ENHANCEMENT OF DIESEL OIL DEGRADATION BY NITROGEN AND PHOSPHORUS FERTILIZERS IN FOUR GHANAIAN SOILS

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ABSTRACT

Nutrient elements, especially N and P, play an important role in the growth of crops and degradation of petroleum hydrocarbons. The present study investigated the effects of nitrogen and phosphorus application on (i) microbial degradation of diesel oil in four Ghanaian soils (Beach sand, Toje series, Nyankpala series, and Oda series) and (ii) growth of maize and cowpea in oil remediated soil. The four soils with no history of contamination were contaminated with diesel oil at 10 g oil/kg soil and amended with single super phosphate (SSP) and ammonium nitrate(AMN) fertilizers separately at 0, 30, 60 and 90 kg/ha. The treated soils were incubated in the laboratory under room temperature and sampled at 10 days interval to monitor the population of hydrocarbon utilizing bacteria (HUB) and the amount of oil degraded. In another experiment, the Toje series was contaminated with diesel oil at 0, 2.5, 5 and 10 g oil/kg soil. The contaminated soils were then amended with SSP and AMN separately at 60kg/ha, allowed to degrade for 30 days, and test crops were grown. Results showed that the two fertilizers stimulated the growth of HUB and enhanced degradation of diesel oil. Increasing the application rate of the fertilizers increased HUB population and enhanced oil degradation. The rates of biodegradation of oil in the soils were in the order Beach > Nyankpala > Toje > Oda. Results also showed that increasing diesel concentration decreased plant growth, however, fertilization improved growth and nutrient uptake. The study (i) confirmed that N and P are important elements needed for microbial degradation of oil and growth of crops in oil contaminated soils and (ii) revealed that beach sand used in the present study has the potential to degrade oil. Further studies are needed to identify and determine the diversity of HUB in the beach sand for future bioremediation along the coast of Ghana.

Keywords: *Degradation, diesel oil, hydrocarbon utilizing bacteria, shoot dry weight, N and P uptake*

INTRODUCTION

It is established that petroleum based products are the major source of energy for industries, transportation, construction and agriculture. Over 2 billion tonnes of petroleum are produced annually worldwide and over 1,300,000 tonnes of petroleum products are introduced into the natural environment worldwide (NAS, 2002). According to Atlas and Philip (2005), environmental contamination with these products introduces a myriad of hydrocarbons that cause a variety of problems.

The effects of petroleum based products on the growth and performance of plants have been reported by many researchers. According to Chaineau *et al*.(1997) and Salanitro *et al*. (2004), the response of plants to diesel oil contamination is negative. These effects have been observed to occur due to the interference in plant uptake of nutrients, the unfavourable soil structure and chemical properties created by oil (McGill and Rowell, 1977; Iwanow *et al.,* 1994; Caravaca and Rodán, 2003).

Biodegradation of petroleum and its products, which exploits the ability of microorganisms to degrade and detoxify these products, has been established as efficient, economical, versatile and environmentally sound treatment (Mehrashi *et al*., 2003). According to Ezeji (2005), biodegradation is possible because the degrading microorganisms have enzyme systems to degrade and utilize the hydrocarbons as a source of carbon and energy.

Bioremediation of oil contaminated soils is considered economic, environmental friendly and more acceptable technique with minimum risk exposure because it is based on natural processes.

There are two approaches in bioremediation: either the contaminated sites are inoculated with specific hydrocarbon degrading microorganisms (bioaugmentation), or the activity of indigenous organisms is enhanced in situ by addition of appropriate nutrients and inducers (Sylvestre and Sondossi, 1994), referred to as biostimulation. Biostimulation of indigenous microorganisms by the addition of nutrient elements such as nitrogen, phosphorous and potassium that are rapidly depleted has been widely used in diesel oil contaminated soils (Perfumo *et al*., 2006).

Research studies documented on biostimulation usually focused on one soil type or soils from same ecological zones, however, little attention is paid to soils from different ecological zones or different soil types. Besides, one of the indicators of a well remediated soil is its ability to allow growth of plants or crops but little work has done in this area of research. Ghana, which is noted for agriculture and has discovered oil in commercial quantities recently, there is little information on biostimulation in her soils. It would therefore be appropriate to research into degradation of oil in her soils and growth of crops. In light of this background and problems associated with the spillage of crude oil or its products the study is aimed at investigating the microbial degradation and growth of maize and cowpea in four different soils contaminated with diesel oil and amended with nitrogen and phosphorus fertilizers.

MATERIALS AND METHODS

Soils used

Soil samples were taken (0-20 cm depth) from four different places in Ghana which had no previous history of petroleum or petroleum product contamination. The soils sampled were Toje series classified as Rhodic Kandiustalf (Eze, 2008), Oda series classified as Eutric Gleysol (Owusu-Bennoah *et. al.,* 2000), Nyankpala series classified as Plinthic Acrisol (Nartey, 1994), and Beach sand classified as Haplic Arenosol (FAO, 1988).Oda series is a forest soil, Toje and Nyankpala series are coastal savannah and guinea savannah soils, respectively and Beach sand from the Bortianor beach near Accra. The Beach sand was included because Ghana has discovered oil in commercial quantities off shore and production has started few years ago. The soils were air dried, passed through a 2 mm sieve and stored for laboratory analyses and experiments. Some physical and chemical characteristics of the soils are shown in Table 1.

Degradation of oil study

The sieved soils were contaminated with diesel oil at 10g oil/kg of soil. The contaminated soils were amended with ammonium nitrate (AMN) and single superphosphate (SSP) separately as source of N and P, respectively at 0,30,60 and 90 kg/ha. The treatments were replicated four times and incubated in the laboratory under room temperature in a complete randomized design arrangement. Soil samples were taken at 10 days interval for the determination of hydrocarbon

Table 1: Some physico-chemical properties of the soils used

Soil properties/Soil	Oda	Toje	Nyankpala	Beach Sand
Sand (%)	62.7	61.5	50.0	99.1
Silt (%)	32.3	13.5	45.0	0.9
Clay (%)	5.0	25.0	5.0	0.0
Texture	sl	cl	sl	s
pH	5.60	5.30	4.80	7.00
OC (%)	0.72	0.56	0.34	0.11
Total N (%)	0.16	0.12	0.07	0.02
Available P (mg/kg)	8.23	4.68	1.92	0.10
Na (cmol/kg)	0.19	0.30	0.23	3.04
K (cmol/kg)	1.86	0.82	1.02	0.09
Mg (cmol/kg)	1.21	0.54	3.48	9.28
Ca (cmol/kg)	3.48	1.03	2.51	10.36
CEC (cmol/kg)	5.72	11.68	6.14	0.21

sl = sandy loam cl = clay loam s = sand

utilizing bacterial (HUB) population and quantity of diesel oil degraded.

The HUB count was estimated using the modified mineral salts agar medium of Mills *et al*. (1978) and modified vapour phase transfer technique of Okpowasili and Amanchukwu (1988). One gramme of soil was sampled, tenfold serial dilution was prepared and 1 mL of appropriate dilution was plated onto the mineral salts agar medium containing 10g NaCl, 0.42 g $MgSO_4.7H_2O$, 0.29 g KCl, 0.53 g KH_2PO4, 0.42 g NH_4NO_4, and 15 g agar in 1L distilled water (adjusted pH= 6.8). The modified vapour transfer technique involved spreading 0.5 ml of diesel (serving as carbon source) on the mineral salts agar medium after setting and allowing to stand for 1 hr in order to let the diesel oil to diffuse into the agar medium before incubating at room temperature for 5 to 7 days. The number of colonies formed was used to estimate the HUB population.

Residual diesel oil in the contaminated soils was extracted using a modified method of Abu and Ogiji (1996). Soil samples were air-dried to constant weight and 5 g were placed into conical flasks and 10 mL chloroform was added for extraction. The residual diesel oil was extracted by gently shaking the flasks for 5 min at 150 rpm on a Kika Labortenik KS501 Digital shaker. Each extract was filtered through cotton wool in a funnel and collected in clean glass containers, closed immediately and analyzed for diesel oil content. Quantitative determination of diesel oil extracted was employed as described by Udeme and Antai (1988). A standard curve of absorbance (520 nm) against varying concentrations of diesel oil in chloroform was drawn after taking readings from a spectrophotometer. The concentrations of diesel oil extracted were calculated from the standard curve. The residual diesel oil data was fitted to the first order kinetics model to calculate the biodegradation rate.

$$C = C_o e^{-kt}$$

Where **C** is the oil content in the soil (gkg^{-1}) at time **t**, $\mathbf{C_o}$ is the initial oil content in soil (gkg^{-1}),

k is the biodegradation rate constant (g oil d^{-1}), and **e** is the specific degradation constant.

Plant growth study

A pot experiment was conducted in the screen house to investigate the response of cowpea and maize to oil contaminated soil fertilized with nitrogen and phosphorus. These test crops are mainly cultivated and consumed in Ghana. The Toje soil was contaminated with diesel oil at different concentrations; 0 g (C0), 2.5g (C1), 5 g (C2) and 10 g (C3) oil/kg soil. The contaminated and uncontaminated soils were amended with AMN (N60) and SSP (P60) separately at a rate of 60kg/ha. The unfertilized treatment (T0) served as the control. The treatments were allowed to stand in the screen house for 30 days to allow degradation to take place. After 30 days, the test crops were sown in the soils for 4 weeks. One week after planting (WAP), % germination was determined and 4 WAP shoot dry weights, total N and P uptake by the shoots were determined. Analysis of variance (ANOVA) was used to test for significant differences among the means at 5% probability level.

RESULTS AND DISCUSSION

Hydrocarbon utilizing bacterial population

The effects of nitrogen and phosphorus fertilization on the population of hydrocarbon utilizing bacteria (HUB) in the four contaminated soils are shown in Figs. 1 and 2. The initial HUB populations in Nyankpala, Oda, Toje and Beach were 5.9 x 10^5, 1.1 x 10^7, 8.4 x 10^6 and 3.3 x 10^2cfu/g soil, respectively. The differences in the initial HUB count in the four soils could be attributed to the difference in nutrient levels (Table 1) as soils with low nutrients, especially N and P, recorded low initial HUB populations. The bacterial growth pattern during the incubation period was similar in the four soils. HUB populations increased to peak values and then declined. The Beach and Nyankpala reached their peak values 20 days after incubation (DAI) whereas those in the Oda and Toje reached their peak values 30 DAI. Sang-Hwan *et al*. (2007) observed similar bacterial growth. Schaefer and Juliane (2007) documented that with increasing time due to soil resistant components with high carbon chain and with less remaining nutrients, the growth of bacteria and oil degradation decrease. In the present study, bacterial populations in Oda and Toje peaking late could be due to higher organic matter, nitrogen and phosphorus in these soils, as compared to Beach and Nyankpala (Table 1), that might had enhanced and sustained bacterial growth much longer in Oda and Toje than the other soils.

Table 2: Biodegradation rate constant (g oil/d) of the various treatments

Treatment	Soils			
	Toje	Nyankpala	Oda	Beach
N0	0.0191a	0.0224a	0.0169a	0.0270a
N30	0.0200a	0.0236b	0.0172ab	0.0290a
N60	0.0204a	0.0241c	0.0176b	0.0300a
N90	0.0210a	0.0244c	0.0185c	0.0310a
Lsd (p = 5%)	0.0023	0.0005	0.0005	0.0046
P0	0.0191a	0.0227a	0.0187a	0.0294a
P30	0.0194a	0.0235b	0.0191a	0.0314b
P60	0.0205b	0.0243c	0.0192a	0.0322c
P90	0.0217c	0.0259d	0.0200b	0.0334d
Lsd (p = 5%)	0.0004	0.0006	0.0007	0.0005

***Means having subscript in common within same column and type of fertilization are not significantly different at 5% probability.*

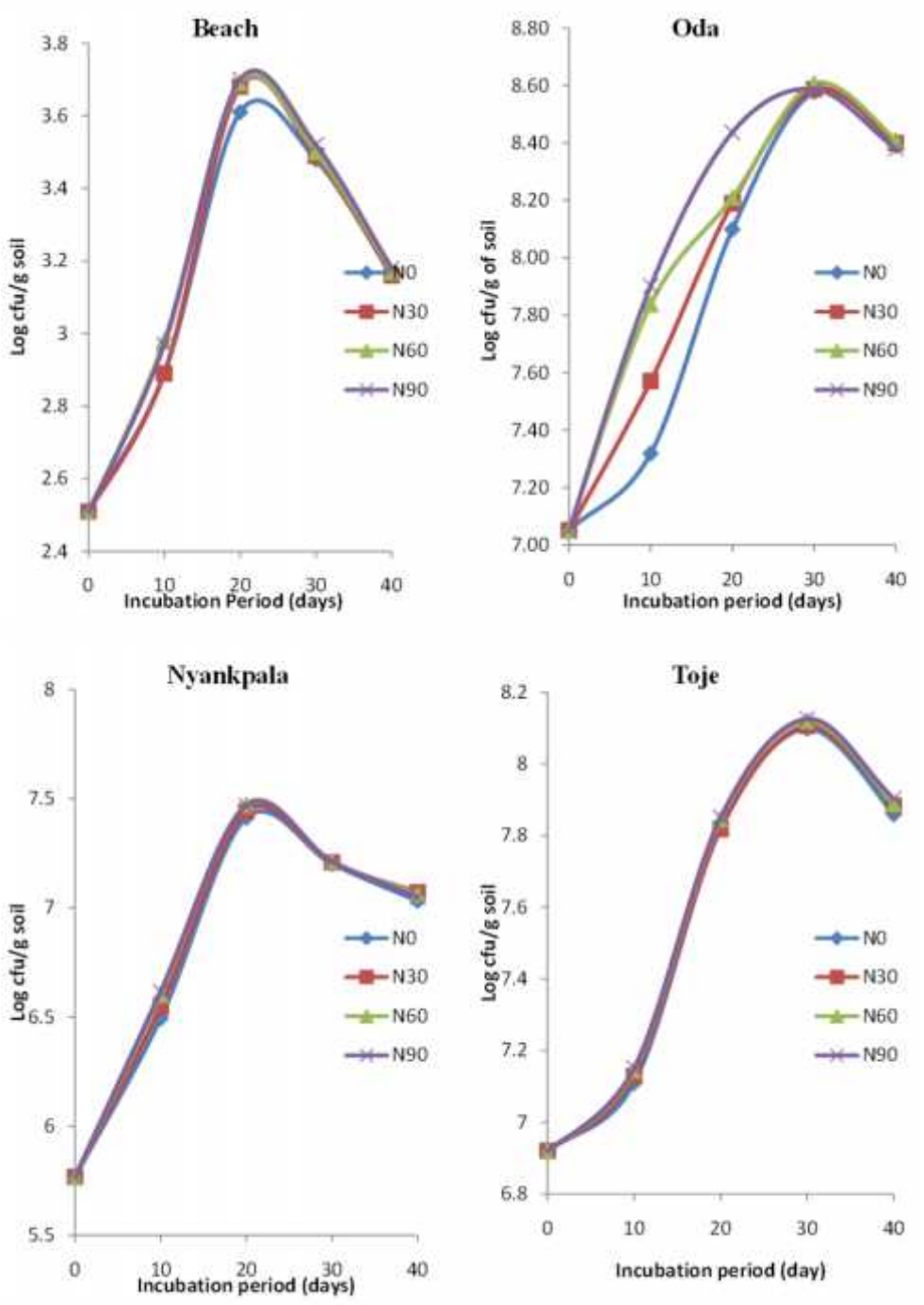


Fig. 1 Response of HUB to different rates of nitrogen

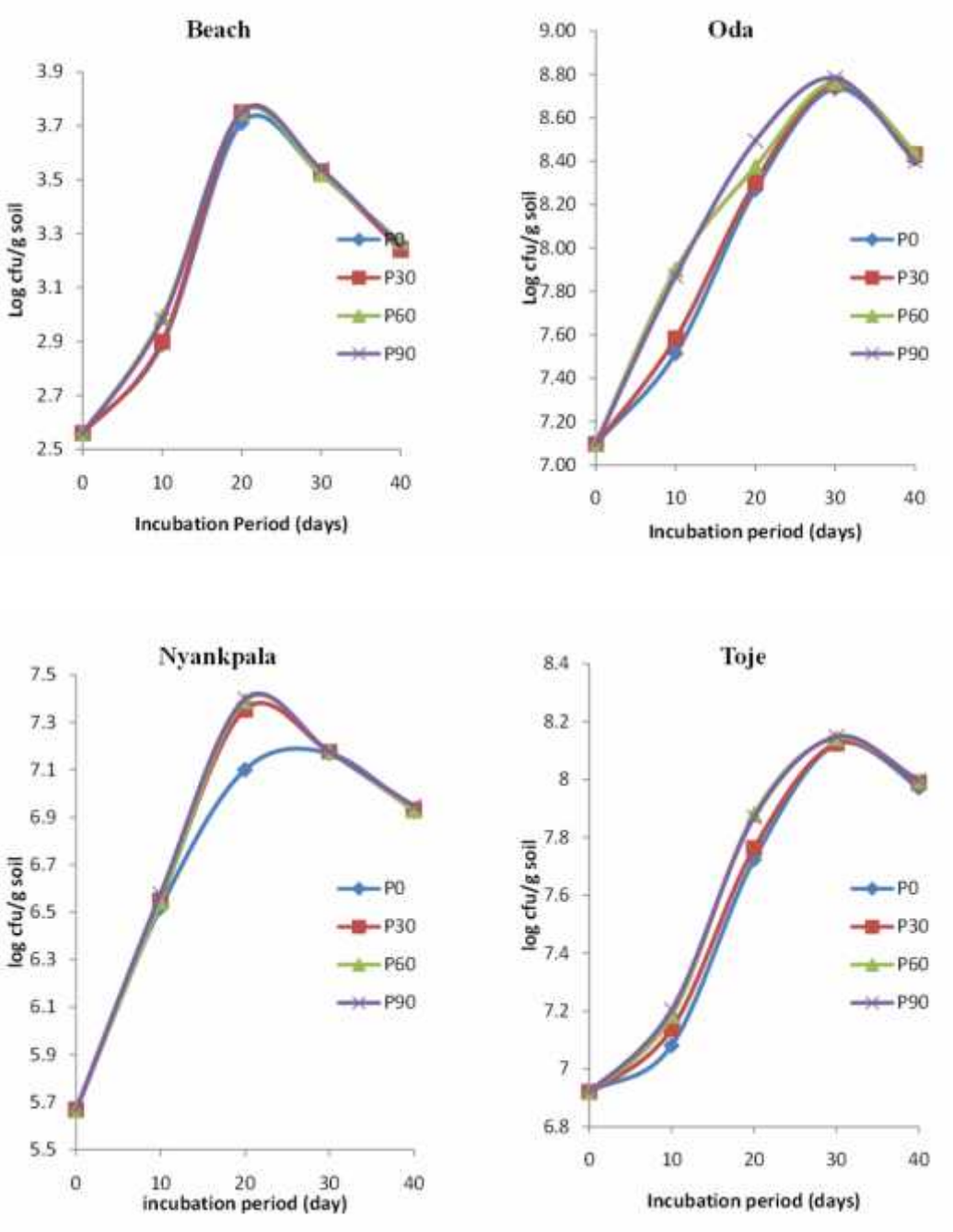


Fig. 2: Response of HUB to different rates of phosphorus

Increasing the application rate of N and P resulted in increased HUB populations. According to McGill (1980), the amounts of N and P present in many oil contaminated soils are limited and these soils are unable to supply soil bacteria with adequate N and P for optimum growth. The present finding agrees with the study by Chorom *et al*. (2010) who observed that application of fertilizers to crude oil polluted soil had a significant effect on soil bacteria growth; and that the average bacterial growth in the treatment samples had a significant difference from bacterial growth in the control. Studies by Joo (2007) also indicated that, nitrogen and phosphorus are necessary nutrients for biodegradation activities because microorganisms require phosphorus as phospholipids in synthesizing cell membranes and for sugar phosphorylation; and microorganisms exploit nitrogen sources to meet their protein and nucleic acid requirements (Odokuma and Akponah, 2010).

Amount of diesel oil degraded

The effects of N and P fertilization on the amount of diesel oil degraded in the contaminated soils are shown in Figs. 3 and 4. There was a general increase in the amount of diesel oil degraded from 10 DAI to 40 DAI for all the soils. The results also showed that as rate of application increased, the amount of diesel degraded also increased. Significant ($p < 0.05$) differences among the treatments were observed between 10 and 20 DAI, but not at 30 and 40 DAI. Using first-order kinetics the rates of biodegradation of diesel oil in the treatments were determined. Results showed that increasing application rate of nitrogen and phosphorus resulted in increased rate of biodegradation (Table 2). The Beach sand had the highest biodegradation rate constants followed by Nyankpala, Toje and Oda series in that order.

The increased biodegradation of oil in the amended soils could be attributed to the increase in HUB population. According to Cooney (1984), nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants, especially nitrogen and phosphorus, and in some cases iron. Atlas (1984) also documented that when a major oil spill occurs, the supply of carbon is dramatically increased and the availability of phosphorus and nitrogen generally becomes the limiting factor. Hence, the addition of nutrients is necessary to enhance the biodegradation of oil pollutants (Kim *et al*., 2004). Studies by Lee and Levy (1991) demonstrated the effectiveness of inorganic fertilizer additions to stimulate the biodegradation rates of oil in sandy beach sediments.

The Beach sand had the highest amount of diesel oil degraded. The present results agreed with the study of Okpokwasili and Oton (2006). The high biodegradation recorded in the Beach sand could be due to free movement or circulation of air since it is sandy in texture (Table 1). According to Al-Aubaidy (2004), aerobic conditions are generally considered necessary for extensive degradation of oil hydrocarbons in the soil environment since major degradative pathways for both saturated and aromatic hydrocarbons involve oxygenases. Studies by Essam *et al*. (2012) demonstrated that aeration plays an influential role in speed and efficiency of the degradation process. Chorom *et al.* (2010) also reported increase in biodegradation of petroleum with the addition of fertilizer and ventilation.

The best two degrading soils were Beach and Nyankpala. The initial HUB populations of these soils were lower than that of Oda and Toje series but they were capable of degrading more oil than Oda and Toje series. This could be due to presence of effective HUB species in the Beach and Nyankpala soils because Leahy and Colwell (1990) documented that oil degradation does not depend on only the population of HUB in the environment but appears to be a function of the genetic make-up of the microbial population of the HUB. It is therefore prudent to identify the HUB in these soils, especially the Beach sand, for future bioremediation.

Percentage germination

Increasing diesel oil concentration resulted in a decrease in the percentage germination of the test crops (Fig. 5). Similar results were reported

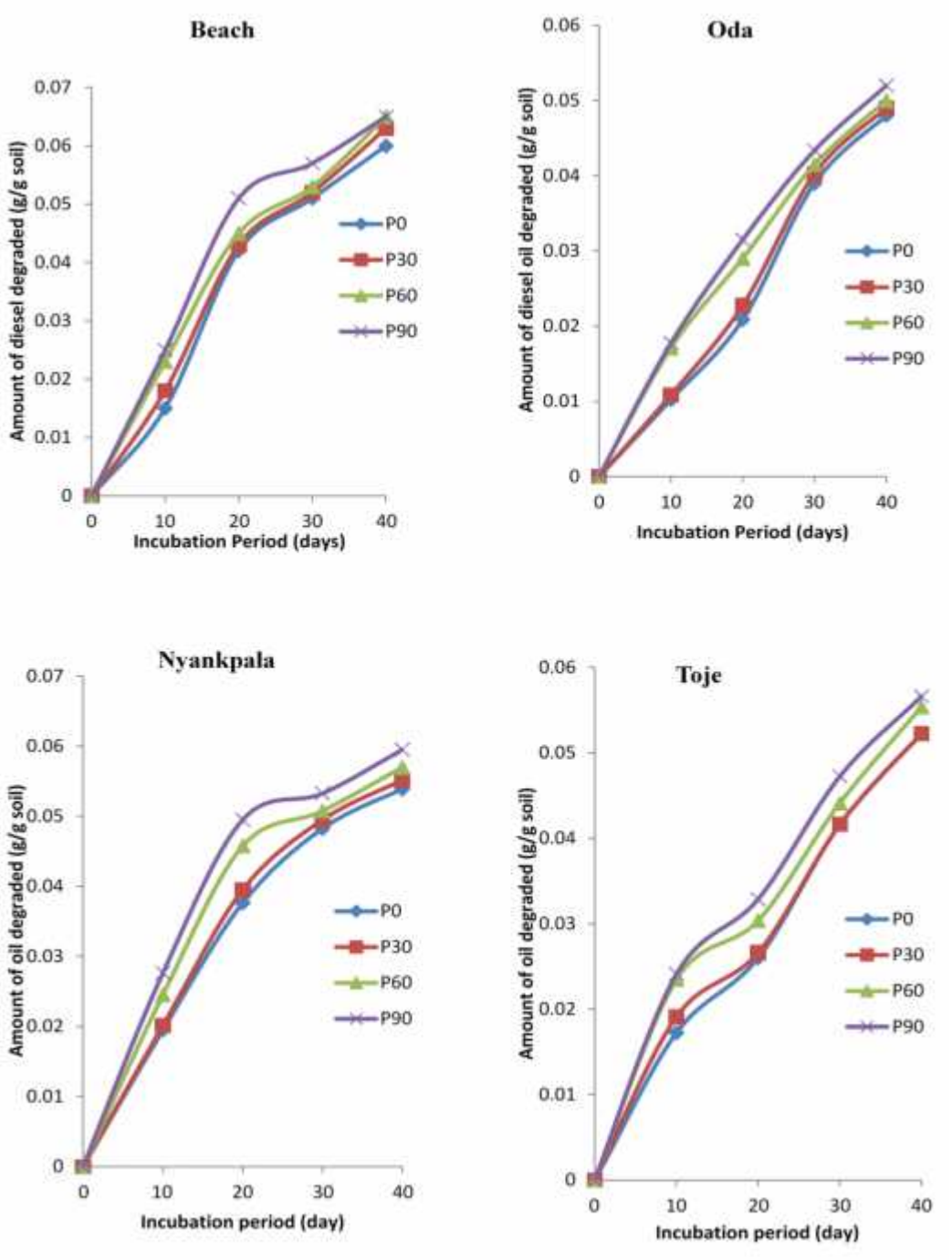


Fig.3: Effect of nitrogen amendment on degradation of diesel oil

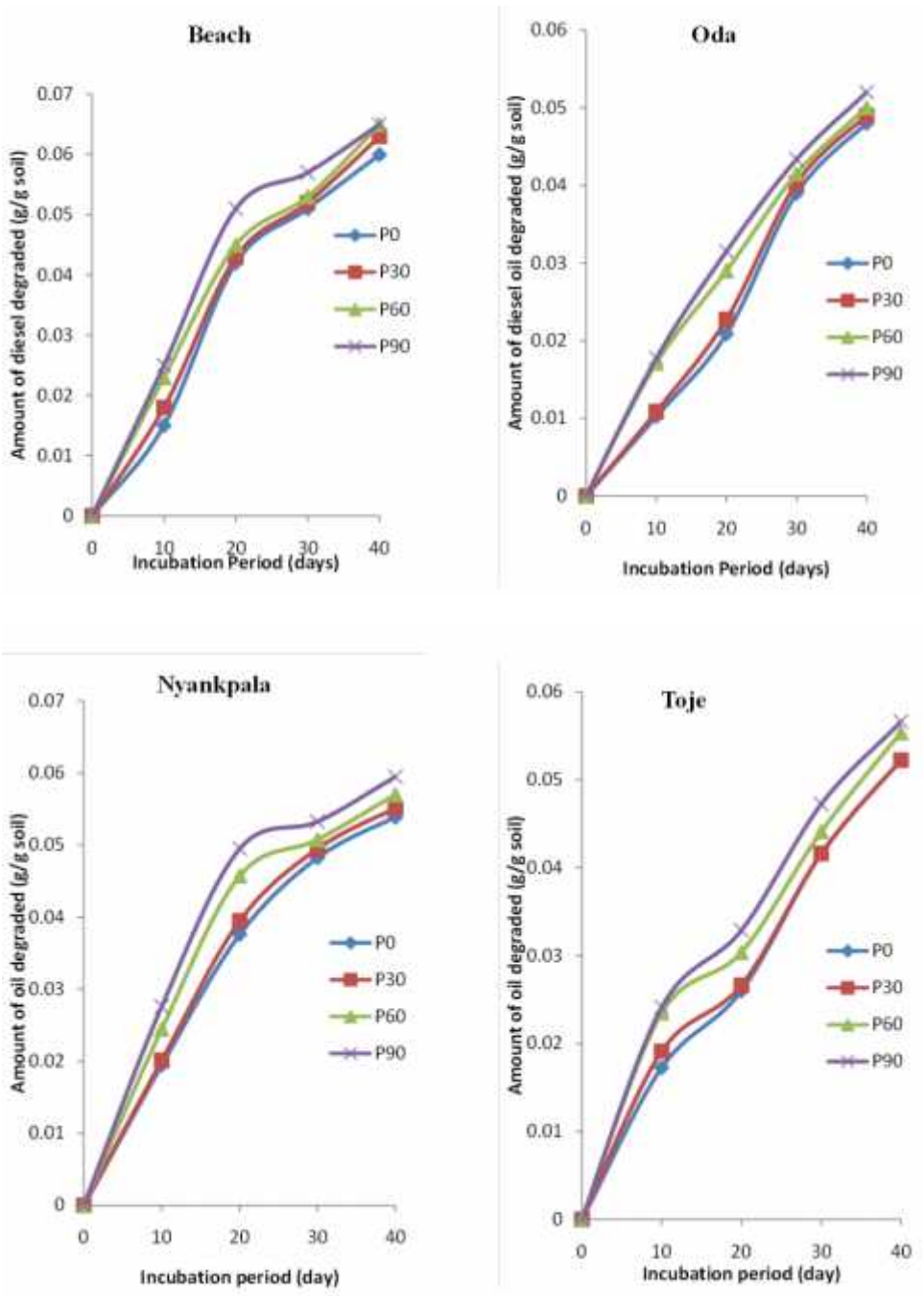


Fig 4: Effect of phosphorus amendment on degradation of diesel oil

by Gallegos-Martinez et al. (2000) who found a reduction in germination between 30-90% in the tropical native Mexican species subjected to soil contamination with crude oil. Campbell and Vavrek (1999) also showed that the number of seedlings germinating in oil contaminated wetland soil declined relative to the uncontaminated soils. According to Adam and Duncan (2002), diesel oil has volatile components that contain light hydrocarbons capable of entering plant seeds easily through the plant cell walls. Bona et al. (2011) also documented that planting in diesel oil contaminated soil could allow diesel oil to cover the seeds producing a barrier to the passage of water and possibly gases to the interior of seeds. At each level of diesel contamination, cowpea had higher percent germination values than that of maize and this could be difference in sensitivity of these crops to oil.

Generally, the application of N and P enhanced germination of both test crops at all levels of diesel oil contamination (Fig. 5). The influence of N and P on germination in the contaminated soils was pronounced in maize and cowpea, respectively. The enhanced germination by N and P fertilization could be attributed to the fact that the fertilizers served as nutrients source for the HUB which enhanced the degradation of the diesel oil and therefore might had reduced the toxic effect of the oil.

Shoot Dry Weight (SDW)

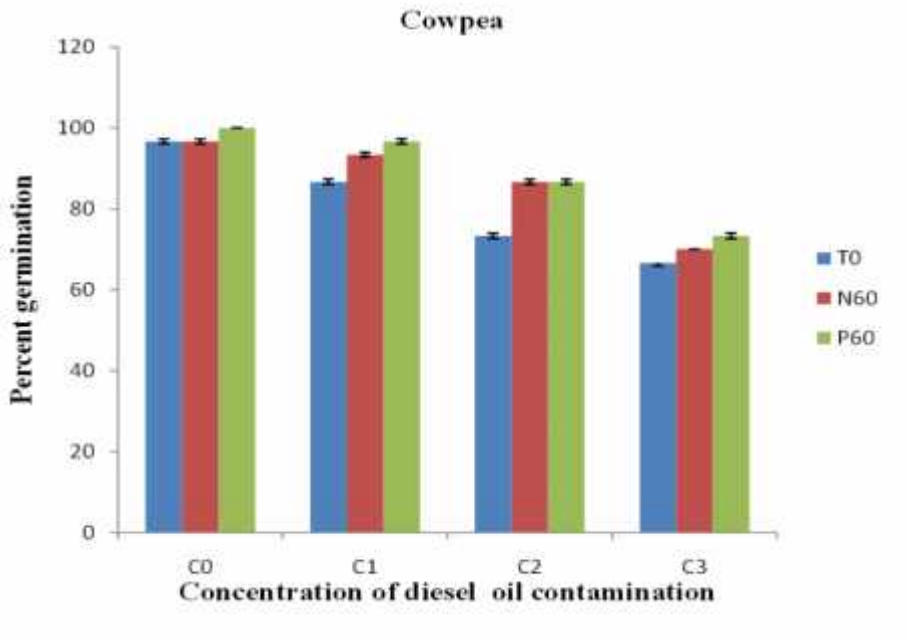
The shoot dry weights (SDW) of the test crops grown in contaminated and uncontaminated soils are shown in Fig. 6. Generally, SDW decreased with increasing level of oil contamination. This shows that crude oil contamination inhibited plant growth and it is in agreement with Agbogidi et al. (2006).

Although the application of nitrogen and phosphorus increased SDW of the test crops in the contaminated soils, the effect was not significant ($p > 0.05$). The toxicity of petroleum hydrocarbons at higher concentrations has been linked to displacement of nutrients and nutrient uptake (Amadi *et al*., 1993); reduction in available phosphorus and total nitrogen (Baker-Coker and Ekundayo, 1995) and interference with soil chemotaxis by crude oil (Rosenberg et al., 1992) culminating in growth retardation (Travern, 1992).

Results also showed that the influence of N on shoot dry weight was more pronounced in maize than in cowpea, while the influence of P was more pronounced in cowpea than in maize. Phosphorus, although not required in large quantities, is critical to cowpea yield because of its multiple effects on nutrition (Adetunji, 1995) and its deficiency is the most limiting soil fertility factor for cowpea production (Reamaekers, 2001). Smyth and Cravo (1990)reported that for optimum growth of cowpea critical levels for soil P was 60 kg P ha^{-1}; a level applied in the present study. The importance of nitrogen for the growth of maize has been extensively investigated. According to Jones (1985) inadequate N availability during the first 2 to 6 weeks after planting of maize could result in reduced yield potentials. Ikisan (2010) documented that nitrogen deficiency in maize at any stage of the growth especially at tasseling and silking stage will lead to virtual crop failure.

Total Nitrogen and Phosphorus Uptake

Uptake of N and P by the test crops decreased with increasing oil concentration (Figs. 7 & 8). N and P fertilization significantly ($p < 0.05$) enhanced N uptake in both test crops in the contaminated soils; however, the enhancement was more pronounced in maize than in cowpea (Fig. 7). N and P fertilization also enhanced uptake of P but it was significant ($p < 0.05$) in maize (Fig. 8). Kayode *et al.* (2008) reported that the presence of engine oil in the soil holds the soil too compact for roots to penetrate and so affects the extent of root growth and nutrient absorption. Many researchers including McGill (1980) observed that available soil nutrients, notably nitrogen are immobilized by soil microorganisms following oil application, and extractable phosphorus levels may also become depressed (Loynachan, 1978).



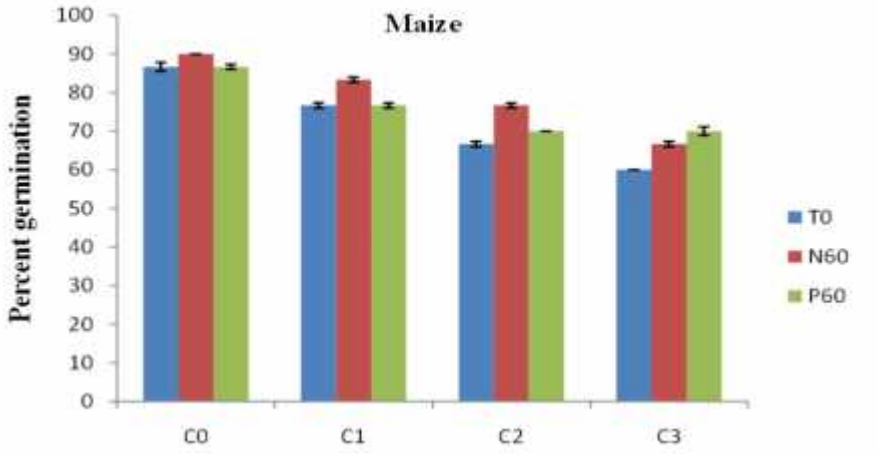
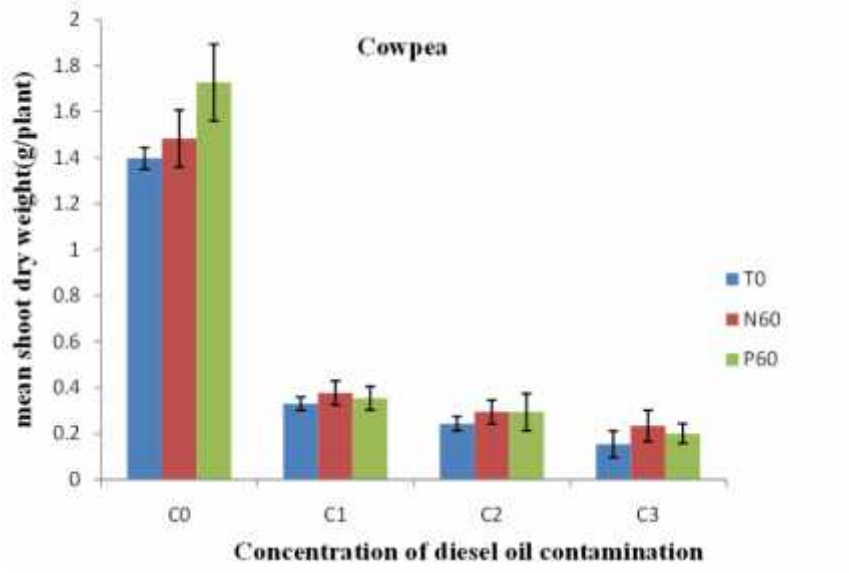


Fig. 5: Germination of cowpea and maize in contaminated and uncontaminated soils amended with N and P.

Note:

- Bars in the figure represent standard errors
- The soils were contaminated with diesel oil at 0 (CO), 2.5 (C.1), 5 (C2) and 10 g oil/kg soil (C3)



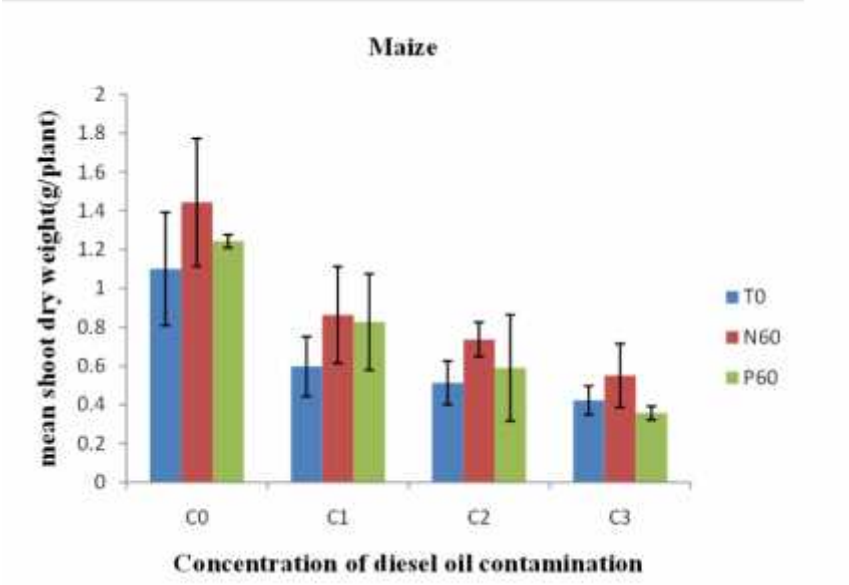


Fig. 6: Shoot dry weights of cowpea and maize in contamination and uncontaminated soils amended with N and P.

Note:

- Bars in the figure represent standard errors
- The soils were contaminated with diesel oil at 0 (CO), 2.5 (C.1), 5 (C2) and 10 g oil/kg soil (C3)

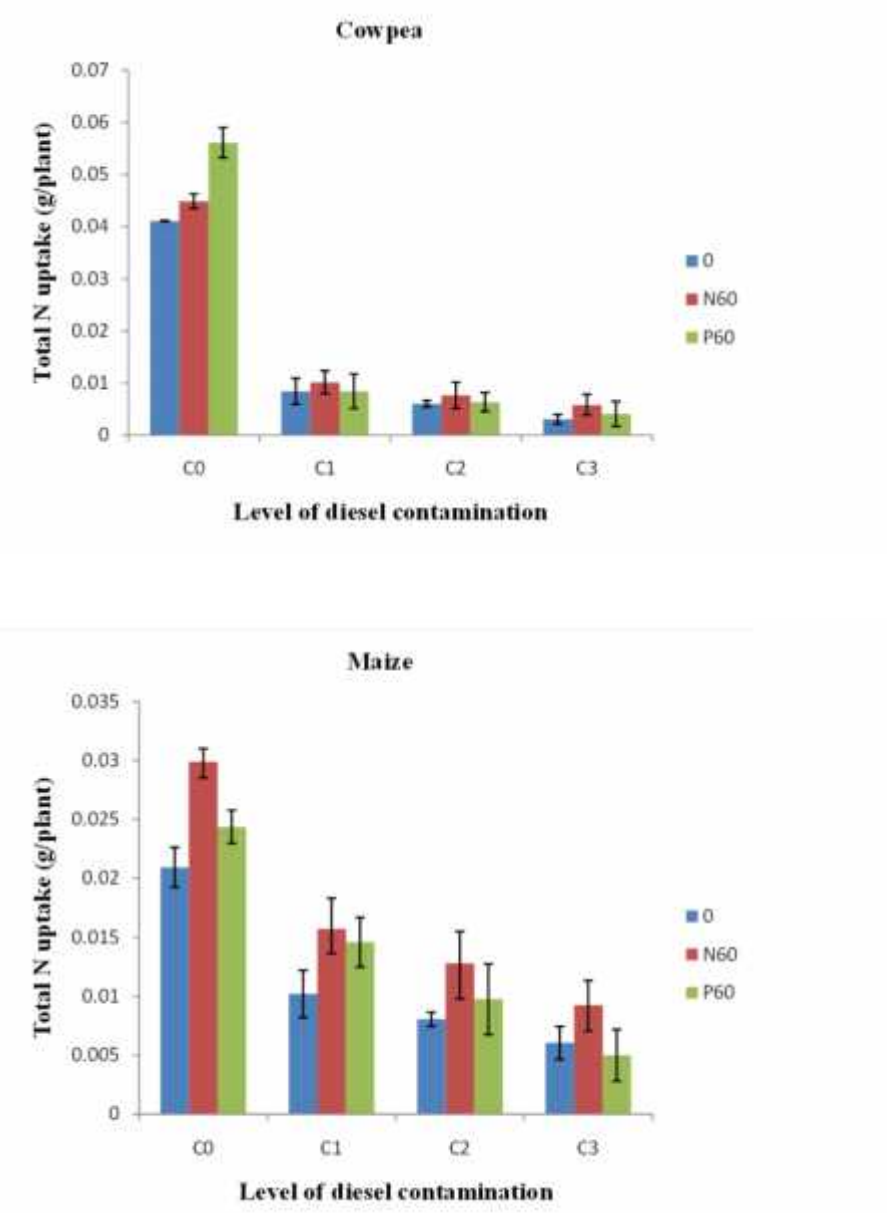


Fig. 7: Total N uptake of cowpea and maize in contaminated and uncontaminated soils amended with N and P.

Note:

- Bars in the figure represent standard errors
- The soils were contaminated with diesel oil at 0 (CO), 2.5 (C.1), 5 (C2) and 10 g oil/kg soil (C3)

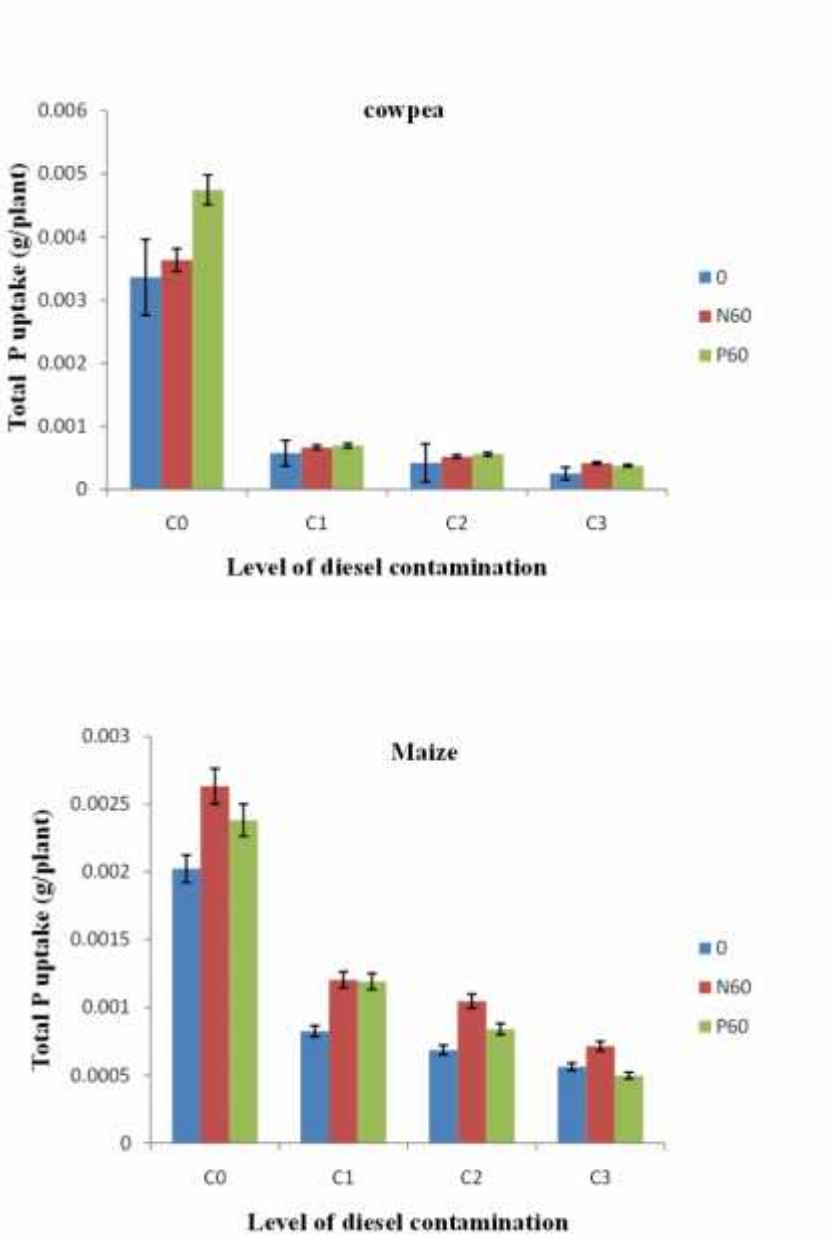


Fig. 8: Total Puptake of cowpea and maize in contaminated and uncontaminated soils amended with N and P.

Note:

- Bars in the figure represent standard errors
- The soils were contaminated with diesel oil at 0 (CO), 2.5 (C.1), 5 (C2) and 10 g oil/kg soil (C3)

CONCLUSION

In the study, increasing the application rate of N and P increased hydrocarbon utilizing bacterial (HUB) population and amount of oil degraded. Beach sand and Nyankpala series had the highest biodegradation rates even though they had the lowest initial HUB population. These soils could contain effective HUB and therefore, further studies needed to identify these microorganisms for future bioremediation in Ghana. Application of N and P also enhanced percentage germination of the test crops in the oil contaminated soils. The application of N and P was more pronounced in maize and cowpea, respectively. Therefore nitrogen and phosphorus fertilizers can be used to restore oil contaminated soils for cultivation of crops.

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