0.11
Metals	25.6	17.2	0.13
Total	149.2	100	0.98

Adapted from Igoni et al; 2007

From Table 3.1, a substantial quantity of the waste stream is non-biodegradable and and therefore cannot be composted. However, in any composting plant, these non-biodegradable fractions (metals, glass, and plastics) are sorted out either manually or mechanically before composting. As was stated earlier, the waste-derived compost used in this research was sourced from United Kingdom. However, given the composition of MSW from the Niger Delta region; it is possible to generate similar compost, which can be applied to contaminated soils in Nigeria, given that backyard composting, have previously been done by farmers at local level.

3.2.3 Crude oil

The Nigerian Bonny Light crude oil (BLCO) with a low sulphur content (0.19%) and density of 0.875g/cm^3 was collected from the oil delivery line located at FPSO Sea Eagle (Floating Production Storage and Offloading), an offshore platform (in the Niger Delta region) owned by Shell Petroleum Development Company of Nigeria. Sea Eagle is located between the coordinates N 0 5° 32'52 and E 005° 43'29.

3.2.4 Seeds

Tomato seeds (Shirley F1 Solanum lycopersicum) were purchased from Justseeds Coedpoeth, Wrexham County Wales UK.

3.3 Experimental set-up for respiration tests

Some researchers have shown that degradation can be monitored using CO_2 production (aerobic metabolism); however, this proved difficult to measure with confidence in the present study, probably due to technical challenges from the equipment (DR4). Basically, aerobic respiration involves a process where food and oxygen are consumed to create energy and new cells while carbon dioxide is released (Lasardi, 1998). Therefore, parameters such as oxygen consumption and CO_2 can be used to determine rate of respiration. In other to relate carbon dioxide production (microbial respiration) and microbial activities to hydrocarbon degradation in soils contaminated with Nigerian crude oil, a biodegradability test was carried out using DR4 apparatus.

3.3.1 Principles of DR4

For the biodegradability test, the major equipment used was the DR4 type apparatus. The DR4 biodegradability test method was devised by Waste Research Centre (WRC) United Kingdom and it is used to measure the production of CO_2 from a solid waste over a four-day period. However, in the present study the incubation period was eight days to allow sufficient time for any degradation of the crude oil. The test material was loaded into a small aerated cylindrical reactor measuring (22cm x 11cm) with a perforated plate at the bottom to distribute the air supply and the respiration was measured by absorbing the carbon dioxide produced in alkali trap bottles (Namkoong *et al.*, 2002). The CO_2 produced was absorbed in one molar concentration of sodium hydroxide (1M NaOH). The experimental set up is shown in Plate 3.1.

There were two traps for absorbing the $CO_{2.}$ The exhaust gases were passed through the two traps in series for absorbing the $CO_{2.}$ A typical volume from trap one bottle was between 500 – 520 ml and close to 200ml for trap two (2). Where the volume was more than in trap 2, perhaps, it might be because of condensation arising from moisture loss during breakdown of organic matters in the compost.

In the preliminary tests, a number of difficulties were encountered using the DR4 apparatus such as inconsistency for carbon dioxide absorbed by the trap bottles and drying up of test materials in the reactor. As a result the method proposed by Stotzky (1960) for measurement of CO_2 was modified and used for future tests. The procedure introduced use of empty reactor to measure ambient CO_2 which served as the control.

The experimental apparatus used comprised four (4) aerated reactors, a humidifier on the incoming air, which also served as CO_2 removal trap as it contains distilled water, and a trap for collecting CO_2 evolved from biodegradation test as shown in Plate 3.1. Most of the CO_2 from the incoming air was removed by passing the air through distilled water in the humidifier. Distilled water was used to avoid any aspirated alkali solution from entering the compost reactor and probably causing harm to the microorganisms. The CO_2 evolved was absorbed in a primary CO_2 removal trap by reacting with 12.5 ml of 4 N Potassium hydroxide (KOH) (Stotzky, 1960, Benyahia *et al.*, 2005, Okolo *et al.*, 2005). The CO_2 collected from empty incubated vessel served as controls during the experiments under soil respiration and it measures the background carbon dioxide. The CO_2 collectors were replaced periodically (every other day to avoid the alkali being too weak for maximum CO_2 absorption) with fresh CO_2 collectors during the incubation period. After CO_2 absorption, excess barium chloride ($BaCl_2$) was used to precipitate out the sodium carbonate (Na_2CO_3) as barium carbonate ($BaCO_3$). Few drops of phenolphthalein indicator were added and the neutralised alkali was titrated with standard hydrochloric acid (1M HCl) directly in the bottle.

The temperature was monitored by inserting a Traceable® long-stem thermometre (VWR International, Pennsylvania USA) into the hole on top of the DR4 reactor as a way of confirming the oven temperature. The temperature reported was the temperature of the reactor and used to control the temperature in the incubator. An aeration rate of 500ml/minute was introduced into the reactor (this ensures large supply of oxygen). The idea of having the secondary trap is to absorb any CO_2 that escaped from trap 1. Flexible tubing connected together all the system elements. The humidifier used in addition to trapping CO_2 from the incoming air, was to raise the water content of the incoming air in order to prevent contents of the reactor drying up. Carbon dioxide was removed from the incoming air so that CO_2 in the exhaust air (flue gases) was attributed entirely to degradation.

Plate 3.1: Laboratory set-up of DR4 apparatus housing more than one reactor

3.3.2 Investigation of the effect of compost addition on degradation of Bonny Light Nigerian crude oil

Different countries have their own intervention values of total petroleum hydrocarbons (TPH) at which soils are regarded as contaminated. Dutch Intervention value of TPH is given as 5 g/kg in soil containing 25% clay and 10% organic matter content. Danish (Zealand) Guidelines define contaminated soils as having TPH, which exceeds 0.2 g/kg of soil. The regulatory body in Nigeria (Department of Petroleum Resources) has specified 5 g/kg soil as their Intervention value (this is similar to that of Dutch). In case of Nigeria, the intervention value is based on existing international value and as mentioned in section 3.5.1, that contamination to the level of 8300 mg gives a reading of 5000 mg oil. In subsequent experiments, contamination values much above this level ranging from 0, 5, 7.5 and 10% (w/w) was applied because this reflected better the actual situation in the Niger Delta.

This preliminary investigation was in two sets using compost obtained from Bardsey, Leeds. In Trial 1; 28/7/10 – 5/08/10, incubation temperature was 35°C and Trial 2, which lasted from 9/9/10 to 17/9/10 had incubation temperature as 45°. The manure-based compost used was horse manure and straw (characteristics shown in Table 3.2). The compost was sieved to particle size of 6.30mm to remove stones and other rough materials that could cause problem during mixing (sieve model- BS 410 manufactured by Endecotts Ltd, London England). The aim as stated above was to measure the carbon dioxide produced as an indication of microbial respiration, which could suggest degradation of the oil.

Table 3.2: Analytical characteristics of the raw materials used for biodegradability test

Analytical characteristics	Compost	Crude oil
Moisture content (% ww)	59	N/A
Dry solids	0.41	N/A
Volatile solids (% dw)	71	N/A
pH	6.9	4.9
Electrical conductivity (µS/cm)	1295	
Carbon (%)	22	84.13
Nitrogen (%)	1.5	0.15
C/N	15	580

*N/A – not applicable

Material preparation and sampling procedure: the soils and compost used for the study were collected as and when needed, no air-dried material was used. The use of moist materials from the field was preferred over air dried soils/compost because a substantial part of the microbial biomass is killed or lost when soils are air dried, stored and rewetted (Rowell, 1994). However, when there was a need for reuse, the remaining material was stored in a sandbag because of high breathability, which allows air to pass through and allowed the material maintain its original texture. Due to the heterogeneity nature of the starting material, care was taken to ensure that the material was as homogenous as possible. The compost was passed through a manual sieve with 6.3mm aperture and large particles were hand-picked out of the samples. The soil was sieved to 2.36 mm particle size.

Compost was mixed with different oil levels and carbon removal was estimated by the production of carbon dioxide. Of the four reactors used; each of the three reactors had 400 g of compost and was mixed with bonny light crude oil at 0.5, 1 and 2% (w/w) respectively that works out to 2, 4 and 8g oil. The fourth reactor had only compost (400 g) and served as control. The 0.5% w/w actually works out the same Percent as the Nigerian's Intervention value. The reactor's size has previously been described (§ 3.3.1). The sample size was in line with the specifications of DR4 (Hobbis, 2006). The temperature of the test mixture was monitored using a thermocouple. Out of the two temperatures applied, 45°C was to reflect extreme temperature. Each of the experimental conditions was replicated three (3) times and the average values taken. The four reactors used in the experimental set up are: Reactor 1 containing 2 g oil, Reactor 2 containing 4 g oil, Reactor 3 containing 8 g oil and Reactor 4 which serves as control (compost only).

During the first experiment of the biodegradability test, the oil (according to the required oil level) was sprayed on the compost using a plastic syringe and then both materials were mixed manually, however in subsequent experiments mixing of material with crude oil was modified after the method developed by (Rezaei *et al.*, 2010) to allow for more homogenous mixing. Crude oil was dissolved in hexane (250 ml of hexane) and after adding to the soil was mixed manually. The spiked mixture was left in a fume cupboard overnight to drive off the hexane. Thereafter, test materials were transferred into small aerated cylindrical reactors.

In all the experiments, the quartering system was used to divide the feedstock to allow for fairly homogenous sub-portions (i.e. feedstock was formed into a heap and divided into four equal portions, two diagonally opposite portions were separated, mixed thoroughly together and formed into smaller heap for quartering). During the 8-day incubation period, there was no measurement of physiochemical parameters such as moisture content and volatile solids and carbon-nitrogen ratio. We believe that opening the reactor might either introduce atmospheric carbon dioxide into the experiment or release the carbon dioxide produced to atmosphere thereby changing the total CO_2 absorbed in the traps. In the first trial, we had challenges in the experimental set up due to problems with the apparatus, which resulted in some difficulties in measuring the carbon dioxide.

3.3.3 Investigation of the effect of compost addition (25%) on degradation of Bonny Light Nigerian crude oil

In this experiment, soil was spiked with crude oil and compost added to enhance degradation of the crude oil since it has greater microbial diversity. Measurements were made of physico-chemical parameters, namely moisture content and C/N ratio. The topsoil used (characteristics shown in Table 3.3) was spiked in the laboratory. Compost used was same as in previous experiment however; compost was added as organic amendment to the contaminated soil. Crude oil degradation was measured by monitoring CO_2 production. The topsoil collected filtered through 2.36 mm mesh (sieve model -BS410/1986 by Endecotts Ltd London, England)**.** The ratio of compost to contaminated soil was one part of compost to three parts of soil. The basic raw material for this test was soil mixed with oil at 0.5% (soil weight). This was prepared as 5 g of oil in 1 kg of soil. The stock was divided into sub-portions of 200 g each. Due to the difficulties experienced in using DR4 apparatus, the titration method of Stotzky (1960) was modified and used.

In this experiment, the moisture level was monitored for the period of 28 days without addition of water. The moisture content kept on reducing to a level that seemed difficult to support microbial activities. Thereafter, in subsequent experiments water was added regularly to make sure the moisture content was not below the recommended range for microorganisms. Elemental analysis for carbon (C) and nitrogen (N) was carried out and the ratio expressed as C/N ratio. The methods used for the elemental analysis has been discussed fully in § 3.5.3. The days of sampling were 3rd, 9th, 16th, 23rd, and 30th of March 2011. The samples used for elemental analysis were sent to the School of Process Engineering, Leeds University for analysis. The samples were collected on the dates mentioned and kept refrigerated at 4°C. This was to make sure we had the required number of samples in order to operate the equipment at full capacity. The four reactors used in the experimental set up are:

Reactor 1 – Containing 0.5% oil in 200 g of material (ratio of compost to contaminated soil is given as 1:3 referred to as 25% compost)

Reactor 2 – same as Reactor 1 (compost – soil 1:3)

Reactor 3 – Compost only (control)

Reactor 4 = soil only (control)

Table 3.3: Characteristics of soil from Bardsey

Analytical characteristics	Soil (from Bardsey)
Moisture content (% ww)	29.6
Dry solids	0.70
Volatile solids (% dw)	23
pH	6.8
Electrical conductivity (µS/cm)	151.9
Carbon (%)	8.49
Nitrogen (%)	0.62
C/N	14

3.4 Degradation of hydrocarbons (n-alkanes fraction) in contaminated soils at 35 and 25°C

The purpose of the investigation was to determine how temperature would enhance degradation of n-alkanes (C_8 – C_{37}) in bonny light crude oil using compost as an organic amendment. In this study, we monitored the treatment of contaminated soils through the application of compost, also using chemical analysis to monitor the level of hydrocarbons remaining in the crude oil. This involves monitoring the contaminated soils to determine any reduction in hydrocarbons (n-alkanes) at the end of 35-day incubation period. A mix ratio of one part contaminated soil to one part compost was used (referred to as 50% compost). Soil was obtained from Selby and compost (made from green and food waste) from TEG composting plant were characterised as shown in Table 3.4.

Table 3.4: Characterization of materials used in the test

Analytical characteristics	Soil (Selby)	3 months-compost (TEG)
Moisture content (% ww)	30	46
Dry matter	0.7	0.54
Volatile solids (% dw)	23	73
pH	7	8.2
EC (µS/cm)	200	1720

Material preparation and sampling procedure: the experimental conditions were set up in duplicates at laboratory scale. The work was carried out at two temperature levels; 35°C and 20°C given the local daytime temperature in the Niger Delta are often around 35°C. A mix ratio of one part of contaminated soil to one part of compost was applied. A kilogram batch of soil was spiked with 8,300 mg of bonny light crude oil, which was dissolved in 250 ml of hexane to enhance homogenous distribution of the crude oil in the soil. Eight Thousand Three Hundred milligram of oil was used given the recovery rate determined in previous experiments discussed in § 3.5.1. A sample taken for standard analysis would report a value of around 5000 mg/kg, which is used in Nigerian legislation).

The experiment was modelled as randomised complete block design as shown in Table 3.5. Five Hundred millilitres (500 ml) amber glass bottles (Plate 3.2) were used to set up the experiment. The sample size, eighty grams (80g) of the mixed material was weighed into the glass bottles and incubated in the oven. The oven temperature was set at 35°C The second batch of the experiment incubated at 20°C was left on bench top in the laboratory. An i. button was used to monitor the temperature in the laboratory. Sampling was done at day 0, 7, 14, 21, and 35. Destructive analysis was used, however before sampling, the contents of the bottle was manually mixed to homogenise the material. Approximately, 30 g of sample were collected for the analyses. Samples from each treatment was extracted and analyses for the hydrocarbon content was carried out using gas chromatography manufactured by Agilent (model 7890A).

Table 3.5: Description of the reactors used in the experiment

Day 0	Day 7	Day 14	Day 21	Day 35
High temperature (35°C) Mix ratio of compost to contaminated soil (1:1)	High temperature (35°C) Mix ratio of compost to contaminated soil (1:1)	High temperature (35°C) Mix ratio of compost to contaminated soil (1:1)	High temperature (35°C) Mix ratio of compost to contaminated soil (1:1)	High temperature (35°C) Mix ratio of compost to contaminated soil (1:1)
Low temperature (20°C) Mix ratio of compost to contaminated soil (1:1)	Low temperature (20°C) Mix ratio of compost to contaminated soil (1:1)	Low temperature (20°C) Mix ratio of compost to contaminated soil (1:1)	Low temperature (20°C) Mix ratio of compost to contaminated soil (1:1)	Low temperature (20°C) Mix ratio of compost to contaminated soil (1:1)

† Random selection of samples were done at days 0, 7, 14, 21 and 35 during the experiment at the two temperature levels

Plate 3.2 : The bottles containing the samples were picked randomly.

3.5 Analyses methods

In order to monitor the progress of the different reactors during various treatments, the feedstock are characterised at regular intervals depending on the experimental design. For the growth trials (described in section 3.6), materials were characterised at the start and end of each experiment. The analyses are summarised as hydrocarbon, physico-chemical, chemical and microbiological analyses. The parameters measured included moisture content, volatile solids, pH, total nitrogen (TKN), electrical conductivity (EC) and total petroleum hydrocarbons. Nitrogen content was measured using Kjeldahl's method and reactive phosphorus by using the ascorbic acid method. The estimation of Total petroleum hydrocarbon (TPH) was by the method of spectrophotometry. For initial TPH reading, sampling was immediately after spiking the soil with the crude oil to determine the original amount of petroleum hydrocarbon.

Where necessary, water extracts were prepared and the ratio of sample to water was one part to ten parts of water. For the analysis of carbon and nitrogen used for determination of C/N ratio, two replicates were analysed (§ 3.5.3). The analysis for total carbon and nitrogen requires air-dried samples and this was done using the fume cupboard with the fan on and at the ambient temperature. The labelled samples were stored, and later sent for analysis to the Chemistry department of the University.

In some of the experimental trials, the moisture level of the soil and spiked materials were low, as a result, water was added to the soil to increase the moisture level. Most of the experiments were under ambient temperature (20°C) except where stated otherwise. An i button was used to monitor both outside temperature and temperature within the reactor (in which case the i. button was dropped inside the reactor) and the temperature data read from a computer. The programme on the i. button was such that it generates readings every five minutes.

3.5.1 Hydrocarbon analyses

One of the challenges in analyzing chemical components of petroleum hydrocarbons is the fact that it is a complex mixture and changes with time after exposure to the environment. Four (4) methods commonly used for analysis are:

3.5.1.1 Gas chromatography (Sugiura et al., 1997, Potter and Simmons, 1998)

This is a common method for extraction of total petroleum hydrocarbons (TPH). Usually, samples are extracted using organic solvents. The extract is analyzed by gas chromatography based on differences in the boiling points of the target compounds present and the compounds are then quantified by comparison to known standards.

3.5.1.2 Infrared Spectrophotometry (Wang and Fingas, 1997, Osuji and Adesiyan, 2005, Osuji et al., 2006)

It is a general belief that spectroscopic methods are more sensitive and accurate than the other methods. The amount of petroleum hydrocarbons remaining is determined by extracting the residual oil with organic solvent (hexane or dichloromethane). The method of infrared spectrophotometry measures the hydrocarbon concentration and read off from the calibration curve i.e. the concentration of the analyte is read from the linear regression analysis of the calibration curve.

3.5.1.3 Gravimetric (Sugiura et al., 1997, Wang and Fingas, 1997)

This method uses difference in weight between the sample and the control. The amount of petroleum hydrocarbons is determined by measuring the weight of sample and the degradation estimated by the decrease in weight compared with that of a control sample. There has been suggestion that the loss of more volatile petroleum fractions can occur especially during measurement.

3.5.1.4 Immunoassay Measurements (Potter and Simmons, 1998)

This method though relatively new is mainly for field measurements and indicates the presence or absence of hydrocarbons. It does not give any information on the carbon range. In addition, there is fear that humic acid from other organic materials could interfere with results.

Total Hydrocarbon (THC) concentration is commonly used by regulatory agencies as a target for cleanup limits, however different methods give varying results due to the fact that they are designed to extract and measure slightly different subsets of petroleum hydrocarbons (Potter and Simmons, 1998). Gas chromatography is widely used because it can give both identification and quantification of hydrocarbons. It can also identify a wide range of hydrocarbons.

There are several incorrect assumptions about analytical methods used for hydrocarbons and these include:

- that the methods measure from the contaminated sites all the hydrocarbons that are petroleum–derived
- the results give exact concentration of petroleum hydrocarbon and
- the result has a linear relationship with the level of risk.

Some methods may not be accurate due to mix up from other organic materials found in the environment for example – animals and vegetable oils and humic material in the topsoil, which can increase concentration of TPH. In this study, use of controls was to limit the variations in results for example measurement of TPH from unpolluted soil and compost served as the controls.

Different authors have used gas chromatography to quantify different hydrocarbons present in soil and sediment (Gustafson *et al.*, 1997, Korda *et al.*, 1997, Jørgensen *et al.*, 2000, Namkoong *et al.*, 2002, Mao *et al.*, 2009, Muijs and Jonker, 2009, Megharaj *et al.*, 2011), however there is a limit to the class of hydrocarbon that a gas chromatography (GC) can and cannot detect and quantify. For example compounds with less than six carbon chain length are mainly volatile and elute before the solvent peak (i.e. they form interfaces from the solvent peak), and so typically they are not measured.

Gas chromatography methods are appropriate for detecting non-polar hydrocarbons with carbon numbers between C_6 and C_{25} or C_{36}; however, crude oil is likely to contain compounds with more than a thousand carbon numbers, as it is a mixture of hydrocarbons. As biodegradation progresses, the pattern of compounds could change such that identification becomes difficult thereby making a GC fingerprint not very conclusive as a diagnostic tool. Some compounds could co-elute forming isomers while others could form unresolved complex mixtures, which may not pass through chromatographic column. Such complex mixtures could even be removed with cleaning agent e.g. silica gel.

Therefore, the use of chemical analysis only for quantifying the amount of residual hydrocarbon in soil after a particular treatment might be useful in terms of evaluating the environmental risks associated with the compounds or meeting up guideline limits by regulatory body. This approach might benefit the oil industries more as well as the regulatory bodies because it would be a pointer to the acceptable limits. However, in terms of the effect on plants' development, a more realistic approach would be to monitor biological indicators such as germination and growth, because of its relatively rapid response. These factors were investigated in § 3.6.

3.5.1.5 Gas chromatography/ flame ionisation detector (GC/FID) - model Agilent 7890A

Principally, a GC has three components namely the injector, column and detector. The sample goes through the injector. On-column injection entails injecting the sample straight into the column. The column shows separation of the components as they come out on the detector. The modes of introduction of sample could be as split or splitless depending on the concentration. In the split injection technique, only a fraction of the vaporized sample is transferred into the column. The reminder of the sample goes out from injection port through the split line vent. In splitless injection technique, most of the vaporized sample is transferred to the head of the column.
The flow rate of the carrier gas (otherwise called mobile phase) is also important. If it is too fast, all the analyte goes into the detector and so no true separation occurs in the column. If it is too slow, the component will interact more and may even form permanent bonds in the stationary phase, which might be physical or chemical. Secondly, the flow rate can affect the total run time. Usually, nitrogen, helium and argon act as carrier gases because they are inert gases.

Temperature is an important factor in using GC/FID given the injector is set at a particular temperature so the analyte can vaporize to enhance its movement by the carrier gas. The lowest boiling point of the analyte normally determines the temperature and so increasing the oven temperature is a gradual process. The detector is set at a temperature higher than the oven (at least 10^oC higher) to allow the analyte maintain its vapour phase. The potential difference is converted to signal and read off from the workstation (a computer).

The chromatogram is representative of the relative concentration of compounds present in the analysed oil .The concentrations could be determined using time of separation or peak areas of the individual components. The chromatogram used in the analyses is on time of separation. Crude oil is a mixture of different compounds and so the components that have less interaction will come out first while those that have more interaction will come out later and read as the peak. Other operations we considered in using GC/FID are:

1. Process of calibration

This involves using n-alkanes of known concentrations (standards) to determine the retention times and peak areas of the individual constituents, which forms a library. The library serves for comparison of retention times of individual target compound in the sample. The first peak that comes out is that of the solvent that dissolved the analyte and for this reason; the solvent used should be inert to the sample whereas the sample should dissolve totally in the solvent. Low boiling point liquids serve as better solvents. Chromatograms with calibrated peaks for the external standard used in the analyses are presented in Figure 3.2 and 3.3.

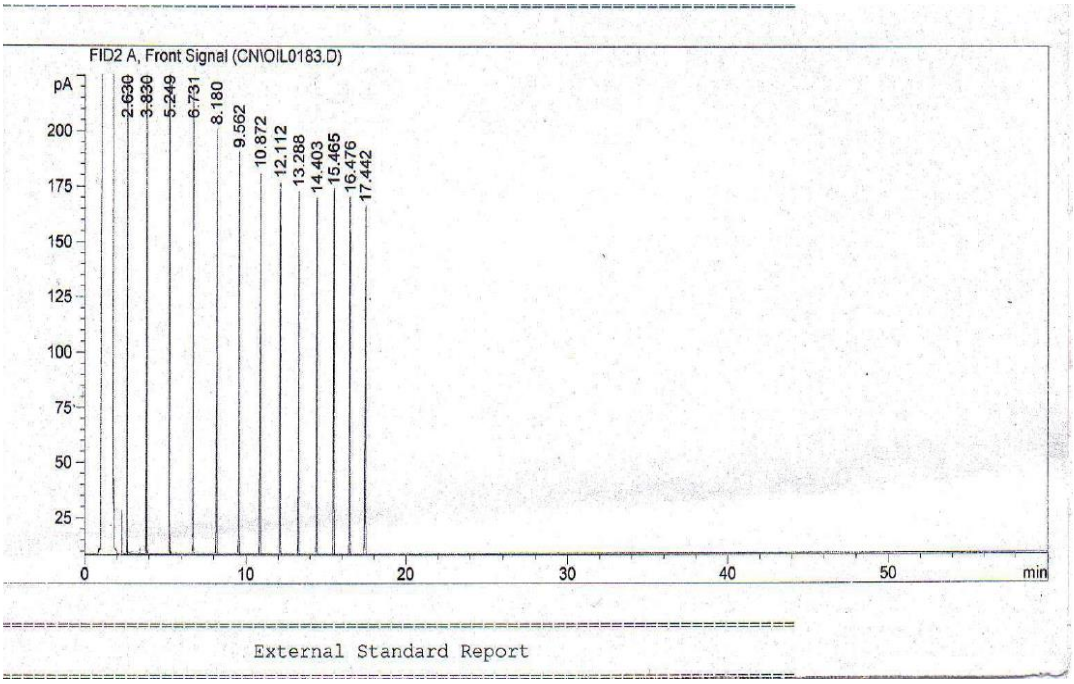

Figure 3.2: Two-part calibrations (C_8 – C_{20}) of n-alkanes standard

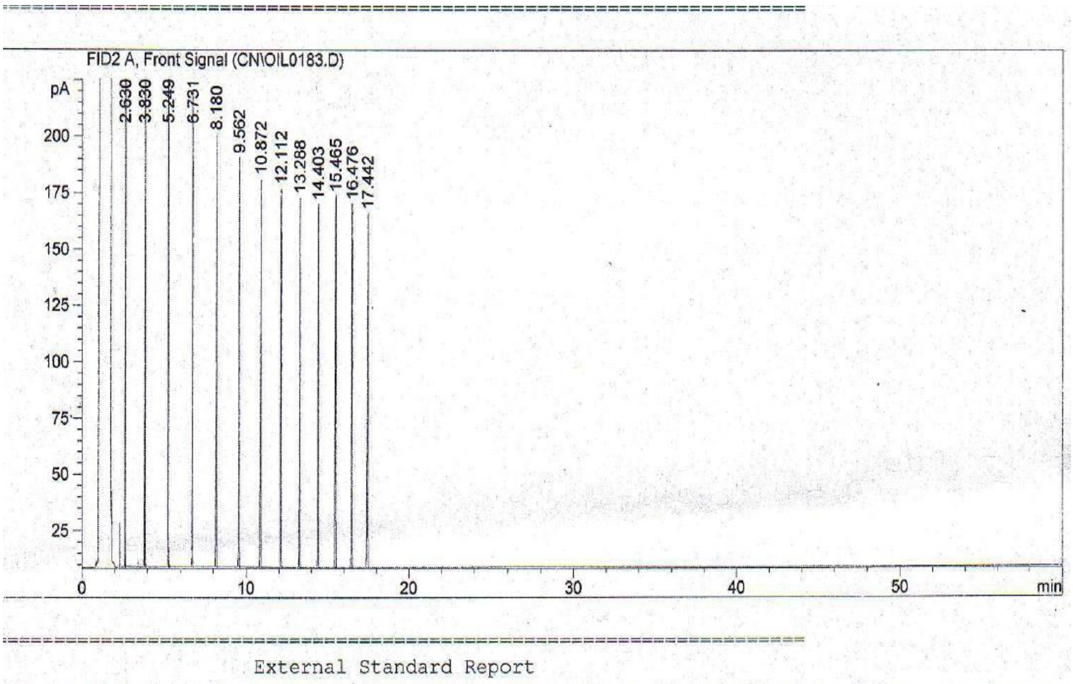

Figure 3.3: Two-part calibrations (C_{21} – C_{40}) of n-alkanes standard

2. GC/Oven program

To arrive at standard procedure, different oven conditions and injection volumes were investigated using split and split less mode. Among factors that could influence the results, are compounds co-eluting and not coming out of the column (i.e. sticking to the column) due to inadequate temperature variation between the oven and the column. A splitless injection of two micro litres was used and the oven conditions as follows; the column temperature was set at 40°C and held for 0.5 minutes, and increased at 10°C /minute to 290°C, and held for 34.5 minutes giving a total run time of 60 minutes. The carrier gas was helium under constant pressure mode but programmed to achieve a flow rate of 1mL /minute. The column specification is Perkin Elmer Elite (DB 5, 30 m long, and 0.25 mm internal diameter).

3. Hydrocarbon Extraction

The use of cartridge against silica-column gel cleanup method (used during preliminary investigation) was preferred because it saves time and has a higher recovery rate with hydrocarbons. ISOLUTE EPH was the brand of cartridge used and is manufactured by Biotage®, Sweden. The basic principle of the ISOLUTE EPH solid phase extraction (SPE) column is the separation of the sample into aliphatic and aromatic hydrocarbons. This is achievable by the use of different solvents as suggested by the manufacturer. Hexane was used to elute the sample for the separation into aliphatic hydrocarbons, which were compounds of interest in this study. The ISOLUTE EPH 5.0 g 25 ml cartridges were used for cleaning of the extracts. Cleaning up involves conditioning the column with 20 ml of hexane before adding the filtrate, after which it was washed out using 12ml of hexane. The cleaned up samples were concentrated using rotary evaporator to approximately 1ml.The concentrated extract was analysed using gas chromatography.

In other to reduce any systematic errors with the cartridge, the sample was cleaned with dichloromethane (DCM) which was the solvent used for the aromatics fraction. The results were compared with that of n-alkanes. The aim was to confirm that the use of hexane as recommended by the manufacturer did not yield compounds other than n-alkanes. However, due to the unavailability of the rotary evaporator in the latter stages of the research, this method was modified and a spectrophotometre (BIOMATE 3-

Thermo Scientific) was used to measure total petroleum hydrocarbons (TPH) in the soil samples (Osuji and Adesiyan, 2005).

4. Recovery Rate

The purpose of defining the recovery rate was to determine the oil recovery efficiency of the method. Three different extractions were done at the threshold value of 5,000 mg/kg soil and the average recovery rate gave 60%, hence a factor of 0.6 was built into the amount of crude oil used in spiking the soil to enable us recover 5,000mg oil/kg. In other words, one kilogram of soil was spiked with 8,300 mg oil. The assumption is that a sample taken for standard analysis would report a value around 5,000 mg/kg, the limit used in Nigerian legislation. Probably, some oil adsorbed to the organic matter in the soil to give the 60% recovery.

5. Preparation of sample used for recovery

A kilogram batch of air-dried soil previously screened to pass 2.36 mm sieve was weighed out. This quantity of soil was mixed with 5000 mg of bonny light crude oil (w/w). The oil was dissolved in 250 ml of hexane before mixing it with soil to achieve a uniform distribution. It was mixed homogeneously in a bucket with a tight lid. The mixture was air dried in the fume cupboard with the fan on to drive off the hexane. Using the quadrant method, three sets of spiked soil (20 g) was weighed out from the mixture. The different volume of hexane used for the extraction was by random selection as shown in Table 3.6. Each volume of hexane was mixed vigorously with the spiked soil. The procedure was repeated on the three replicates. After ten minutes, it was filtered through using Buchner's flask and funnel and volume measurements taken.

Table 3.6: Volume of extract used to determine recovery rate

Sample weight (g)	Volume of hexane (ml)	Final volume of extract (ml)	Unaccounted volume lost (ml)
20	30	12	18
20	50	32	18
20	110	93	17

6. Rate of biodegradation

This was studied as a function of time. Sampling immediately after spiking (day 0) was used to estimate the initial concentration. TPH was extracted using 10ml of acetone/hexane mixture at 1:1 volume, however the fraction containing aliphatic hydrocarbons was separated using ISOLUTE EPH cartridges and analysed accordingly. The identification of hydrocarbons of interest was based on their retention time and by comparing them to those of external standard used.

7. Modification of method for measurement of TPH

As earlier pointed out in (§3.5.1), the method used for the extraction of hydrocarbon was modified due to the unavailability of a rotary evaporator for the purpose of concentrating the samples before analysing with the GC. In subsequent experiments during the growth trials (§ 3.6), the hydrocarbon content was extracted using a spectrophotometer, the method adopted by (Osuji and Adesiyan, 2005, Adekunle, 2011). In their method, twenty grams of sample material was dissolved in 50 ml solvent mixture of (1:1) acetone: Dichloromethane. The extract was passed through silica column gel (2 g) capped with 1 g sodium sulphate ($NaSO_4$). One millilitre of the extract was diluted with nine millilitres (9ml) of the solvent mixture. The extracts were placed in cuvette wells and hydrocarbon contents in the samples were calculated after reading optical density of the extract at 425 nm in a spectrophotometre (BIOMATE 3 Thermo scientific). The hydrocarbon contents were determined from the calibration curve (Figure 3.4) by plotting absorbance against concentration obtained from dilute standard solutions of bonny light crude oil and multiplying by the dilution factor and the linearity of the curve id defined by 0.807.

The TPH in soil extract obtained from the calibration curve was related to soil sample weight and expressed as mg/kg. By relating the difference between the initial and final concentrations of TPH in the soil, the biodegradation efficiency for TPH was calculated.

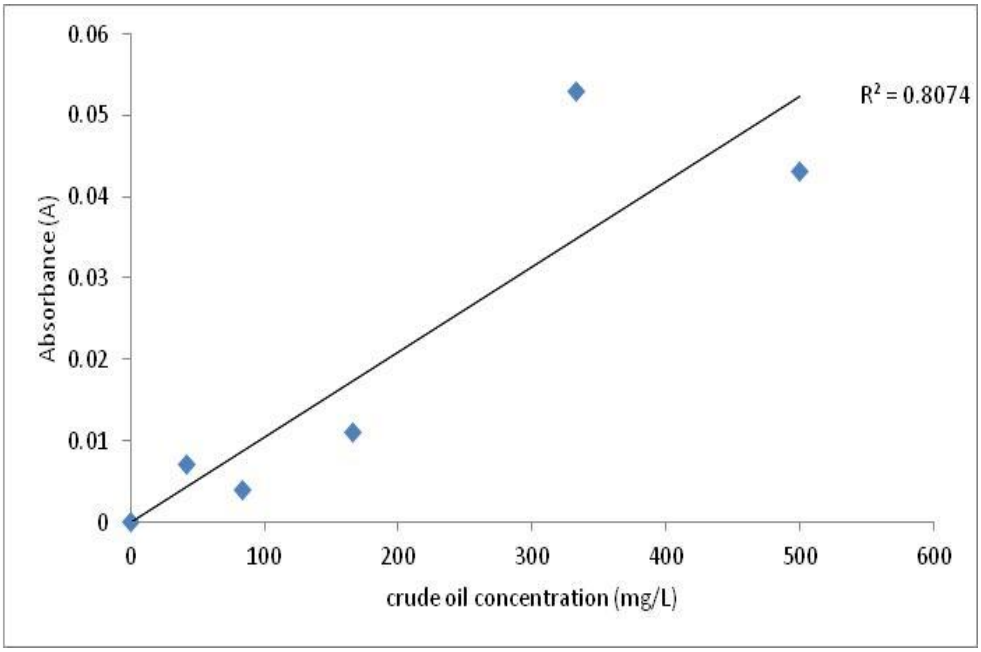

Figure 3.4: Calibration graph for the determination of soil total hydrocarbon using UV-visible spectrophotometry

3.5.2 Physico-chemical analyses

3.5.2.1 Moisture content

Moisture content (% ww) was determined as the weight loss after the sample (10 -20g) had been dried in an oven to constant weight at 100-110°C for 24 hours (Fogarty and Tuovinen, 1991). The oven model was Gallenkamp Hotbox oven with fan size 2.

3.5.2.2 Volatile solids

Volatile solids content (% dw) was determined by the weight loss after ignition of the oven dried sample in a muffle furnace (Carbolite AAF 1100) at 550°C for 2 hours according to standard methods (APHA, 1992). The volatile content is measured as the dried residues of the sample after organic material are burnt at temperature of 550°C.

3.5.3 Chemical analyses

3.5.3.1 Total carbon

Flash combustion (Thermo Flash EA 1112 series) was used for the analysis of samples for carbon and nitrogen. Dried samples (2 to 3 mg) were homogenized and weighed into a tin capsule. The tin capsule enclosing the sample falls into the reactor chamber (at

900°C) where excess oxygen is introduced. The gaseous mixture is passed through the column for separation and is analyzed The quantities are detected using a Thermal Conductivity Detector and then compared with standards to determine the percentages of carbon and nitrogen.

3.5.3.2 Total nitrogen

Total nitrogen was determined by the combustion method as described in § 3.5.3.1, however, during the growth trials (§ 3.6), TKN was determined by the Kjeldahl digestion technique at the start and end of the planting periods. Depending on the anticipated nitrogen, 50g sample was dissolved in 500ml of distilled water and 50ml of the solution was digested using Buchi Distillation unit and then followed by titration. The formula below was used for calculation;

Mg TKN = (A –B) X 280/V

Where A= volume of titrant for sample

B = volume of titrant for control

V = original volume of sample

3.5.3.3 Phosphorus

Phosphate-phosphorus was measured in soil extracts by the ascorbic acid reduction method (Ekundayo and Obuekwe, 1997). A calibration curve was obtained by measuring the absorbance of dilute phosphate standard solutions from the spectrophotometer at a wavelength of 880nm. By plotting absorbance against concentration, a calibration curve was obtained (Figure 3.5), defined by linearity 0.922. This test was only carried out during growth trials (refer to § 3.6) to estimate phosphorus in both uncontaminated and recovered soil.

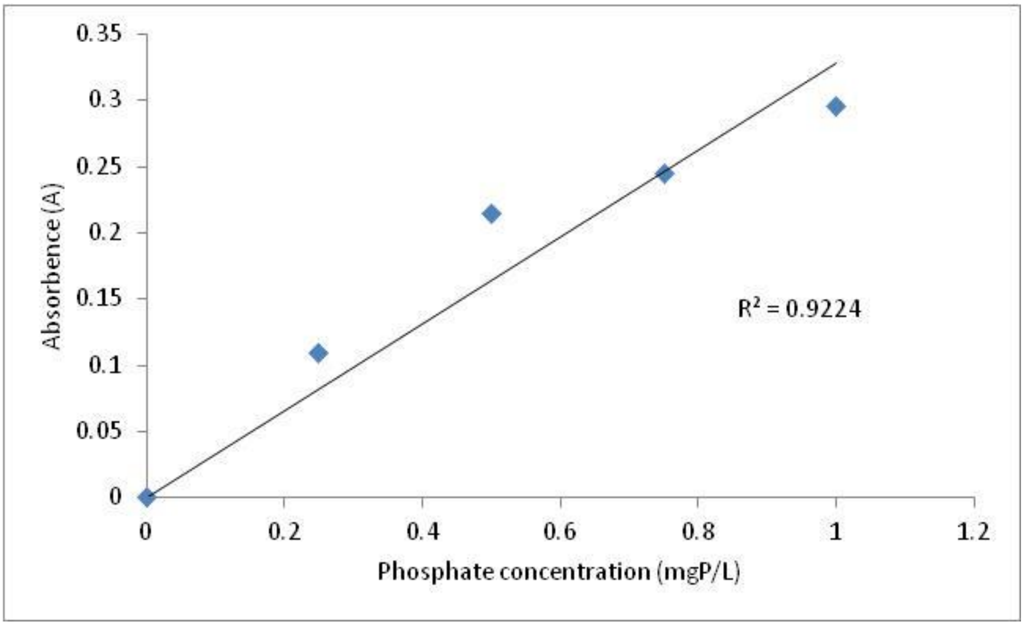

Figure 3.5: Calibration graph for the determination of phosphate using UV-visible spectrophotometry

3.5.3.4 pH and Electrical conductivity (EC)

Generally, pH affects both biological and chemical processes. The pH and electrical conductivity of the soil/compost extracts were determined according to standard methods (APHA, 1992). This was done using calibrated HQ 40d portable pH, conductivity meter manufactured by HACH, U.S.A. Ten grams of the sample was placed into a 200 ml beaker, and then 100 ml of distilled water was added and mixed using a mechanical shaker at medium speed for fifteen minutes (dilution 1:10). Each probe was inserted into the beaker and the values read off the instrument once it had stabilised. The pH meter was calibrated with standards at pH 4 and pH 7. A higher value of electrical conductivity is associated with high concentration of dissolved salts. The values of electrical conductivity are in micro siemens per centimetre (µS/cm).

3.5.4 Microbiological analysis

The aim was to determine the total bacterial population in feedstock as well as monitor the effects of crude oil on microbial activities. This analysis was a one off experiment during the growth trial 1. The total heterotrophic bacterial population was counted once a week for the period of 5 weeks. About one gram of sample (compost/soil) was weighed into 10 ml of ringer’s solution forming a suspension. Serial dilution was 10-

fold dilution, 0.1 ml of each dilution was measured out and the suspension was spread on nutrient agar plates using wedge-shaped spreader. Working from the least dilution, it was spread evenly on the entire plate using the spreader, and the plates were labelled according. (Adekunle, 2011).

The plates were kept in an upside position and incubated overnight at 37°C. We made use of a colony counter to count the different colonies. Plates between 30 – 300 colonies were counted while plates with less than 30 colonies or above 300 colonies were discarded. The colonies were multiplied using the dilution factor to obtain the number of bacteria per gram of soil and expressed as CFU/g (i.e. colony forming unit per gram of dry weight).

3.6 Growth trials using tomato plants

As discussed fully in chapter six (page 118), the use of chemical analyses to identify environmental risks associated with hydrocarbons does not necessarily show the impact of crude oil on plants. This is a major concern of the local farmer in the Niger Delta region, as he wants to go back as soon as possible to the contaminated land for farming which is the source of his livelihood. Therefore, in terms of the effects of oil spillage on plants' development, a more realistic approach would be to monitor biological indicators such as seedling germination and growth and biomass yield of plants. The level of oil at 5000 mg /kg soil (0.5% w/w) is the limit used in Nigerian legislation for identifying oil-contaminated land but at this relatively low level, it is unlikely to affect many of the indigenous plants in the Niger Delta. In this research, contamination values much above this level were investigated ranging from 0, 5, 7.5 and 10% (soil weight) as this was found to better reflect the actual situation in the Niger Delta.

A number of workers have proposed use of indices such as seed germination and growth of vegetable crops on crude oil contaminated soils as evidence of recovery of the crude oil impacted soil. For example seeds of tomatoes (Poulik, 1999), water cress (Nwachukwu, 2001), hot pepper and tomatoes (Anoliefo and Vwioko, 1995), and a variety of other plants (Udo and Fayemi, 1975, Ekundayo *et al.*, 2001, Adekunle, 2011) have been investigated. In this study, germination of tomato seeds were used because of their relatively rapid response.

It seems that contaminated soil at the lower level of 5,000 mg/kg can be treated using mineral fertilizer given results from previous investigations on the Niger Delta as shown in Table 2, however this is not the case at the inhibitory level of 5 – 10% (w/w) oil contamination. The growth trial was to evaluate the impact of compost at these inhibitory oil levels. Different mix ratios of compost were applied to crude oil contaminated soil. Growth trials with tomato seeds (Solanum lycopersicum) were used to assess the impact of compost by evaluating parameters such as germination, plant height and biomass yield.

Material preparation and sampling procedure: Topsoil, not previously exposed to oil contamination was passed through a 2.36 mm sieve. Compost was also screened through a 6.3 mm sieve to remove large particles. Soil was spiked with bonny light crude oil at different oil levels ranging from 0 - 10% w/w. This was done by adding 0 g, 50 g, 75 g and 100 g of crude oil in trays containing 1000g of sandy loam soil. Each quantity of crude oil was mixed thoroughly with the soil using hand and divided into pots each containing 500g of spiked material depending on the mix ratios.

The treatment comprised compost at different mix ratios. Duplicate trays were set up for each treatment along with the controls (no oil). The controls were either soil only, compost only or compost/soil. Approximately 6 - 10 tomato seeds per treatment were grown in plastic trays measuring 220 (L) x 170 (W) x 50 mm (deep). Seeds were planted at a depth of about 10mm. After planting the trays were covered with plastic sheet (Plate 3.3) and the sheet was taken off after 70% of the control germinated based on the report of British Standards Institute (BSI) for assessing plant response in composted material (Bsi, 2005). The analytical characteristics of the starting mixture for the different experiments in growth trials are presented in the result section (Table 6.1 pg. 118).

The percent germination was calculated as the sum total of number of seeds that germinated after 14 days (Njoku *et al.*, 2008), and the value "0" given to non-germinated seeds.

$$\% \text{ germination} = \frac{\text{number of seeds that germinated}}{\text{number of seeds grown}} * 100$$

The plant height was measured at the end of planting using a metre rule and the biomass of the plant was determined by measuring the above ground biomass of the plants using a weighing scale (model – Mettler PJ 3600 Delta Range®). The different stages in the germination and development of the tomato seedlings are shown in the result section (chapters 4 - 6).

3.6.1 Determination of the effects of BLCO on tomatoes planted in soils contaminated at 10% oil level (w/w) using two compost mix (scoping study 1)

Preliminary assessments were made to determine the impact of Bonny light crude oil on agricultural topsoil This was to allow for structured experiments during the research. . The preliminary experiment helped to establish the oil inhibitory level. In this preliminary experiment, the light bulbs in the laboratory provided a daily cycle of 12-hour illumination to the seedlings. However, improved light source was used during subsequent tests (refer to Plate 3.4 on pg. 98). The improved light in the indoor growth chamber consists of four mercury lamps suspended in an open topped- frame. The lamps provided a daily cycle of 16 hours of illumination and 8 hours of darkness to reflect the longer daylight experienced during summer. The experiment lasted for 28 days (01/05/2012 – 28/05/2012) and the experimental design as follows:

I. Soil without oil (soil)
II. Soil + oil at 10% oil level w/w (soil 10%)
III. Compost without oil (compost)
IV. Compost + oil at 10% oil level w/w (compost 10%)
V. Compost/Soil mix 1:1 at 10% oil level (50 % compost)
VI. Compost/Soil mix 1:3 at 10% oil level (25% compost)
VII. Compost/Soil mix 1:1 at 10% oil level (50 % compost)
VIII. Compost/Soil mix 1:3 at 10% oil level (25 % compost)

At the end of incubation period (28 days after planting), biomass was measured as yield above ground. The oil contamination level refers to the soil before the addition of any compost. In reality, contamination levels will be diluted.

Observation

The soils in the different pots dried up and this made the soils crack maybe due to low moisture level because at this stage wetting was done every two days.

3.6.2 Determination of the effects of BLCO on tomatoes planted in soils contaminated at 10% oil level (w/w) using two compost mix (scoping study 2)

In this experiment, compost was applied at the rate of 25 and 50% as in § 3.6.1 however, wetting of the plants was on a daily basis. The start date was 01/06/2012 and lasted for 28 days. Germination in the treatment trays picked off seven days after planting but in the previous experiment, the seedlings did not germinate until 14 days after planting.

3.6.3 Determination of the effects of BLCO on tomatoes planted in soils contaminated at 0, 5, and 7.5% oil level (w/w) using one compost mix ratio (Plant growth 1)

For this study, contamination levels much above the intervention values of 5,000 mg/kg soil given by the Nigerian government was applied as this reflected better the actual situation in the Niger Delta. Spiking of topsoil with crude oil was at the following concentrations 0, 5, and 7.5% w/w in other to evaluate the effects of oil on the tomatoes and the efficacy of compost in the degradation of soil TPH. The experiment started on 04/07/2012 and lasted for 35 days

Material preparation and sampling procedure: the mix ratio is made of one part of compost to one part of contaminated soil (1:1) referred to as 50% compost and the controls were (a) soil, (b) compost and (c) compost/soil (without oil) 1:1. Watering of the plants was daily and physico-chemical parameters (such as pH and electrical conductivity) measured once a week throughout the planting duration. Enumeration of total heterotrophic bacteria was also carried out, according to the methods described in § 3.5.4. At the end of planting period, biomass was measured as yield above ground.

3.6.4 Determination of the effects of BLCO on tomatoes planted in soils contaminated at 0, 5, 7.5 and 10 % oil level (w/w) using three compost mix ratios (Plant growth 2)

This experiment was more of a confirmatory investigation on the effects of crude oil on plants at all the oil levels considered in this study (0, 5, 7.5 and 10% w/w), in addition to determining the efficacy of different compost mix ratios as a treatment for the contaminated soil with respect to the degradation of soil total petroleum hydrocarbons. The mix ratios of compost to contaminated soils are one part of compost to one part of contaminated soil (50% compost), one part of compost to three parts of contaminated soil (25% compost), and three parts of compost to one part of contaminated soil (75% compost). In reality the addition of compost will dilute the contamination levels. In the experiment, the controls are compost/soil 1:1; compost/soil 1:3 and compost/soil 3:1. The start date for the experiment was 17/09/2012. At the end of planting period (35 days), biomass was measured as yield above ground.

3.6.5 Determination of the effects of BLCO on tomatoes planted in soils contaminated at 0, 5, 7.5 and 10 % oil level (w/w) using two compost mix ratios (Plant growth 3)

In this experiment, topsoil used was from the Niger Delta, Nigeria. It was more of a comparative study to understand the effects of oil contamination from same petroleum products on different soils. The mix ratios of compost to contaminated soils are 1:1(50% compost) and 1:2 (33% compost). The controls were soil only, compost only and compost/soil. We obtained topsoil from Bardsey, Leeds as well (the use of soil samples from the Niger Delta was to make the research relevant to the region). The experiment, which took off on 15/01/13, lasted for 35 days after which biomass were measured as yield above ground.

3.7 Data analysis and Statistics

The germination was expressed as percentage of the control Analyses of variance for physico-chemical parameters were performed and correlation were used to identify significant variations and relationships (Adekunle, 2011). The results were analysed using the a software for statistical package known as IBM SPSS Statistics 19.

Plate 3.3: Day 1 showing the trays covered with plastic sheet to reduce any interference with germination. The plastic sheet was removed after 70% germination was observed in the control trays

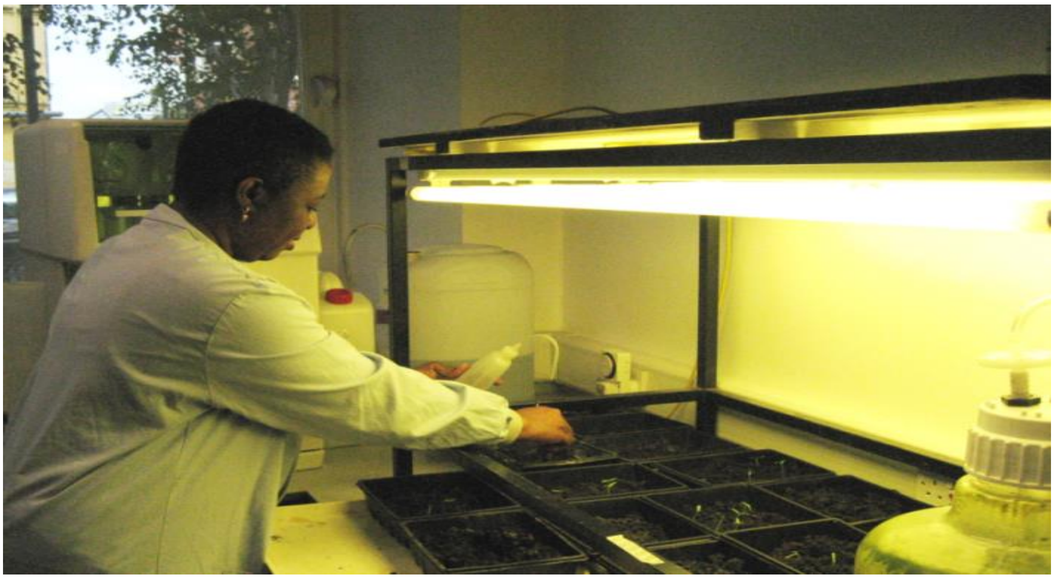

Plate 3.4: The light chamber used for the growth trials and the researcher monitoring the experiment

4 Results and Discussions (respiration tests)

4.1 Introduction

The results in this experimental work are reported chronologically to present the flow of ideas and thoughts during the course of the research. However, we reviewed results and as ideas developed, some new tests were introduced and a number of modifications made to others. There were variations in the materials' composition because sourcing of the materials was from different locations. Chapter 3 explains the philosophy behind the methods adopted in this research, consequently the sets of parameters analysed differed from one experiment to the other. However, as suggested in the literature some basic tests such as moisture content, volatile solids, pH, and electrical conductivity were analysed for all the feed materials in order to characterise them. Where not shown in the core of the thesis, the results are shown in the appendices.

4.2 Respiration tests

The objective of the tests is to determine the biodegradability of bonny light crude oil by relating changes in carbon dioxide production as a function of time to allow for monitoring microbial activities (respiration). Summary of experimental details are in § 3.3.2 and 3.3.3. The two temperatures (35°& 45°C) applied were chosen since the 35°C was expected to reflect daytime temperature in the Niger Delta region and 45°C to reflect extreme temperature. Crude oil was used as the hydrocarbon of interest because whenever there is oil spillage in the region, it is the raw crude oil that contaminates the soil simply because most of the oil wells in the region are located onshore (right on the farmland as previously shown in Plate 1.2 pg. 14). Bonny Light Nigerian crude oil is light with low sulphur content (0.19%). It contains both saturated aliphatics and aromatics with typical composition of approximately 57% n-alkanes, 29% aromatics and 14% resins and asphaltanes by weight (Odeyemi and Ogunseitan, 1985). This high percentage of n-alkanes contributed largely to studying the degradation of this group of hydrocarbons during the current research § 3.4.

4.2.1 Effects of compost on degradation of hydrocarbons in Bonny light crude oil at 35°C

The purpose of using DR4 was to relate carbon dioxide production and microbial activity to the degradation of the hydrocarbon in crude oil. However, this method is sensitive to (1) gas leakages and (2) using the appropriate strength of alkali for absorption of CO_2 produced. Even though we took care in setting up the apparatus, the results (Figure 4.1 – 4.4) obtained showed variation in the volume of CO_2 produced, thus we can relate carbon dioxide production to microbial activities however, the CO_2 production is not a good indicator of the degree of microbial degradation of crude oil in soils. Due to this challenge, the results from the second experiment at 45°C was not analysed as a result of the inconsistency in carbon dioxide produced.

The values used for the daily CO_2 production were from the first CO_2 collector (trap bottle) because of the negative values observed when the control value was deducted from the sum of carbon dioxide from both collectors. Perhaps the negative values show the inconsistency of CO_2 production during the breakdown of oil. This factor could suggest that CO_2 production may not be an adequate tool to measure the biodegradability of the oil. However, for the graph representing the cumulative CO_2 production (Figure 4.4), values from both traps were used. Respiration is a measure of the readily available biodegradables in a substrate. There is an observable difference in terms of respiration between the samples at different oil levels, though the negative values were more noticeable at 2 g oil level. At these low levels of oil concentrations, it is possible that the carbon dioxide produced was low when compared to that from the control and so the variations is regarded as background noise. This variation could explain the negative values in CO_2 production earlier observed, even though the values were regarded as relative numbers in plotting the graphs.

Secondly, because oil is slow to degrade and given the low concentrations at which oil was applied (0.5, 1 and 2% w/w of compost respectively) maybe the CO_2 production was not noticeable. The same control value of CO_2 was taken out of all the test reactors. For the different values of CO_2 produced within the reactors, the values of the blank were taken away so as to measure the amount of CO_2 produced from the oil i.e. to account for CO_2 from other sources such as other indigenous soil organic (Alexander, 1994). Even though from the graphs we can assume a continuous production of CO_2 however, CO_2 evolution is not a conclusive tool in this case to measure the degradability of the crude oil. This conclusion is applicable to Trial 2 as negative values were also recorded. An estimate of CO_2 production rate of soil is given as 216 to 360 mg CO_2 /m^2.day (Wickland *et al.*, 2010). This is empirical relationship and depends on soil temperature and moisture content. Some of the tests at this stage were not very definitive and so as we work through, some new tests were introduced.

4.2.1.1 Visual

Visual examination of the reactors at the end of the eight days revealed that the material remaining in the reactors was soggy. Perhaps, this relates to the tendency of fresh compost having more biodegradable material. As it continues to degrade there are changes in structure of the organic material causing the pore spaces to collapse. As a result, less gaseous exchange will take place and there is tendency for water retention, which could make the feedstock soggy in appearance.

4.2.1.2 Physico-chemical parameters

As mentioned in § 3.5.1, physico-chemical parameters such as moisture content, volatile solids and C/N ratio were only measured for the feed material at the start of the experiment. We presume that opening the reactors could introduce variation in the CO_2 reading either by allowing in atmospheric CO_2 or escape of the CO_2 generated.

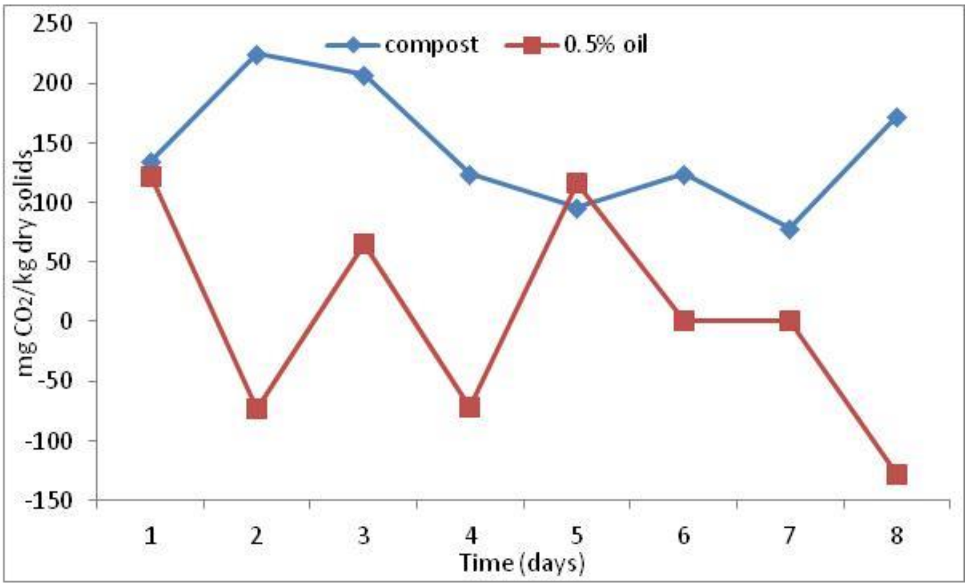

Figure 4.1: The daily CO_2 productions over a period of 8 days at 0.5% oil w/w (35°C)

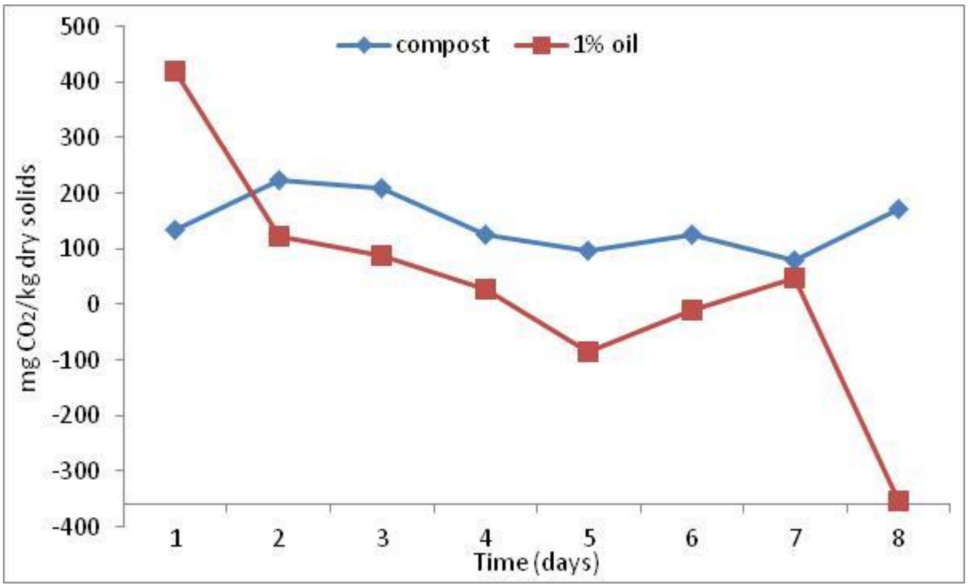

Figure 4.2: The daily CO_2 productions over a period of 8 days at 1% oil w/w (35°C)

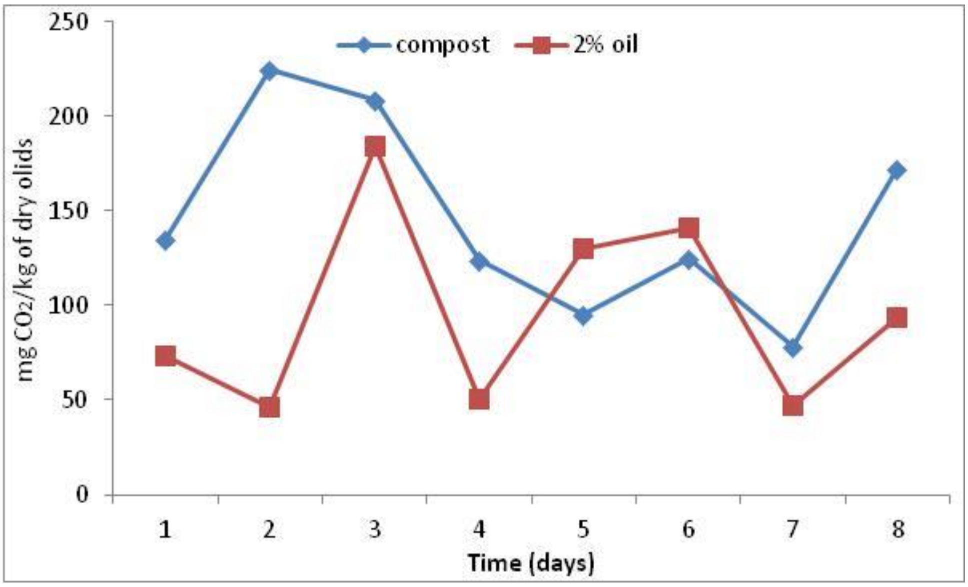

Figure 4.3: The daily CO_2 productions over a period of 8 days at 2% oil w/w (35°C)

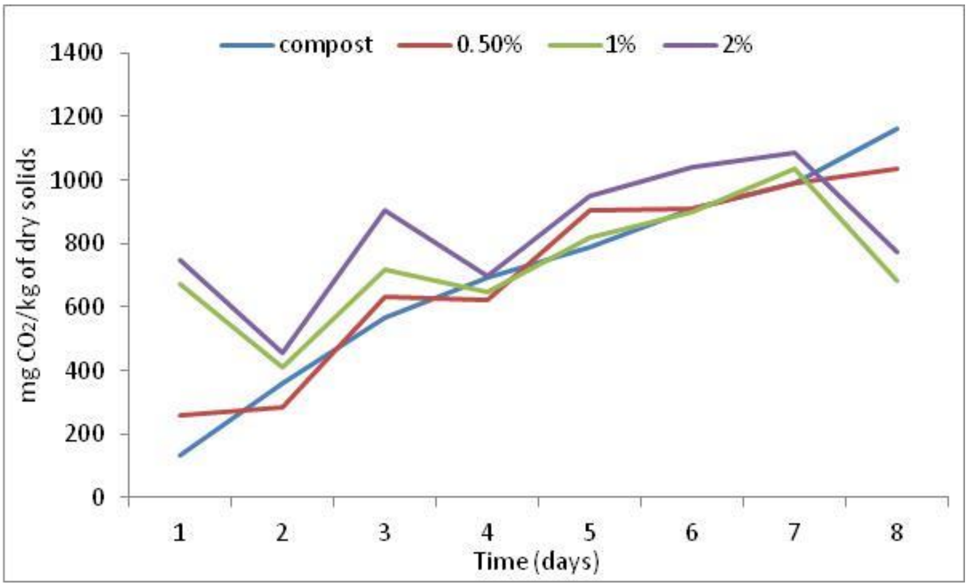

Figure 4.4: Cumulative CO_2 productions over a period of 8 days at 0.5, 1& 2% oil w/w and compost (control)

4.2.2 Degradation of Bonny light crude oil by evaluation of CO_2 production at 25% compost

Technically, this experiment fine-tuned the method for measurement of carbon dioxide produced during degradation of the crude oil based on the method proposed by Stotzky (1960). The modification was introduced due to difficulties experienced in using the DR4 apparatus of which have been addressed in § 3.3.1 and to allow for investigating the sensitivity of some selected parameters (moisture content, volatile solid and carbon-nitrogen ratio) for monitoring the condition of the biological process during the incubation period.

Carbon dioxide (CO_2) readings were taken every three days and the trap bottles containing the alkali changed. This was to make sure that the carbon dioxide does not saturate the alkali. The formula shown in Equation 4.1 was used to calculate the amount of CO_2 evolved from titrimetric data:

$$\text{Milligram C or } CO_2 = (B\text{-}V)\,NE \qquad (4.1)$$

Where:

B is the volume of acid required to titrate the alkali in CO_2 collectors from control vessel (ml)

V is the volume of acid required to titrate the alkali in CO_2 collector from treatment reactors (ml)

N is the normality of acid (1)

E is the equivalent weight. If expressed in terms of carbon, E is 6 or if expressed as CO_2, E is 22.

In this experiment, soil was spiked with oil at 0.5% (w/w) and compost, which served as organic amendment. The test condition was compost/soil 1:3 i.e. one part of compost to three parts of soil and the controls were soil without oil and compost without oil. Compost was added at the rate of 25% (w/w). The cumulative CO_2 production over time is shown in Figure 4.5. A higher CO_2 production was recorded in the reactor containing soil only. The high CO_2 production in soil probably would have come from the more active microbial population in the soil as shown by its higher respiration.

The CO_2 values from compost (control) were very low probably as a result of its low microbial activity due to its age (about 5 months old). Analytically, from the plot (Figure 4.5), we can say that probably the addition of such a low concentration of oil did not make much impact and so any difference in CO_2 production was just background noise. Soil without the addition of oil produced more CO_2 than at 25% compost (i.e. 50 g of compost to 150 g of spiked soil). In reality, the contamination level will be diluted from initial 0.5% w/w to 0.13% oil level, thus one can suggest that probably there was no oil breakdown or it was very negligible. Perhaps, the oil was not used as a substrate by the microorganisms, which therefore low microbial activities.

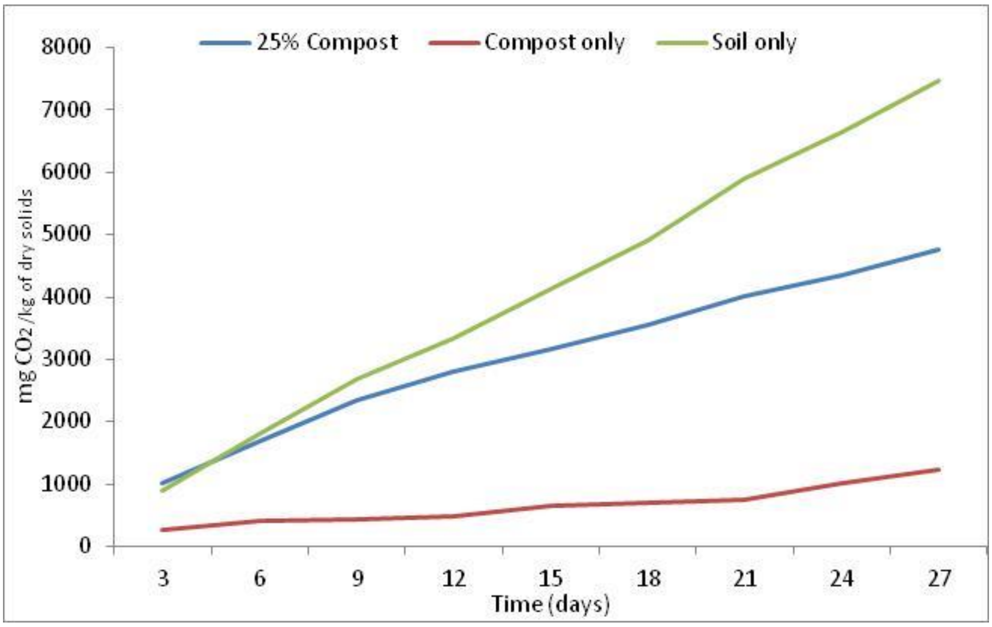

Figure 4.5: Cumulative CO_2 production at 0.5% oil (35°C) over a period of 27 days

4.2.2.1 Carbon dioxide emission

The carbon dioxide emission for the first three days was calculated using initial values of CO_2, (mg CO_2/kg of dry solids) produced from the test reactors (25% compost) and the controls (soil and compost without oil) because measurements were taken at three days interval during the experiment (values are shown in Appendix D). In order to determine the effecett of the oil on the production of carbon dioxide, a series of calculations can be carried out using the results from the 'control' experiments in which no oil was included. The results of the control test will show the amount of carbon dioxide produced by the soil or compost alone and this can be compared to the amount of carbon dioxide produce by the soil, compost and oil mixtures. An example of the calculation is shown below:

Example:

Ratio of compost to soil used	1 part compost: 3 parts soil
Mass of soils used	150 g
Mass of compost used	50 g
Amount of CO_2 produced by 200g soil	126 mg CO_2/kg ds
Amount of CO_2 produced by 150g soil	(126/200)*150 = 94.5 mg CO_2/kg ds
Amount of CO_2 produced by 200g Compost	23 mg CO_2/kg ds
Amount of CO_2 produced by 50g Compost	(23/200)*50 = 5.75 mg CO_2/kg ds

Therefore, summing together the combined CO_2 produced from the controls (i.e. CO_2 from 150 g of soil and 50 g of compost) = (94.5 + 5.75) CO_2/kg ds = 100.25 CO_2/kg ds

However the actual mg CO_2 produced from the test reactors is 147 CO_2/kg ds (averaged from the two reactors 254 CO_2/kg ds/2). Therefore, theoretically approximately 46.75 CO_2/kg ds is not accounted for by the soil and compost alone and may be attributed to the degradation of the oil.

4.2.2.2 Moisture content

The monitoring of the moisture levels in the four reactors used was done every two weeks through the incubation period and the variation is shown in Table 4.1. The initial moisture content and volatile solids of the feed material used in Reactors 1 and 2 were 29.4% and 38.4% respectively, below this level probably there will be a drop off in microbial activity. Therefore, the initial moisture level does not serve as optimum for microbial activity. Optimum moisture content in the range of 50 to 60 % is suitable for composting and other microbial activities (Tiquia *et al.*, 1996, Liang *et al.*, 2003).

Table 4.1: Moisture content and volatile solids for the four reactors

		Reactor 1	Reactor 2	Reactor 3	Reactor 4
Day 1	MC (% ww)	29.4	Do	Do	Do
	VS (% ds)	38.4	Do	Do	Do
Day 14	MC (% ww)	31.2	41.1	39.1	11.1
	VS (% ds)	23.2	24.1	30.9	22.5

†Reactor 1= Reactor 2 (25% compost i.e. one part of compost to three parts of contaminated soil), Reactor 3 = compost only and Reactor 4 = soil only.

An increase in moisture level (calculated as the percent of the initial moisture content of the feed stock) was observed two weeks after incubation, from 29.4% to 31.2% in Reactor 1 and 41.1% in reactor 2. This is attributable to the process of mineralisation, which produces water, and carbon dioxide leading to the suggestion that the initial high level of CO_2 produced at the early days of incubation could be from microbial respiration. Even though the two test reactors had the same contents and were exposed to the same experimental conditions, the increase in moisture level was not the same (Reactor 1 had 1.8% increase and Reactor 2 had 11.7%). This demonstrates that microorganisms could respond differently to the same environmental conditions. Liang *et al.* (2003) revealed that there is limited knowledge as to fully understanding the interactions between environmental variables during microbial respiration. The fluctuation in moisture content can affect microbial activities. The use of distilled water as humidifier in the experiment will presumably increase the humidity of the incoming air.

2. C/N ratio

The elemental analysis of carbon and nitrogen done once a week (for 3 weeks) was used to calculate the C/N ratio. As expected, compost had a higher C/N value than the soil, it ranged from 15.1 – 17.3 with an average of 16. The mean values of C/N ratio for the other feedstock over the three weeks were as follows, 14.9 for soil, 13.6 at 25% compost (Rep 1) and 15.8 for the second replicate. The variation in the C/N ratio (Figure 4.6) was probably due to the feedstock undergoing biological decomposition.

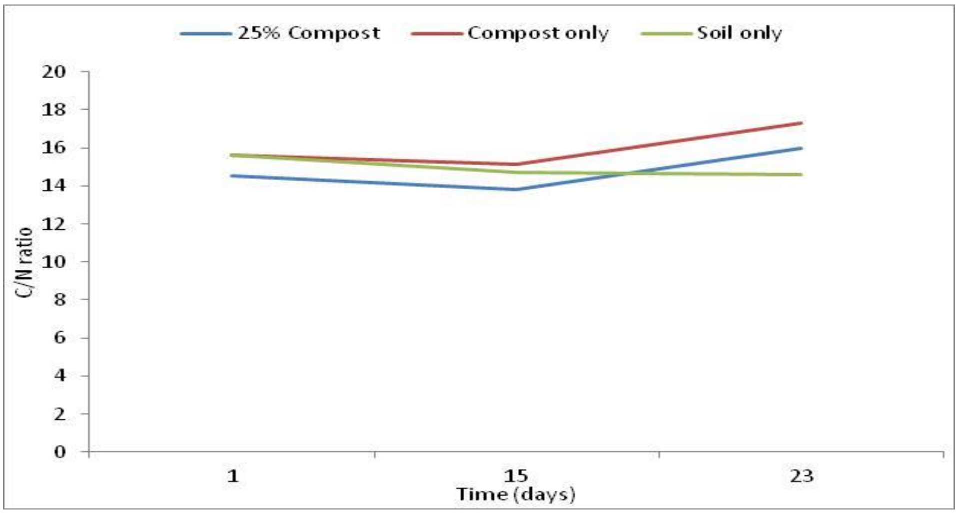

Figure 4.6: Carbon to nitrogen ratio of different reactors at 25% compost and 0.5% oil level w/w. The measurement was every two weeks.

4.3 Summary of the respiration tests

A number of observations were made during the different experiments on crude oil biodegradability such as the inconsistency in the trend of CO_2 evolved probably as a result of the complexities in measuring CO_2 produced especially with DR4 apparatus. It is also possible to have negative values if the CO_2 produced from the test reactors is seen as background noise due to its low concentration relative to the CO_2 produced from the controls (without oil). Another possible reason for this is inhibition of microbial activities due to the toxic nature of crude oil.

Other associated problems as noticed in the preliminary test were gas leakage from the reactors used and the interruption of compressed air supply in the laboratory. The compressed air used for aeration was sometimes turned off from the mains supply without prior notice. One of the challenges that could arise from lack of aeration in a closed system is anaerobiosis which could develop and produce CO_2 together with gases such as hydrogen (H_2), methane (CH_4). Accumulation of these gases in a closed system for a long term experiment could possibly retard microbial activities. The compressor supplying the air is not oil free even though it has in-built filters for cleaning. However, one cannot rule out a slight possibility of contamination by the compressor. With respect to strength of alkali used, there is no standard concentration. Franco *et al.* (2004) used 20ml of 0.5M NaOH in their reactor but Namkoong *et al.* (2002) used 25ml of 4N NaOH. The volume and strength of alkali used generally depends on how active the substrates or materials were. The concentration of alkali should be such that not more than two-thirds (2/3) of the alkali will be neutralized by the CO_2 collected in the trap bottle. In one of the experiments not reported, so much CO_2 produced was produced that all of it could not be absorbed by the NaOH perhaps the alkali was saturated. It might be that the compost used for that trial was very active. This demonstrates one of the many limitations of using CO_2 produced as a measure of hydrocarbon degradation. Even though the production of CO_2 was irregular when 2g oil was used, which is equivalent to 0.5% oil levels by soil weight. Odu, (1972) reported that very light contamination of 0.5% by soil weight stimulated CO_2 production.

We investigated as a side test the use of a solvita® kit, which provides a measure of the microbial respiration rate in contaminated soils (Woods End Laboratories, 2012). The solvita® kit is a simple test kit for the determination of the activity of a soil sample and was used to determine the CO_2 evolution levels of test materials using a colour code. It evaluates the level of CO_2 present after 4 hours of closed respiration without addition of air. The CO_2 colour chart provides ordinal number scale of CO_2 rates in eight colours representing a concentration range, with 8 indicating a low CO_2 level and 1 a high CO_2 level. The samples used were soil and compost spiked at different oil levels (0, 5, and 7.5%) w/w. The results obtained from the solvita® test kits are shown in Table 4.2.

The results show that the overall respiration rate (CO_2 evolution) of the soil samples were extremely low with the colour chart value of eight (8) for both soil without oil and at 5% oil level. At a Soil 7.5% oil level by weight, the colour code was seven (7). The fact that the colour chart value decreased from eight (8) down to 7 at 7.5% oil level shows slightly increased respiration rate which could be attributed to the higher oil level.

The overall respiration rates on addition of compost are slightly higher ranging from five to six (Table 4.2). This increased respiration could be due to the presence of the consortium of microorganisms in compost. Table 4.2 shows more information on the quantitative indication of how the, numerical relates to respiration rate.

The results obtained for the compost/soil mixture were similar to those of the soil alone although overall the values indicated a much higher respiration rate with values of 6, 5 and 5 for the control, 5% and 7.5% samples respectively. In general, soil without oil and soils contaminated at different oil levels had higher colour codes indicating lower respiration however, when compost was added it showed a lower colour code which means increased CO_2 production and hence an increased microbial activity

Table 4.2: Solvita® test results.

	Colour number scale	CO_2 rates indicated by Solvita® test	Test conditions (% oil w/w)	Colour code
High CO_2	1	> 20% CO_2	Soil 0%	8
	2	15%	Soil + 5% Oil	8
	3	7.5%	Soil + 7.5% Oil	7
	4	4%	Compost 0% Oil	5
	5	2%	Compost + 5% Oil	6
	6	1%	Compost + 7.5% Oil	6
	7	0.5%	Compost/Soil 0% Oil	6
Low CO_2	8	0.2 or ambient (typical soil levels)	Compost/Soil + 5% Oil	5
			Compost/Soil + 7.5% Oil	5

The use of the solvita® kit could be appropriate in the field as a quick way of determining respiration in any contaminated soils because it eliminates the laboratory experience. This could serve as an alternative to the DR4 for researchers given that the rig would be difficult to set up in the field due to the technicalities involved.

5 Degradation of Hydrocarbons

5.1 Degradation of hydrocarbons (n-alkanes) in contaminated soil at 20 and 35°C

In §3.4, two temperature levels (20 and 35°C) were used to investigate how temperature would enhance degradation of n-alkanes (C_8 – C_{37}) in bonny light crude oil using compost as an organic amendment. The incubation period lasted for 35 days; however, the extraction of the hydrocarbons was only possible for the first 14 days due to breakdown of the equipment.

The analyses of hydrocarbons were based on groupings according to the carbon length. Five fractions namely; C_8 – C_{10}, C_{11} – C_{15}, C_{16} – C_{20}, C_{21} – C_{25}, and C_{26} – C_{37} similar to Chang *et al.* (2011) who in their investigation of petroleum biodegradation using a pilot scale experiment summarised total petroleum hydrocarbon into four fractions based on the carbon length. C_6 –C_{10} represents the volatile fraction, C_{10} –C_{16} (semi-volatile), C_{16} –C_{34} (non-volatile) and C_{34} – C_{50} (heavier compounds). They also observed that the majority of the petroleum hydrocarbon contaminants were in the F2 (C_{10} –C_{16}) and F3 (C_{16} –C_{34}) fractions while the F4 fractions (heavier compounds) were present at trace levels.

The topsoil used was spiked with 8,300 mg of oil/kg soil. This level was to account for the recovery making it equivalent to a value of 5000 mg in the laboratory as stated in the Nigerian intervention value (§3.5.1). This low oil level represents the Nigerian intervention value even though in later experiments 10% w/w oil level was used which falls into the lower limit of studies by Ijah and Antai (2003) since they spiked at 10 – 40% v/w. During the preliminary experiment, we established 10% oil level (w/w) as inhibitory to plant growth and this formed the limit for the present study. Analysis of the hydrocarbon extracted after 14 days showed C_{21} – C_{25} to have the highest degradation (Nwankwo, 2012). Ijah and Antai (2003) observed that hydrocarbon components C_{14} –C_{32} were extensively reduced during field trials with Nigerian topsoil contaminated with 10 - 40% (v/w) of Nigerian crude oil.

Physico-chemical parameters of the different feedstock used for this experiment are shown in Table 5.1. The range of acceptable pH for crude oil polluted soil is generally

5.5 to 6.5 (DPR, 1991) however, in the present experiment, the pH of the contaminated soil was 7.17. From Table 5.1, an increase in pH was more noticeable on the addition of compost rather on the addition of the oil; perhaps, the oil level was too small for any noticeable change in pH to occur. In the later experiments when soils from the Niger Delta were introduced, the pH of the contaminated soils ranged from 5.8 to 6 (refer to chapter 6 pg 118). The increase in moisture level could be due to addition of oil and compost, because the moisture content for spiked soil only was 37.2% in comparison to soil without oil, which was 30%. Tolerance of organisms differs with respect to variation in pH and as such, pH is a key factor in soil-microbial interactions.as a result during one of the growth trials discussed fully in § 6.4.3, the pH was monitored over the 35 day planting period. The pH determines the availability of nutrients for plant growth and maintenance. A pH range of 6 to 8 has been reported as suitable to enhance microbial activity during biodegradation of crude oil contaminated soils (Gogoi *et al.*, 2003). Osuji and Adesiyan (2005) also reported that neutral to alkaline soil influenced the carbon mineralisation and breakdown of organic matter positively.

Table 5.1: Physico-chemical parameters of the different components used in the experiment for monitoring the effects of two temperature levels (20 and 35°C) on degradation of n-alkanes

Sample ID	pH	Moisture content (%)	Organic matter content (%)	C/N ratio	Density (kg/m^3)
Soil	7	30	23	13	1300
Compost	8.2	46	73	12.8	600
Bonny light oil	4.9				835
Spiked soil (with compost)	7.9	42.9	40.1	15	
Spiked soil (without compost)	7.2	37.2	23	13	

The organic matter content (OMC) of the spiked soil on addition of compost was 40.1% while the spiked soil without addition of compost was 23%. The addition of compost increased the OMC by 17.1%. Similarly, the C/N ratio increased on the addition of compost to 15 in comparison to spiked soil without compost, which were 13. One would have expected that the C/N ratio would have increased on addition of oil however; at an addition rate of 8300 mg/kg i.e. 0.8% by weight, it would not have been detectable.

The amount of oil which is 8300 mg/kg of soil added was after building in an average recovery rate of 60% to the threshold value of 5000 mg/kg. We had assumed the n-alkanes recovered to be same as the n-alkanes degraded because the recovery rate was consistent given the number of trials carried out. The method used for sampling was destructive analysis i.e. randomly picking samples and discarding the contents thereafter. The initial amount of n-alkanes in the spiked soil at 0.8% oil level was 652 mg/kg ds. The amounts of n-alkanes remaining after 14 days at the two temperature levels are shown in Figures 5.1 and 5.2. The choice of n-alkanes as hydrocarbon of interest is because Bonny light crude oil contains approximately 57% n-alkanes. At 35°C, the amount of n-alkanes remaining in the spiked soils with addition of compost (50% compost) was 16.12 mg/kg ds and the amount of n-alkanes remaining in the spiked soils without addition of compost was 50.4 mg/kg ds. We assumed that the big difference might have to do with the effect of compost on oil, which enhanced degradation. At 20°C, the amount of n-alkanes remaining in the spiked soils with addition of compost was 60.14 mg/kg ds and the amount of n-alkanes in spiked soils without addition of compost was 91 mg/kg ds. In general, there is increased degradation at 33°C and higher rate on addition of compost.

Although the amount of n-alkanes in the controls i.e. soil and compost without oil was negligible (1.33 mg/kg ds and 2.23 mg/kg ds respectively), the standard practice of taking away the control values from the test mixtures was applied. This suggests that the amount of n-alkanes from the organic materials in the soil and compost did not add much to the total n-alkanes remaining after 14 days incubation period. The amount of n-alkane remaining on treatment with compost was higher at 20°C (60.14mg /kg ds) than at 35°C (16.12 mg/kg ds) suggesting that higher temperature had affected the degradation rate of n-alkanes. Mesophilic organisms survive better at temperatures around 30 to 40°C and are known to have the metabolic capability to decompose a wider range of organic compounds (Lasardi, 1998). Probably at 35°C, the temperature had favoured higher degradation of n-alkanes than at 20°C and the use of matured and not aged compost may have helped the microbial population. Usually, the mesophilic bacteria dominate matured compost at maturity. Similarly, it is possible that the indigenous microbial population in the soil were mainly mesophilic organisms, and were more active at a higher temperature of 35°C resulting to more degradation of n-alkanes at 35°C than at 20°C.

Compost provides higher consortium of microorganisms that can feed on the hydrocarbon as substrate. The limitation to hydrocarbon degradation comes when respiration is low and the microbes have to choose a readily available substrate in other not to use up their little energy for anabolism; it is likely that compost will be the substrate of choice. To meet this challenge, there is need to work out the appropriate mix ratio of compost (organic amendment) to soil when treating contaminated land.

Igoni *et al.* (2007) reported that the Niger Delta region has an annual rainfall of 2,280 mm with a maximum temperature of 30°C. They identified the two main seasons as wet and dry seasons. The driest months occur from December to March. Given that the temperature in the Niger Delta region in the dry season is around 35°C, it might be better to carry out bioremediation treatment during the dry season rather than the wet season since it does not involve additional cost. The high annual rainfall would probably increase the moisture level, this could lead to lack of aeration due to the saturation of the air pores with water, and this could affect the performance of the aerobic microorganisms and the entire bioremediation process.

Biodegradation was observed in this study as oil was applied at 0.83% w/w, similarly Bossert and Bartha,(1984) reported that application of oil at 0.5 - 10% rate (w/w) encouraged extensive biodegradation. However, Leahy and Colwell (1990) reported that at 10% w/w or above 15% w/w oil contamination - an inhibitory effect on microorganisms occurs. This observation was true in later tests when oil was applied at a rate of 10% soil weight. Results from the growth trials (chapter 6) revealed total inhibition of plant growth at 10% oil level. The present results might also suggest that n-alkanes could be highly biodegradable. According to Leahy and Colwell ,(1990) the breakdown of hydrocarbon is more at higher temperatures due to increased rate of enzymatic activities. This is in line with most microbial processes where the rate of reaction increases with rise in temperature although limits apply depending on the microorganisms.

The heterogeneity of different crude oils also influences its biodegradability and that of its individual components. In this study, n-alkanes of C_{21}-C_{25} carbon length had the highest degradation followed by C_{16} –C_{20}. Microorganisms preferentially utilise certain types of molecules. In this case, perhaps compounds with long carbon chains is converted to short chains hence the reduction obtained in this group. This observation is in agreement with the findings of Ijah and Antai, (2003). They reported rapid degradation among alkanes of carbon length C_{14}-C_{32} on Nigerian light crude oil in contaminated soil after a period of 12-months. According to Atlas (1981), the size and type of hydrocarbon molecule play a key role in determining how they undergo microbial degradation. He revealed that alkanes C_{10} –C_{24} degraded faster but that very long chain alkanes are more resistant to microbial degradation. The n-alkanes in the range of C_{21} -C_{25} of the present study had the highest amount of degradation (refer to Table 5.2).

This reduction is attributed to biodegradation even though evaporation could have contributed. In this research, the loss of volatile fraction of the Bonny light crude oil was investigated. The results are fully discussed in § 8.1. However, as part of sample preparation, we exposed the spiked material overnight in the fume cupboard and presumably; the lighter fractions would have evaporated alongside hexane before the laboratory trials took place.

Table 5.2: Hydrocarbon reduction by chain length.

Carbon chain length	Amount of n-alkanes degraded (mg/kg)	
	35°C	20°C
C8 - C10	2.919	0.064
C11 –C15	50.742	14.641
C16 –C20	186.149	19.655
C21 – C25	384.792	44.248
C26 – C37	153.059	18.626

In summary, the addition of compost improved the degradation of n-alkanes especially the fraction C_{21}-C_{25}, also the result of the analysis showed differences for n-alkanes remaining at 20°C and 35°C. From this study, bioremediation of contaminated soil could yield better results if done during the dry season when the temperature is higher however, there is concern about moisture level, which could result in the soil drying out and inhibiting the action of the microorganisms.

Temperature has a significant effect on microbial activities and degradation rates. As earlier mentioned the amount of hydrocarbon biodegraded was estimated as the mass of n-alkanes remaining.

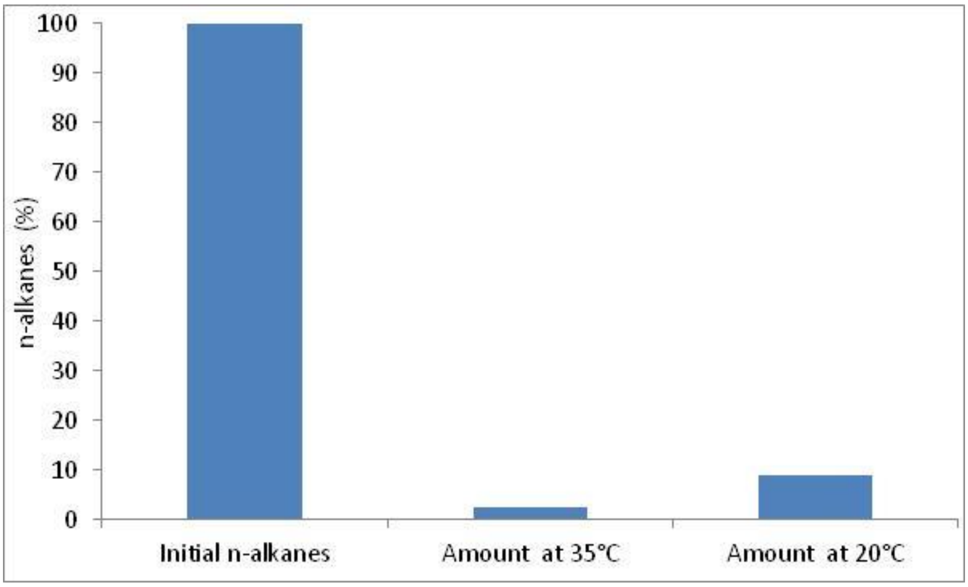

Figure 5.1: Amount of n-alkanes (%) remaining at 14 days in contaminated soil with 50% compost

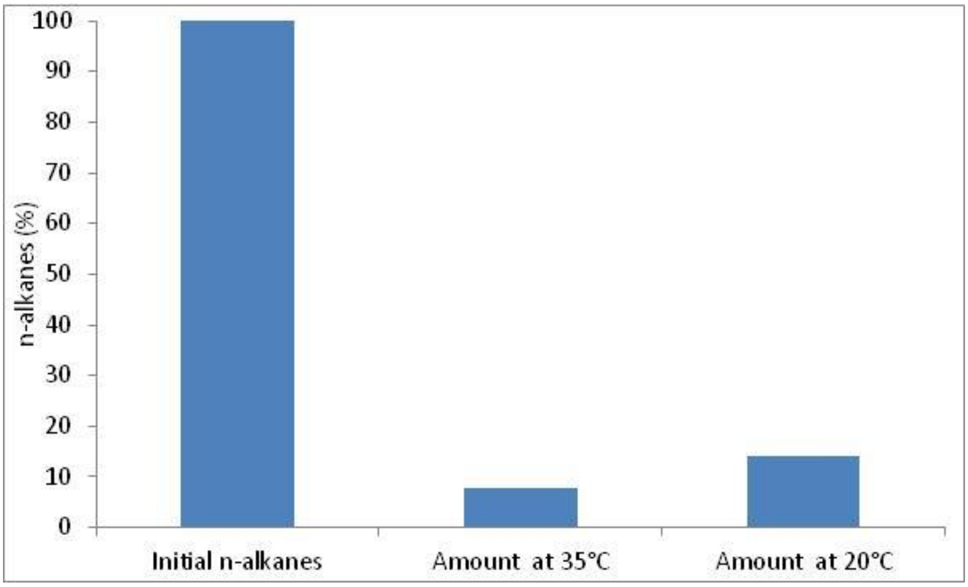

Figure 5.2: Amount of n-alkanes (%) remaining at 14 days in contaminated soil without compost

6 Growth Trials

6.1 Results of the growth trials

Table 6.1: Physico-chemical parameters of the feedstock used in various growth trials during the research

Experiments	Type of feedstock	Moisture content (% of ww)	Volatile solids (% of ds)	pH	EC (µS/cm)
Scoping study (1 & 2)	Soil (Selby)	26	9	7.4	29
	Compost (TEG)	34	34	8.4	685
Plant growth 1	Soil (Selby)	29.1	9.4	6.9	82
	Compost (Rufforth)	43.7	25	8.5	890
Plant growth 2	Soil (Bardsey)	22	8	7.7	36.5
	Compost (Bardsey)	43	31	8.4	1004
Plant growth 3	Soil (Niger Delta)	6	3.7	6.3	46.3
	Soil (Bardsey)	25	8.9	6.5	152
	Compost	42	32	8.3	1295
After spiking at different oil levels (Niger Delta Soil)					
	5%	5	5.6	5.9	71
	Soil 7.5%	7	6	6.0	113.6
	Soil 10%	9	6.8	5.8	109

The use of total petroleum hydrocarbon (TPH) for assessing hydrocarbon components in any spillage site as well as evaluating their risk to human with respect to their level of toxicity and carcinogenity was established by a group known as the total petroleum hydrocarbon working group (TPHWC). The group was set up in United States of America to look at the different guidelines used by different regulatory bodies and then to come up with more robust ways of assessing TPH (Edwards *et al.*, 1997, Gustafson *et al.*, 1997, Potter and Simmons, 1998). Although TPH is a gross measure of petroleum contamination, nevertheless it shows that petroleum hydrocarbons are present in the sampled media.

This research departs from traditional approaches of using chemical analysis to quantify the risks associated with crude oil contamination rather it attempts to examine how oil spillage affects crop production and develop better ways to help a local farmer recover the contaminated soils to enable him put the land back to agricultural use. This is used as a basis for examining the effects of oil spillage on the traditional economies of the local farmer (in this case farming) especially in the Niger Delta region as he wants to go back as soon as possible to farming the contaminated land. This approach allowed us to address more sustainable cleanup options in the Niger Delta region and areas of similar contexts. Using parameters such as pH, electrical conductivity, total nitrogen (TN), phosphorus, total heterotrophic bacterial count, and germination index could be useful to assess the microbiological resilience of contaminated soils and their potential capacity to degrade hydrocarbons in the crude oil. Also, the ability of the restored soil to grow plants. In addition, we assessed the effects of crude oil contamination on the growth of tomatoes after the addition of organic amendment in form of compost. The materials used included compost from a wide range of organic wastes as well as soil samples from the UK and the Niger Delta as previously shown in Table 6.1. Selected experimental set-ups during the growth trials in this research are shown in Plates 6.1 to 6.4.

Plate 6.1: The controls (no oil) recorded over 80% germination. First tray to the left is soil only and second tray to the right contains 50% compost

Plate 6.2: Test trays containing contaminated soil, the seedlings are struggling and less robust unlike the controls in Plate 6.1. The tray to the left contains 5% oil w/w, the tray to the right contains 7.5% oil w/w and the tray behind contains 10% oil w/w

Plate 6.3: Treatment trays containing 50% compost, on addition of compost to the contaminated soil, germination and growth were enhanced (compare with plate 6.2)

Plate 6.4: Control trays containing different ratios of compost/soil at day 35. The mix ratio at the front left is 75% compost while the trays to far right contain 50% compost.

6.2 Scoping study 1 (effects of BLCO on the growth of tomato plants at 1:1 and 1:3 compost/soil ratio

This investigation was at 10% oil level (w/w). We used germination and growth trials to assess the plant's response to crude oil contamination because they are relatively cheap test, and affordable by farmers. The information is useful for determining the potential of the contaminated land for plant growing and the potential benefits of using organic compost. Germination alone cannot fully explain the dynamics of nutrient availability in compost for plants' use, however a combination of germination, seedling growth and fruiting can provide this information. The planting periods in the present study ranging from 28 to 35 days did not allow for the fruiting stage, which could be an option to investigate during further studies. It might provide conclusive evidence of how compost improves crop production on contaminated soils. Reference will be made to the application rate of compost in this experiment as 50% and 25% compost to reflect compost/soil at 1:1 and 1:3 ratios respectively.

6.2.1 Qualitative Results

In this first Trial, visual examination at the end of the planting period revealed the soil dry and cracked up (Figure 6.1); this was at the preliminary stage when wetting was not daily. It is possible the dryness could have resulted from low moisture level that prevailed during this particular test. One of the requirements for seedling emergence if the soil is dry is an increase in moisture level. It is also possible that the oil displaced both the air and some water in the pores during contamination leading to the cracking of the soil. Factors that can inhibit plant growth include inhibition to moisture, which can affect nutrient uptake as well as photosynthesis. This exclusion of air could have been detrimental to both plants and microorganisms.

There was also yellowing of the leaves after the second week of planting. The leaves are important organs of plants because they are the central site for photosynthesis. The leaf cells house the organelles (chloroplasts) which absorb the light energy for photosynthesis. Therefore, proper formation of leaves is important to the manufacturing of carbohydrates such as sugars, which are very helpful for the full development of the plants. The yellowing of the leaves eventually led to death in some of the plants while in some, the plants were able to recover quickly maybe in that case they were more tolerant of the oil contamination.

Figure 6.1: Soil looking dry and cracked up during the preliminary studies, perhaps the reduced water content contributed to cracking of the soil and plant death

6.2.2 Seed germination

The weekly counting of the seedlings revealed that germination increased and was highest on 14th day after planting for each treatment. The germination count calculated as percentage of number of seedlings that sprouted at day 14 compared to the total number of seeds sown. The percentage germination increased more in the controls (no oil) than in the contaminated soils (Table 6.2). One hundred percent inhibition was observed for soil contaminated at 10% oil level (without treatment) similar to observations by (Udo and Fayemi, 1975) who recorded 100% reduction in maize yield at 10.6% level of oil contamination and 44% germination at 6.8% oil level on comparison to the controls. We observed that after the germination stage, the number of survivals reduced before the end of the planting period for the controls (Figure 6.2) and the treatments (Figure 6.3).

The term ‘die-back’ was used to express this phenomenon of eventual death of germinated plants due to some constraints on their development. Talarposhti *et al.* (2005) suggested that after germination, poor performance in later stages could be be attributed to lack of available nutrients for sustained growth. The ‘die-back’ could be due to a number of different factors such as nutrients and effects of oil on the soil. Other workers have also observed reduction in number of survivals after seedlings' germination on crude oil contaminated soils (Udo and Fayemi, 1975, Kuhn *et al.*, 1998, Ekundayo *et al.*, 2001, Adoki and Orugbani, 2007b, Adekunle, 2011). Udo and Fayemi (1975), attributed this reduction to the effect of crude oil on the seed's embryo. The soil first absorbs the oil and once in contact with the seed surfaces then spreads to the embryo thereby affecting the physiological functions within the seed. However, this study did not examine such factors rather the investigations focused more on finding optimum mix ratios that would improve soil fertility to allow the local farmer return to farming his contaminated soils within the shortest possible time.

A different logical interpretation to the reduction after germination could be that during seedling development, the seeds use energy from itself and on exhausting the energy, it dies off and sprouts into new seedling. After germination, the seedlings start the search for food from the surrounding environment and if the environment is not supportive enough the seedlings will eventually die off. Such adverse environments can be as a result of oxygen depletion in a hydrocarbon contaminated soil or more likely due to utilization of nutrients by resident microorganisms because increased metabolic activities are going on to breakdown the hydrocarbon.

From the farmer's viewpoint, his main concern is to get higher yield from his farm therefore, we suggest that after germination of seedlings on the contaminated soil, it is advisable to add other forms of supplement to support plant growth.

Table 6.2: Growth parameters of tomato recorded during the scoping studies (1 & 2)

Treatments	Germination count (%) & No. of survivals at 14 days		Average Plant height (cm) at 28 days of planting	
Controls (no oil)	1	2	1	2
Soil	100	100	4.1	4.1
Compost	100	100	3.8	3.8
Compost/Soil 1:1	100	100	3.9	3.9
Compost/Soil 1:3	90	90	4.0	4.0
Test conditions (with oil w/w)				
Soil 10%	0	0	0.00	0.00
Compost 10%	70	67	2.4	2.3
50% Compost/Soil	50	100	2.3	2.8
25% Compost/Soil	40	67	2.7	2.6

† Plant height for the controls (no oil) ranged from 3.8 to 4.1 cm and for the test combinations ranged from 2.3 to 2.8 cm. There was little variation observed between the two trials.

The biomass measured as yield above ground is shown in Figure 6.4. An average of 491 ± 159 mg was highest in the two control pots, which contain one part of compost to one part of soil (compost/soil 1:1) and an average of 309 ± 69 mg in the second pot (compost/soil 1:3). Given the high organic matter in the compost, perhaps the compost is able to supply nutrients that are lacking in the soil and thus improved yield. On the addition of oil (10%), the biomass yield (70 mg ± 0.71) was more at 25% compost than at 50% compost (40 ± 36 mg).

Compost has higher microbial diversity and so at 1:1 mix ratio, it is possible that the microorganisms were more and therefore more demand on available nutrients and oxygen resulting in their depletion, which could adversely affect plants ’growth more than at mix ratio of 1:3. The addition of compost also diluted the contamination levels to 5 and 7.5% at 50 and 25% compost/soil mix respectively. The higher biomass recorded at 25% compost might suggest that the higher hydrocarbons present at the 7.5% diluted oil level provided more substrate for the plants and microorganisms.

The biomass yield from soil at 0.5% w/w oil level which was the intervention value given by the Nigerian government at which a cleanup is mandatory was 129 mg. This biomass value (approximately 53% of the control) was below the baseline value of 245 mg recorded from soil not contaminated with crude oil. Only 30% of the seedlings survived up to day 28. Thus, the low yield from oil-contaminated soil at the Intervention value has demonstrated the adverse effect of crude oil contamination on plants even at low concentrations. This indicates that there is need for the contaminated soil to receive treatment even at this relatively low oil contamination to enhance yields. In a similar manner, Udo and Fayemi (1975) reported a 30% dry matter yield reduction in growth of maize at 1% w/w crude oil contamination on comparison with the control.

With respect to safety limit, probably the choice of 5000 mg/kg soil by the Nigerian government as the intervention value could have been because of the adverse effects as observed in the germination of seedlings. Such result can be a valuable tool in helping policy makers in Nigeria on issues relating to regulations on cleanup of contaminated soils in the country.

Similarly Kuhn *et al.* (1998) investigating the effects of Kuwait crude oil on the growth of tomatoes observed that at various oil levels ranging from 0.48% to 2.4% by soil weight, the plants were all affected. At 0.48% w/w, approximately 75% growth relative to the control was recorded however, the seedlings did not survive after 35 days. At 0.60% w/w, there was 75% growth however, none of the seedlings survived to 10 days while at 1.2% and 2.4%, all the seedlings died within 6 days of planting. The trend suggests that at higher oil concentration, plant growth was adversely affected. We can draw inference from the above results that crude oil contaminated soils should be treated before cultivation irrespective of the low oil level. Such treatment could be in the form of compost application depending on the best mix ratio defined in this study.

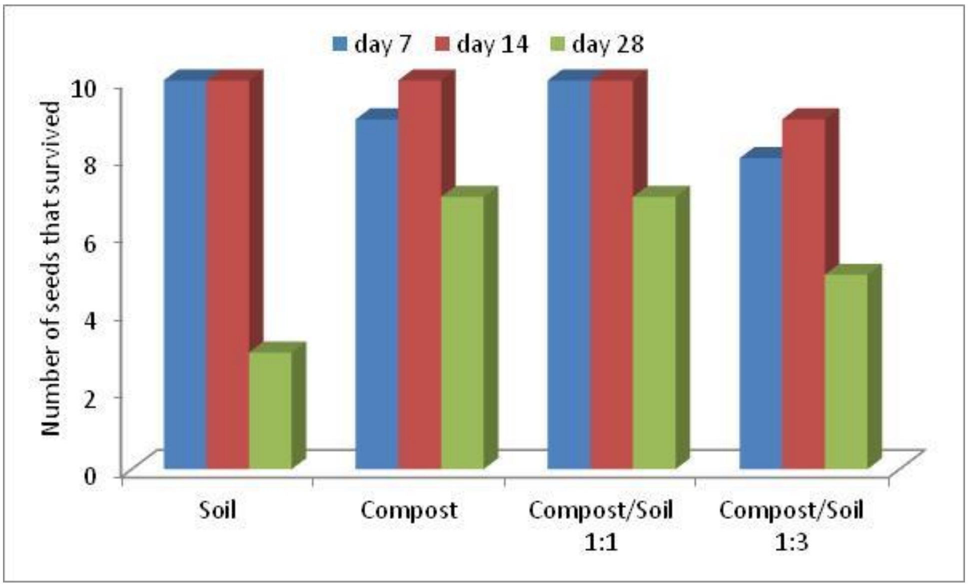

Figure 6.2: The numbers of seedlings (controls) that survived over 28 days, counting was every week. The soil samples were from Selby.

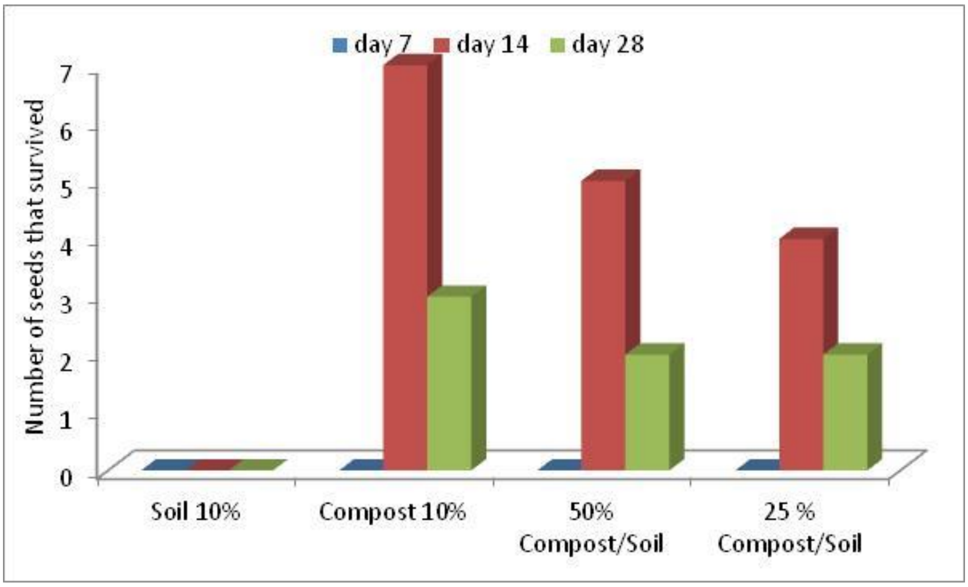

Figure 6.3: The numbers of seedlings that survived on treatment with compost over 28 days, counting was every week. The soil samples were from Selby and there was total inhibition at soil 10% oil level.

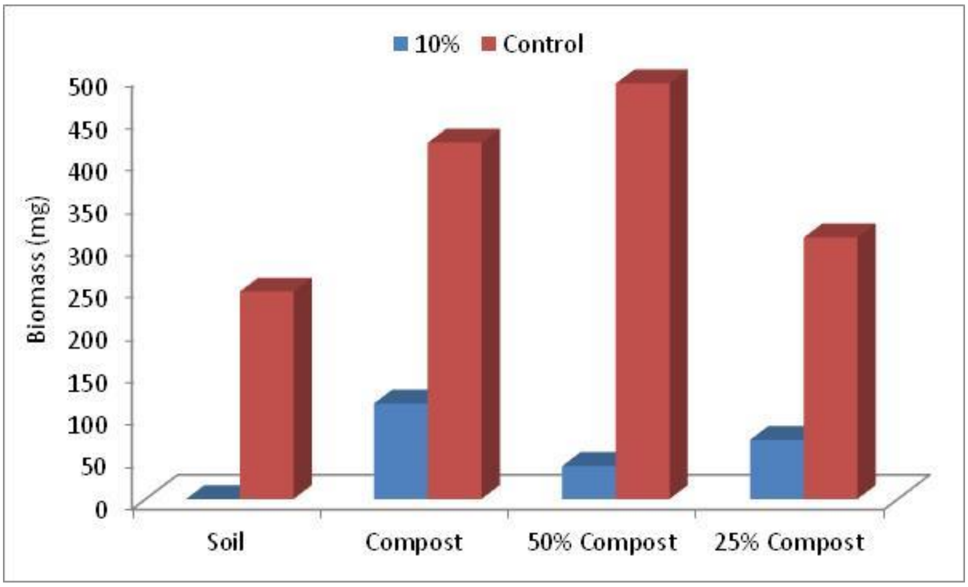

Figure 6.4: Above ground biomass after 28 days of planting at 10% oil level

6.3 Scoping study 2 (effects of BLCO on the growth of tomato plants at 1:1 and 1:3 compost/soil ratio

The investigation, which was at 10% oil level (w/w), was to allow for more observations at this preliminary stage.

6.3.1 Qualitative Results

Visual examination of the shoots during the planting period revealed shoots from the controls and pots containing compost as treatments to be thicker in appearance. It could be that the absence of oil in the controls and the presence of compost in the spiked soil allowed the seedlings to develop better.

6.3.2 Analytical Results

There was monitoring of the moisture level in this experiment as a precautionary measure to understand the effects of water, on seed germination. Moisture content of the spiked materials was determined immediately after spiking. The moisture level of the feedstock was built up to 50% after spiking to make sure the seedlings had sufficient moisture. There was not much variation in the pH at the start and end of the experiment. The initial pH for the treatments ranged from 7.4 to 7.8 and the pH after harvesting was 7.3 to 8.2. The test trays (Compost 10%) had the highest pH value of 8.3.

6.3.3 Nitrogen content

The nitrogen content of the soil at the start of the experiment was 0.18%, on addition of oil at 5 and at 7.5% oil level, the nitrogen content increased averaging 0.4%. For treatments containing compost, the initial nitrogen content ranged from 0.4 to 0.7% and averaging 0.3% at the end of the experiment. Although the total nitrogen in the mixture containing compost was low, different studies have revealed variation in the total nitrogen content of compost.

Kapetanios *et al.* (1993) in their study reported that the total nitrogen content of compost produced from Greek domestic refuse ranged from 1.55 to 2.14%. Similarly Adoki and Orugbani (2007b) reported a range of 0.08 to 0.16% for total nitrogen in crude oil contaminated soil treated with effluent from a fertilizer plant. Osuji and Adesiyan, (2005) reported 0.08 to 0.12% as values for total nitrogen for crude oil contaminated soil in the Niger Delta region.

It could be that the type of waste used as feedback contributes to the final nitrogen content of the compost. For example, compost made from feedstock containing woodchips will be low in nitrogen. The nitrogen content of plant tissues is approximately 1 to 6% (Nie *et al.*, 2011). Given the low nitrogen content of the compost and as a recommendation, the farmers should supplement with other sources of mineral nitrogen for an enhanced plant growth.

6.3.4 Seed germination

Six tomatoes seeds were planted in each tray in this experiment due to unavailability of seeds. Some of the seeds did germinate but experienced dieback afterwards (Figure 6.5). The yellowing of leaves became obvious 21 days after planting and eventually the affected plants wilted. It is possible that the lower intensity of light energy during the scoping study 1 and 2 affected both germination and survival of the seedlings. At this preliminary stage, the light bulbs provided only 12-hour illumination because the light was switched on at 6am and off at 6pm. However, a higher intensity of light energy as well as longer duration was available in subsequent experiments. Mercury lamps delivered a daily cycle of 16-hours of illumination and 8-hours of darkness to reflect the longer daylight in the Niger Delta region.

Light is very important to plants for photosynthesis, a process through which carbohydrates are formed. Carbohydrates are energy sources used for the formation of leaves, flowers and fruits thus the higher biomass yield observed in later experiments could be due to better light.

The relative performance did not show marked difference for similar treatments at diluted oil content of 2.5%. For example in plant growth experiments 1, 2 and 3 at 5% initial oil level with 50% compost treatment (compost/soil 1:1), the biomass yield was 130, 181, 178 and 138 mg respectively (Table 6.5 pg. 171). Similarly, at 10%, initial oil level and 50% compost application, the biomass yield was 113 and 126 mg respectively in plant growth 2 and 3. However, at other diluted oil concentration difference in relative performance was observed. For example at 7.5% initial oil level and 50% compost treatment, the biomass yield was 55, 146, 266, and 71 mg respectively for plant growth 1, 2 and 3. The increased number of seedlings that germinated and survived on addition of compost suggests the ability of compost to improve yield.

An analysis of the data from both experiments showed that the above ground biomass represented previously in Figures 6.4 and Figure 6.6 were similar but more importantly it showed that tomatoes cannot grow in soils containing 10% crude oil since 100% inhibition to seed germination was observed at 10% oil level.

In summary, germination and growth could be an evidence of restoration of contaminated soils for use in agricultural purposes; also, it demonstrates the benefit of compost treatment in terms of potential crop yield compared to the use of contaminated soil without treatment.

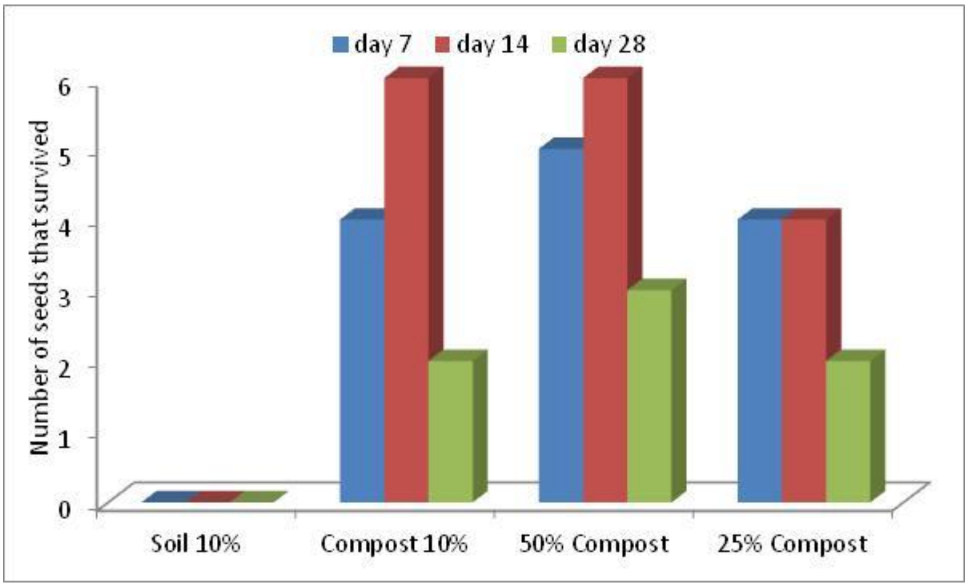

Figure 6.5: The numbers of seedlings that survived on treatment with compost over 28 days, counting was every week (scoping study 2).

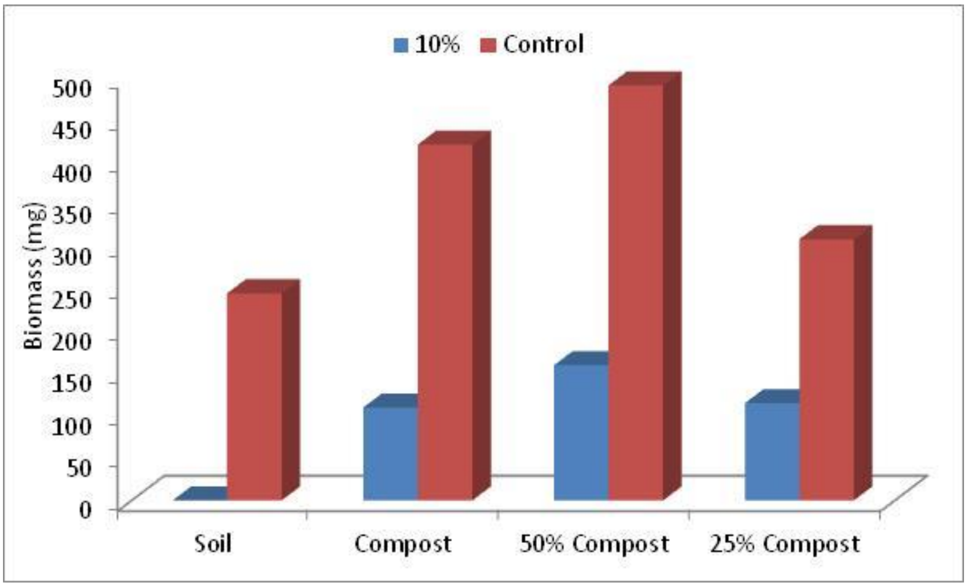

Figure 6.6: Above ground biomass after 28 days of planting at 10% oil level (scoping study 2).

6.4 Effect of BLCO on biomass yield of tomatoes at 0, 5 and 7.5% w/w using compost-soil mix at 1:1 (growth trial 1)

6.4.1 Qualitative Results

From visual observation, the treatments that had a combination of soil and compost had thicker shoots. The highest average plant height shown in Figure 6.7 was from the controls, soil (5.2 cm) compost only (5.5 cm) and 50% compost (4.7 cm). Generally, plant height decreased with increasing oil level showing the effects of oil contamination on plants' development. The seedling emergence in soil at 7.5% oil level did not show any germination until day 14. Given there was no compost at Soil 7.5%, perhaps the crude oil adversely affected both the soil and microorganisms and restricted growth. Another possible explanation could be that the microorganisms having recovered from the initial effects of oil (described as a lag phase) were able to utilize the hydrocarbons as their carbon source and such microbial degradation allowed for seedling emergence after 14 days.

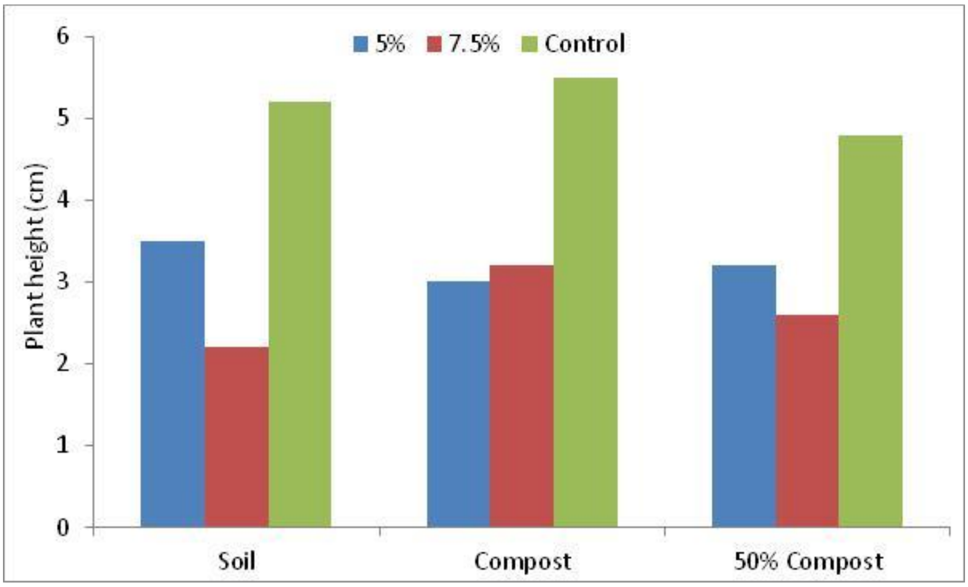

Figure 6.7: Average plant height 5 and 7.5% oil level measured at day 35. The soil samples were from Selby

6.4.2 Analytical Results

Treatments with compost generally increased pH and electrical conductivity (Figures 6.8 and 6.9). The variation in electrical conductivity on addition of compost suggests the presence of ions due to more nutrients in the compost. A higher value of electrical conductivity is associated with high concentration of dissolved salts. The range for electrical conductivity was from 85 to 878 µS/cm. It is generally acceptable that when values for electrical conductivity exceed 2000µS/cm it could be a concern especially for sensitive plants (Lasardi, 1998). The conductivity of all samples in the present research remained below the 2000 µS/cm value. The electrical conductivity determines the salinity of the soil and if it is within the acceptable range. The soil after treatment was analysed for pH and electrical conductivity. There was a significant difference ($p < 0.05$) in pH at different oil pollution levels, though the mean pH was within the acceptable range for microbial and plant growth. On spiking the soil with crude oil and after compost treatment, the observed pH was in the range 6.9 – 8.2.

Zuofa *et al.* (1988) working on the Niger Delta soils observed that there was an increase in soil pH with exposure to higher oil levels. They revealed that oil tended to buffer the soil pH towards neutral and that when soils are low in organic matter, the changes in pH are more noticeable. In this experiment, the pH of the unpolluted soil was 6.9, and at Soil 5%, the pH shifted to 7.1. On addition of compost (compost/soil 5%) to soil at 5% initial oil level, the pH shifted slightly to 7.6, perhaps the higher organic matter content (34%) of the compost contributed to the change in pH. As earlier pointed out in § 2.4.1.1, plants differ in their tolerance to changes in pH especially after crude oil spillage. Probably the extent to which oil pollution affects any soil largely depends on the composition of the oil and the soils original physiochemical properties.

6.4.3 pH

pH is one of the major variables that affects soils properties because it is one factor which determines not just the availability of nutrients for plant growth but also the fate of soil pollutants and their movement . Osuji and Nwoye, (2007) reported that the soil physicochemical conditions such as pH were affected after oil spillage. For example, in their study the pH of the unpolluted soil was 5.6 and the pH of oil impacted soils both at surface soil and subsurface soil was 4.9 and 5.1 respectively which is in contrast to the findings of Zuofa *et al.* (1988) that oil tended to buffer the soil pH towards neutral. According to their report, low pH affects solubility of minerals and that acidic soils have high concentrations of soluble aluminium and manganese, which are toxic to plants and could mean low soil fertility.

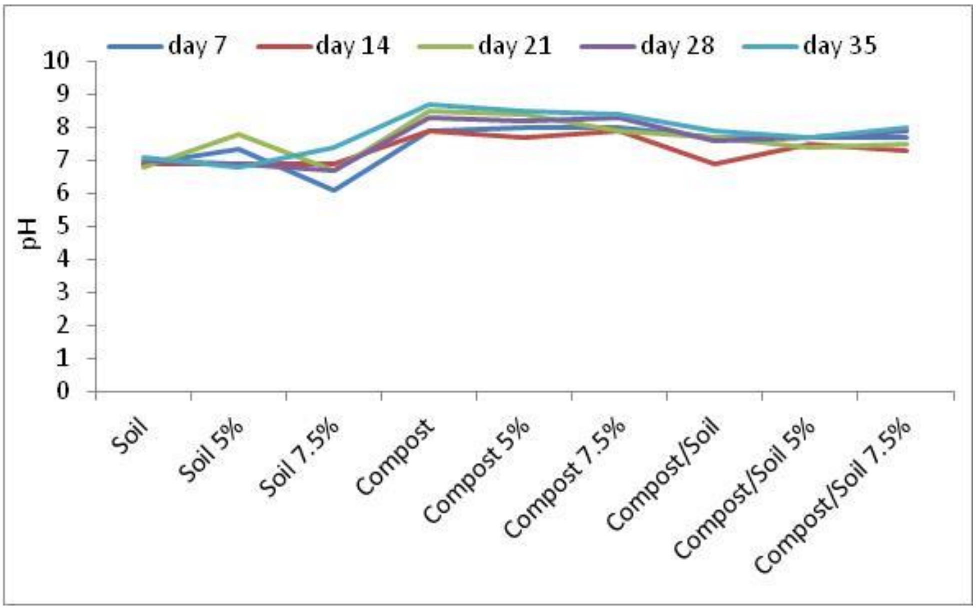

Figure 6.8: Variation of the pH at 5 and 7.5% oil level, the soil samples were from Selby

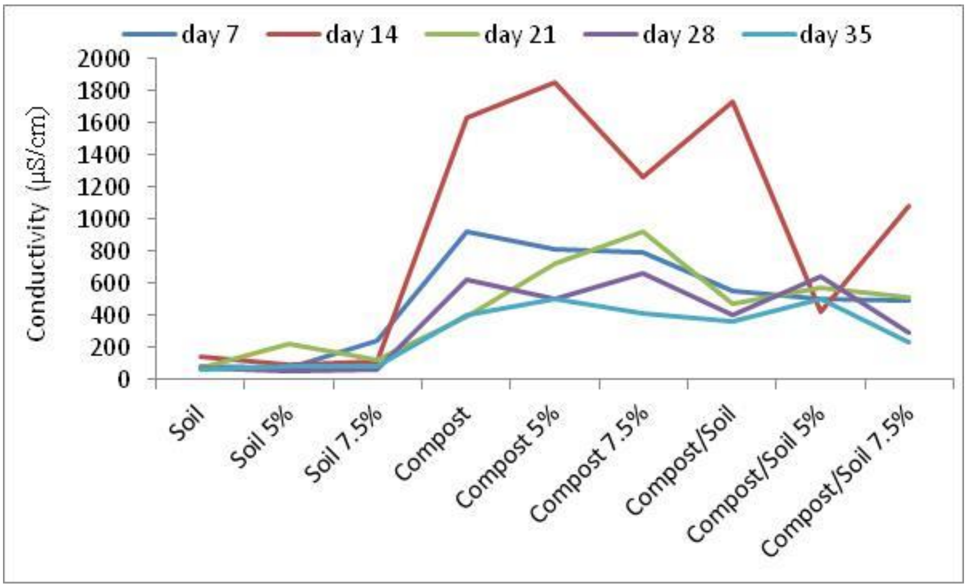

Figure 6.9: Variation in conductivity at 5 and 7.5% initial oil level, the soil samples were obtained from Selby

6.4.4 Nitrogen content

The TKN values were the average of the two replicates. The initial TKN values (Figure 6.10) of the test combinations was in the range 0.3 to 0.7% dry weight and the final values in the range 0.04 to 0.3% ds. Nitrogen is one of the primary constituents needed by plants for development. The difference in values between the controls and crude oil impacted zones could be due to depletion of oxygen in a hydrocarbon-contaminated environment.

Ekundayo *et al.* (2001) investigated the effect of nitrogen on growth and yield of preplanting maize on crude oil contaminated soils obtained from the Edo, in the oil producing Niger Delta region of Nigeria. The nitrogen content was 0.21% and 0.16 for the control and the polluted soil respectively (before planting). Seven weeks after planting, the nitrogen (% dry weight) for the control soil (0.20%) did not vary much and polluted soil was reduced to 0.05%. Ekundayo and Obuekwe (1997) similarly observed a low nitrogen content after investigating how oil spillage affects physico-chemical properties of a polluted site. The ranges were from 0.023% (dry weight) and 0.075% (dry weight) in the impacted and control zones respectively even though these low values make their result from the study questionable. This is to say that soil characteristics' could be site specific and that contaminated soils should be treated based on the soils' qualities.

The 5% Compost had a higher nitrogen content of 0.7% when compared to compost at 7.5% oil, which had a value of 0.3%. A similar trend was observed at both oil levels for compost/soil mixture. Perhaps the higher biomass with the compost at 5% oil (153 mg) in comparison with a yield of 110 mg with compost at 7.5% oil is related to the higher nitrogen content in compost 5% initial oil level.

Variation in results could also be due to the sampling as there is possibility of the feedstock forming aggregates when not mixed uniformly. As a precautionary measure, subsequent sample preparations were done using the quartering system to ensure that we had a homogenous mix.

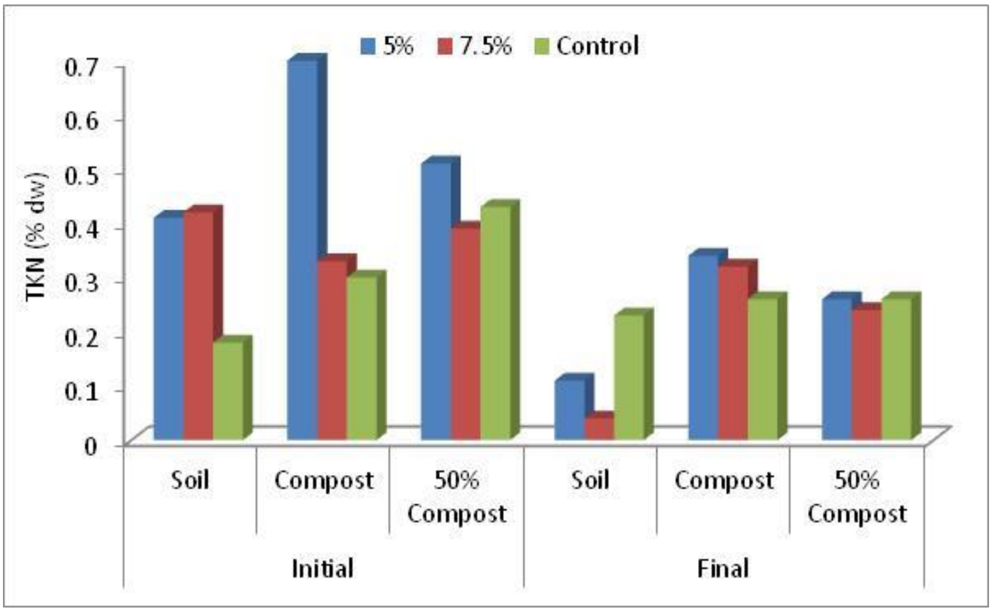

Figure 6.10: Nitrogen measured as TKN at the start and end of growth trial 1. The mix ratio of compost to contaminated soil was 1:1 (50% compost).

6.4.5 Seed germination

The trends in germination at the different oil levels are shown in Figures 6.11 to 6.13. For the controls i.e. Soil, Compost and Compost/Soil without oil, germination was sustained until about 20 days, after which the number of survived plants started reducing. Compost/Soil had the highest number of survivals with Soil having the least survivals. It seems the soil used in plant growth 1 was not a very good growth media given that the original soil (i.e. soil not contaminated with crude oil) produced only 161 mg as above ground biomass when compared to the combination of soil and compost, which yielded 628 mg above ground biomass (Figure 6.14). The compost did reduce the toxic or unfavourable effects for upwards of 14 days after which die-off became noticeable, possibly due to depletion of nutrients in the compost. In terms of crop management, the results indicate that the application of compost might be useful in the seedling soil bed because it is likely to produce seedlings that will tolerate oil pollution.

In general, above ground biomass was better at treatments with 5% initial oil level than with treatments at 7.5% initial oil level showing that the tomato plants were affected more at higher levels of oil contamination. Compost 5% recorded higher biomass (153 mg) than Compost 7.5% (110 mg) which could be due to the high nitrogen in the initial feedstock. However, with Soil 7.5% the above ground biomass was 33 mg as against 22 mg at Soil 5%. A possible explanation could be that at Soil 7.5%, the toxicity of oil is expected to be higher and as the microbial floral were dying, they were adding to the pool of organic carbon and through the process of carbon cycle were converted to inorganic carbon for use by the surviving plants resulting to better plant' development.

Given the die-off of plants was after 15days on average, there is need for further treatment that will sustain the plants after germination such as supplementing with other forms of fertilizers.

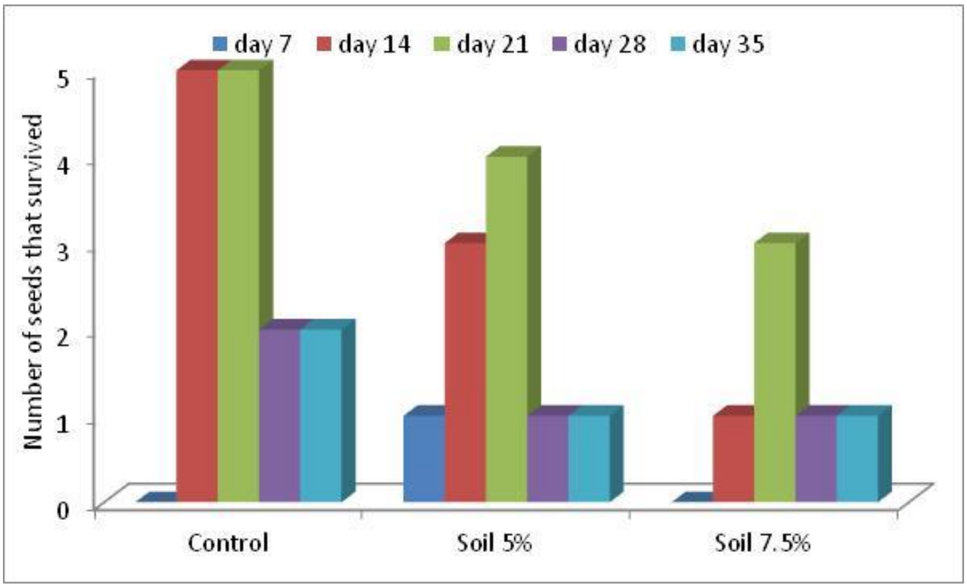

Figure 6.11: The numbers of seedlings that survived in soil at different oil levels over 35 days. Counting was once every week for 5 weeks

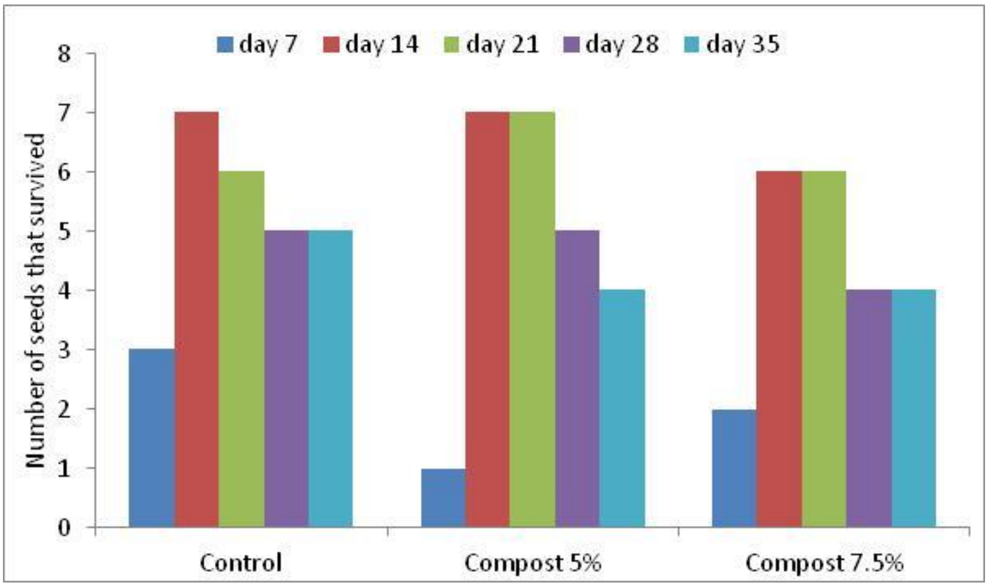

Figure 6.12: The numbers of seedlings that survived in compost at different oil levels over 35 days. Counting was once every week for 5 weeks

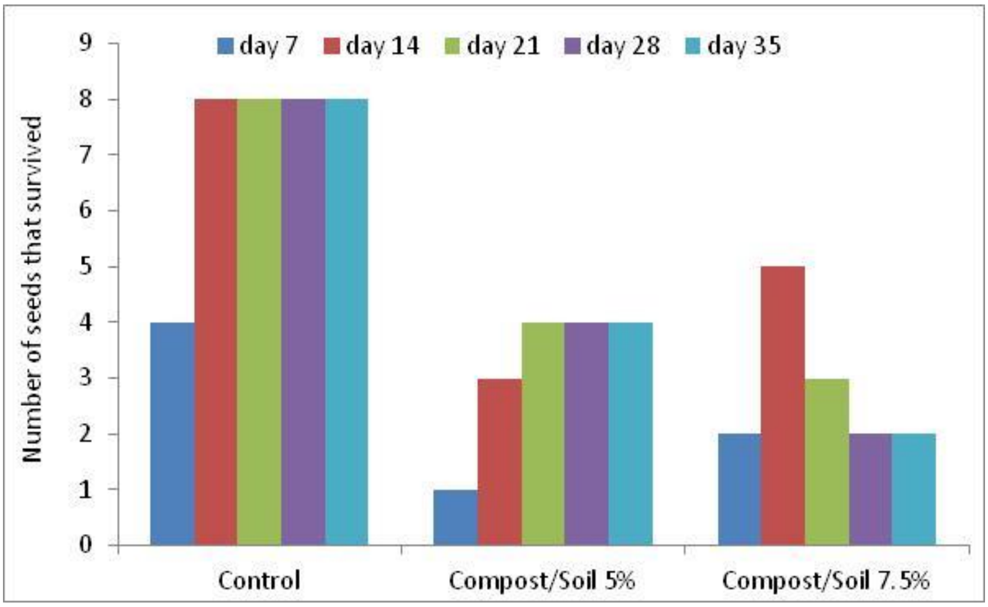

Figure 6.13: The numbers of seedlings that survived in compost/soil at different oil levels over 35 days. Counting was once every week for 5 weeks

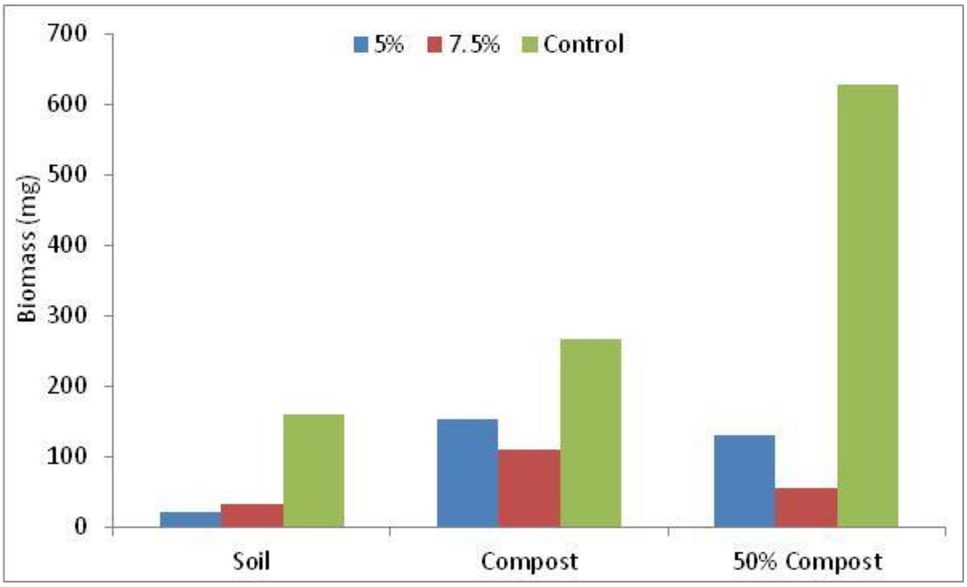

Figure 6.14: Above ground biomass after 35 days of planting at different oil level. The addition of compost to the contaminated soil at 1:1 mix ratio reduced the oil level

6.4.6 Microbial population during bioremediation

John *et al.*(2011) and Odu, (1972) reported that crude oil contamination in soil can negatively affect the microbial densities and activities even at lighter contamination. At the same time, they emphasized that the impact of crude oil contamination depends mainly on the property of the original soil and tolerance of the plant exposed to contaminated soils.

In this experiment, we monitored the effects of crude oil on microbial population at 0, 5% and 7.5% initial oil level by soil weight for the different treatments (Figures 6.15 to 6.17) having done it as a one off experiment. An initial increase in the total heterotrophic bacterial count was observed in soil without oil and soil with 7.5% oil, round about 14 days after planting. The fall in count for the oil-free soil could be due to depletion of nutrients in the soil by the microorganisms since there was no oil to act as substrate (refer to Figure 6.15). The effect of oil on the total heterotrophic bacteria seemed to be similar at both Soil 5% and Soil 7.5% only that an initial drop in total count was observed at higher oil level (7.5% wet weight). However, these increases were not sustained until the end of planting period suggesting the effect of crude oil on the microbial population.

The 5% Compost and 7.5% Compost as well as the control had a similar pattern of growth. The 7.5% Compost was more stable though it can be said that all treatment conditions peaked round about day 14. Higher values of total microbial counts were observed when the feedstock was compost spiked at different oil levels than soil spiked at similar levels. This suggests that compost consists of greater diversity of microorganisms more than soil and the synergistic relationship would have contributed to more efficient breakdown of the hydrocarbons in the oil.

A similar growth pattern was observed for Compost with 5% oil and Compost with 7.5% oil up to day 21, probably it goes to show possible variation in microbial response on addition of compost to the contaminated soil.

For Compost + Soil (Figure 6.17), the growth was at peak until day 21 and at Compost + Soil with 7.5% initial oil level, there was an observed decline in growth afterl day 14 of planting. However, even with the decline at day 21 it still showed concentrations as high as those in the other two samples with compost.

To summarize the section, as the breakdown of organic materials proceed, it is expected that nutrients will be depleted and eventually affect microbial growth. Perhaps, there was increase in soluble chemically oxidized organic matter available to microorganisms at the onset of metabolism, which could lead to initial peak in microbial population. As the microorganisms adjust to the new environment, they could produce enzymes that will help in biochemical conversion of large molecules to simpler ones (Das and Chandran, 2011). This results in production of more substrates, which can as well benefit surrounding organisms that are not able to utilize such complex molecules.

With respect to the total microbial count, higher biomass was observed at 7.5% Soil. Soil at 7.5% (w/w) had the highest microbial population with a peak value of 7.97E +07 cfu/g dw on day 14 reducing to 2.8E+07 cfu/g dw on day 21 and stabilizing with a slight increase through day 28 which ended with a value of 6.2E+07 cfu/g dw. At 5% (w/w) oil level, the initial high microbial levels of 7.3E+07 cfu/g dw was not stable and slowly decreased to the end of the trial.

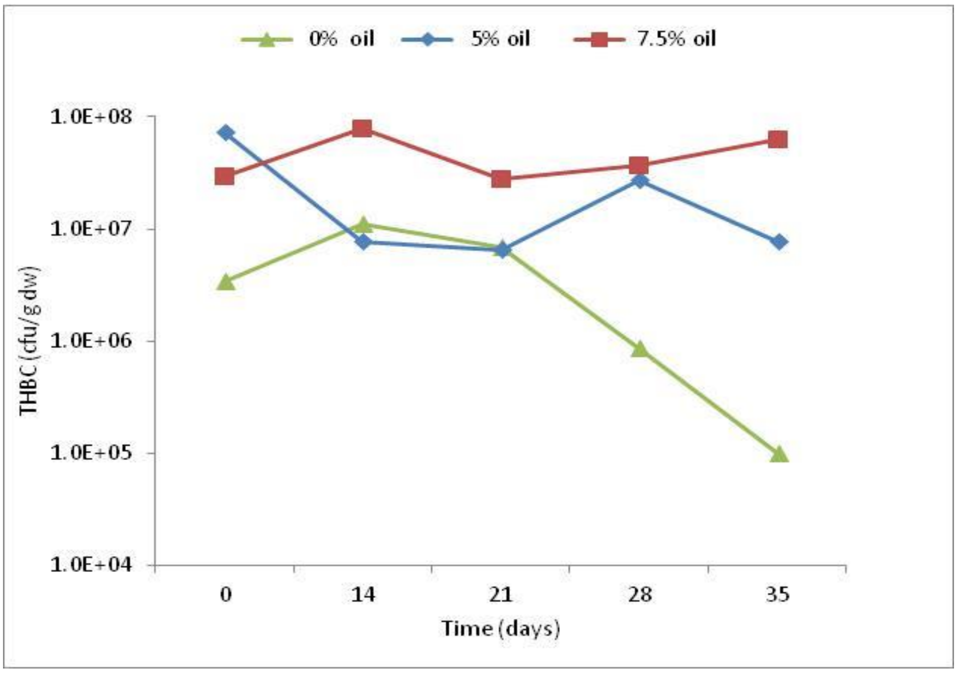

Figure 6.15: Total heterotrophic bacteria count in soil samples at different oil levels. Counting was done once every week for 5 weeks.

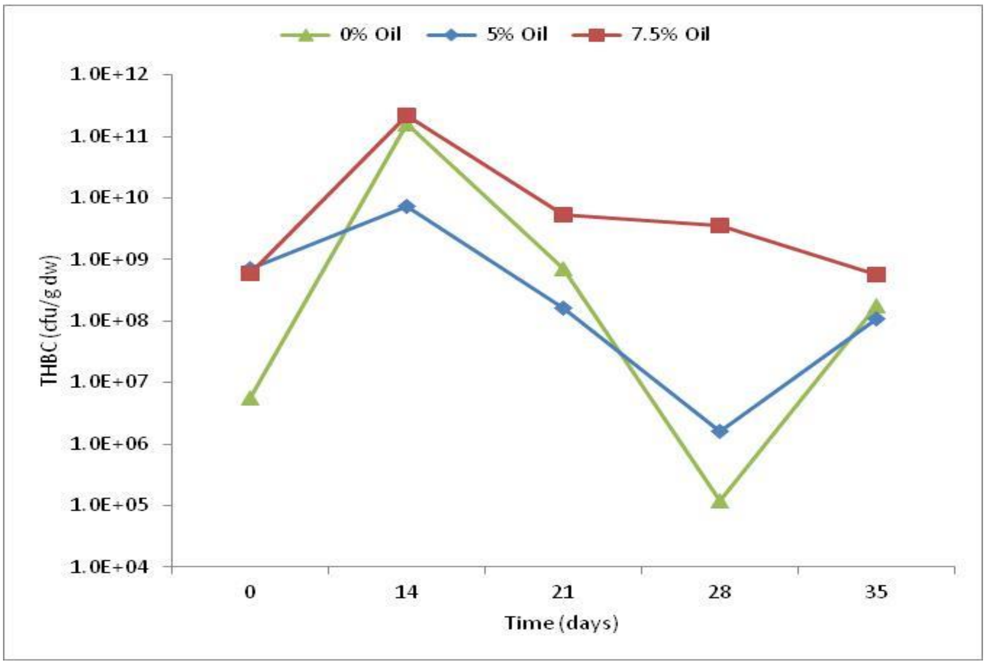

Figure 6.16: Total heterotrophic bacteria count in compost samples at different oil levels.Counting was done once every week for 5 weeks.

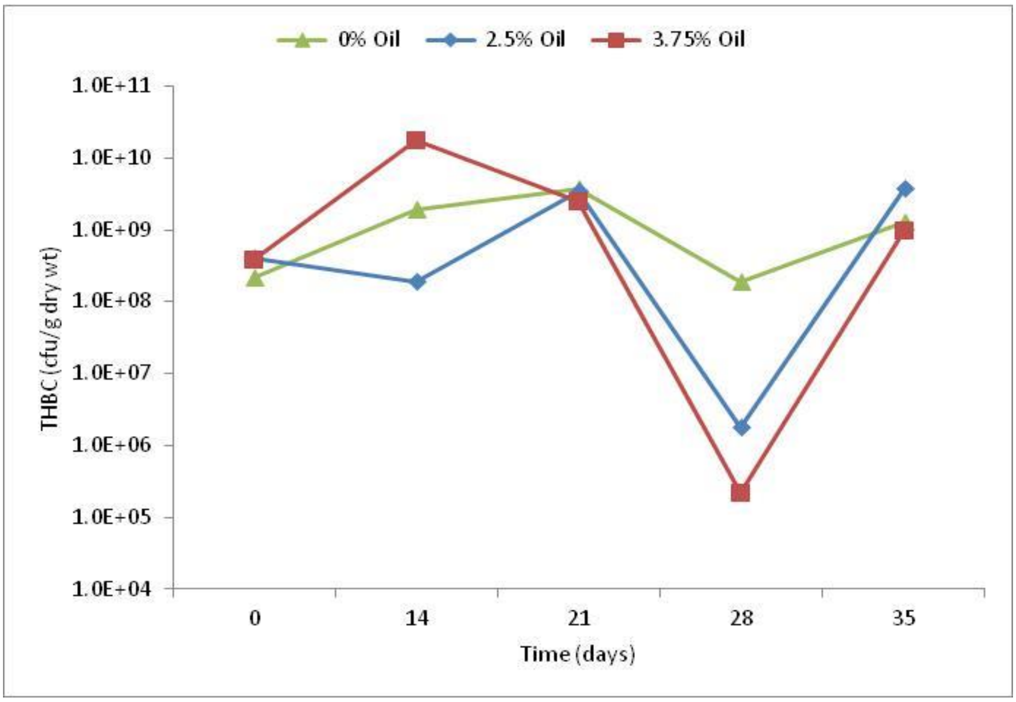

Figure 6.17: Total heterotrophic bacteria count in compost/soil samples at different oil levels. Counting was done once every week for 5 weeks.

6.5 Effect of BLCO on biomass yield of tomatoes at 0, 5, 7.5 and 10% w/w using compost-soil mix at 1:1, 1:3 & 3:1 (growth trial 2)

6.5.1 Qualitative Results

The different ratios of compost to soil at 1:1, 1:3 and 3:1 is referred to as 50%, 25% and 75% compost application respectively. Visually, the surface of the test trays containing 10% initial oil with 50 and 75% compost application were cracked regardless of the daily wetting. It is possible that at such high level of oil contamination, the pore spaces trapped the oil, which reduced the penetration of water and nutrients. This could influence root penetration and eventually lead to death. For example, with 25% compost at 10% initial oil level; there was total inhibition to plant growth although this mix ratio produced the least diluted oil level of 7.5%. This could explain the predicament of the local farmers living in areas affected by oil spillage and the effect of crude oil on their crop production, which invariably affects their mainstay livelihood.

Plant height was measured as above ground height at the end of the 35-day planting period as shown in Figures 6.18 and 6.19 for both the controls and treatment. These include plant heights for the unpolluted soils and treatment combinations with compost.

The controls ranged from 4.1 to 7.5 cm and the treatments from zero (0) to 3.0 cm. There was no growth at 10% oil level with 25% Compost application. The difference in height demonstrates the deleterious effects on plants' development due to oil contamination in soils. The average height with 50% Compost at 5% initial oil (2.8 cm) was higher than with 50% Compost at 7.5% initial oil (1 cm) and 50% Compost at 10% initial oil (1.9 cm). This difference also reflected in the higher biomass recorded with 50% Compost at 5% oil. Probably, plants grown at 5% initial oil level better tolerated the oil concentration. Contrary to expectation, in the second replicate of 50% Compost at 7.5% initial oil level, three out of the eight seeds planted germinated and then died off by day 28; hence, the plant height value was 0 cm. Perhaps, this difference shows the challenges in understanding fully the interactions between plants, microorganisms and their environment especially in the presence of stress, which in this case is the oil. It could also be indicative of the inherent variability in nature with respect to metabolic activities in the soil.

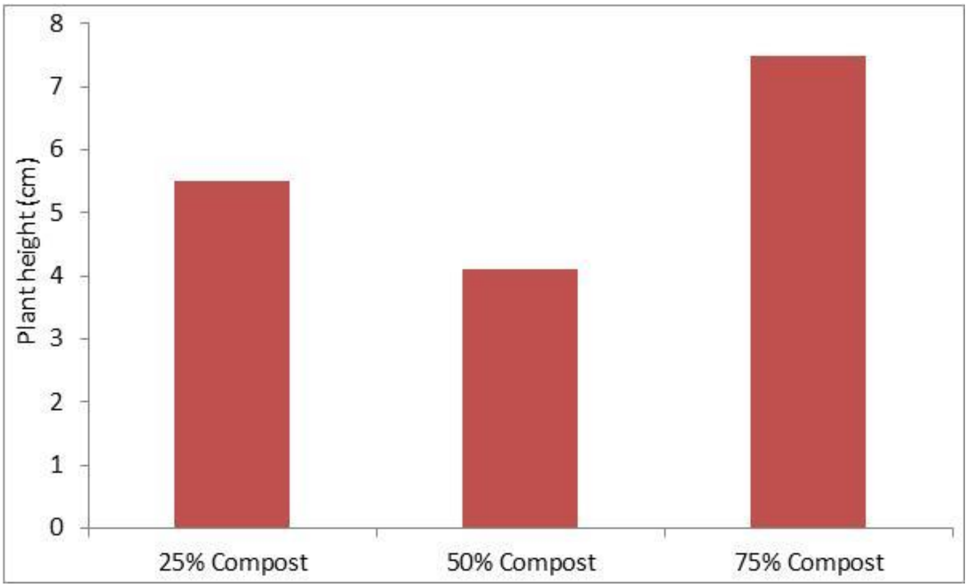

Figure 6.18: Average plant height (controls) at different compost application after 35 days planting period

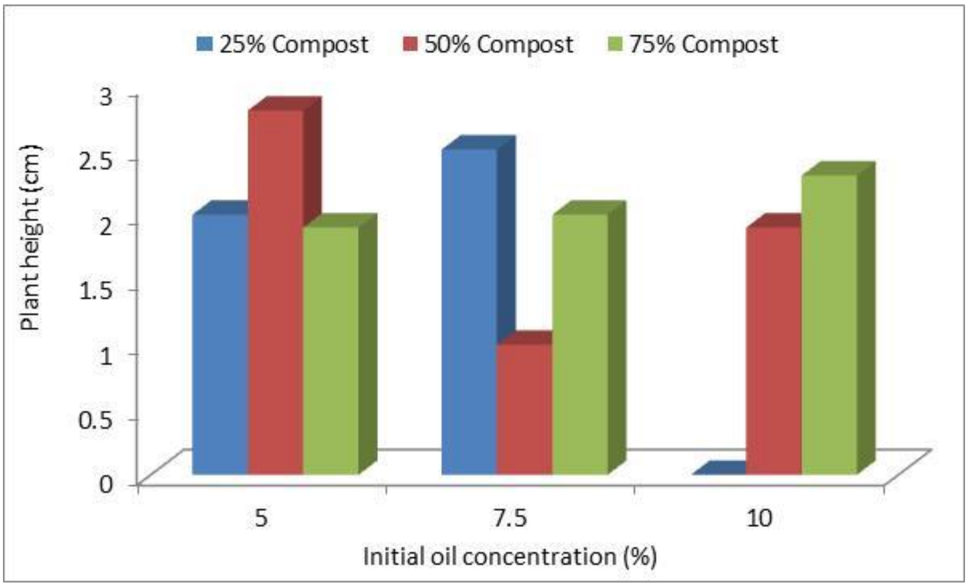

Figure 6.19: Average plant height at different compost application after 35 days planting period

6.5.2 Analytical results

The range in pH was 6.8 to 8.7 at the end of planting period, with an average of 8.4 for the treated soil. This alkaline range falls within the acceptable range for most soil microorganisms. The range in electrical conductivity for the treatments (i.e. trays containing compost) at the end of the planting period was 227 – 807 µS/cm in comparison to the initial value of 1000.4 µS/cm for compost. The decreasing values of electrical conductivity could be because of depletion of nutrients in the mixture since electrical conductivity increases with higher concentration of dissolved salts.

6.5.3 Nitrogen content

Total nitrogen was determined by the Kjeldahl digestion method explained fully in § 3.7.2. The analysis showed initial TKN values to be 0.06 and 1% dry weight for soil and compost respectively. The initial readings for Soil with 10%, 7.5% and 5% oil were 0.06, 0.07 and 0.07% dry weight respectively. This did not show any variation in nitrogen content on addition of oil at the different levels since the nitrogen level in the crude oil is very low. Odeyemi and Ogunseitan (1985) reported that Nigerian bonny light crude oil which was used for this study had little nitrogen content (0.075% by weight). This is in agreement with the report of Udo and Fayemi

(1975) as their work revealed that concentration of nitrogen did not vary much with the level of oil pollution while investigating the effect of Nigerian light oil on growth of corn. They investigated the effect of oil pollution (0 to 10.6% by soil weight) on germination and nutrient uptake of corn. The soil was analysed after harvesting the crop, similar to our procedure and the total nitrogen was 0.078%.

At the end of the present study, nitrogen ranged from 0.03 to 0.08%. The treatment combinations having mix ratios of three parts of compost to one part of contaminated soil (3:1) showed higher value for TKN perhaps due to the presence of more compost in the mix. Adoki and Orugbani (2007b) reported a range of 0.08 to 0.16% for total nitrogen in crude oil contaminated soil after being treated with effluent from a fertilizer plant

Nutrients are vital in supporting microbial activities. Across the literature, various authors have recognised that nitrogen and phosphorus could be limiting during the bioremediation process (Atlas, 1981, Leahy and Colwell, 1990, Ayotamuno *et al.*, 2006, Osuji and Nwoye, 2007, Adoki and Orugbani, 2007a, Adoki and Orugbani, 2007b). We suggest that these nutrients could be supplemented using other forms of manure however application should be done with caution to prevent any negative effects arising from an excess nutrients. Walworth and Ferguson, (2008) criticised the fact that most authors had concentrated on the deficiency of nitrogen in soils without much attention being paid to the harmful effects when excess levels of nitrogen are applied. They suggested that no more than 2000 mg N kg^{-1} soil water be added irrespective of the contaminant concentration. However, if higher levels of nitrogen are needed, it should be split into smaller repeated applications. Therefore, there is need for proper management of nitrogen during bioremediation processes.

6.5.4 Phosphorus

At the end of experiment, the range of phosphorus in the present RUN was from 31.9 to 122 mgP/kg dw. The phosphorus content of the feedstock at the start of the experiment was 51 mgP/kg dw and 85 mgP/kg dw for soil and compost respectively. On addition of oil, the phosphorus level decreased with increasing oil quantities reaching 39.7, 32.3 and 19.8 mgP/kg dw at 5, 7.5 and 10% oil level respectively. It is not very clear whether dilution of oil on addition of compost contributed to the reduced phosphorus. It was observed that phosphorus values were highest at the 3:1 mix ratios of compost to contaminated soils. Perhaps growth factors are less optimal in soils than in compost and probably the conditions were made worse in crude oil contaminated soils. Osuji and Adesiyan (2005) reported phosphorus in the range of 4 -80mg/kg for contaminated soils from Isiokpo in the Niger Delta region, although the soil used in this experiment was obtained from United Kingdom.

There is no consensus regarding the limitation of petroleum biodegradation due to available nitrogen and phosphorus even though several reports support the addition of external sources of nutrients in other to accelerate biodegradation of crude oil in soil (Odu, 1978, Atlas, 1981, Antai, 1990, Leahy and Colwell, 1990). The present study did not investigate nutrient uptake by plants. In general it is thought that in the presence of excess phosphorus that biodegradation should not be limited due to the low solubility of phosphorus (Walworth and Ferguson, 2008, Pau Vall and Vidal, 2013).

6.5.5 Total petroleum hydrocarbon (TPH)

The monitoring of the changes in TPH concentration was at the start and end of the planting period. Total petroleum hydrocarbons are complex components and they show a gross measurement of the petroleum hydrocarbons present in the sampled media. Of the total petroleum hydrocarbon in the Bonny light crude oil, approximately about 57% is n-alkanes (Odeyemi and Ogunseitan, 1985). In the present study, the TPH content of the spiked soil at 10, 7.5 and 5% oil level were 79, 73 and 88% of the calculated values of 100,000 75 000 and 50 000 mg/kg soil respectively. The difference between the calculated values and the measured values could be from a number of factors. We had established that the lighter components were lost through volatisation; secondly, we obtained an average recovery rate of 60% from the analytical procedure. Sampling techniques could also introduce variations for example, improper mixing of the samples even though the quartering system was used to obtain representative sample.

Therefore, we did not expect to find 100% TPH when analysing the spiked samples. However, we need to be aware that the study was not just about measuring the residual hydrocarbon but monitoring and evaluating the efficacy of compost as a treatment option particularly in terms of its enhancement of plant growth. In this research, availability of equipment played a role in determining the method used for the quantification of hydrocarbon (UV spectrophotometer).

The mix ratio of three parts of compost to one part of contaminated soil (75% Compost), produced high TPH removal reaching 68, 89, and 59% at 10, 7.5 and 5% initial oil level suggesting that the addition of compost could have contributed to the TPH removal due to its microbial load. However, we need to point out the effect of dilution on addition of compost to the contaminated soil for example, on addition of 75% Compost at 10%, 7.5% and 5% oil level, there was dilution effect to 2.5%, 1.9% and 1.25% oil levels respectively. Therefore, it is possible the effects caused by the dilution with compost had actually reduced the oil concentration to a trigger level at which plants were able to grow. This in part at least could explain the higher biomass values for the different oil levels at the mix ratio of three parts of compost to one part of contaminated soil because at this mix ratio, the lowest dilution was attained (Table 6.3).

At a mix ratio of one part of compost to three parts of contaminated soil (25% Compost), the effect of dilution factor on addition of compost for example, at 10%, 7.5% and 5% oil level in the soil was 7.5%, 5.6% and 3.75% respectively. Ijah and Antai (2003) worked with oil at 10% and above and reported that at this oil level, microorganisms were negatively affected even with addition of mineral salts. By originally spiking soil at 10% oil level, we achieved this level of oil contamination however; the dilution factor played a role in reducing the actual oil contamination.

The mix ratio of three parts of compost to one part of contaminated soil (i.e. 75% Compost) at 5% oil level produced the highest dilution of 1.25%, in addition to 59% TPH removal. However, in terms of the quantity of compost needed it would be better to treat contamination level approximately 5% with a 50% compost application rate (compost/soil 1:1) even though at 1:1 mix ratio, the diluted concentration was 2.5% and TPH removal was 57%. The difference between the two mix ratios in terms of TPH removal is negligible (2%) but makes a difference in terms of volume of compost needed.

At 7.5% oil level, the 50% compost application rate reduced the oil contamination to 3.75% and produced the highest TPH removal (96%). The dilution factor is similar to the 3.75% diluted oil concentration produced at with 25% Compost at 5% initial oil level even though in this case the TPH removal was only 45%. The difference could be in the amount of readily degradable organic matter present at the different mix ratios, it is possible to have more organic matter with 50% Compost at 7.5% initial oil level than with 25% Compost at 5% initial oil level. Soil contaminated at both 5 and 7.5% initial oil without the addition of compost had 30 and 39.4% TPH removal respectively. This suggests that the addition of compost resulted to more breakdown of the hydrocarbon.

At 10% oil level, after dilution with treatment at 75% Compost, the oil concentration was reduced to 2.5% similar to the dilution in 50% Compost at 5% initial oil level. However, a higher TPH removal of 68% was attained with 75% Compost at 10% initial oil level in comparison to 57% TPH removal in 50% Compost at 5% initial oil level. At a mix ratio of one part of compost to three parts of contaminated soil (25% Compost) with 10% initial oil level, the least dilution of 7.5% was achievable. The 68% TPH removal demonstrates that after the initial effect of the oil, dilution would have played a role in reducing the oil toxicity and the surviving microorganisms were able to utilise the oil and cope with the stress introduced by the presence of oil although the soil itself did not recover quickly to support plant growth. Perhaps, this level of oil concentration adversely affected the soil such that it did not trigger plant growth hence 100% inhibition to growth was recorded (refer to Figure 6.23 pg. 154).

Table 6.3: A summary of Total Petroleum Hydrocarbon (TPH) removal (%). The addition of compost contributed to the dilution of the oil concentration and this effect would have contributed to the overall TPH removal.

Growth conditions (oil concentration by soil weight)	Mix ratio of compost to contaminated soil	Expected oil concentration after dilution (%)	TPH degradation (%) at different diluted oil level
10%	1:1	5	13
	1:3	7.5	64
	3:1	2.5	68
7.5%	1:1	3.75	96
	1:3	5.6	74
	3:1	1.88	89
5%	1:1	2.5	57
	1:3	3.75	45
	3:1	1.25	59

6.5.6 Seed germination

In growth trial 2, the effect of different mix ratios of compost to contaminated soil on the germination and growth of tomato seeds planted at various oil levels was investigated. The different mix ratios of compost to contaminated soils used were 1:1, 1:3 and 3:1. This rationale was to determine an approximate mix ratio of compost that would enhance plant growth at the oil levels investigated. This will help in identifying a better way of treating the contaminated soil to improve yield. Earlier we had proposed that due to the abundance and availability of the biodegradable material suitable for composting, the product could serve as a bioremediation material in the region. This method will also contribute to a sustainable way of waste management. The down side is that if the appropriate amount of compost to be mix with the soil is not determined it might be a case of over-dilution of the contaminated soils and so availability of compost could become a limiting factor during the bioremediation process. What we were trying to determine was the minimum amount of compost we could add to the contaminated soil that will enhance the germination and growth of seedlings.

In other to conserve space, the trend in the number of seeds that germinated and survived is shown in appendix E (Figure E.1 – E.4). There was a general improvement in seedling germination and growth when compared to growth trial 1. In the present experiment higher number of plants survived than in the previous growth trial 1.

Germination counts per tray were taken and replicates were averaged. At 5% oil level, the percentage germination after 14 days of planting was 50, 75 and 81% at mix ratios 1:1, 1:3 and 3:1 of compost to contaminated soil respectively. At 7.5% oil level, the percentage germination was 69, 63 and 75% at mix ratios 1:1, 1:3 and 3:1 of compost to contaminated soil respectively. At 10% oil level, the percentage germination was 12, 0 and 56% at mix ratios 1:1, 1:3 and 3:1 of compost to contaminated soil with the highest percentage germination recorded at a mix ratio of 3:1 and none at 1:3.

Germination is the first step of seedling development in plants. Germination of seedlings is very important when considering the impact of the treatment on contaminated soils even though the farmer can apply other forms of fertilizer during the life cycle of the plants to improve yield. Heeb *et al.*(2006) reported that appropriate nutrient supply is an important component that will help in high yield, however, the effects of nutrients were not considered in the present research. It will be interesting to carry out such investigations during future work with emphasis on nitrogen and phosphorus as they are two of the three macro nutrients needed mostly by plants.

Biomass yield: the average aboveground biomass for 50% compost was higher at (5% oil level) than 7.5 and 10% oil at the same mix ratio. This falls within expectation of our visual observation during the planting period because at 5% oil, the plants were robust with healthy leaves. The leaves play very important role during photosynthesis. The leaves produced by the plants at 5% oil level were normal in appearance when compared to some of the leaves at higher oil levels, which were chlorotic.

The above ground biomass of the different compost/soil mix ratios are shown in Figures 6.20 – 6.23. Results show that the different mix ratios had an effect on biomass yield even though the difference in yield is not proportionate to the compost added. The analysis of the average biomass from different mix ratios at the three oil levels revealed that 75% Compost produced higher biomass of 462, 245.5 and 113.5 mg at 5, 7.5 and 10% oil levels respectively. These values are more than the average biomass yield of 181, 146, and 113 mg at 5, 7.5 and 10% respectively at 50% Compost.

Given the volume of at 75% compost (3:1 mix ratio), it can be concluded that regardless of the higher biomass observed, the effect could largely be due to dilution as it is possible that the compost had masked the oil from having any harmful effect on both the microorganisms and plants. The level of dilution to the oil concentration shown in Table 6.3 revealed that the least dilution of 7.5% was attained at 10% initial oil level for 25% Compost (one part of compost to three parts of contaminated soil) and it seems this oil level is above the trigger level for plant growth. The highest dilution effect was at 75% compost (for all the oil levels). We think that applying compost at the rate of 75% is not economical; it would be advisable to consider applying compost at the rate of 50% (1 part of compost to 1 part of contaminated soil). In general, addition of compost to the contaminated soils improved yield even though variation was observed in some replicates. This variation is worthy of investigation during further work.

In a different experiment, compost was applied as mulch at 10% oil level and compost 1: soil 1 mix ratio. Compost was applied after three weeks of planting at 10% and 20% (soil weight), which came to 50 g and 100 g respectively. A higher aboveground biomass of 970 mg and 1110 mg respectively were obtained in comparison to 185 mg from a similar test condition without reapplication of compost. This method of applying compost as mulch was on a limited number of pot experiments within the limits of the present research, probably if the plants were allowed to reach fruiting stage, the biomass yield from such application might be more conclusive.

A look at the different mix ratios of compost to contaminated soil at 5% oil level revealed that the highest average biomass of 462 mg was at 75% compost application rate. In addition, the 75% compost produced the highest diluted oil concentration of 1.25%. Similarly, at 7.5 and 10% oil levels, the 75% compost application rate, which produced the highest oil dilution, also yielded a higher above ground biomass. At 5% and 10% oil levels, the 25% compost rate (compost/soil 1:3) produced the least dilution reaching 3.75 and 7.5% of oil respectively. Similarly, at this mix ratio, the biomass yield was lower recording 139 mg and no growth at 10% initial growth. This shows that at higher oil levels of 7.5% and above, the development of plants is adversely affected. The effect reduced on addition of compost. We can conclude that under the conditions investigated, applying compost to contaminated soil at 50% rate (compost/soil 1:1) will probably be the most effective in terms of productivity and compost use.

SUMMARY OF KEY RESULTS

At initial contamination levels of 5%, 7.5 % and 10%, the presence of compost improved yield and growth of the tomato seedlings. The observed impact was higher at 75% Compost even though much of this would have been the result of dilution, which reduced the oil concentration to the minimum because the highest dilution was recorded at this mix ratio. In addition, compost at various mix ratios achieved part dilution of the different oil concentration and allowed the soil to reach a trigger level at which plants were able to grow.

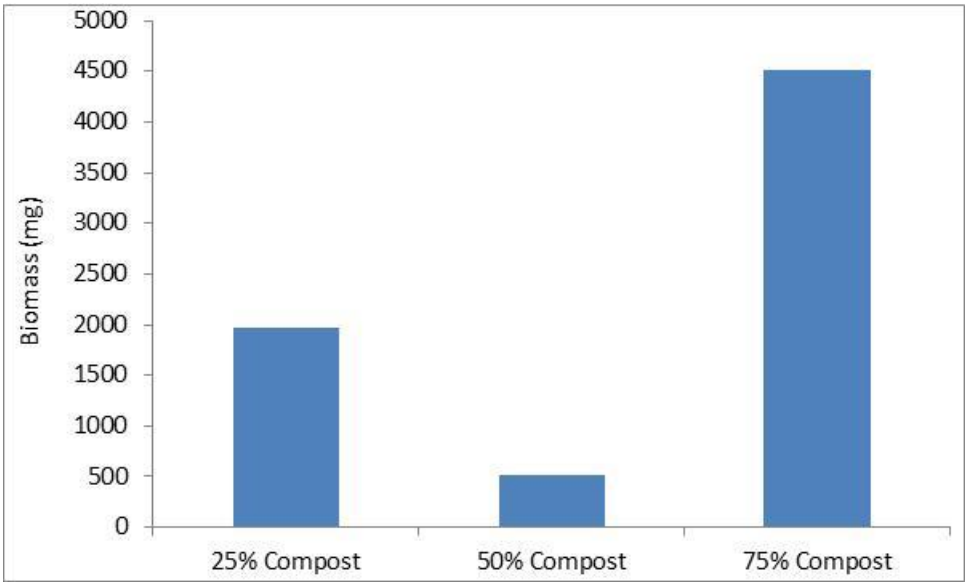

Figure 6.20: Above ground biomass at different compost application rate (controls) after 35 days of planting. The soil samples were from Selby

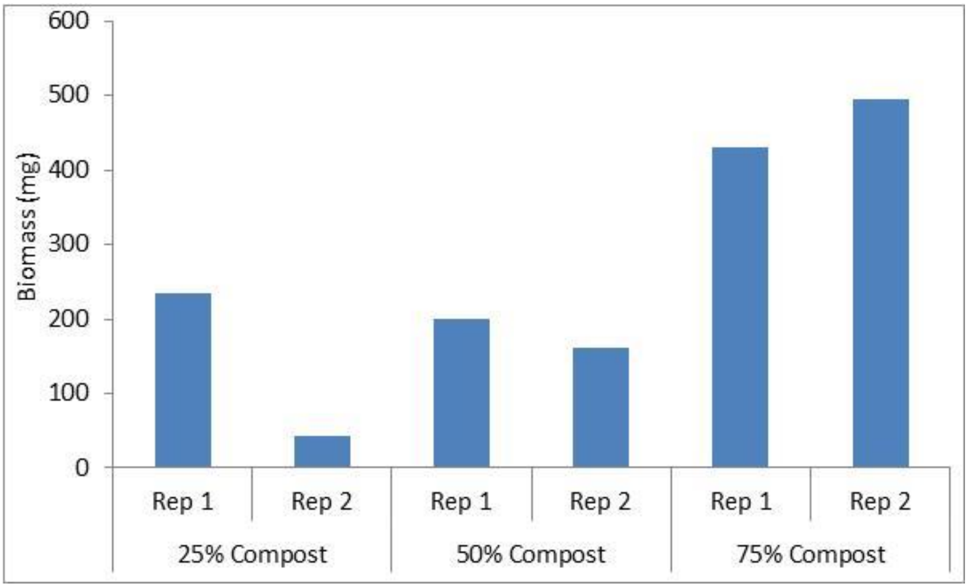

Figure 6.21: Above ground biomass at different compost application rate after 35 days of planting at 5% initial oil concentration. The soil samples were from Selby

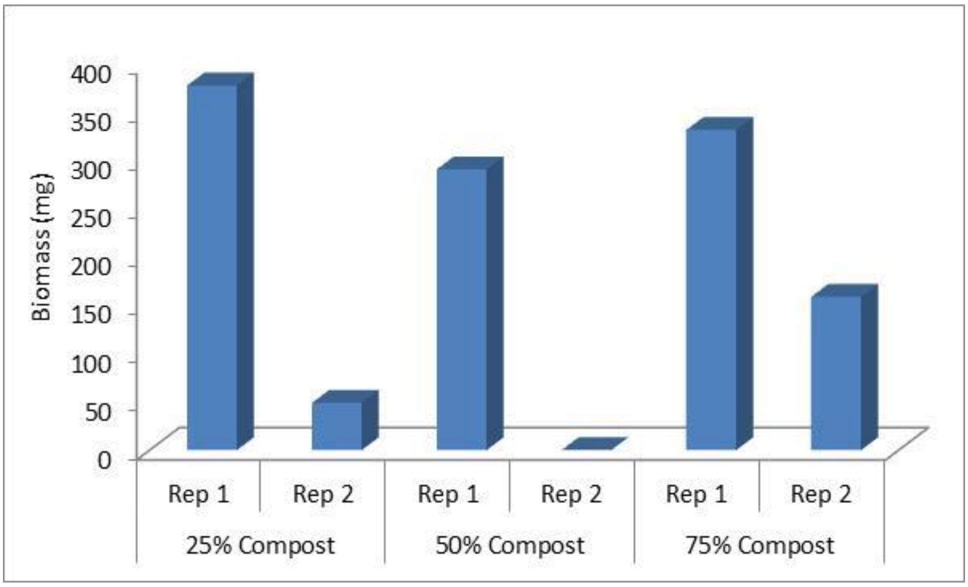

Figure 6.22: Above ground biomass at different compost application rate after 35 days of planting at 7.5% initial oil concentration. The soil samples were from Selby

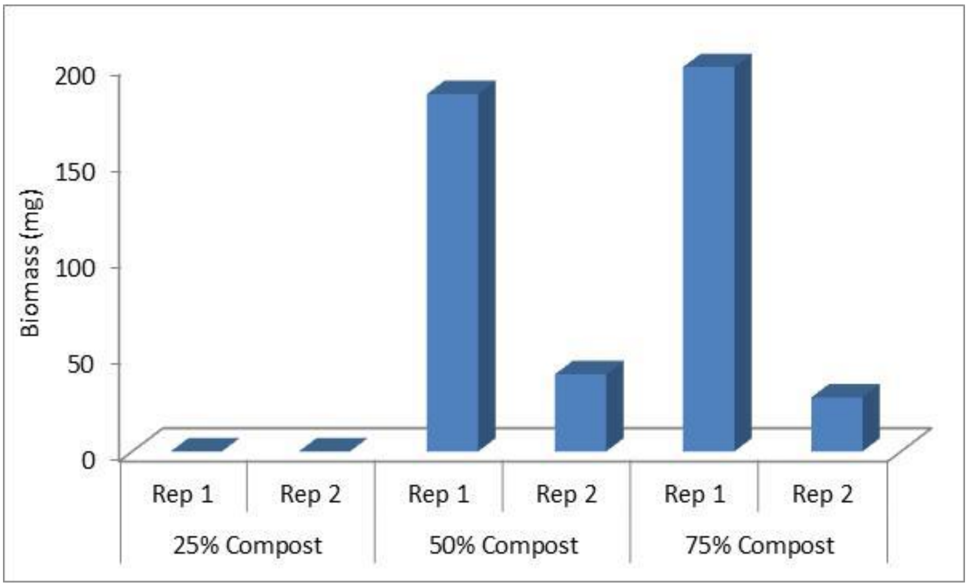

Figure 6.23: Above ground biomass at different compost application rate after 35 days of planting at 10% initial oil concentration. The soil samples were from Selby

6.6 Effect of BLCO on biomass yield of tomatoes at 0, 5, 7.5 and 10% w/w using compost-soil mix at 1:1& 1:2, introducing soil from Niger Delta (growth trial 3)

6.6.1 Qualitative Results

The different mix ratios of compost to contaminated soil in this experiment were at 1: and 1:2 and referred to as 50% and 33% compost application respectively. The results from this experiment were more of a comparison of two different soils, one from the Niger Delta and the other from Bardsey, Leeds (referred to as N/D and UK soil respectively). This has helped us understand the effects of oil contamination from same petroleum products on different soils. Secondly, the use of soil from the Niger Delta region was to make the findings of the research relevant to the region. The soil from the Niger Delta can be described as coarse –textured silt soil with low water holding capacity therefore having more tendencies to lose nutrients from leaching (Igoni *et al.*, 2007). This is comparable to fine textured soils such as loam or clay loam typical of the soil from Bardsey. The soil from the N/D without any form of oil pollution supported germination of the tomato seeds for the first week reaching 33% and 50% germination for the two replicates but the seedlings did not survive up to the second week (14 days). However, on addition of compost to the unpolluted soil at a mix ratio of one part of compost to one part of soil (1:1), there was enhancement of germination and growth in replicates 1 and 2 reaching 85% and 100% respectively at the end of the first week and replicate 1 reaching 100% by the end of the second week.

The N/D topsoil was fetched from farmland in Rivers State Nigeria, which was already prepared for planting. The agricultural practice in the region is described as ‘clear and burn’ a method that exposes the bare soil to the first rain which tends to wash away the nutrients. In contrast, the soil obtained from Bardsey supported germination and growth reaching 83% and 100% respectively on day 14 for the two replicates. The difference in the qualities of the two soils with respect to the organic matter contents (OMC) could have influenced their response to oil contamination. The N/D soil contained 3.7% OMC and the soil from Bardsey had OMC value of 9%.

Measurement of plant's height was on day 35 at the end of planting period. The control pots for Niger Delta soils in which there was no oil contamination did not record any growth hence no plant height was recorded, possibly the low organic matter content of the soil from Niger Delta (3.7%) may have contributed to the low productivity. Similarly at 5%, 7.5% and 10% oil levels, there was 100% growth inhibition and as such no plant height was recorded (Figure 6.24). In a different pot experiment where the control soil was amended with compost, growth was enhanced yielding an average plant height of 10.5, 10.3 cm respectively for replicates one, and two (results not shown). However, in the control pots containing soil from Bardsey without any oil contamination, the average plant height was recorded as 4.3 cm (Figure 6.25). In the case of this control, compost was not added, therefore the growth observed shows that different soils differ in their productivity.

We observed that the average plant height at 50% compost with 2.5% diluted oil level was higher than the remaining pot experiments for both the UK soil and N/D soil. The average height for 50% Compost at 2.5% diluted oil concentration for the N/D and UK soils was 2.05 ± 0.07 cm and 3.35 ± 0.07 cm respectively. Similarly, for the N/D soil with 5% oil level at compost/soil ratio of 1:2, the average height was 2.0 ± 0.28 cm. We recognised the two mix ratios produced quite different diluted oil concentrations, for example at 50% Compost with 5% initial oil level, the dilution oil concentration was 2.5% and at mix ratio of compost/soil 1:2 (33% Compost) with 5% oil level, the dilution effect was 3.3%. Nevertheless, the conclusion we want to draw is that oil contamination affected the plant height.

As observed earlier, there was total growth inhibition in Niger Delta soil at the three levels of oil contamination without addition of compost as well as the unpolluted soil (i.e. control soil without oil). However, addition of compost to contaminated soils enhanced soil productivity.

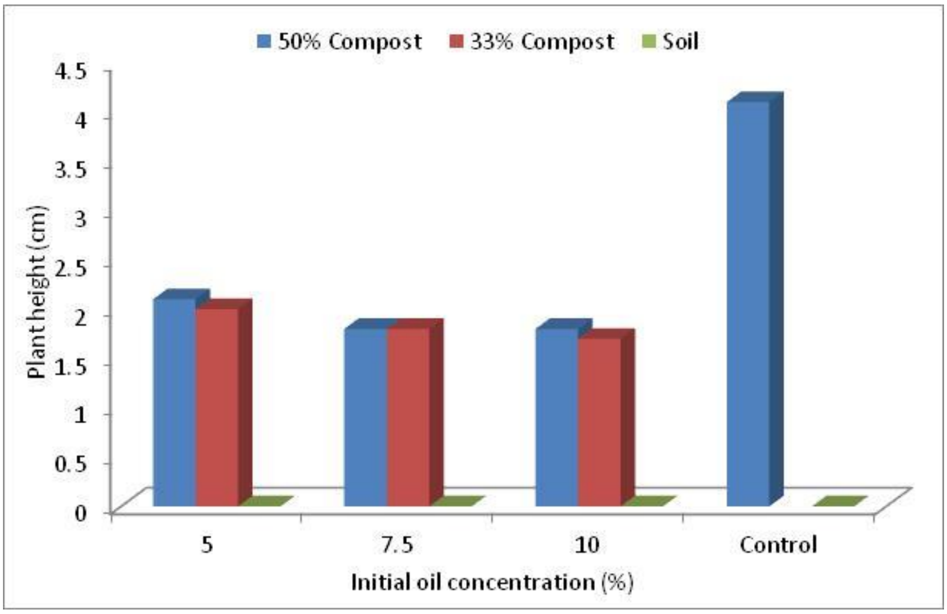

Figure 6.24: Plant height at 35 days of soil samples from the Niger Delta showing different mix ratios of compost to contaminated soil

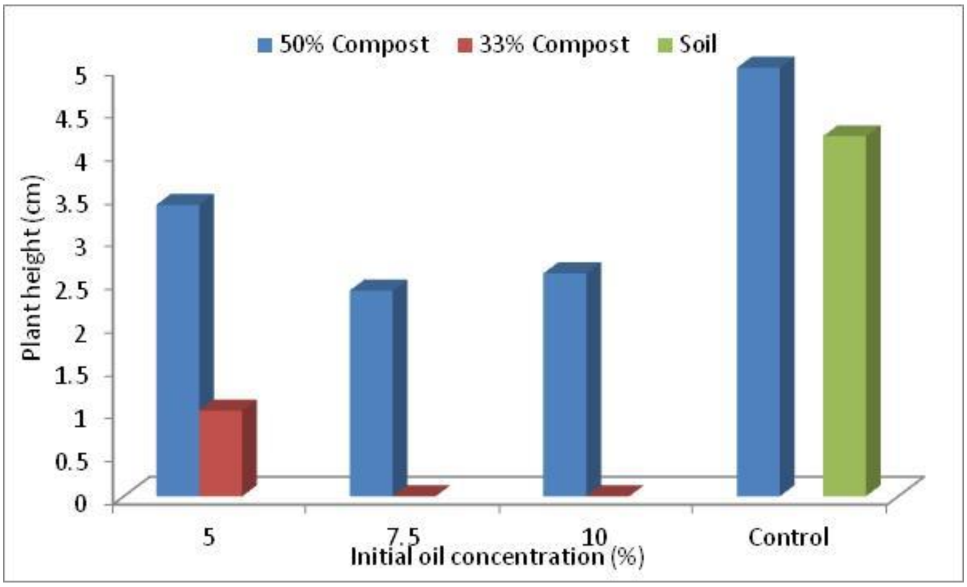

Figure 6.25: Plant height at 35 days of soil samples from Bardsey showing different mix ratios of compost to contaminated soil

6.6.2 Analytical results

Analysis of variance (ANOVA) was used to compare means. Even though it is possible for population means to be equal, sample means are hardly equal, there will be differences due to sampling variation. Therefore, using ANOVA helps to compare the variability between and within groups. Analyses of variance for physico-chemical parameters were performed. The correlations were calculated between selected parameters and soil types over time. P-value was defined at 0.05. In general, treatment with compost increased soil pH and electrical conductivity for both soils types (Figures 6.26 to 6.29). The software used is IBM SPSS Statistics 19 for Windows®. The data used for the analysis is presented in Appendix F. The contaminated soil was defined by the oil levels for example in categorising the N/D soil numbers were assigned from 1 to 10 to allow us perform the analysis. Similar soil classification based on level of oil contamination was used for UK soil.

1) Soil (without contamination or treatment)
2) Soil 5% oil (soil with 5% oil level (w/w) and without treatment)
3) Soil 7.5% oil (soil with 7.5% oil level (w/w) and without treatment)
4) Soil 10% oil (soil with 10% oil level (w/w) and without treatment)
5) Compost 1: Soil 1 5% oil (compost/soil mix at 1:1 ratio and 2.5% diluted oil)
6) Compost 1: Soil 1 7.5% oil (compost/soil mix at 1:1 ratio 1:1 and 3.75% diluted oil)
7) Compost 1: Soil 1 10% oil (compost/soil mix at 1:1 ratio 1:1 and 5% diluted oil)
8) Compost 1: Soil 2 5% oil (compost/soil mix at 1:2 ratio and 3.3% diluted oil)
9) Compost 1: Soil 2 7.5% oil (compost/soil mix at 1:2 ratio and 5% diluted oil)
10) Compost 1: Soil 2 10% oil (compost/soil mix at 1:2 ratio and 6.7% diluted oil)

The hypothesis tested states that there is no significant difference in mean pH for the contaminated soils (i.e. soils types were defined by the various oil levels) and the incubation days. The ANOVA table (Table 6.4) gives F statistics = 12.76, $p < 0.001$ for incubation days; 229, $p < 0.001$for contaminated soil and 2.72, $p = 0.001$ for combination of incubation days* contaminated soil, $r = 0.978$. This suggests that there is a strong correlation between soils contaminated at different oil levels, days of incubation and pH in the experiments using the soil from Niger Delta. Therefore, oil levels in contaminated soils, incubation period as well as their interaction can be used to explain the difference in pH. The nature of these differences was not explored in the current research. Soil pH and soil electrical conductivity gave a moderate positive correlation with germination of tomato seeds: r = 0.748, p = 0.001 for pH and r = 0.209, p = 0.037 for electrical conductivity. A moderate positive relationship exists between the two variables, r = 0.567 for contaminated soil and r = 0.322 for days of incubation (Table not shown). For the soils from Bardsey, there was no statistically difference (p = 0.01) at 5% probability levels, however r = 0.947 which shows a strong correlation between contaminated soil, days of incubation and pH. Electrical conductivity showed moderate correlation for the variables i.e. seed germination (r = 0.079) and days of incubation (r = 0.549). Electrical conductivity as a feature of the soil measures the ions in soil aqueous phase and can affect the soil properties that affect crop productivity such as salinity level and organic matter.

Table 6.4: Summary of ANOVA (N/D soil) for dependent variable pH, correlation between pH, electrical conductivity and germination

Tests of Between-Subjects Effects

Dependent Variable:ph

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	109.159[a]	49	2.228	45.116	.000
Intercept	5854.086	1	5854.086	118556.567	.000
Days	2.520	4	.630	12.760	.000
Soil	101.805	9	11.312	229.084	.000
days * soil	4.833	36	.134	2.719	.001
Error	2.469	50	.049		
Total	5965.714	100			
Corrected Total	111.628	99			

a. R Squared = .978 (Adjusted R Squared = .956)

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.748[a]	.559	.555	.70841

a. Predictors: (Constant), germination

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	62.447	1	62.447	124.436	.000[a]
	Residual	49.180	98	.502		
	Total	111.628	99			

a. Predictors: (Constant), germination

b. Dependent Variable: ph

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.209[a]	.044	.034	218.44067

a. Predictors: (Constant), germination

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	212817.383	1	212817.383	4.460	.037[a]
	Residual	4676200.052	98	47716.327		
	Total	4889017.436	99			

a. Predictors: (Constant), germination

b. Dependent Variable: EC

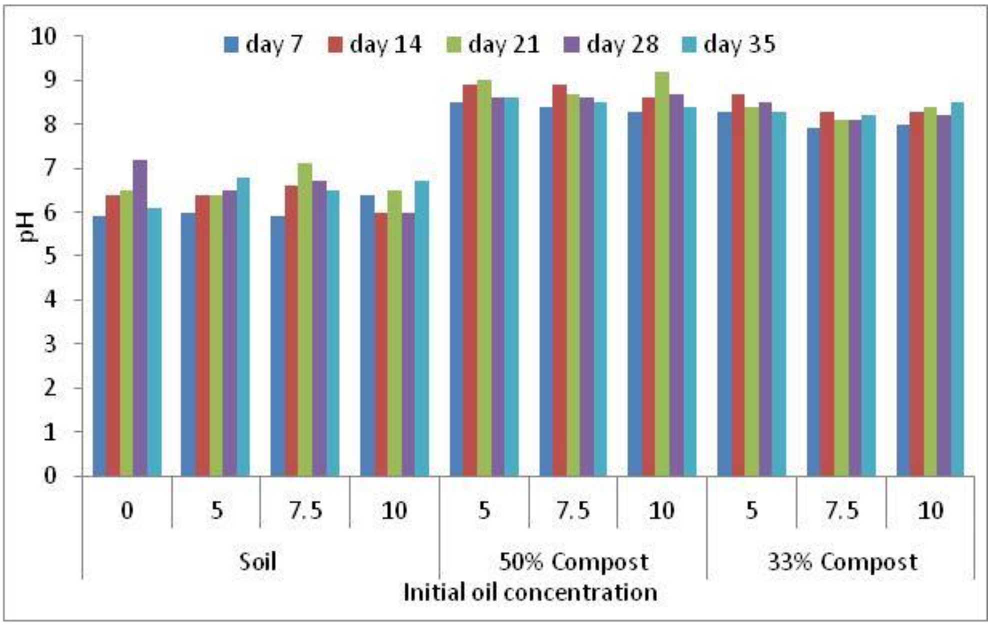

Figure 6.26: Variation of the pH over 35 days of soil samples obtained from the Niger Delta. Measurements were every week for 5 weeks.

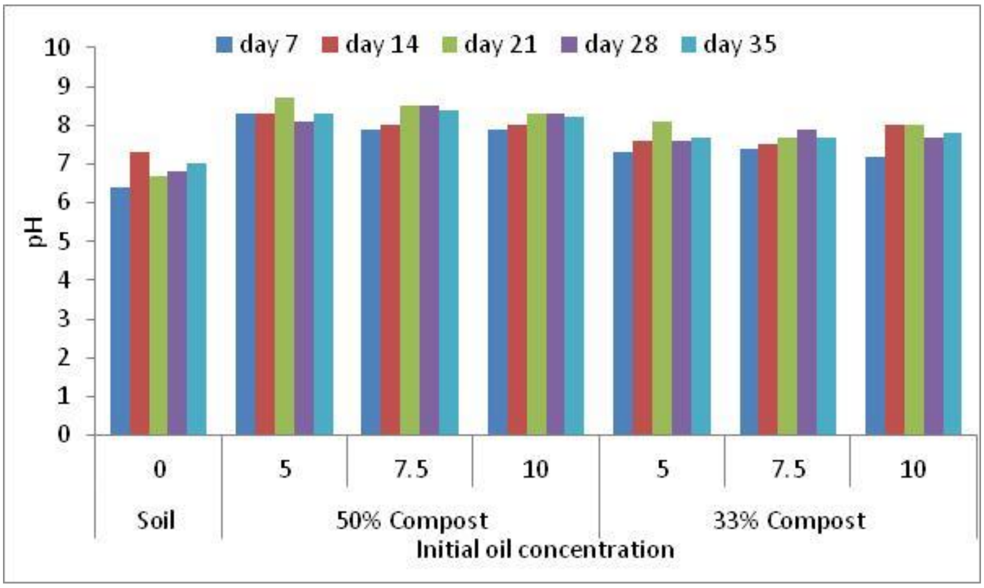

Figure 6.27: Variation of the pH over 35 days of soil samples obtained from Bardsey. Measurements were done once every week for 5 weeks

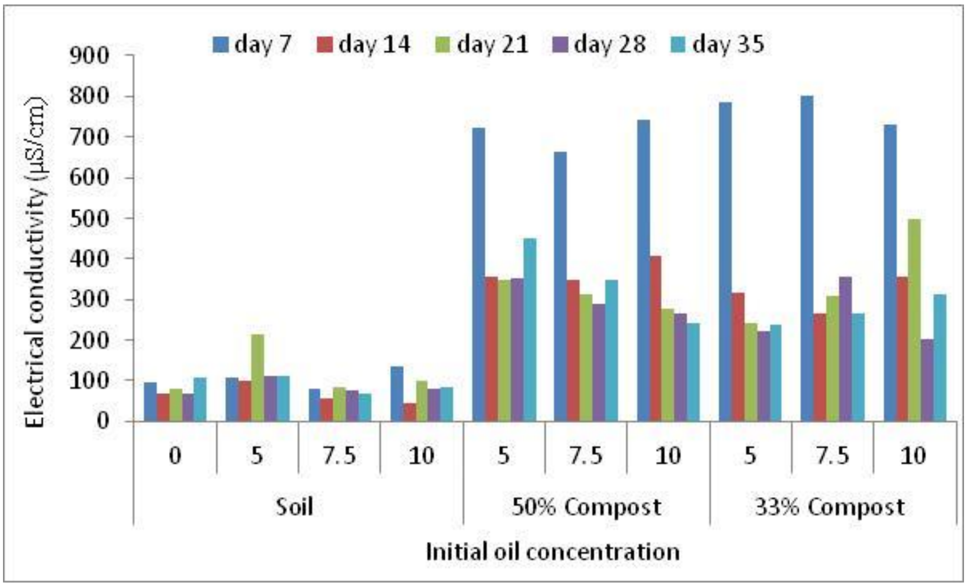

Figure 6.28: Variation in conductivity over 35 days of soil samples obtained the Niger Delta. Measurements were done once every week for 5 weeks.

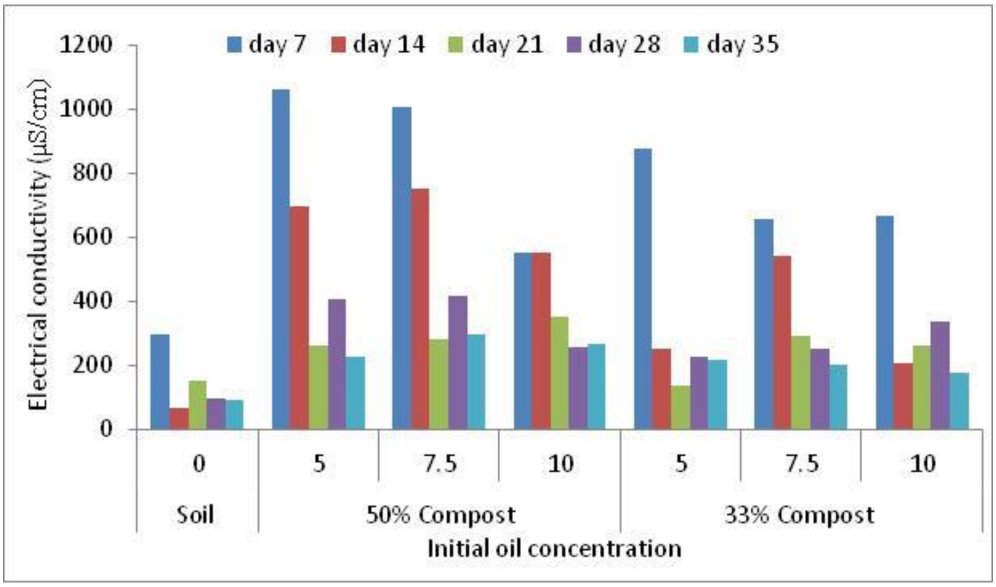

Figure 6.29: Variation in conductivity over 35 days of soil samples obtained from Bardsey. Measurements were done once every week for 5 weeks.

6.6.3 Nitrogen content

Measurement of TKN (% dw) for the feedstock used in plant growth 3 revealed that compost had a high nitrogen content of 0.1% in relation to the other feedstock which were in the range; 0.02 to 0.06% dw. Addition of crude oil at different oil levels to the soil did not affect the TKN of the spiked soil because the nitrogen in the crude oil is negligible. In the end of experiment, the contaminated soils (both Niger Delta and UK) with addition of compost showed higher values of TKN (Figure 6.30). Perhaps the high nitrogen content of the compost contributed to the high TKN in the treatment pots. The trend in TKN of UK soil with 7.5 and 10% oil level and compost-soil ratio 1:1 showed TKN reducing with increasing oil contamination recording 0.04 and 0.08% dw at 10 and 7.5% oil level respectively. By contrast, the Niger Delta soil recorded higher TKN at 7.5 and 10% oil level than at 5% oil level on addition of 50% Compost. Probably, the way and manner that crude oil can affect a particular soil differs from the other.

Niger Delta soil had a lower TKN values and it further reduced after the planting period perhaps the low nitrogen contributed to the total growth inhibition observed in N/D soils without compost treatment at 5%, 7.5% and 10% oil levels. However, on addition of compost growth was observed. It is important to recognise that dilution of the contaminated soil because of the addition of compost could have played a role in reducing the oil contamination to a trigger level, which allowed plant growth. The UK soil recorded nearly steady TKN values after planting; probably this could be the reason for sustained growth in the uncontaminated UK soil (i.e. without oil contamination). The reducing trend of TKN in UK soil on contamination with crude oil may also be the reason why the average biomass yield at 50% Compost was reducing with increasing oil level reaching 138, 71 and 64 mg respectively at 5, 7.5 and 10% oil level (Figures 6.42 – 6.44). The values of TKN was higher in N/D soil with 7.5% oil level at compost to soil ratio 1:1 and at 10% oil level reaching (0.12 and 0.11% dw respectively) than at 5% oil level (0.07% dw). With respect to average biomass, yield was higher at 7.5% and 5% (266 and 178 mg respectively) than at 10% (126mg). For full details see Figure 6.35 – 6.37. Probably, the effect of higher oil level (10%) on plants was more and as a result masked any observable impact from availability of nitrogen.

In general, the test conditions with compost-soil ratio 1:1 had higher values of TKN. Different factors could be responsible for the variation in nitrogen content such as differences in sampling approach, or heterogeneity of feedstock (even though large particles were removed to reduce heterogeneity to a minimum). As the level of nitrogen and phosphorus are reducing, it could be an indication that they serve as fertilizers or food to the microorganisms that degrade the petroleum hydrocarbon.

Nitrogen is the key nutrient limiting plant growth and productivity in soils (Nie *et al.*, 2011). It is not very clear how petroleum contamination impacts on the ability of plants to use different forms of nitrogen however, many studies have focused on the effects that nitrogen addition has on plants growing in petroleum-contaminated soils

Organic Nitrogen content estimated using Kjeldahl's nitrogen is commonly referred to as TKN. The organic nitrogen is the form that is available to soil microorganisms for use, even though for plants it is converted to inorganic form before use. For compost, even though it is possible to measure the total nitrogen present, it is difficult to determine its availability to crops in the laboratory, and a local farmer might not even have the capacity to measure available nitrogen routinely.

In view of the above, there might be need to supplement the nitrogen in case of nitrogen-deficient soils. Nitrogen can be lost from agricultural soil either by soil leaching or through runoff especially in areas with high rainfall intensity. Nitrogen also needs to be applied with caution, when nitrogen input to soil is more than the crop needs , there is a possibility of excess nitrates polluting surface or ground water as well as reducing the activity of the microorganisms in the soil. The nitrogen contents in plants' tissue ranges from 1 to 6% (Ros *et al.*, 2005). For plants to use organic nitrogen sources, it must be converted to inorganic forms before they become available to plants either as ammonium (NH_4+) or nitrate (NO_3-).

In the present study we measured nitrogen as total Kjeldahl's nitrogen (TKN) which measures the sum of organic nitrogen. One of the mechanisms of conversion of organic nitrogen found in soil organic matter and manure to inorganic nitrogen is through the process of mineralisation by the actions of bacteria. The bacteria breakdown the organic matter and releases ammonium nitrogen, this ammonium nitrogen increases with increase in microbial activities. Because the nitrogen is subject to different degrees of transformation, this will also determine the availability of nitrogen to plants (O'Leary *et al.*, 2002). However, this research did not investigate different ways of nitrogen transformation.

As mentioned previously (§ 2.4) inorganic nitrogen in the form of nitrate nitrogen and ammonium nitrogen are readily available to plants. However, before plants use nitrate it is converted to ammonium ions and the process is energy consuming, the energy would otherwise have been used for the plant's productivity.

When nitrates are used as fertilizer, they can easily be leached out of soil and this makes them difficult to be retained in the soil. This is one of the disadvantages of using mineral fertilizers. The mechanisms responsible for the loss, gain and transformation of nitrogen are complex and as such not covered in the present study.

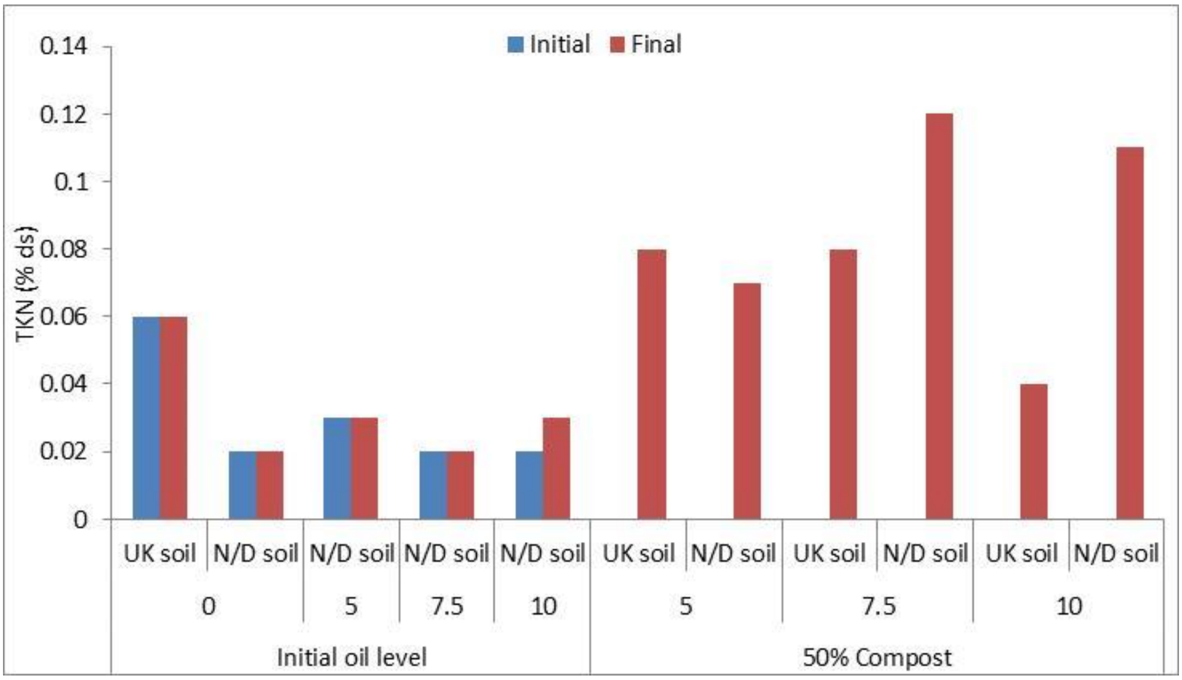

Figure 6.30: Nitrogen measured as TKN at the start and end of planting period. The mix ratio of compost to contaminated soil is 1:1.

6.6.4 Phosphorus

At the end of 35-day planting period, extractable phosphorus was measured in neutralized aliquots (10 ml) of soil extract using the ammonium molybdate-ascorbic acid method. Concentrations of phosphorus were not significantly different between treatments ($P = 0.001$). Available phosphorus in the end of the investigation was decreasing with increasing oil level. This is similar to the observation in plant growth 2. Experimental conditions using soils from Niger Delta recorded 57.9, 27.2, and 15.2 mgP/kg dw at 5, 7.5 and 10% oil level respectively at compost- soil ratio 1:1. We can say that phosphorus was lost from the system. For the experimental conditions using

UK soil, the values of phosphorus were 70.1, 53.7 and 45.5 mgP/kg dw at 5, 7.5 and 10% oil level respectively at compost- soil ratio 1:1. Osuji and Adesiyan (2005) reported a range of 4 to 80 mg/kg as phosphate in crude oil contaminated soils in Isiokpo area of the Niger Delta region. The values observed from the results analysis from N/D soils was within the limit. It is possible that the decrease in phosphorus is associated with the greater mineralisation of organic matter during biodegradation.

Available literature on phosphorus in the soil is concerned mostly with the factors that govern phosphorus mineralization rather than phosphorus build up in soil (Brady, 1977). Soil organic matter is made of carbon, oxygen, hydrogen, nitrogen, sulphur and phosphorus, however the other constituents except for phosphorus are added to the soil basically from the atmosphere but for phosphorus it is mainly from the parent material (Brady, 1977). Microorganisms can convert soil organic phosphorus into inorganic phosphorus by process of mineralization. The variation in phosphorus can be affected by a number of factors depending on whether the organic phosphorus is closely bound to carbon, nitrogen and sulphur of the humus or whether it is present as independent organic phosphorus. However, the present research did not include fate of phosphorus in the soil. There is little understanding about the contribution of organic phosphorus compounds and their combination with organic matter with respect to the variation in the phosphorus content of soil organic matter. Other contributing factors include; the hydrolytic enzymes which generally control the the rate at which various substrates are degraded, the amount of total petroleum hydrocarbon added, availability of nutrients, type of soil and difference in number and type of microbial population (Rivera-Espinoza and Dendooven, 2004).

6.6.5 Total petroleum hydrocarbon (TPH)

Result analysis monitored the removal rate of petroleum hydrocarbons at the end of 35 days planting period. Crude oil is a mixture of different components of various concentrations. For the purpose of comparison, the removal rate of TPH was measured as the percentage of its initial concentration within the petroleum biodegraded in the soil. There was a marked decrease with time in the percentage of total petroleum hydrocarbon in all the soil samples. The percentage removal was lowest in crude oil contaminated soils without addition of compost. Similar results have been reported in the literature (Namkoong *et al.*, 2002, Van Gestel *et al.*, 2003, Okolo *et al.*, 2005, Ayotamuno *et al.*, 2006, Adekunle, 2011).

After five weeks of bioremediation, the percentage reductions for Soil at 5%, Soil 7.5% and Soil 10% oil level without addition of compost were 30%, 39% and 32% respectively. For the treatment cells (i.e. test conditions with addition of compost), TPH measurement was done at only compost/soil ratio of 1:1 because that was the preferred mix ratio with respect to biomass yield.

The TPH analysis for the test conditions using Niger Delta soil with 50% Compost at 10%, 7.5% and 5% oil levels were 34, 62 and 86% respectively. Similarly, the TPH contents of the test conditions using UK soil recorded 32, 91 and 62% with 50% Compost at 10%, 7.5 and 5% oil levels respectively. In general, the lowest removal was recorded at 10% oil level and the highest at 7.5% oil level. Probably at 10% oil level, the microbial population could not cope with the level of toxicity but at 7.5% the hydrocarbon would have been utilized as carbon source for the microorganisms hence an increase in the metabolic activities, which can be translated to more breakdown of hydrocarbon during respiration. As stated earlier (Table 6.3), we need to recognise the dilution effect from the addition of compost to the contaminated soil, which could have helped reduce the final oil concentration thereby causing the oil level in the contaminated soil to reach a trigger level where it allowed plants to grow.

Ayotamuno *et al.* (2006) reported a range of 50 to 95% reduction in degradation of total hydrocarbon content of a Niger Delta soil spiked with crude oil in which different amounts of mineral fertilizer were applied as treatment. The experimental period was for six weeks. Adekunle, (2011) reported a reduction in total petroleum hydrocarbon of 40 to 75.87% when using composted municipal wastes after incubation period of three (3) weeks. However, the incubation period in the present study was for 5 weeks.

The results indicate that the application of compost increased the removal rate of the hydrocarbons because the hydrocarbon removal from contaminated soil without treatment was lower. This was reflected in the biomass regardless of the intial germination in the contaminated soils. Figure 6.31 to 6.34 shows the trend in germination. There was no survival at the end of 5 weeks planting period (35 days). The biomass yield for the cells that received compost as treatment revealed that yield was decreasing with increasing oil level. The biomass yields were 178, 266 and 126 mg at 5, 7.5 and 10% oil level for the Niger Delta soil and 138, 71 and 64 mg at 5, 7.5, and 10% oil level respectively for the UK soil.

6.6.6 Seedling tests

There was inhibition at 10% oil contamination in the present study similar to the observations made by (Udo and Fayemi, 1975) who reported that the higher the oil pollution levels, the lower the performance of maize seedlings. They recorded no germination of maize seeds at 10.6% oil level (w/w). They recorded 44% germination of maize grains at 6.8% oil contamination by soil weight. Adekunle (2011), accordingly observed 100% toxicity to maize germination on a diesel-contaminated soil but on the contrary in crude oil contaminated soil, the toxicity was reduced to 13.33%.

The trend in poor germination was not different when soils from the Niger Delta were spiked with crude oil as depicted in Figures 6.31 to 6.34. There was growth inhibition at 7.5 – 10% oil contamination. The poor germination and survival of seeds at 5% oil level was almost the same except at day 21, one of the replicates recorded 17% germination even though it did not survived in the end of planting period. On addition of compost to the test conditions, 50% increase in overall germination was observed after 5 weeks. The results demonstrated that application of compost in crude oil contaminated soils enhanced the soil and sustained germination and growth.

Exposing tomato seeds to increasing crude oil concentrations may have adversely affected their germination and biomass yield. It is advisable to reintroduce the use of compost for the local farmers in the Niger Delta region not just for treatment of crude oil contaminated soils but even for routine farming especially as high cost of mineral fertilizer drives them to reuse the contaminated land without any form of treatment. As earlier mentioned in the literature review, local farmers in the region had previously practised backyard composting but in the recent times, the use of mineral fertilizers has taken over even though it is neither cheaper nor readily available. Therefore, reintroducing compost as organic amendment seems a more sustainable option in the region.

In the absence of extensive waste management in the Niger Delta region, the feasible option for the organic fraction of the municipal solid waste is composting even though there is no record of commercial production of compost in Nigeria (Adekunle *et al.*, 2010, Adekunle, 2011). A fifty-kilogram bag of nitrogen-phosphate-potassium fertilizer (NPK) costs an average of twenty to twenty-four pounds in the open market in Nigeria. The cost of compost made locally for example; a 50 kg bag of cow manure is available at less than five pounds (£5) from animal farms. A comparison of the prices of fertilizer and manure on per kg of nitrogen is demonstrated in the example below.

Example:

50 kg of fertilizer	= £25/bag
Assume 20% N as dry solids	= 0.2 x 50 kg
Price/ kg N	= £25/10 = £2.5/kg
Compost at £5/50 kg (50% moisture)	= £5/25 kg dry solids
Assume 2% N	= 0.02 x 25 kg
Price/ kg N	= £5/0.5 = £10/kg

Even though the price of nitrogen per kilogram is lesser in the fertilizer than the compost however, the emphasis on compost is not all about nitrogen content. Compost is not seen as fertilizer but as soil conditioner, it affects soil structure and absorption. We had mentioned in § 7.1.1 (pg.181) that in certain circumstances, there will be combination of compost and fertilizer depending on what the plant's needs are.

At oil contamination levels of 5% and 10%, the presence of compost improved yield, also varying the mix ratios of compost to contaminated soil did affect the growth of the tomato seedlings. In the case of contamination at 7.5% oil level, the mix ratio of compost to contaminated soil did have an observed impact on yield with higher yields at higher compost ratios (Table 6.5).

Namkoong *et al.*(2002) investigated the effects of different mix ratios of compost and sewage sludge on diesel contaminated soils. The ratios of contaminated soils to organic amendments were 1:0.1, 1: 0.3, 1:0.5 & 1:1) on wet weight basis. Their results were analysed using kinetic models and the conclusions were similar to our findings. The use of mature compost as amendment when compared to soil only experiment gave more active degradation of TPH about 98.4% at 1:0.5 mix ratio which is equivalent to 33% compost at oil level (1% w/w) given that the diesel was applied at 10 000 mg/kg soil.

In the present study at compost/soil 1:1, which is approximately 50% compost before dilution (2.5%) by the compost, 86% removal of TPH was observed at 5% oil level w/w. Perhaps, the difference in percentage degradation is due to low level of contamination in their study (1% oil w/w as against 5% in the present study). Namkoong *et al.* (2002) reported that higher mix ratio of compost did not amount to higher degradation rate. They concluded that the carbon source in the amendment material should not be preferable to the microorganisms rather than the microorganisms using carbon from the target contaminant. In essence, the appropriate mix ratio will help to avoid such an occurrence i.e. the amendment should not be acting as a competing energy source.

In the present study, use of compost did not impose the danger of a competitor because the level of degradation in compost-addition experiments was higher when compared to soil-only experiment. Therefore, we recommend doing a feasibility pilot study using the recommended mix ratio of 50% Compost.

Even though comparison of different studies on biodegradation of TPH have been made above, it is worth noting that Van Gestel *et al.* (2003) highlighted the challenges of making comparisons of bioremediation treatment in contaminated soils because of the difference in soil types, target contaminants and experimental times. In addition, they reported that microorganisms are specialized differently in the way and manner they breakdown hydrocarbons and the type of petroleum hydrocarbon they breakdown.

Table 6.5: Summary of the biomass yield from the different experiments, note that the experiments have different dilution effects and was at different times, the chart is to give a picture of the impact of oil on yield at different oil levels.

Experiments	Mix ratio of compost to contaminated soils (C+S)	Oil levels by material weight (w/w)	Oil concentration (%) after dilution with compost	Average biomass yield (mg)
Plant growth 1	C + S 1:1	5%	2.5	130
	C + S 1:1	7.5 %	3.75	55
Plant growth 2	C + S 1:1	5 %	2.5	181
	C + S 1:3	5 %	3.75	139
	C + S 3:1	5 %	1.25	462
	C + S 1:1	7.5 %	3.75	146
	C + S 1:3	7.5 %	5.6	214
	C + S 3:1	7.5 %	1.9	246
	C + S 1:1	10 %	5	113
	C + S 1:3	10 %	7.5	0
	C + S 3:1	10 %	2.5	114
Plant growth 3 (Niger Delta soils)	C + S 1:1	5 %	2.5	178
	C + S 1:2	5 %	3.3	194
	C + S 1:1	7.5 %	3.75	266
	C + S 1:2	7.5 %	5	109
	C + S 1:1	10 %	5	126
	C + S 1:2	10 %	6.7	109
Bardsey soils	C + S 1:1	5 %	2.5	138
	C + S 1:2	5 %	3.3	7
	C + S 1:1	7.5 %	3.75	71
	C + S 1:2	7.5 %	5	0
	C + S 1:1	10 %	5	64
	C + S 1:2	10 %	6.7	0

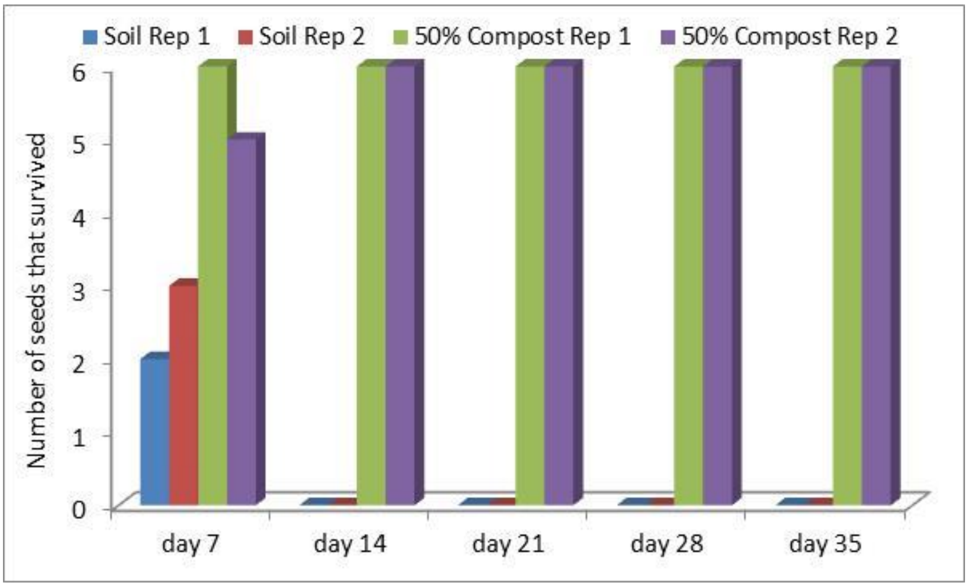

Figure 6.31: The numbers of seeds that survived (controls) over 35 days. Counting was once every week for 5 weeks and the soil was from the Niger Delta

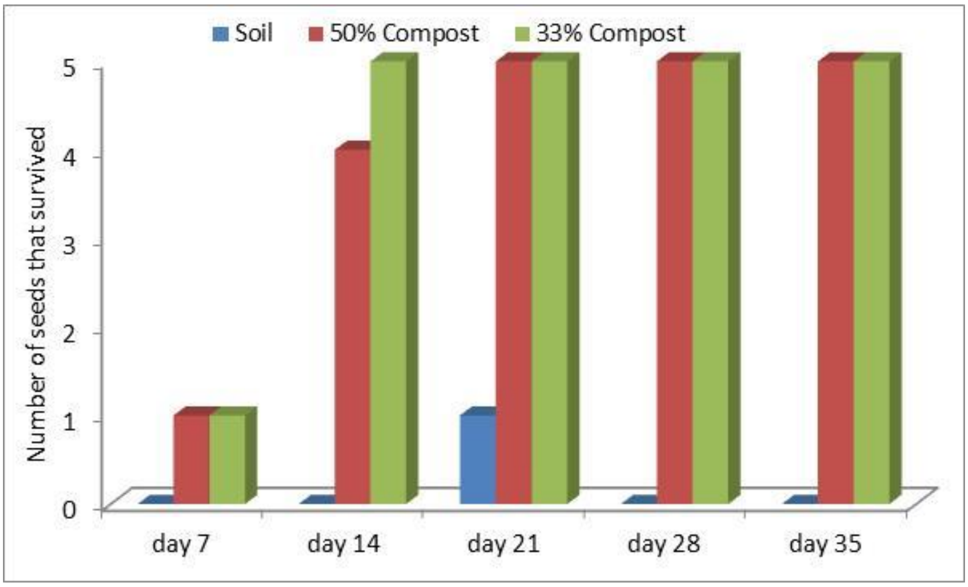

Figure 6.32: The numbers of seeds that survived at 5% initial oil level over 35 days. Counting was once every week for 5 weeks and the soil was from the Niger Delta

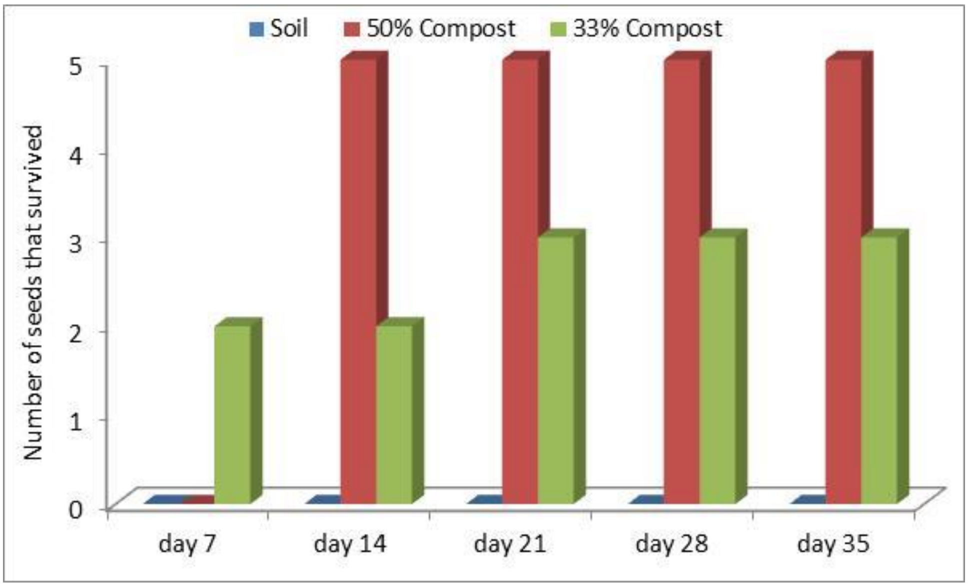

Figure 6.33: The numbers of seeds that survived at 7.5% initial oil level over 35 days. Counting was once every week for 5 weeks and the soil was from the Niger Delta

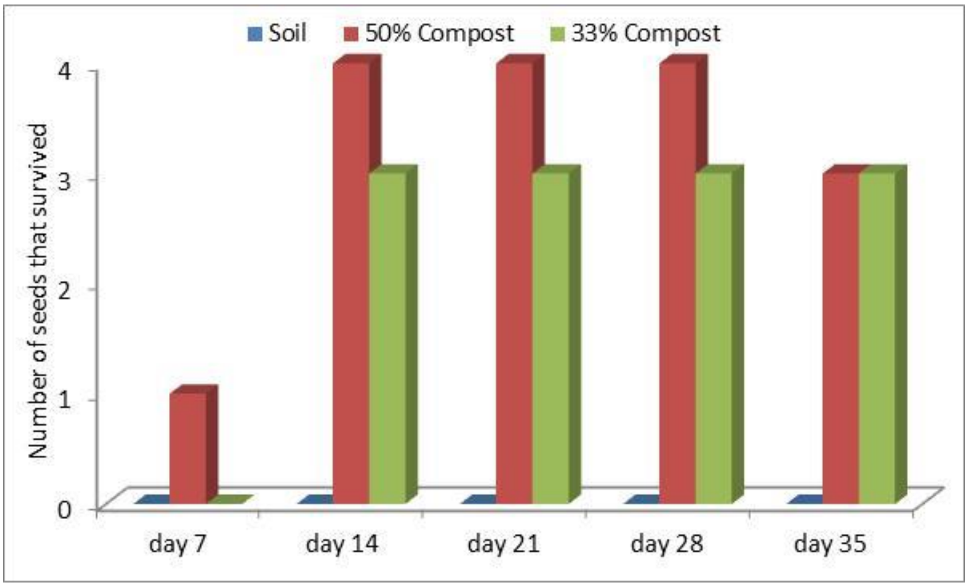

Figure 6.34: The numbers of seeds that survived at 10% initial oil level over 35 days. Counting was once every week for 5 weeks and the soil was from the Niger Delta

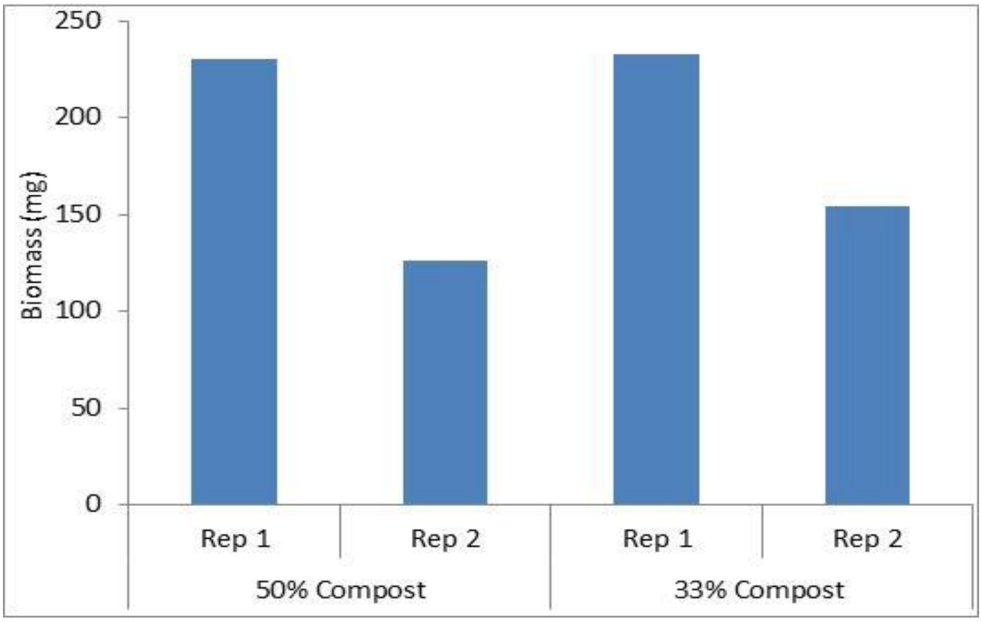

Figure 6.35: Above ground biomass of soil samples from the Niger Delta after 35 days at 5% initial oil level

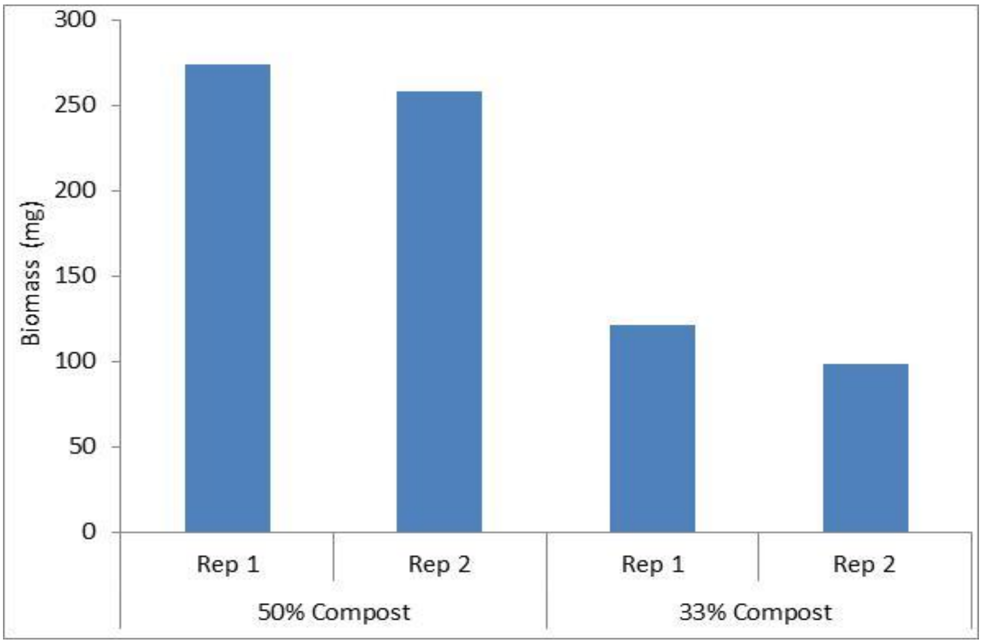

Figure 6.36: Above ground biomass of soil samples from the Niger Delta after 35 days at 7.5% initial oil level

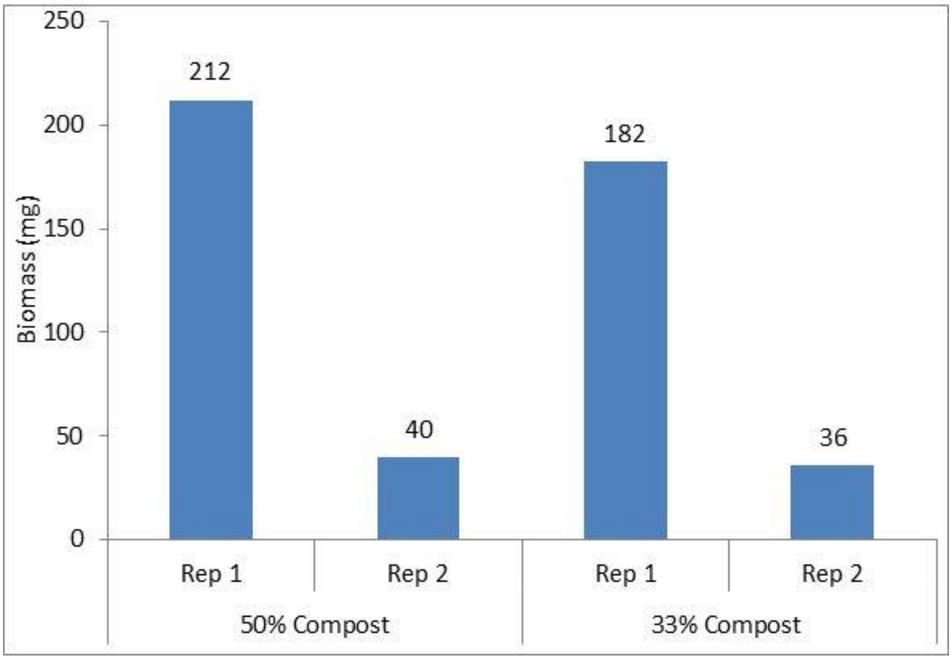

Figure 6.37: Above ground biomass of soil samples from the Niger Delta after 35 days at 10% initial oil level

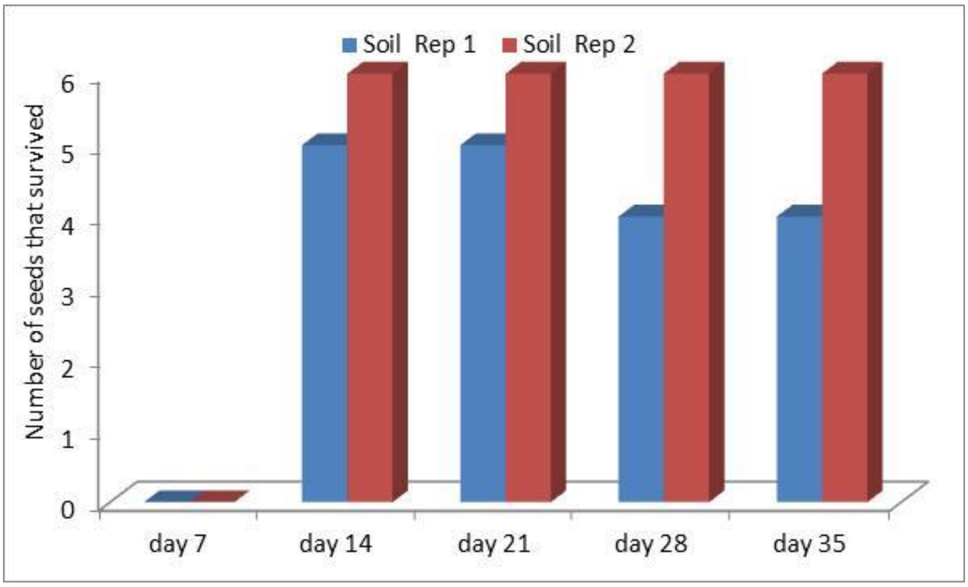

Figure 6.38: Germination and survival of seeds (control) during 35 days planting period. The soil was from Bardsey

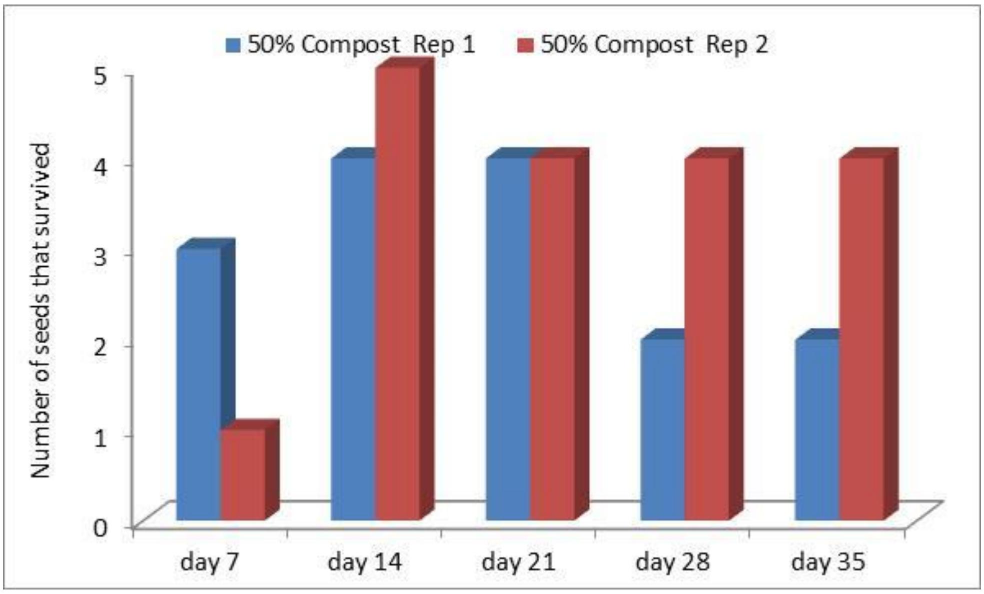

Figure 6.39: Germination and survival of seeds during 35 days planting period at 5% initial oil (w/w). Soil was from Bardsey

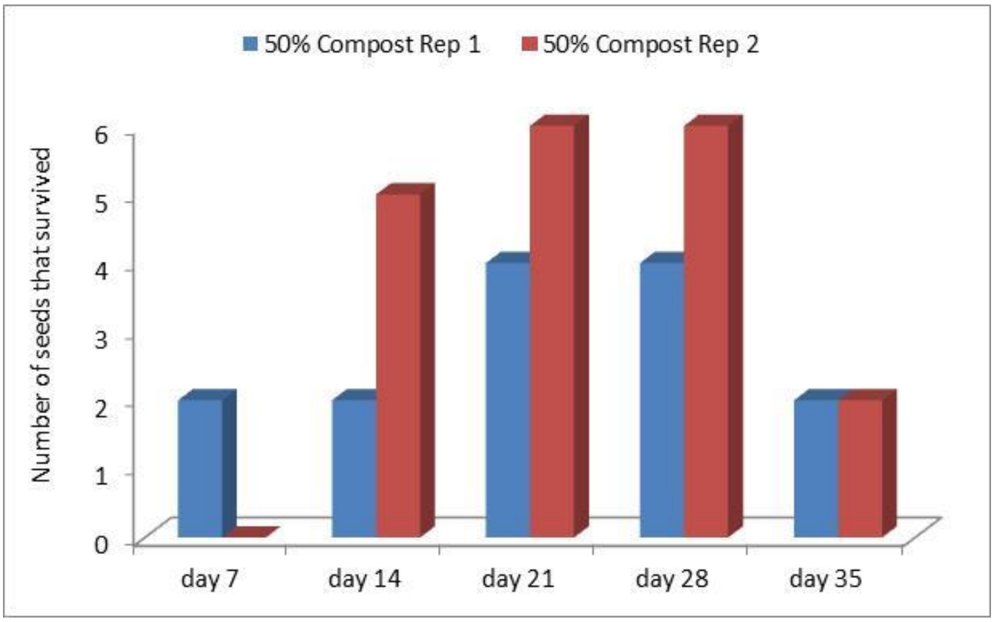

Figure 6.40: Germination and survival of seeds during 35 days planting period at 7.5% initial oil (w/w). Soil was from Bardsey

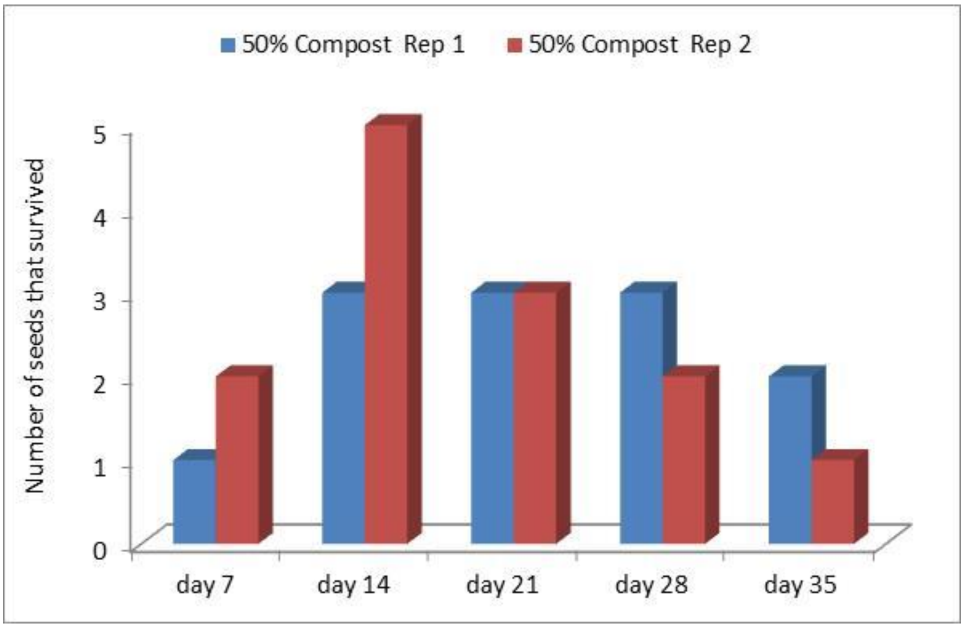

Figure 6.41: Germination and survival of seeds during 35 days planting period at 10% initial oil (w/w). Soil was from Bardsey

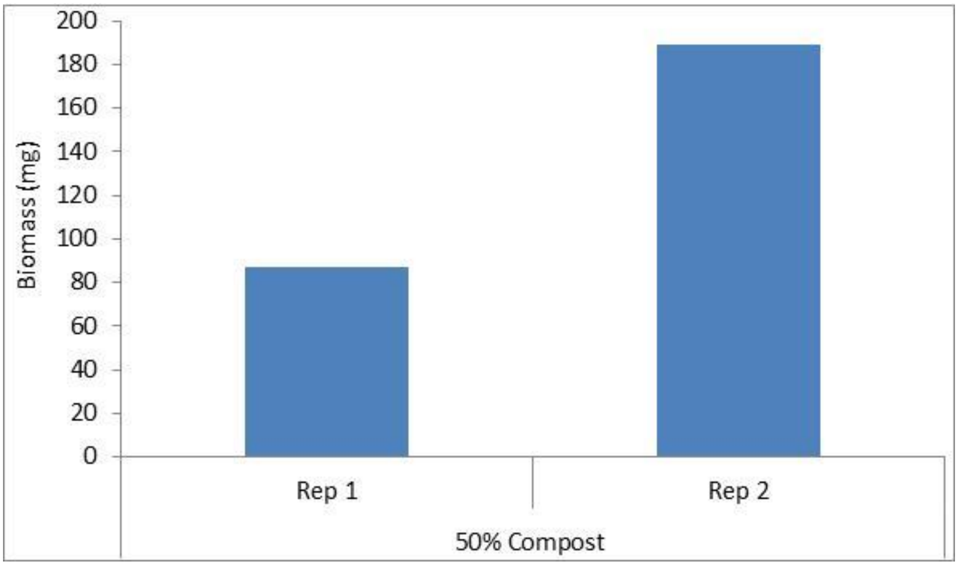

Figure 6.42: Above ground biomass of soil samples obtained from Bardsey at 35 days planting period at 5% initial oil level

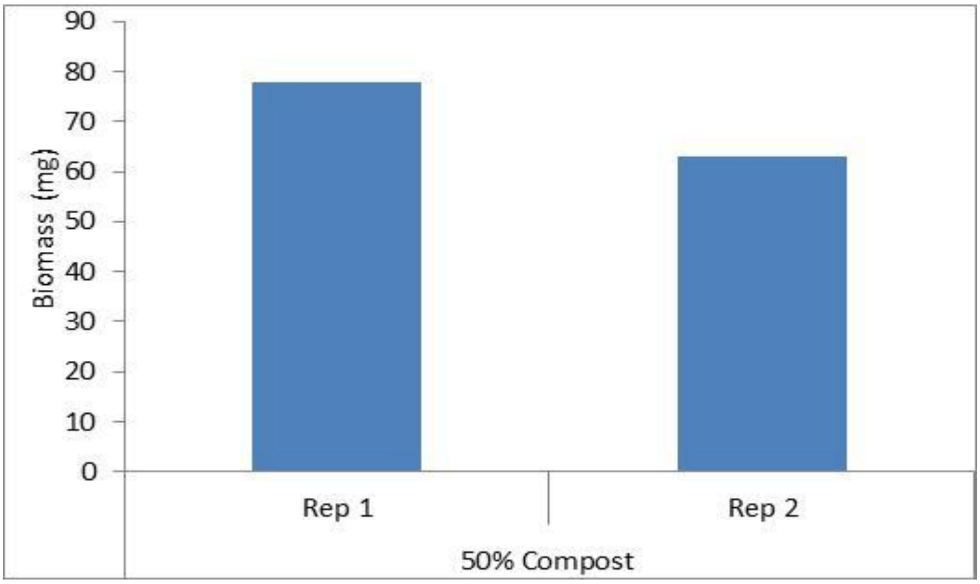

Figure 6.43: Above ground biomass of soil samples obtained from Bardsey at 35 days planting period at 7.5% initial oil level

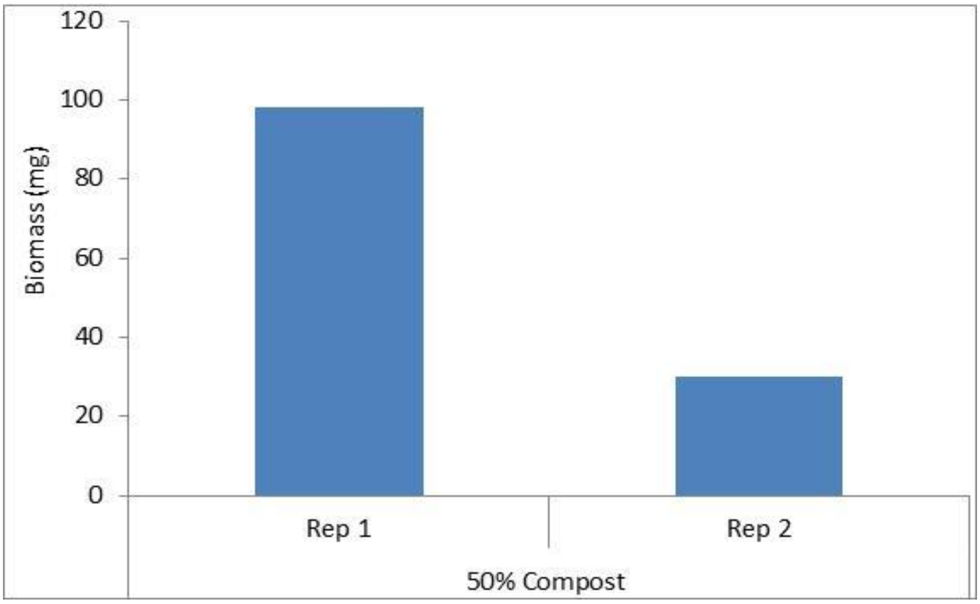

Figure 6.44: Above ground biomass of soil samples obtained from Bardsey at 35 days planting period at 10% initial oil level

7 Treatment Protocols for the Niger Delta and Areas of Similar Contexts

7.1 General overview of the treatment protocols

The situation that currently exists within the Niger Delta for the restoration of oil-contaminated land is rather ad hoc and there is no guidance provided for either the oil companies or local farmers as to how to deal with such soil. In response to this lack of guidance, a number of observations from the present study were used as the basis for developing treatment protocols that could be used by the oil companies and local farmers. These protocols are set of procedure to be put in place after an oil incident. For full discussion on availability of compost in the Niger Delta region, refer to § 1.8.

For the purpose of this research, crude oil contaminated sites were grouped into three categories, bearing in mind likely situations that might arise in real life. These are non-recoverable, recoverable and unpolluted reference zones. The non-recoverable zone comprises (a) burnt contaminated sites (due to fire incidents after an oil spillage) and (b) sites affected by major spills and not recoverable biologically in a reasonable time (generally for contamination levels greater than 10% oil by soil weight). The recoverable zone assumed to fall below the ten percent oil level would receive treatment in the form of compost application at different mix ratios of soil-compost and the unpolluted reference zone, which is the control. We assume that no matter how bad the oil pollution is, some areas not polluted will serve as the reference zone.

The land use in the Niger Delta is such that within the larger family, the land sharing is per householder. On average, an extended family consists of 5 to 7 householders and the extended family owns the land as a partnership. Typically, the land comes to one hectare per householder. The householder is able to make maximum use of the land through the practice of mixed cropping and sometimes he borrows a piece of land from the community for use in a particular planting season. Depending on the yield from the harvest, it serves the family first and any surpluses are sold in the market to earn some cash for other household needs.

Assuming there was a piece of land affected by an oil spill; usually the farmer abandons it or farms on the unaffected area. In the Niger Delta region, the local farmer's knowledge about crude oil contaminated soils is limited to what he can see, usually the waxy appearance of the soil after an oil spillage. Depending on the intensity of the oil spillage, the farmers are not bothered about the effect of the oil on the crops. They farm on the affected land and only if there is inhibition to germination would they realise the extent of damage to the land and abandon it.

Other occasions that the contaminated land is abandoned are during legal actions when the affected farmers want to prove that the contaminated land was not good for farming. Depending on the severity of the oil spillage and if a fire after the incident, will determine whether the contaminated land is abandoned. A typical example is Ebubu community in the Eleme local government area of Rivers State in the Niger Delta region. In this case, the entire contaminated land was burnt and charred making it an abandoned site since 1998. Our intention is to determine how much of such abandoned land is recoverable by applying the proposed treatment protocol (i.e. reclamation of contaminated soil).

The protocol recognises that for any crude oil contaminated land, the degree of contamination is likely to vary across the site. The land is divided into segments depending on the level of contamination. A series of trials will involve using vegetable crops, which the farmer is likely to plant. These vegetables in the Niger Delta would commonly include tomatoes, hot pepper, okra and maize. The treatment consisting of different compost mix ratios would then be used to assess seedling emergence and subsequent plant yields.

For the local farmer who might not come to terms with specific measurement units, the amount of compost needed will be quantified using buckets or barrows as these are measures, which the farmer will understand better. For example, a standard bucket in Nigeria is equivalent to 20 litres. The contaminated land will be dosed in buckets per square metre to quantify the approximate volume of compost to be applied.

The protocol does not intended to be complex, we proposed the use of vegetable crops in the cases where the root zones are shallow and roughly, the mixing depth will be 150 mm. It is common in the literature for topsoil samples to be collected to depths of approximately 150 mm, probably because of the shallow root systems of many vegetable species. Ren *et al.*(2010) described the root zone for tomatoes as 0 to 300 mm. A surface depth of 15cm (150 mm) has mostly been used on studies involving the use of agricultural topsoil. The following researchers used a depth of 100 to 150mm (Anoliefo and Vwioko, 1995, Osuji and Nwoye, 2007). Other researchers have used a greater mixing depth of 300mm (Ebuehi *et al.*, 2005, Ayotamuno *et al.*, 2006, Adoki and Orugbani, 2007b).

It is important to get involved with the farmer in order to deliver the protocol effectively and to get feedback. The testing of the protocol on the farms in the Niger Delta forms the next phase of the work and is discussed in the chapter on Further Work at the end of the thesis.

7.1.1 Variation in results of different mix ratios of compost to contaminated soils in the present research

This result for this part of the study was in line with expectations due to variations in the composition of the compost and soil because we used different batches of both materials from different locations in different experimental trials. The key point in the protocol is to expect that there will be differences in both the soil and the compost but to use an evaluation method that takes account of these. The use of seedling tests for vegetable crops to assess the recovery of a crude oil contaminated soil should cover expected variations in both the Niger Delta and similar areas facing such oil pollution. In some cases, we advise the use of nutrient supplements (organic or mineral fertilizer depending on the cheaper alternative) for the seedlings as they grow to enhance yield. The ranges of nutrient requirements of some plants in the region are shown in Table 7.1. These nutrient values are the minimum requirements in the soil for the specific plant growth.

Table 7.1: Summary of soil nutrient concentrations (NPK) observed in farm crops used in the Niger Delta and acceptable values for agricultural soils.

Species	Total nitrogen (TN) %	Phosphorus (PO_4^{-3}) mg/kg	Potassium (K) ppm	Source
Maize (Zea mays)	0.078 – 0.095	14.7 – 17.9	28.5 – 39.4	(Udo and Fayemi, 1975)
Okra, Maize and fluted pumpkin	0.08	7.02 – 12.28	0.06 meq/100g soil	(Adoki and Orugbani, 2007b)
Tomatoes	0.07 – 0.11	15.2 – 57.9		Present study

Table 7.1 shows concentrations of macronutrients from previous studies both from study and control areas within the region. It was observed that the plants grew well with these levels of nutrients.

Before an effective protocol is developed, it requires an understanding of the needs of the different groups affected. In this case, it includes the oil companies, the government, the farmers and the community at large. Some of the technicalities might be challenging for the farmers and the community to understand, therefore in a real situation we might engage the services of agricultural extension officers within the local council to assist in training the farmers. The agricultural extension officers are employees of the local council. In addition, the participation of the local farmer in the cleanup will need some sort of funding which the oil company should finance, as this might be another opportunity for them to redeem their image within the oil communities.

The proposed protocol will be in two parts. Part (a) designed specifically for the oil companies or the government due to the technicalities involved and part (b) will not have the complexities of chemical analysis making it easier for the local farmer to apply (the focus of this research).

The different oil levels (0 -10%) at which this study was carried out included a maximum of 10% which is regarded as the upper limit based on the range of values from the literature at which agricultural topsoil has been spiked with crude oil. Anoliefo and Vwioko, (1995) investigated the effects of lubricating oil on tomatoes and hot pepper at 0 to 5% w/w and Udo and Fayemi (1975) applied crude oil on maize at 0 to 10% oil w/w. In reality, especially for excessive spills, oil levels have been found to be higher for example Osuji and Adesiyan, 2005 reported a spill in Ogbodo-Isiokpo, Rivers State which released 1.9 x 10^6 Litres of crude oil over a land area covering 15 900m^2 which worked out to be 41.6% oil level w/w at a planting depth of 150 mm. Therefore, a spilled site will be categorised based on anticipated levels of crude oil contamination.

This protocol is applicable in countries with less advanced technology especially in the developing countries where (a) the cost of labour is low and (b) financial constraints might not allow government or local authorities to invest in the high technology methods available in more advanced nations. Even though the research was laboratory based, it has led to a better understanding of the issue of oil pollution, its effects on crop yields, which formed a basis for developing a treatment protocol that will allow an end user to grow crops on contaminated soil.

Despite that, previous investigations in Nigeria have shown the efficacy of bioremediation of contaminated soils using microorganisms, mineral and organic fertilizers; no such studies produced treatment protocols to enable a local farmer get involved in the clean-up of his contaminated soils using appropriate technology.

7.2 Proposed protocol for the oil companies

The protocol described below is for the oil companies and comprises six steps namely; environmental analysis, generation of waste derived-compost, determination of contamination level, treatment of contaminated soil, farm trials following treatment of contaminated soil and full use of treated soil for cultivation.

1. Environmental analysis: for every spill, the offending companies can possibly determine the volume of crude oil that has spilled and hence the mass of the oil could be determined. From the area and depth of land covered by the spill, the volume of the affected land can be determined and the percentage of contamination estimated. It is important to note that the depth of oil penetration could vary across the contaminated site depending on various factors such as the soil texture and concentration of spilled oil (oil spreading may not be uniform).

For the purpose of this protocol, 150 mm depth is assumed as the planting depth as referenced by Odu, (1972). He investigated the extent of hydrocarbon contamination in the Ogoni Niger Delta and reported that even in heavily contaminated areas; the crude oil penetration was limited to the top 150mm of soil. Anoliefo and Vwioko (1995) also considered similar planting depths of 100 – 150 mm. These planting depths were limited to vegetable crops as they mostly have shallow root systems. The depth for crops with deep roots could vary as demonstrated by Essien and John (2010) who reported 300 - 600 mm as the root zone depth of certain tropical crops.

2. Generation of waste derived compost: this is achievable by using the simple windrow method. As earlier stated, there is abundance of organic waste in the region. The raw materials are gathered into piles of 1.5 – 2.5 m high (the lower height is applicable where the pile systems are small and turned by hand). The turning of the piles is periodical, to allow aeration. Following the initial rapid composting period (2 – 3 months), the compost matures for upwards of two further months. For compost application, areas with contamination levels above 10% might need repeated applications of compost, which should be reviewed seasonally as the yield, might improve over time. We assume that ready to use compost will be available for the treatment and possibly the oil companies hold supplies in readiness.

3. Mapping out contaminated site: the area of contaminated soil will be subdivided into different sections to determine the contamination level given the oil pollution will not be evenly spread. This will be done initially based on a visual assessment so that the more polluted areas are separated from the less polluted ones.

4. Treatment of contaminated soil: apply a 1:1 mix ratio of compost to contaminated soil as treatment, based on the laboratory results.

5. Farm trials following treatment of contaminated soil: crop choice will involve planting a number of crops (such as maize, tomatoes, okra and hot pepper which are local to the region) on the affected soil and monitoring germination, growth and biomass yield for a period of 28 days.

6. Full use of treated soil for cultivation: the section of land with the best yield is cultivated. The crops to be grown are determined by the planting season. Compost could be re-applied to sections of land with low growth. The remainder of the soil with poor

yield would receive repeated applications over a period of years to enhance the performance.

As mentioned earlier, the polluting companies are likely to contribute to the financial cost of implementing the treatment protocol for instance in setting up of a pilot composting plant. As part of the outreach team, we could be responsible for monitoring and evaluation of the treatment operations. The steps shown above might be exclusive for the oil companies and government sector where they chose to be involved. In order to achieve a high success rate during implementation, Figure 7.1 shows the recommended project life cycle of the treatment protocol for the oil companies.

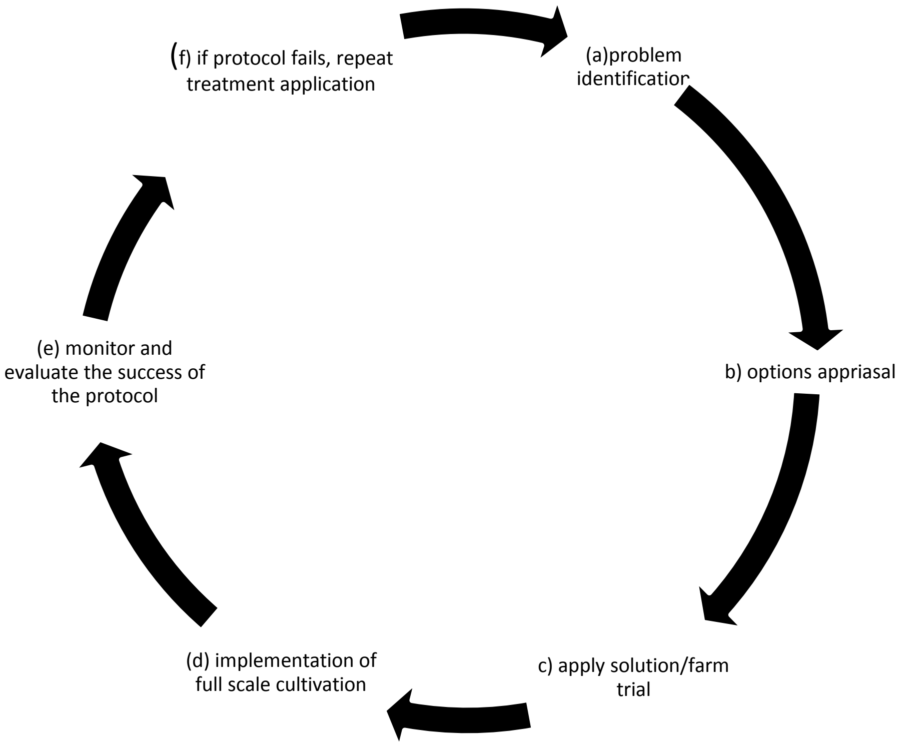

Figure 7.1: A systematic diagram for the project cycle of the protocol designed for the oil companies and government.

Equations 7.1 to 7.7 are used to define a hypothetical case of a contaminated soil covering an area ($\boldsymbol{A_s}$) and volume ($\boldsymbol{V_s}$), and the volume of compost ($\boldsymbol{V_c}$) to be applied at a mixing depth of ($\boldsymbol{H_s}$), at a mix ratio of one part of compost to one part of contaminated soil.

Annotations

$\boldsymbol{V_s}$ = Total volume of contaminated soil

$\boldsymbol{V_c}$ = Total volume of compost required for the treatment of total volume (V_s) of contaminated soil

$\boldsymbol{A_s}$ = Surface area of contaminated soil

$\boldsymbol{H_s}$ = The mixing depth of contaminated soil

$\boldsymbol{V_{si}}$ = Volume of contaminated soil sectioned out for batch treatment

$\boldsymbol{V_{ci}}$ = Volume of compost required for batch treatment of (V_{si}) volume of contaminated soil

$\boldsymbol{A_{si}}$ = Surface area of contaminated soil sectioned out for batch treatment

$\boldsymbol{\bar{\rho}_s}$= Average density of contaminated soil

$\boldsymbol{\bar{\rho}_c}$ = Average density of compost

$\boldsymbol{m_s}$ = Mass of contaminated soil

$\boldsymbol{m_{si}}$ = Mass of contaminated soil sectioned out for batch treatment

$\boldsymbol{m_c}$ = Mass of compost for treatment of $\boldsymbol{m_s}$ **(**kg) of contaminated soil

$\boldsymbol{m_{ci}}$ = Mass of compost for batch treatment of $\boldsymbol{m_{si}}$ **(**kg) of contaminated soil

7.2.1 Total Volume of Contaminated soil to be treated

This is deduced from the relationship; volume = area × height and mass = density × $v$olume

$$V_s = A_s \times H_s \tag{7.1}$$

$$V_s = \sum_{i}^{n} V_{si} = \sum_{i=1}^{n} A_{si} H_s \tag{7.2}$$

And,

$$m_{si} = \bar{\rho}_s \times V_{si} = \bar{\rho}_s \times A_{si} H_s$$

$$m_s = \bar{\rho}_s \times V_s = \bar{\rho}_s \sum_{i=1}^{n} A_{si} H_s \tag{7.3}$$

7.2.2 Batch Treatment of contaminated soil with compost

Given the higher yield from the experiment was at mix ratio of one part of compost to one part of contaminated soil (by weight), let us assume it to be 1 kg of contaminated soil to 1 kg of compost,

Mass of compost ($\boldsymbol{m_{ci}}$) required for batch treatment of mass of contaminated soil ($\boldsymbol{m_{si}}$) is

$$\boldsymbol{m_{ci}} \equiv \boldsymbol{m_{si}} \text{ (Based on weight)}$$

Therefore**,**

$$\bar{\rho}_c \times V_{ci} = \bar{\rho}_s \times V_{si} \tag{7.4}$$

$$V_{ci} = \frac{\bar{\rho}_s \times V_{si}}{\bar{\rho}_c} \tag{7.5}$$

$$V_{ci} = \frac{m_{si}}{\bar{\rho}_c}$$

Therefore, total volume of compost needed for the treatment of the contaminated soil at a 1:1 mixing ratio is,

$$V_c = \sum_{i=1}^{n} V_{ci} = \sum_{i=1}^{n} \frac{m_{si}}{\bar{\rho}_c} = \sum_{i=1}^{n} \frac{\bar{\rho}_s \times A_{si} H_s}{\bar{\rho}_c} \tag{7.6}$$

$$V_c = \frac{\bar{\rho}_s}{\bar{\rho}_c} \sum_{i=1}^{n} A_{si} H_s \tag{7.7}$$

However, the oil company or the farmer wants to know the volume of compost to add per square metre and not the amount to add per cubic metre even though he has to assume a depth as well.

Let us assume the following,

Density of soil ≡ 1300 kg/m^3 (1 kg of soil will be of volume 1 kg/1300 kg/m^3 = 0.00076 m^3)

Density of compost ≡ 600 kg/m^3 (1 kg of compost will be of volume 1 kg/600 kg/m^3 = 0.00167 m^3)

To calculate the area of contaminated soil at a planting depth of 50mm (0.05 m) since using soil at a depth of 0.15 m will consume so much compost,

Area x depth = volume

Area = 0.00076 m^3/0.05 m

Area = 0.0152 m^2

At compost-soil ratio 1:1, 0.00167 m^3 of compost is needed on 0.0152 m^2 of contaminated soil.

Therefore, a square metre of contaminated soil will need compost of volume; (0.00167 m^3/0.0152 m^2) = 0.11 m^3 equivalent to 110 litres of compost which can be represented as 5.5 standard buckets in Nigeria where one standard bucket is equivalent to twenty litres. Overall, the volume of compost per square metre of contaminated soil at 0.05 m depth is 0.11m^3/m^2. The addition of compost at a depth of 50 mm though less than 150 mm will give a greater depth for the plant root. It is feasible to repeat compost application over a period of years by applying at the above rate. Depending on the soil response, we can review application of compost on yearly basis.

The oil companies can adopt the above formula as specifications to their local contractors who have little or no expertise thereby encouraging a more sustainable clean up method.

7.3 Treatment protocol for the local farmer

In order to get the farmers to use this protocol, the procedure will entail the following four steps: feasibility of the scheme, mapping out the contaminated site, sourcing of organic matter/compost, compost application and cultivation.

Step 1: feasibility of the scheme

This involves meeting with the oil producing community to determine the influencing factors, which include the village residents, the village chiefs and the farmers, as well as identifying the contaminated sites.

Step 2: map out the contaminated site

The local farmer has limited knowledge about the extent of crude oil contamination on a site. However, we will rely on his experience of knowing the contaminated sites that have potential for recovery based on the waxy appearance and intensity of spreading.

As earlier shown in § 7.2, rather than using soil at a depth of 0.15 m which will consume so much compost, start the first year with a depth of 50 mm (0.05 m) of compost and evaluate yield. It might not get a full crop but then we can repeat the treatment in the following year. The farmer needs to mix the soil thoroughly with the compost for the top 50 mm of soil.

Therefore, a square metre of contaminated soil will need compost of volume 0.00167 m^3/0.0152 m^2) = 0.11 m^3 equivalent to 110 litres of compost which can be represented as 5.5 standard buckets in Nigeria where one standard bucket is equivalent to twenty litres.

Step 3: Sourcing of organic matter or compost

This will include locally available materials and if necessary, a pilot composting plant will be set up since there is abundance of biodegradable waste in the region. The operations will need labour; we suggest that families that benefit from the scheme should supply the labour even if funding comes from the government and the oil companies. Mostly in community projects, people can only get involved when they are beneficiaries.

The amount of daily waste (1,580 tonnes) in the region that is compostable has been fully discussed in § 1.8. Therefore, at a density of 600 kg/m^3, the amount of contaminated land that is treatable using this volume of waste at 5.5 buckets/m^2 is approximately 24,000 m^2.

Step 4: compost application and cultivation.

Apply and mix compost at the rate of 5.5 buckets/m^2 across the recoverable parts of the sites and plant local species such as maize, okra, tomatoes and hot pepper.

For a local famer, the steps are shown in Figure 7.2 and 7.3:

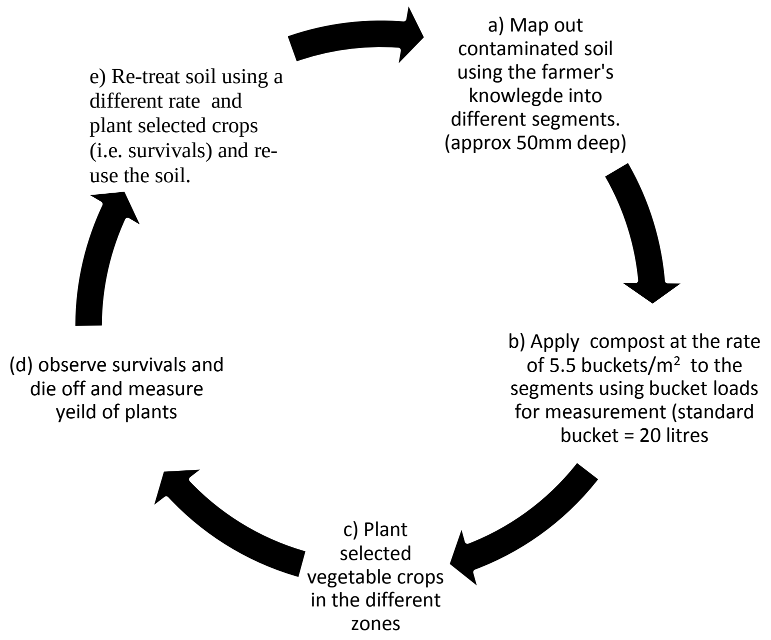

Figure 7.2: Treatment of contaminated soil and re-use for agriculture by the local farmers

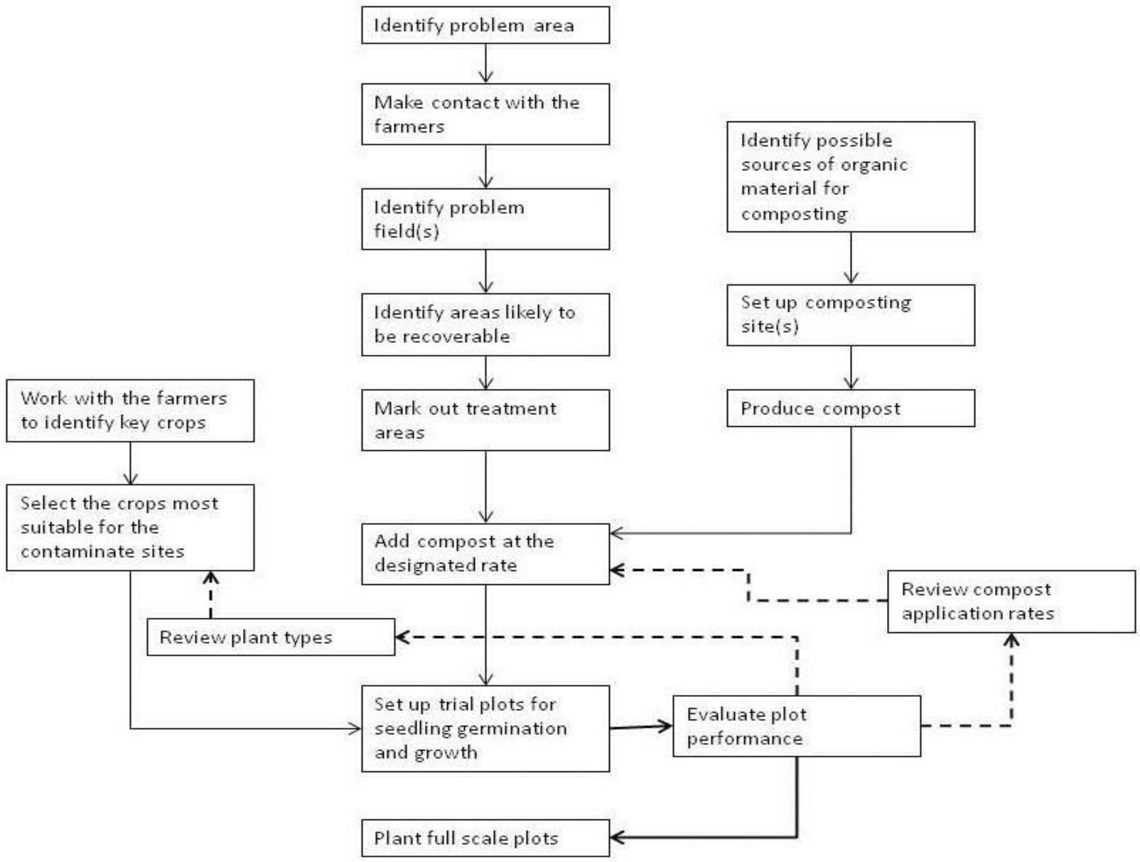

Figure 7.3: Methodology for contaminated land reclamation

As mentioned earlier (§ 7.1), due to the limited availability of land in the region, the local farmer practices mixed cropping. He plants different crops on the land in any planting season for example he may choose to plant maize, yam, okra and pepper in one year and plant cocoyam, tomatoes and spinach the next year. The idea behind the practice is to reduce the nutrient requirement for the different crops on the land so that the soil fertility will not be too low to support growth. The treatment proposed in Figure 5.2 will help him recover the contaminated soil for reuse. The approach is cyclic because of the continuous use of the land by the farmer, bearing in mind that he could plant different crops each planting season.

The application of compost is expected to enhance soil fertility over time. In the end, the farmer should be able to increase the yield from the farm not just for his family's use but also be able to sell off the excess to improve his income. The application of the treatment protocol would enhance production in comparison to little or no growth which was observed after oil spillage.

7.4 Justification for seedling test

The seedling test is used because it helps measure both germination and to a lesser extent yield which are key elements to producing a viable crop for the local farmer. Its relatively rapid response means that the local farmers can assess the likely response of their soil without having to use some of the more sophisticated analytical tools used by the government and the oil companies. Since germination is the first stage in the development of plant, thus if seeds did not germinate then it means that one cannot evaluate plant's response to oil pollution.

7.5 Project Model

The stakeholders in this research include the oil community (both residents and farmers), the government and the oil polluters. The stakeholders should work together in order to benefit from this protocol. The relationship between the stakeholders can be visualised in the diagram below (Figure 7.4)

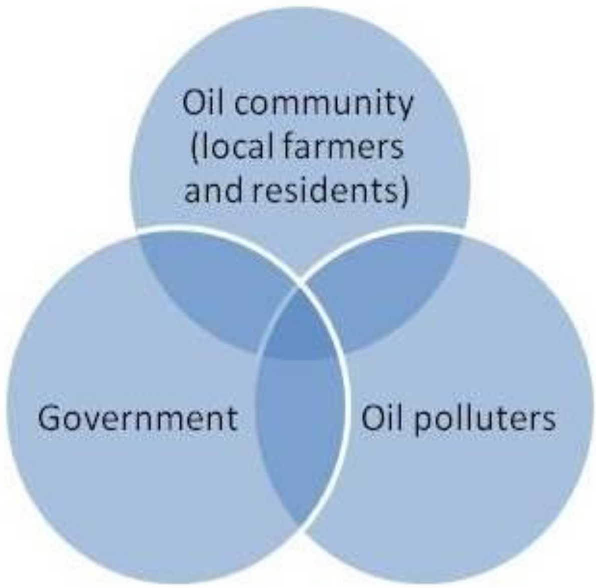

Figure 7.4: Relationship between the stakeholders in an oil contaminated site

One of the ways towards improving the environment in the Niger Delta region is getting all the stakeholders to work together; the government, oil companies, farmers and the local residents who might not be farmers. The government should provide enabling environmental policies, while the companies should give their expertise, and finances. The people own the resources being exploited and will outlive both the government and the companies and so the oil community should be made responsible especially in relation to the sabotaging of oil installations such as when people break into the oil pipes to steal crude oil. The local residents need support from the law enforcement agencies to help prevent this wilful destruction of oil installations. More importantly, the government should be firm in making oil polluters accountable for environmental pollution.

Part of the future work will be to collaborate with oil companies and explore the possibility of setting up a pilot composting plant. This could be set up very close to the refuse dumpsite. Usually, the refuse scavengers sort the plastics and metals, which they market to manufacturing companies. The organic fraction could be used as composting materials. This could create a new form of employment for the locals. One of the challenges to tackle will be that of setting up a local composting plant due to the public's perception that refuse needs to be disposed of immediately. There would be a need for reorientation to awaken the environmental consciences of the local community within the region.

7.6 Economics, design and cost

The use of compost for bioremediation of crude oil contaminated soil does not require sophisticated technology; however, there is a challenge to successful application of the technology. Some of the challenges are site specific in other words; the challenges will come up at the start of the actual bioremediation process at a given site. As observed by Essien and John, (2010) one of the limitations is the fact that it is difficult to achieve the good results obtained from laboratory studies when the techniques are applied in the field.

Therefore, the important criteria to be considered in practice are, the mix ratio of compost to be applied, the concentration of crude oil in the given site and the selection of the key crops. A bioremediation protocol not well designed will have poor growth and will not be able to achieve the desired goal. There is need for a pilot test before initialising a full bioremediation protocol on a contaminated site.

7.6.1 Selection of key crops

There are many papers and reports which deal extensively with different crops and their tolerance to crude oil contamination (Odu, 1972, Udo and Fayemi, 1975, Odu, 1978, Zuofa *et al.*, 1988, Poulik, 1999, McCutcheon and Schnoor, 2003, Merkl *et al.*, 2005, Gbadebo and Adenuga, 2012). It is evident that selection of wrong species can affect the bioremediation process. Therefore, it will be part of our duty to advise the farmer on the local crops most tolerant to the oil contaminated conditions as shown in Table 7.2.

Based on Table 7.2, we would advise the farmer to plant maize, pumpkin and cassava on the recovered contaminated land. We are aware that the farmer might be lucky to have uncontaminated land as well so the sensitive, but important local crops would be grown on the uncontaminated part of his land. The present research has shown that tomatoes are sensitive plants.

Table 7.2: A summary of different local crops in the Niger Delta and their planting cycle

S/N	Crop	Time of planting		Maturity	Sensitivity to oil
		Early planting	Late planting		
	Maize	January - April	August	3 – 4 months	sensitive
1.	Tomatoes	March - May	August - September	3 – 4 months	Very sensitive
2.	Okra	March - May	August - September		Very sensitive
3.	Pepper	May – July		3 months	Very sensitive
4.	Pumpkin	March - May	August – October	2 - 3 months	sensitive
5.	Cassava	January – December		12 – 13 months	Less sensitive to crude oil due to their deeper root system (perhaps the effect of the compost will be limited in this group as we will only be mixing at 50 mm)

7.6.2 External factors

There are environmental constraints associated with any crude oil contaminated sites. Factors such as temperature, climate and season might influence the application of a bioremediation treatment on a given contaminated site. These factors might also affect the germination and growth of selected crops and extent of biodegradation of the petroleum hydrocarbons. In practice (i.e. real situation), the length of the rainy season, the pattern of rainfall and temperature will have a strong influence on selected crops as these factors will affect the moisture content of the soil. In general, the time chosen for bioremediation will influence the decision about the type of crops to be grown whether it is the season for such selected crops.

The two main seasons in the Niger Delta are wet and dry seasons with about 70% of the annual rainfall falling between April and August, a small amount of rainfall is recorded between September and November and the dry season is between December and March

(Igoni *et al.*, 2007). Other soil properties considered include texture and bulk density, which influences water infiltration and retention. Igoni *et al.*(2007) described the soil from the Niger Delta as poorly drained silt clays mixed with sand. Uzoije and Agunwamba, (2011) investigating the physiochemical properties of soil from various sites in the Niger Delta region reported that soil of high bulk density may not be good for cropping because the root penetration will be poor and plant growth will be affected due to compaction of the soil. Bulk density gives a good estimate of soil physical properties. They revealed that the volume of the crude oil in the soil also affected soil properties. Before the start of any treatment during farm trial, it is recommended that the soil texture be characterised as well.

7.6.3 Selection criteria

This aspect is more applicable to companies and experts. We recognised that sometimes treatment of contaminated sites might need a combination of remediation techniques. A proper knowledge and understanding of the site properties and its impact on plants and hydrocarbon degradation is very important (Table 7.2 gives a list of local crops and their oil tolerance levels).

7.6.4 Intervention and target values

There is no universal intervention value, as variations do exist among different countries. It is a function of the legislation in a particular country. For example the Dutch Intervention value for TPH is 5 g/kg in soil containing 25% clay and 10% organic matter content, while Danish (Zealand) guidelines classes a soil as contaminated if TPH exceeds 0.2 g/kg and in Nigeria, the Intervention value is given as 5000 mg/kg soil and sediments (i.e. 0.5% oil by soil weight). Therefore, in terms of chemical analysis, the risk that will be associated with TPH in any contaminated soil before and after clean-up will depend on the intervention and target values of a particular country. Such analysis may not give a complete picture of the effects of petroleum hydrocarbons on plants without doing seedling emergence tests. In this study, we showed that there was very little impact on seedling emergence at 0.5% oil level.

8 Discussions and Conclusions

A review of the literature from major databases reveals a growing concern by scientists to understand better ways of treating soils contaminated with petroleum products. There are two approaches to this quest (1) an environmental engineering view (2) public health and human development view

The former approach monitors the reduction of the hydrocarbons in the contaminated sites to match the guideline limits; mostly the regulatory bodies and industries favour this approach. The emphasis here is on quantity and type of hydrocarbons remaining after the clean-up i.e. the residual hydrocarbons. However, this is not good enough a parameter in assessing whether the recovered soil is fit for agricultural purposes.

It is the researcher's opinion that research should not be methodologically led; rather choice of methodology should reflect the imminent research question that the research intends to answer, which in this case was providing a user focused method as a clean-up option for local farmers in the Niger Delta region of Nigeria.

Therefore, from the public health and human development point of view, the study wanted to explore simple method for recovering crude oil contaminated land, which contributes to the traditional source of livelihood of the local people of the Niger Delta. They are subsistent farmers and produce for their consumption. Oil spillages have contributed mainly to shortage of land in the region, as a result reuse of untreated contaminated soils affect yield, and contribute to the problem of food security.

None of the previous studies has come up with any treatment protocol that will allow a local farmer within the Niger Delta region and areas of similar context to be able to recover the abandoned contaminated land.

8.1 Overall discussions and summary

The data presented in this research were limited to laboratory experiments. The options in the protocols have been included after due consideration of technical limitations in the laboratory bearing in mind that results from field demonstrations might vary. Firstly, the amount of data from the present study made it challenging to determine the optimum conditions for the treatment of crude oil contaminated soil. Secondly, there is a need to conduct farm trials in the context of the Niger Delta to ascertain the applicability of laboratory data to site application. We propose the use of selected contaminated sites in the Niger Delta for the farm trials.

Despite the limitations mentioned above, we recognise other sustainable environmental benefits from the treatment options such as replacement of mineral fertilizers with the use of products from composting.

The use of different compost feedstock was investigated in this research. Compost made from combination of food waste, green waste and organic fraction of municipal solid waste as well as manure-based compost was used during plant growth trials. However, there was not observable difference in the biomass yield. As presented in Table 6.5, the biomass yield from similar mix ratios at the same contamination levels did not vary so much. For example in plant growth 2 and 3 at 50% compost for 10% oil contamination, the biomass yield was 113 and 126 mg respectively. Therefore, the source of compost did not make a difference.

The different trends in germination and growth of tomato seeds showed different ways oil spillage affects plants regardless of the plants having same parent seeds. In our judgement, factors such as types of crude oil, soils, seed varieties and oil level could influence plants' response to oil contamination.

Variation in germination, growth and biomass yield of plants on unpolluted soils in this research have shown that soil quality can affect productivity. For example, the soil obtained from the Niger Delta did not sustain growth of tomato seeds after the initial germination in comparison to soil from Bardsey, UK (Figures 6.31 and 6.41), even though this judgement is based on the results from the laboratory scale under conditions been investigated. Further work in the field is necessary to establish factors responsible for such variation.

A gradual increase in the number of germinated plants and survivals occurred between 12 -20 days after planting before the plants started dying off. Perhaps, the death was due to the toxic effect of the oil on plants as well as microorganisms. Possibly the oxygen depleted environment caused by the presence of the oil and use of other electron acceptors such as nitrates led to reducing environmental conditions and probably affected plants' use of nutrients and their physical development which eventually resulted in the death of the plants. Another possible explanation might be that, as some of the microorganisms die off, the pool of organic carbon increases and through the process of carbon cycle. The remains are converted to inorganic carbon for the plants and so growth is enhanced but time comes when the inorganic carbon becomes limiting and the plants started dying after growth has reached its peak.

Perhaps, the undulating trend in germination of the seeds relates to the organic decomposition in the soil. As decomposition takes place, carbon dioxide is given off which is used by the plants for photosynthesis. However, when diminishing return sets in and decomposition reduces, it invariably affects the CO_2 production which affects the process of photosynthesis and eventually could led to the death and reduction in the number of plants that survived at the end of planting period.

Osuji and Nwoye, (2007) suggested two types of decomposition in an oil spillage site namely (1) the decomposition of petroleum hydrocarbons and (2) the decomposition of soil organic matter. The action of indigenous heterotrophic bacteria could affect both processes. Even though the presence of crude oil could have initially stimulated the microbial population (Figure 6.20 & 6.21), it is possible that their multiplication could not cope with the influences from environmental factors as well as the soil's physico-chemical characteristics altered by the oil contamination. A number of factors can influence changes in soil properties. However, chemical changes in soils are somewhat easier to study than biological changes, which are controlled mainly by microbial activities. Such environmental factors include, but are not limited to, availability of substrate, nutrient supply, and aeration.

This undulating trend in germination is linked to the volatile fractions of the crude oil, which seemed to be more toxic. Probably as the volatile component evaporates over time, the toxicity reduces and the soil is able to regain itself and support growth until other variables set in. In this present study, we investigated loss of the volatile fraction of the bonny light crude oil by exposing crude oil samples to the open air in the fume cupboard for 35days. After the first week, 21% of the initial mass was lost, and then reduced to 6, 2.5, 2.4, and 1.9% respectively for the next four weeks. Exposing the crude oil in this manner represents the worst-case scenario because in the present study, oil was mixed with the soil. Also in life application, oil spillage usually occurs in media such as on land or water, therefore we can attribute the degradation in this study to microbial degradation. There was significant difference ($P < 0.05$; using one-way ANOVA) for the recorded values of volatilisation losses over the 5 weeks. The high percentage loss during the first week is largely due to the volatile components of the hydrocarbons.

Another possibility from the viewpoint of germination is viability of seeds. In future, it is worth testing the viability of the seeds before planting by using the floating method in which case non-viable seeds float on top of water. Although the labels on the seeds used for the study reads; ‘all seeds are viable’ it is possible that conditions during storage could affect such product.

8.2 Other parameters

8.2.1 Oil level

Effects of different concentrations of crude oil on soil have been studied by various workers to understand the extent to which agronomy characteristics in addition to germination, growth and yield of crops are affected by such contamination (Udo and Fayemi, 1975, Ekundayo and Obuekwe, 1997, Kuhn *et al.*, 1998, Talarposhti *et al.*, 2005, Ayotamuno *et al.*, 2006, Osuji and Nwoye, 2007, Adoki and Orugbani, 2007b, Adekunle, 2011). Adekunle *et al.* (2010) used maize (Zea mays L.), cowpea (Vigna unguiculata) and spinach (Amaranthus hybriddus) to measure crude oil resistance by investigating such indices as seed germination, plant height and leaf number. Of the three crops used except for Z. mays, the other two crops recorded 100% inhibition in seed germination. Similarly,Adekunle (2011) used a different petroleum product (diesel) and reported 100% toxicity to seed germination (Z.mays) in a diesel-contaminated soil without any form of treatment. The result from diesel contamination was comparable to crude oil polluted soil without any treatment, which supported growth but recorded about 13% toxicity to seed germination.

This just demonstrates the diversity of the effects of petroleum pollution on plants, which could depend on factors such as type and concentration of oil, plant species and cultivar used. The knowledge could be exploited in oil impacted regions during bioremediation to enable the locals to cultivate the best plant that would grow on the recovered soil.

The studies mentioned above on oil contamination have used oil levels ranging from 0% to 10.6% w/w. In some cases, total inhibition was observed at given oil levels but in some other cases growth was recorded even at higher levels, which shows variation in the ways crude oil affects plants.

In the present study, the analysis of results showed a similar trend. For example in growth trial 1 (Figure 6.17), at 5% oil level (Soil 5%), the growth inhibition was 86% when compared to the uncontaminated soil and 79.5% inhibition at 7.5% oil level (Soil 7.5%). However, on addition of compost to the contaminated soil at 1:1 mix ratio, the growth inhibition reduced to 5% and 32% at 5 and 7.5% oil level respectively compared to the control (uncontaminated soil). The possible explanation why there was higher yield at 7.5% oil level could be that the microorganisms were able to use the hydrocarbon as substrate even at that level while at 5%; probably the use of the oil was masked by the soil, which unfortunately did not support the plant growth as much.

Still on the response of plants to different oil levels, Kuhn *et al.* (1998) reported seedling death within six days of planting at 1.2 and 2.4% crude oil contamination. Similarly Anoliefo and Vwioko (1995) reported no germination of hot pepper and tomatoes at 5% oil but Udo and Fayemi, (1975) reported 44% germination for maize seeds at 6.8% oil application. This call for a pilot test to determine what could be termed a safety level of oil contamination for growing any local species of plant.

8.2.2 Mix ratios

Addition of compost to contaminated soils was at various mix ratios to determine the appropriate mix ratio under the conditions investigated. This will help in identifying a better way of treating the contaminated soil to improve yield. In growth trial 2, we investigated three different compost/soil ratios of 1:1, 1:3 and 3:1,also referred to as 50%, 25% and 75% Compost. Given the volume of compost used at 3:1 mix ratio, we concluded that regardless of the higher biomass observed, the effect might be due to higher dilution of the contaminated soil by the compost. It is possible that the compost had masked the oil from having any harmful impact on the plants and microorganisms. Therefore, to reduce wastage of compost which might be limiting in the region, it might be more economical to use the 1:1 mix ratio because the difference in yield at 3:1 mix ratio of compost to contaminated soil was decreasing with an increasing oil level. For instance at 5, 7.5 and 10% oil level, the average biomass yield was 462, 245 and 114mg respectively on applying 3:1 mix ratio of compost/soil (Figure 6.21 to 6.23). We observed an average of 113 and 114 mg biomass at 10% oil level for 1:1 and 3:1 mix ratio respectively. Given, crude oil spillage in the region usually occurs as major spill, it means that whatever the biomass gain of applying a 3:1 mix ratio at lower oil levels this will be lost at a higher oil level. In general, addition of compost to the contaminated soils improved yield but varying the ratio of compost did not have such a big impact on both growth and biomass yield at higher mix ratios. Therefore, we recommend 50% compost application rate.

8.2.3 Plant height

Analysis of the shoot height showed that plants from treatments did not attain as great a height as the controls. There exists extensive evidence from literature that other researchers made similar observations of the wide variation in plants' height with respect to oil contamination (Anoliefo and Vwioko, 1995, Kuhn *et al.*, 1998, Ekundayo *et al.*, 2001, Adoki and Orugbani, 2007b, Adekunle, 2011). Anoliefo and Vwioko,(1995) investigated the effects of spent lubricating oil (1 to 5% w/w) on two vegetable crops namely hot pepper (Capsicum annum. L) and tomato (Lycopersicon esculentum) at eighty-four days planting period. The effects were more on the tomatoes. They reported plant height in contaminated soils in the range of 1.47 to 2.43 cm and the control (uncontaminated soil) was 5.23 cm, there was suppression of growth at 4% and 5% oil level.

In the present study, the plant heights for contaminated soils were between 1.0 and 3.3 cm while the controls (uncontaminated soil) were 4.1 to 7.5 cm at a planting period of twenty-eight to thirty-five days. Adekunle (2011) investigating bioremediation of soil contaminated with Nigerian petroleum products (5% v/w) of spent engine oil similarly observed difference in height of plants in control and treatments. Plant height was among the indices she used to assess the toxicity of oil to germination of maize. The experiment lasted for 90 days. She observed plant height in the control i.e. soil without oil or compost to be 33.8 cm within one week of germination and 46.7 cm in the second week. In comparison, the plants grown in diesel contaminated soil with compost as treatment recorded only 7.6 cm, the first week and 38 cm on day 14. This shows that toxicity of petroleum product on plants' height could vary. This variation is dependent on such factors as type of oil, climatic conditions, treatment, planting period as well as species of plant.

8.2.4 Light intensity:

In the preliminary experiments, it was difficult to have light intensity that could supply energy for sixteen hours as proposed by British Standards Institute (Bsi, 2005). Despite the initial germination, 14 days after germination, the plants turned yellowish and started dying off. We recognise that other factors could have contributed to the yellowing of the leaves. At the end of twenty-eight days, an average of three plants was remaining in the unpolluted soil (Figure 6.2 – preliminary experiments) but in subsequent experiments on improvement of light intensity higher number of plants survived. The non-survivals would have suffered from the effects of insufficient light energy on the plants or a combination of other factors such as poor moisture level and availability of nutrients. Nakaune *et al.*(2012) observed that poor light quality especially in greenhouse experiments can influence the quality of germinated seedlings. Generally, light is seen as the primary factor that limits plants' growth before nutrients (Nakaune *et al.*, 2012). During subsequent experiments in the course of the research, in addition to improved light energy, which provided sixteen hours of light and six hours of darkness to reflect summer periods, wetting of the plants was done more frequently than was the case during the scoping study. In addition, the planting period was longer (35 days). The damping and yellowing of the leaves became noticeable 21 days after planting in later experiments but in scoping studies, yellowing of the leaves appeared 14 days after planting.

Damping-off is a problem especially when seeds are grown indoors or in screen house. Mostly, it has to do with high humidity and poor air circulation. Also low levels of light and temperatures could be contributing factors. Damping-off is a common problem noticed in vegetable seedlings such as cucumber, tomatoes, cress and cabbage (Talarposhti *et al.*, 2005, Lazcano *et al.*, 2008). Lazcano *et al.*(2008) observed twenty-five percent damping-off on cucumber seedling in pots treated with peat moss. It is possible this phenomenon affected the growth of plants during the preliminary stages before an improved light source was made available.

8.2.5 Salinity and Electrical conductivity

McCutcheon and Schnoor, (2003) reported that soils contaminated with high petroleum hydrocarbons are high in soluble salts (expressed as electrical conductivity). Electrical conductivity of values higher than 2000 µS/cm is high; however, different plant's species can tolerate high salinity. The authors suggested that toxicity of contaminated soils to plants do not come only from high salt concentration, but that metals and organic compounds can also be toxic to plants at some level. Volatile organic hydrocarbons are toxic to plants; however, the extent of volatilization determines its toxicity. Electrical conductivity relates to the presence of nutrients, which form ions in soil water. In other words, it is a measure of the total amount of dissolved ions in water.

It is likely that a fraction of total dissolved solids and nutrients such as ammonium-nitrogen and ammonium nitrate from fertilizers could add to the electrical conductivity of a medium.

Generally, it is belief that plants need nutrients in the right proportion for growth and that excess of nutrients could be fatal to plants. According to Adu *et al.*(1994) while investigating the response of a plant (Dactyloctenium aegyptium) to salinity in Southern Nigeria soils, they reported that it is the action of the nutrients on water that causes stress to plants rather than the nutrients themselves. The mechanism is due to the action of salt in water. Salt reduces the water potential thereby making the water less available to plants (a phenomenon known as reduced hydration). Presence of salt can be from fertilizers and dissolving soil minerals. They revealed that when a plant suffers salt stress, which can occur due to lack of proper water and nutrient management, it can result in reduced growth, small fruit and decreased yield. Their findings showed that reduction in growth was due to ion toxicity within the leaves, the plant however had tolerance to reduced hydration caused by high salinity. However, in the present study the values of electrical conductivity were within the acceptable range of less than 1200 µS/cm above which it becomes inhibitory to the plants. Therefore, for the purpose of this study we do not tie poor growth to reduced hydration.

To the best of our knowledge few studies have been done on salt tolerance of local plants in Nigeria however having a knowledge of electrical conductivity of the treated contaminated soil could help us to judge from the general effects of salinity on plants how good the recovered soil is for agricultural purposes. During the present study, the values for electrical conductivity were within the acceptable range of less than 2000 µS/cm. Real farm trial of crude oil contaminated soil is recommended for future studies as well as evaluating the suitability of the recovered soil for agriculture. In that case, local plants could be grown on the recovered soils.

8.2.6 pH

Different plant species are sensitive to different ranges of pH, while some could be tolerant of very low pH; other plants thrive better in a neutral or alkaline pH. Therefore, it is a question of monitoring the soils' pH and the local species that can be grown even though some investigators have reported the use of liming for acidic soils (Osuji and Adesiyan, 2005).

Liming of soils may not be the best approach when it comes to soils from the Niger Delta region because of the high intensity of rainfall around 1500 to 4000 mm as described by (Nwilo and Badejo, 2007) and this means the soil can easily erode. In other words positively charged ions such as calcium, potassium and sodium will leach out of the soil and be replaced by hydrogen ions causing short-term changes in pH. In addition, the farming practice in the region is such that the land is cleared of any weeds or bushes close to the start of farming season and these weeds are set on fire (usually towards the start of dry season). This practice known as slash-and-burn (Babanyara *et al.*, 2010), exposes the bare soil to the first rains, and this tend to wash away the soil nutrients. Rather than liming, if compost is introduced it will slowly release its nutrients as well as act as a buffer which makes it a better alternative to be used in the region.

Neutral pH favours bioremediation activity by bacteria in the soil and carbon mineralisation (Atlas, 1981, Bossert and Bartha, 1984, Osuji and Adesiyan, 2005) . In agreement with their investigations, the pH recorded from the test conditions (6 - 9) in the present research was within the acceptable range for bioremediation.

8.2.7 Nutrients

Inorganic nitrogen and phosphorus have been reported as limiting bioremediation of petroleum hydrocarbons in the soil (Zuofa *et al.*, 1988, Antai, 1990, Zhou and Crawford, 1995, Adriano *et al.*, 1999, Ijah and Antai, 2003, Adoki and Orugbani, 2007a, Adoki and Orugbani, 2007b). In other words, as bioremediation progresses in the absence of external source of nitrogen and phosphorus, the microorganisms will utilise available nitrogen and phosphorus in the soil to a point of depletion and it becomes limiting. It is easier to focus attention on the nutrient requirement of plants without considering the nutrient requirements of the hydrocarbon degrading microorganisms; this imbalance might underestimate the total nutrient requirement especially when using nutrient supplements. Therefore, any nutrient supplement should take into consideration the requirements for both plants and microorganisms.

8.3 Monitoring recovery of soil as indicated by growth parameters

Assessment of the recovery potential of the soil was by planting tomato seeds in crude oil contaminated soils, both with treatment and without. The number of germination counts and survivals served as growth parameters for monitoring response to various treatments. The soils were analysed for some physico-chemical characteristics. The values obtained in this research could form guidelines for characterising the suitability of recovered crude oil contaminated soils for agriculture in the Niger Delta region and similar contexts.

Summary:

One of the challenges to face in introducing local farmers to the use of compost is their fear of perceived lower yield from crop production on application of compost in comparison to addition of conventional mineral fertilizer. This notion may not be very wrong as mineral fertilizer releases its nutrients faster because it contains more readily available mineral nutrients than the organic fertilizer, which releases its nutrients slowly. On the contrary, it is not very advisable to apply mineral fertilizer in the Niger Delta region given the intensity of rainfall, which increases the chances of washing away the nutrients from mineral fertilizers. To sell the ‘use of compost’ there is need for a local composting facility within the reach of the local farmer because such local production will help bring prices down.

Secondly, there is possible objection to the use of compost, especially compost made from refuse, due to problems of metal toxicity, risks associated with pathogens and presence of small glass particles. However, Adekunle *et al.* (2010) investigated the feasibility of converting, through composting, waste materials generated from households, agricultural waste and kitchen wastes into compost in Nigeria. From their investigations, plant assay using the resulting compost as an additive revealed that values of heavy metal absorbed in plant roots and leaves did not exceed the critical phytotoxic values. Therefore, the use of local waste to generate compost in the Niger Delta region does not indicate the likelihood of any problems arising from metal toxicity. Local farmers need to be made aware of such information to reduce their fears.

Germination and early development of seedlings could be under genetic control (Lazcano *et al.*, 2008). However, it is important to draw attention to the fact that environmental factors and developmental conditions could modify appearance and behaviour of seedlings. Therefore, seeds of the same variety may not behave alike as was seen in some of the duplicates in the results from seedling tests (Figure 6.3 & 6.4). This has more to do with phenotypes, though reasons for such morphological changes were not part of the present research.

We have demonstrated that seeds are able to germinate in oil-contaminated soil with the addition of compost. Some seeds eventually died from upwards of 2 weeks after planting, we suggest that farmers could introduce mineral fertilizers at some point during treatment of the crude oil contaminated soils as a nutrient supplement to the plants even though the research did not investigate the efficacy of mineral fertilizer. Perhaps such application could enhance the biomass yield.

However, the major benefit from the application of compost is the impact on the crude oil contaminated soils, which allowed for initial germination and growth of seeds when compared to cultivating contaminated soils without addition of compost, which did not allow germination.

It is possible that quality of soils played a role in the biomass yield of the plants; this assumption is based on the physico-chemical properties of soils from different locations used in this study. Mostly, the unpolluted soil obtained from Bardsey had a higher biomass with an average of 1113.5 mg in comparison to the soil samples from the Niger delta in which the plants died off before the end of planting. However, on addition of compost to the Niger Delta soil at 50% application rate, the yield was highest producing an average biomass of 12760 mg. The different soils used in this research might be location specific and so exhibit some variation in characteristics.

The findings of this study revealed that addition of compost to crude oil contaminated soils make such contaminated soils useful for agricultural purposes.

8.4 Legislation in Nigeria (environmental regulation)

Under the law, clean-up of oil spills whether on land or waterways is mandatory. The oil pollution bill, which became an Act in 1990, provides guidelines for government and oil industries in matters relating to oil spill, prevention, mitigations and clean up. Part of the ‘Act’ states that compensation is paid to affected parties in addition to providing adequate financial resources for clean-up. Laudable as this ‘Act’ was, it is the case of someone having to put the wheel of the law into motion. Many kickbacks are going on within the government and private sectors and these practices have complicated matters. courts’ proceedings and injunctions are not always followed to the latter and this has left many cases of oil spillage unresolved thereby making the aggrieved parties have no trust in the rule of law.

There is need for the oil companies to use the cleaner technology, considering the environmental and social cost of oil exploration in the region. The use of cleaner technology would mean low incident of oil spills. Therefore, fewer people lose their traditional sources of livelihood because of oil exploration while alternative sources of livelihood are available for displaced citizens. For example Ojimba and Iyagba, (2012) analysed how crude oil contamination on soils affected the traditional economy of some oil communities in Rivers State, Nigeria. Their results showed that; (a) the average farm size used for crop production was one hectare per farmer in crude oil contaminated farms which was lower than the 1.17 hectares for non-polluted farms, showing that crude oil spillages and acquisition of farmlands for construction of oil wells and installation of oil facilities have reduced the farm sizes. (b) The average crop yield after harvest from crude oil polluted farms was 385 kg while 551 kg was from non-polluted farms confirming reduced yield on crude oil polluted farms. (c) The farm income realized from produce in crude oil polluted farms was $26 less than the amount from non-polluted farms. This shows that income declined on farms affected by oil spillage, one of the negative consequences of oil exploration and production on lands. Unfortunately, the government's inability to set minimum standards for oil companies and address the environmental issues has boosted the level of negligence by the oil multinational companies and indirectly contributed to environmental degradation. In the context of this paradox, this is termed as 'biting the finger that fed you'.

The Niger Delta Development Commission (NDDC), established in 2000 has the following among its duties:

- monitoring and evaluation of Niger Delta to assess any environmental degradation
- to prepare plans for the physical development of Niger Delta; and
- to make sure that funds earmarked for any developmental projects in Niger Delta are not siphoned off for other purposes..
- However, there has been lack of political will by the government officials to implement the law. Therefore, for functional policies, the government should take into consideration the needs of the community, to reduce cases of over or under compensation. There is need to evaluate the 'losses' in terms of the environment, livelihood and health of the affected people. What this means in reality is that the amount of compensation received by a household will be determined by the type of loss peculiar to that household. Example of such losses may include (1) was the land acquired by the oil company for right of way to their oil installations (2) are the economic trees removed for safety of oil installations (3) was the farmland affected by oil spillage just to mention but a few.

Secondly, there is need for a reorientation programme for the local people of the Niger Delta region. The current practice whereby the locals are more interested in the financial compensation should not be the only emphasis. They should be aware of the values of a sustainable environment and the consequences of their action to the future generation.

8.5 The way forward

One of the best mitigation measures against pollution is that of prevention i.e. being proactive. Oil multinational companies should put in place proper cheeks and balances with respect to the general conditions of their oil installations as well as the technical staff operating these oil installations because spillages have been attributed to flow line/pipeline leakages, hose failures, blowouts, and tanker loading systems. For oil wells with joint ownership by two or more companies, their memorandum of understanding (MOU) should state which part each party plays in the case of any spillage so that it will not be the case of two elephants fighting and the grass suffering.

In the case of the Niger Delta, the challenges of sabotage-induced spills (i.e. wilful destruction of the oil installations) can take on such intervention as involving the local people in safeguarding the oil installations (especially the land installations). The intervention could be in form of neighbourhood watch, instituted through the local authorities and would work hand in hand with the police. This aims at people reporting immediately any form of pipeline' vandalism. However, people can only get involved if they are confident of their safety by the police.

Communities can also serve as their own watchdogs over oil installations, but in this case, rewards should be in form of developmental benefits. Such benefits would go to a community without a case of sabotage-induced spills over a stipulated period. Such a scheme directed to communities rather than individuals could serve as motivation.

Sadly, the oil companies are more interested in their profits, share values and shareholders and so each time production goes down, it is the local farmers and oil communities at large that suffer. Little goes back to the communities from which the oil comes. This phenomenon cuts across the oil communities in nations as diverse as Venezuela, Brazil and Nigeria.

In conclusion, the policy framework with respect to oil pollution should not just be about obligatory financial compensation from the oil polluters, but government should also take into consideration the needs of the oil community affected by the oil spillage, which in most cases affects their traditional economies. Therefore, impact assessment of any oil spillage should not be based on using only chemical analysis of petroleum hydrocarbon for evaluation of associated risks rather it should include the damages done to their traditional economies (such as farming and fishing), environment (soil, flora and fauna) and health.

8.6 Interventions required (from the oil companies)

This could be in the form of improving the material circumstances of the oil communities. One of the driving forces for youths' wilful destruction of oil installations is poverty even though poverty is not an excuse for criminality. As reported by Ebenso, (2012) despite the huge external revenue from crude oil sales between 2002 and 2007, there is widespread poverty in Nigeria with about 70% of citizens still below the poverty line as at 2007. Multinational oil companies can sponsor short-term projects such as skill acquisition programme as part of their corporate social responsibilities, with such programmes aimed at alleviating poverty within the oil communities. This type of programme already exists in some oil communities. However, most of the beneficiaries of this programme, in oil communities where it exists are not able to establish themselves after the training due to lack of finance.

One of the ways of overcoming this challenge is for the oil companies to pay stipends either through agencies or banks to the trainees that successfully complete their programme. Such money will be available for acquisition of equipment for the trainee by the agency and any balance payable to the person. Such interventions will not only help them to be self-reliant but will indirectly help them take care of the needs of their families. In addition, it will help them build better positive social identities.

8.7 Recommendations for research, policy and practice

Recommendation 1: Education of the lawmakers on the challenges of re-using contaminated farmland.

The findings from this research would influence positively on policy making in Nigeria as it provides a better understanding on how oil spillage affects soil and crop production, which indirectly undermines food security. Results from this study showed how reusing crude oil contaminated soil affected crop yield. Therefore, without such knowledge it will be difficult for the lawmakers to grasp the turmoil of the affected persons. Such information packaged as short courses should be available for the lawmakers to attend. We can say that the public sector has little or no understanding of the impact of oil pollution. There should be collaboration between the Ministry of Environment and Agriculture with respect to policy making so that guidelines will not just be numbers and figures rather they should reflect outputs from applied researches.

Recommendation 2: Investigation of institutional corruption in the oil sector

In this research, we identified high level of politicking in the government sector that allowed public officers to embezzle revenues from crude oil sales in addition to acquisition of oil blocks with impunity regardless of the federal and state laws. This has broadened the widespread poverty and social inequalities within the country especially in the Niger Delta region. There is need for a research to understand how economic, political and legal factors influenced institutional corruption. This act of public aggrandisement has also given the Multinational oil and gas companies the impetus not to take responsibility for the environmental degradation caused by oil exploration and production.

The Multinational oil companies are guilty of corrupt practices. There have been instances where they instigate different factions of the community to disrupt developmental projects so that they can withdraw from such ventures sighting internal conflicts by the parties affected as the reasons for withdrawal. There should be very strict sanctions against any oil company that is involved in such practices which might be paying penalties or stopping them from operations in that community.

Recommendation 3: Change of behaviour for the oil communities.

The law stipulates clean-up in case of oil spills as well as payment of financial compensation to the affected persons. Evidence abounds how some influential people in the oil communities would ask for total monetisation of the damages caused by the oil spillage rather than allowing the oil companies to clean up the contaminated soil. There should be an outreach programmes to educate the oil community residents that financial compensation is not the only solution to the challenges of oil spillage. Such information should be very simple and made available at no extra cost. It could be in the form of posters written in the local language for clarity.

The outcomes from the research could be summarised as follows:

1. This study brings out more understanding of the effects of re-using crude oil contaminated soils for vegetable crops, especially tomatoes and the relevance of low cost bioremediation technology in improving above ground biomass. This protocol applies particularly to Nigeria where labour cost is very cheap.
2. This research is user focused, so it will build on existing collaboration between the oil companies and the oil communities with the intention of increasing the amount of arable land. The treatment protocol will educate local farmers on their role in sustaining the environment especially for the future generation by using compost as treatment option for enhancement of bioremediation of crude oil contaminated soils.
3. The research has demonstrated that we can categorise contaminated soils using both physico-chemical and biological indicators such as pH, electrical conductivity and germination to assess restoration of contaminated soils for agriculture.

9 Major conclusions and further works

This chapter draws together the various sections of the thesis bearing in mind the aims and objectives (§ 1.7) of the research; and summarizes the findings that emerged from the study. The overall aim of this research was to determine the feasibility of using compost for the restoration of soils contaminated with Nigerian Bonny light crude oil. The purposes of this research include returning the contaminated land to agricultural use. Therefore, successful restoration will be assessed by the ability of the soil to sustain plant growth and improve yields.

The findings of this research suggest that the addition of compost to soils contaminated with Nigerian Bonny light crude oil can results in improved seed germination and increased yield. In terms of the more detailed objectives (§ 1.7) that this research set out to achieve, a number of conclusions can be drawn as follows:

- The laboratory results show that it is possible to relate carbon dioxide production to microbial activities in soils contaminated with Nigerian Bonny light crude oil. However, the results also show that carbon dioxide production is not a good indicator of the degree of microbial biodegradation of crude oil in soils.

- The laboratory results show that respiration measurement techniques such as DR4 and Solvita® kit do not provide a reliable and cost effective soil respiration-monitoring tool that can be used by government institutions or the oil companies.

- The results show that the addition of compost improved the degradation of n-alkane and more specifically the fraction C_{21} to C_{25}. This is seen as a welcome development since Bonny light crude oil, which is the type of crude oil from the region, comprises approximately 57% n-alkanes. Factors such as temperature, carbon, nutrient quality and availability affect microbial degradation of hydrocarbons and there is a complex interaction between these factors, difficult to explain.

- The addition of compost to contaminated soils and treatment at elevated temperatures proved to be of limited success. A comparison of the degradation of hydrocarbons at 20°C and 35°C showed a 5% increase in degradation at 35°C. Although this increase in biodegradation is small it would still suggest that carrying out bioremediation during the hotter season would result in a small improvement in degradation rates at no additional cost.

- From the outset, the success of the bioremediation of contaminated soils was to be measured using a range of seed germination and plant yield parameters. The work carried out as part of this study show that of the various biological indicators based on plant growth that were used in this work (seed germination, plant height and biomass yield), seed germination was the most useful as it showed the combined effects of crude oil on the soil and plants physiological features.

- The use of aboveground biomass as a measure of yield may be a limitation of the method used in this study since in this case it did not allow the tomato plants to reach fruiting stage, which may be a more realistic approach for measuring yield.

- The results from this research suggest that the damage that occurs to plants growing in crude oil contaminated soils was due to a combination of different factors taking place in the soil. Under the conditions investigated, a mix ratio of compost to contaminated soil at 1:1 was a better option than the other mix ratios for treatment of contaminated soil at 10% oil levels and below.

- Although the use of compost improved seed germination, there was no observable difference in the biomass yield of tomato plants by using different compost feedstock (manure based compost, green and food waste).

- The use of compost, as a bioremediation material for treatment of crude oil contaminated soil is a cheaper option. This is against the background that given the cost of and accessibility to inorganic fertilizers especially in Nigeria when compared to the abundance of biodegradable material for composting,

- Treatment protocols for contaminated soils were developed for the use of the oil companies and local farmers based on observations from the study such as response of tomato plants to the different mix ratios. The application of this treatment protocol is expected to improve soil recovery and reduce the present use of ad hoc methods for treatment of contaminated soils in the Niger Delta region.

The focus of this research is the use of compost to reduce oil contamination in soils; thus, the study did not investigate parameters that determine compost quality with respect to intended use such as presence of pathogens, state of maturity or stability, organic matter, carbon content and nutrients. This can be included in future work as part of feasibility studies for composting of locally generated organic waste in the Niger Delta region and areas of similar contexts.

9.1 Suggestions for further work

There are challenges to this research in terms of real application. We suggest that a more robust experiment could be set up as farm trials, using similar experimental procedure but at a larger scale with local plants specific to the region. As part of the outreach team, we could be responsible for monitoring and evaluation of the treatment operations. In view of that, the data presented in this thesis could serve as preliminary results. The results from the studies show that addition of compost improved performance. However, given the differences observed in biomass yield of some replicates (§ 6.5.6) at higher oil levels (7.5 & 10% initial oil level), there is need to further investigate this variation in future works.

Other important factors in future work include:

I. the effectiveness of compost addition for example should compost be added as repeated application.

II. other operational parameters for example the time of the year and season of farming, the mode of application of the compost either mixed after application or just left on top as mulch.

Part of the future work will be to collaborate with oil companies and explore the possibility of setting up a pilot composting plant. This could be set up very close to the dumpsite. The organic fraction of the waste serves as composting materials, such project will create a new form of employment for the locals.

In addition, we suggest that the government of Nigeria should build composting plants especially in areas where there is availability and abundance of biodegradable waste such as the Niger Delta region. This will provide the local farmer with compost for treatment of the contaminated soil to quicken land reclamation for return to food production without necessarily involving financial resources to do it.

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List of Abbreviations

@ - at

BLCO – Bonny Light Crude Oil

C – Compost

C/N – Carbon to Nitrogen ratio

CFU – Colony forming unit

CO_2 - Carbon dioxide

DEFRA – Department for Environmental, Food and Rural Affairs

DPR – Department for Petroleum Resources

DR4 – 4-day Dynamic Respiration

DS – dry weight

FEPA – Federal Environment Protection Agency

GC/FID – Gas chromatography /Flame Ionisation Detector

IYC – Ijaw Youth Council

MEND – Movement for the Emancipation of the Niger Delta

MOSOP – Movement for the Survival of Ogoni People

MSW – Municipal Solid Waste

N/D – Niger Delta

NDDC – Niger Delta Development Commission

NDPVF – Niger Delta Peoples' Volunteer Force

OG – Oil and grease

OM – Organic matter

pH – Potential d'Hydrogene

S – Soil

SPDC – Shell Petroleum Development Corporation

THBC – Total Heterotrophic Bacteria Count

TPH – Total Petroleum Hydrocarbon

UK – United Kingdom

USEPA – United States Environmental Protection Agency

V/W – Volume by weight

W/W – Weight by weight

WW – Wet weight

Appendices

Appendix A: Top World Oil Producers, Exporters, Consumers and Importers, 2006 (Millions of Barrels per day)

Producers[1]	**Total oil production**	**Exporters**	**Net oil exports**	**Consumers**	**Total oil consumption**	**Importers**	**Net oil imports**
1. Saudi Arabia	10.72	1. Saudi Arabia	8.65	1. United States	20.59	1. United States	12.22
2. Russia	9.67	2. Russia	6.57	2. China	7.27	2. Japan	5.10
3. United States	8.37	3. Norway	2.54	3. Japan	5.22	3. China	3.44
4. Iran	4.12	4. Iran	2.52	4. Russia	3.10	4. Germany	2.48
5. Mexico	3.71	5. United Arab Emirates	2.52	5. Germany	2.63	5. South Korea	2.15
6. China	3.84	6. Venezuela	2.20	6. India	2.53	6. France	1.89
7. Canada	3.23	7. Kuwait	2.15	7. Canada	2.22	7. India	1.69
8. United Arab Emirates	2.94	8. Nigeria	2.15	8. Brazil	2.12	8. Italy	1.56
9. Venezuela	2.81	9. Algeria	1.85	9. South Korea	2.12	9. Spain	1.56

10. Norway	2.79	10. Mexico	1.68	10. Saudi Arabia	2.07	10. Taiwan	0.94
11. Kuwait	2.67	11. Libya	1.52	11. Mexico	2.03		
12. Nigeria	2.44	12. Iraq	1.43	12. France	1.97		
13. Brazil	2.16	13. Angola	1.36	13. United Kingdom	1.82		
14. Iraq	2.01	14. Kazakhstan	1.11	14. Italy	1.71		

Appendix B: Number of oil spill incidents and the quantity spilled (1976-1998)

S/NO	Year	Number of Spill Incidents	Quantity spilled (barrels)
1	1976	128	26,157.00
2	1977	104	32,879.25
3	1978	154	489,294.75
4	1979	157	694,117.13
5	1980	241	600,511.02
6	1981	238	42,722.50
7	1982	257	42,841.00
8	1983	173	48,351.30
9	1984	151	40,209.00
10	1985	187	11,876.60

11	1986	155	12,905.00
12	1987	129	31,866.00
13	1988	208	9,172.00
14	1989	195	7,628.161
15	1990	160	14,940.816
16	1991	201	106,827.98
17	1992	367	51,131.91
18	1993	428	9,752.22
19	1994	515	30,282.67
20	1995	417	63,677.17
21	1996	430	46,353.12
22	1997	339	59,272.30

23	1998	390	98345.00
	Total	**5724**	**2,571,113.90**

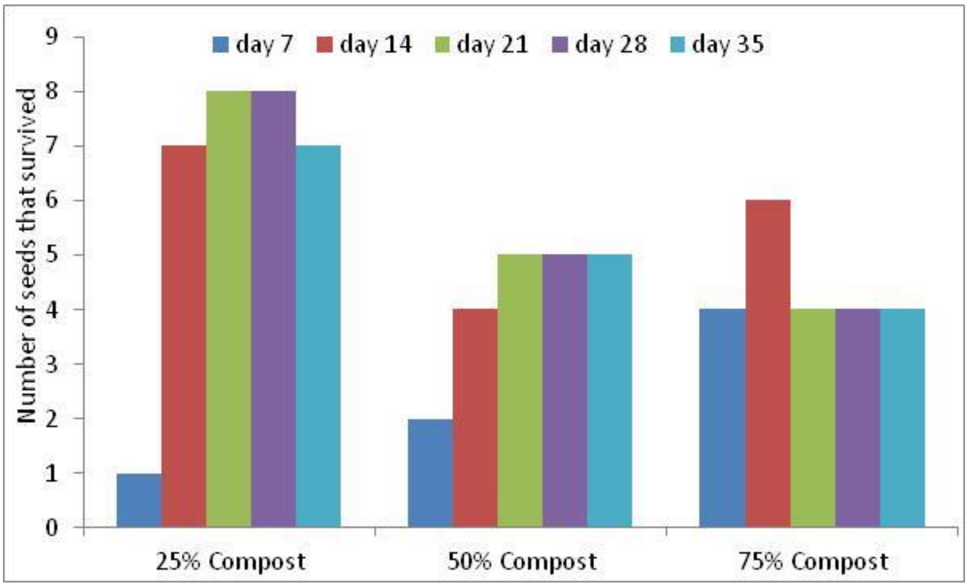
Appendix C: Titre values at 35 and 45^{0}C as well as the cumulative CO_2 production from the different reactors

		TRIAL 1(28/07/10 - 5/(	At 35 degrees										
Time (days	Carbon dioxide (mg)				Actual CO2 produced (mg)			Actual CO2 produced (mg)			Actual CO2 produced (mg)		
	2g oil(Reactor 1)	4g oil (Reactor 2)	8g oil (Reacto	control (compost o	Reactor 1	*2.5(kg) -mg/kg	cumulative CO2	Reactor 2	*2.5(kg)	cumulative CC	Reactor :	*2.5(kg)	cumulative CO2
1	448	737	400	329	119	297.5	297.5	408	1020	1020	71	177.5	177.5
2	476	668	593	548	-72	-180	117.5	120	300	1320	45	112.5	290
3	571	594	687	508	63	157.5	275	86	215	1535	179	447.5	737.5
4	232	327	351	302	-70	-175	100	25	62.5	1597.5	49	122.5	860
5	347	150	360	233	114	285	385	-83	-207.5	1390	127	317.5	1177.5
6	304	293	441	304	0	0	385	-11	-27.5	1362.5	137	342.5	1520
7		237	237	191		0	385	46	115	1477.5	46	115	1635
8	295	76	511	420	-125	-312.5	72.5	-344	-860	617.5	91	227.5	1862.5
At 45 degrees													
Time (days	Carbondioxide (mg)				Actual CO2 produced (mg)								
	2g oil(Reactor 1)	4g oil (Reactor 2)	8g oil (Reacto	control (compost o	Reactor 1	*2.5(kg)	cumulative CO2	Reactor 2	*2.5(kg)	cumulative CC	Reactor :	*2.5(kg)	cumulative CO2
1	928	1051	1336	1259	-331	-827.5	-827.5	-208	-520	-520	77	192.5	192.5
2	772	1095	1172	685	87	217.5	-610	410	1025	505	487	1217.5	1410
3	710	813	995	624	86	215	-395	189	472.5	977.5	371	927.5	2337.5
4	325	748	325	223	102	255	-140	525	1312.5	2290	102	255	2592.5
5	421	569	445	421	0	0	-140	148	370	2660	24	60	2652.5
6	261	590	338	338	-77	-192.5	-332.5	252	630	3290	0	0	2652.5
7	377	327	455	476	-99	-247.5	-580	-149	-372.5	2917.5	-21	-52.5	2600
8	518	469	444	642	-124	-310	-890	-173	-432.5	2485	-198	-495	2105

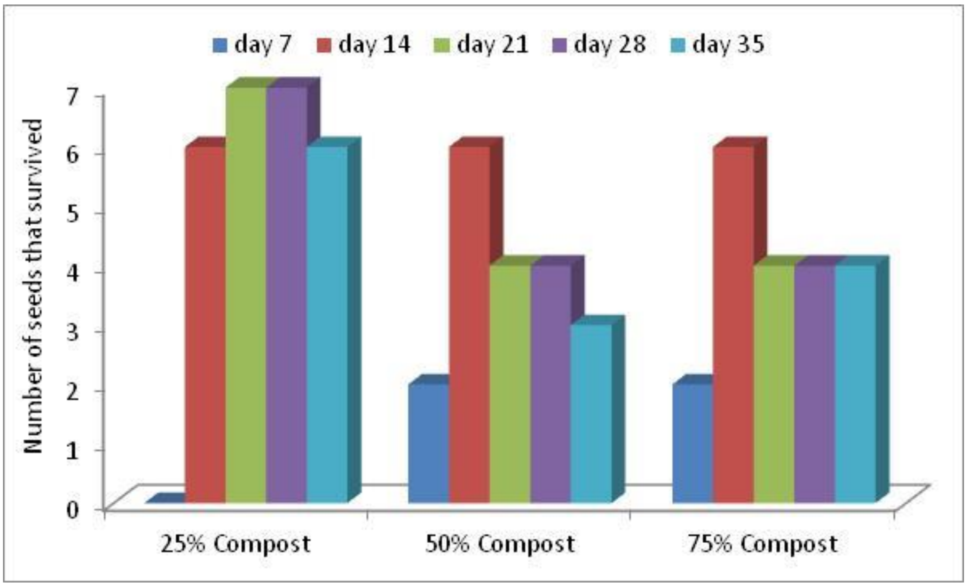
Appendix D: Daily carbon dioxide (CO_2) production measured every 3 days for 27 days (RUN 1 at 35^0C)

	Compost + Soil (1:3) Replicate 1		Compost + Soil (1:3) Replicate 2		Compost only		Soil only	
DAYS	mg CO2/kg ww	mg CO2/kg ds	mg CO2/kg ww	mg CO2/kg ds	mg CO2/kg ww	mg CO2/kg ds	mg CO2/kg ww	mg CO2/kg ds
3	204.6	129	198	125	55	23	180.4	126
6	336.6	212	336.6	212	81.4	33	363	254
9	470.8	297	464.2	292	85.8	35	534.6	374
12	558.8	352	563.2	355	96.8	40	666.6	467
15	633.6	399	631.4	398	132	54	827.2	579
18	715	450	704	444	138.6	57	981.2	687
21	825	520	781	492	149.6	61	1179.2	825
24	902	568	836	527	171.6	70	1328.8	930
27	983.4	620	1071.4	675	215.6	88	1493.8	1046

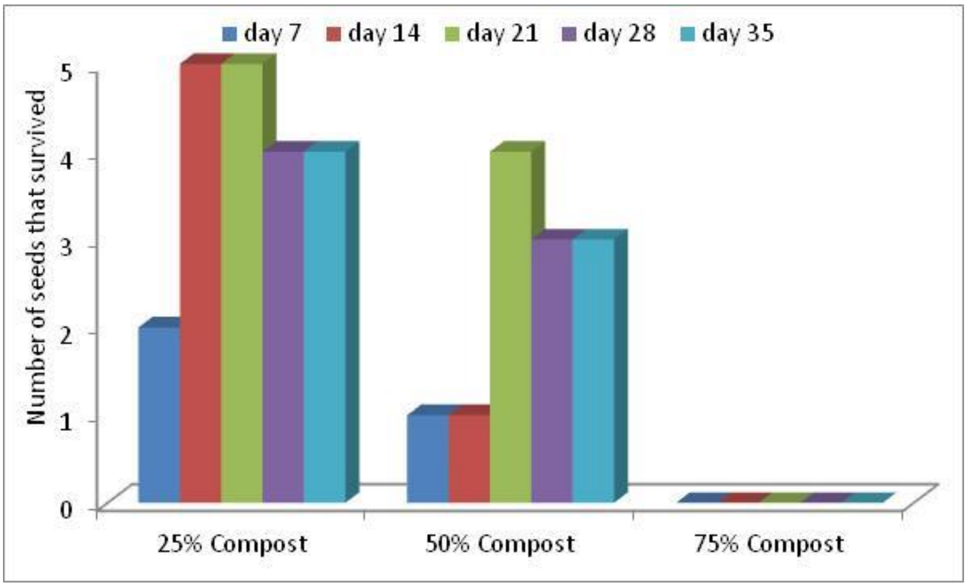
Appendix E: Germination and growth of seeds (Growth trial 2)



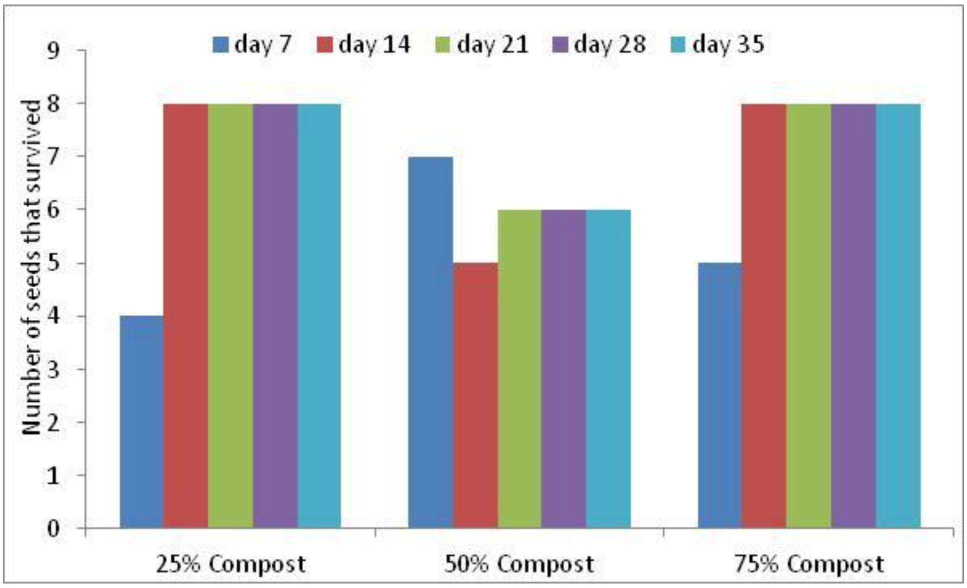
E.1: The numbers of seedlings that survived in compost at 5% initial oil level over 35 days. Counting was once every week for 5 weeks, soil was from Bardsey



E.2: The numbers of seedlings that survived in compost at 7.5% initial oil level over 35 days. Counting was once every week for 5 weeks, soil was from Bardsey



E.3: The numbers of seedlings that survived in compost at 10% initial oil level over 35 days. Counting was once every week for 5 weeks, soil was from Bardsey



E.4: The numbers of seedlings that survived (controls) over 35 days. Counting was once every week for 5 weeks, soil was from Bardsey

Appendix F: Typical data set from SPSS software (ANOVA [dataSet1] IBM SPSS Statistics)

days	PH	EC	soil	germination
10	5.9	111.8	1	2
14	6.84	33.2	1	0
21	6.27	97	1	0
27	6.86	67.6	1	0
35	5.9	104.5	1	0
10	5.86	79.9	1	3
14	5.98	105.5	1	0
21	6.7	61.8	1	0
27	7.6	71.2	1	0
35	6.27	109.6	1	0
10	5.99	98.7	2	0
14	6.61	33.9	2	0
21	6.38	156.4	2	0
27	6.37	125.7	2	0
35	7.02	114.9	2	0
10	5.95	119.2	2	0
14	6.28	164.7	2	1
21	6.32	268	2	1
27	6.55	95.2	2	0
35	6.67	111.6	2	0
10	5.9	101.7	3	0
14	6.45	33.1	3	0
21	6.97	89	3	0
27	6.89	54.6	3	0
35	6.83	62.3	3	0
10	5.98	60.5	3	0
14	6.75	79.4	3	0

days	PH	EC	soil	germination
21	7.28	77.1	3	0
27	6.49	96.7	3	0
35	6.24	76.2	3	0
10	6.45	134.5	4	0
14	5.96	43.1	4	0
21	6.56	100	4	0
27	6.07	81.6	4	0
35	6.97	83.3	4	0
10	6.4	134.5	4	0
14	6	43.1	4	0
21	6.45	100	4	0
27	6	81.6	4	0
35	6.4	83.3	4	0
10	8.45	812	5	1
14	8.95	420	5	5
21	9.01	259	5	5
27	8.45	446	5	5
35	8.46	317	5	5
10	8.45	634	5	0
14	8.86	289	5	3
21	8.94	434	5	4
27	8.8	258	5	4
35	8.64	588	5	4
10	8.52	700	6	0
14	9.07	268	6	6
21	8.67	351	6	5
27	8.37	250	6	5
35	8.45	279	6	5

days	PH	EC	soil	germination
10	8.23	624	6	0
14	8.73	427	6	4
21	8.75	277	6	5
27	8.84	325	6	5
35	8.51	421	6	5
10	8.31	529	7	0
14	8.67	437	7	4
21	9.15	278	7	5
27	8.77	265	7	5
35	8.6	250	7	5
10	8.25	959	7	1
14	8.57	374	7	4
21	9.18	273	7	3
27	8.63	265	7	2
35	8.21	234	7	1
10	8.21	803	8	0
14	8.83	162.9	8	5
21	8.63	207	8	5
27	8.45	209.5	8	5
35	8.37	298	8	5
10	8.35	768	8	1
14	8.5	470	8	4
21	8.22	276	8	4
27	8.57	235	8	4
35	8.24	175.7	8	4
10	7.97	665	9	0
14	7.95	354	9	1
21	8.02	252	9	3

days	PH	EC	soil	germination
27	8.07	446	9	3
35	8.12	297	9	3
10	7.89	941	9	3
14	8.56	179.1	9	3
21	8.08	365	9	3
27	8.17	269	9	3
35	8.19	237	9	3
10	8.1	535	10	0
14	8.36	201.5	10	4
21	8.77	687	10	4
27	8.17	193.6	10	4
35	8.45	301	10	4
10	7.92	928	10	0
14	8.24	512	10	2
21	8.11	306	10	2
27	8.31	207.9	10	2
35	8.45	325	10	1