

Bioremediation of diesel polluted soils with *Eichhornia crassipes* (water hyacinth)

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Abstract

Diesel oil contamination is a growing environmental concern in most crude oil processing regions of the world. This study assessed the efficacy of both fresh and powdered *Eichhornia crassipes* (water hyacinth) as potential biostimulants in the remediation of diesel oil contaminated soils using three test concentrations (50 g, 100 g and 150 g) and a control (0 g). The remediation process was monitored by assaying the total organic carbon (TOC), total petroleum hydrocarbon (TPH), and soil pH before and after amendment with the fresh and powdered *E. crassipes* for 90 days. The result showed increase in soil pH, TOC, TPH and volatile matter (VM) in comparison with the control due to soil contamination by diesel oil. However, there was a significant reduction ($p < 0.05$) in soil pH and TOC with the introduction of fresh and powdered *E. crassipes* at different concentrations. Contaminated soil amended with 100 g of fresh *E. crassipes* showed the highest TOC loss (59.7%) alongside soil amended with 100 g of powdered *E. crassipes* (47.36%) while the control showed the least TOC loss (0.91%). Similarly, soil TPH decreased significantly across all concentrations after amendment ($p < 0.05$). Overall, soil amended with fresh *E. crassipes* showed higher TPH loss than soil amended with powdered *E. crassipes*. This study reveals the potentials of using *E. crassipes* in the remediation of diesel oil contaminated soils. Above all, we demonstrate that fresh *E. crassipes* is a potentially stronger biostimulant than powdered *E. crassipes*.

Keywords: bioremediation; biostimulant; diesel oil; *Eichhornia crassipes*; volatile matter

Introduction

Soil contamination has become a global challenge due to the increasing demand for petroleum-derived substances, causing indiscriminate accidental spillage of petroleum-based substances into the soil. These substances become persistent and harmful when released into the environment which may be during the processing, distribution, and daily use of petroleum and petro-chemical products (Ayotamuno *et al.*, 2006). Soil contaminated with petroleum-derived products, such as diesel oil can pose detrimental effects to the surrounding water table, including the flora and fauna that depend on the soil ecosystem for support (Sztompka, 1999; Ikhajagi and Anoliefo, 2012; Osawaru *et al.*, 2013).

Diesel is a specific liquid fuel used in diesel engines, with ignition that occurs without emitting sparks (Chang, 2000). It is a crude oil-derived product that consists of 75% saturated hydrocarbons and 25% aromatic hydrocarbon (Ghaly *et al.*, 2013). Its daily usage encompasses heavy engineering equipment, such as motor vehicles and generators (Ogbo *et al.*, 2009). However, diesel contamination of soil and aquatic ecosystem constitutes a major environmental problem in oil producing nations, especially Nigeria. Diesel has been documented to affect seed germination and plant growth, leading to low agricultural yield and food shortage (Jerome *et al.*, 2016). Diesel contaminated soils could pose potential risks for human habitation and the establishment of agricultural steads due to increased expose and the associated bio-accumulation of harmful hydrocarbon compounds from underground water and agricultural products (Ogbo *et al.*, 2009).

Previous studies have shown the ecological hazard associated with crude oil and several remediation approaches for contaminated soils (Njoku *et al.*, 2012; Ikhajiagbe *et al.*, 2019; Tang and Angela, 2019; Ikhajiagbe and Ogwu, 2020). This includes physical approach that involves the incineration and excavation of contaminated soils (Tang and Angela, 2019), and the chemical approach, which rely on reduction-oxidation and hydrolysis-neutralization reactions to treat contaminated soils (Lim *et al.*, 2014). Nevertheless, both approaches are labor intensive and expensive (Nriagu *et al.*, 2016). Preferably, the biological approach has been used for remediation (*sensu* bioremediation). This process involves the use of living organisms, such as microbes or plants (*sensu* phytoremediation), which can be efficient and ecologically friendly (Njoku *et al.*, 2012). These organisms are known to transform the physicochemical properties of the soil following several techniques (Adam and Duncan, 2002; Collins, 2009).

Existing bioremediation techniques are natural attenuation, bioaugmentation and biostimulation (Njoku *et al.*, 2012; Sheppard *et al.*, 2019). These techniques rely on the underlying functionality of increasing the soil organic matter contents and decreasing soil pH for improved outcomes. In some instances, organic matter is added directly to the contaminated soil or indirectly in compost form as a biostimulant (Ogbo *et al.*, 2009). Several plants, such as *Peperomia pellucida*, *Alternanthera brasiliana* and *Eichhornia crassipes* have been investigated for high organic matter content and biostimulation potential for remediation of diesel oil contaminated soils (Ogbo *et al.*, 2009; Sheppard *et al.*, 2019). *E. crassipes* is used extensively in phytoremediation (Udeh *et al.*, 2013). In a recent study, it was shown to out-perform *Peperomia pellucida* and *Alternanthera brasiliana* in a model diesel oil contaminated soil (Ikhajiagbe and Anoliefo, 2012). Despite these studies, there is still a dearth of information on which plant and plant forms show biostimulation efficacy in improving remediation processes of contaminated soils. Therefore, this study was conducted to assess the biostimulation efficacy of fresh and powdered forms of *E. crassipes* as an effective agent in remediating diesel oil contaminated soils.

Materials and Methods

Study site

This study was conducted in the botanical garden of the University of Lagos, Nigeria (6.5157° N, 3.3886° E). Field collection of large quantities of *Eichhornia crassipes* was conducted along a riverside in the Bariga Local Government Area (LGA) of Lagos State [6.5391° N, 3.3849° E] by handpicking the plant during the daytime. Using a clean treated gallon, about 40 l of diesel oil was purchased from the Nigeria National Petroleum Cooperation (NNPC) petrol station at Palm Groove in Shomolu LGA, Lagos State. The chemical composition was in line with (Chang, 2000).

Soil preparation and physicochemical analysis

Using a 10 m soil auger, 50 cm of loamy soil samples were obtained directly from the botanical garden. Physicochemical parameters of both the control and diesel contaminated soils were determined prior to usage for amendment following standard procedures of the Association of Official Analytical Chemists (AOAC),

2005. Soil pH was measured using a pH meter (Model PHS-3C). Soil total organic carbon (TOC) and organic matter (OM) were determined following the methods of Nelson and Sommers (1983). Total petroleum hydrocarbon content (TPH) and volatile matter (VM) were assayed as described by Ayotamuno *et al.* (2006).

Plant preparation

Freshly harvested *E. crassipes* stems were shredded in pieces (3/4 x 20) using a standard table knife after washing with clean tap water following Ayotamuno *et al.* (2006). Next, 5 kg of the shredded *E. crassipes* was air-dried, ground into powder, then stored in 5 ml ethanol treated bottles for further experimental use. A similar 5 kg of the fresh *E. crassipes* was applied directly to the contaminated soil following the method of Swamps (2013).

Soil contamination

Exactly 21 kg of loamy soil was air dried, sieved with a standard mesh and then contaminated with 750 ml/g w/w of diesel oil. Contaminated soils were placed in 9 plastic pots and amended with 50 g, 100 g and 150 g of fresh and powdered *E. crassipes* alongside a control (0 g). The content of each pot was turned twice a week to ensure proper aeration. Soil moisture content was maintained at a 60% water holding capacity threshold by the monitored addition of distilled water. The set-up was developed in three replicates using a randomized block design and incubation period of 90 days at room temperature (± 25 °C). Similar physicochemical analysis as detailed above was conducted on the contaminated soils during and after this period.

Bioremediation analysis

Bioremediation assessment was done by comparing outputs of the baseline physicochemical analysis before and after diesel soil contamination. Upon the introduction of the fresh and powdered *E. crassipes* as amendments, soil pH and TOC were determined at 30, 60 and 90 days after contamination (DAC), while TPH was determined at 1, 45 and 90 DAC. Percentage TOC and TPH loss were also quantified and comparatively assessed.

Statistical analysis

Data were analyzed as mean and standard error following a two-way analysis of variance (ANOVA) IN SPSS (8th edition). The statistical significance of the values obtained was based on a p-value of (0.05).

Results and Discussion

Baseline physicochemical parameters

The baseline physicochemical content of the control soil (CSS) and diesel contaminated soil (DCS) measured after contamination indicate that diesel remarkably altered the test soil properties (Table 1). Soil pH increased significantly in the DCS (6.49-7.12; $P < 0.05$). The high soil pH was likely caused by the hydrophobic nature of diesel and its potential to induce drought in soil surface layers. This could aggravate salinization and thereby increase pH values in comparison to the control (Collins, 2009; Njoku *et al.*, 2009). Also, the TOC, TPH and VM significantly increased in diesel contaminated soils ($p < 0.05$). However, OM significantly decreased ($p < 0.05$). This was likely caused by a reduction in the exchangeable anion/cation acidity (Ikhajiagbe *et al.*, 2019). Similarly, OM was observed declining in soils with high pH (7.5) which was associated with reduction in activities and aggregation of beneficial rhizospheric bacteria (David *et al.*, 2019).

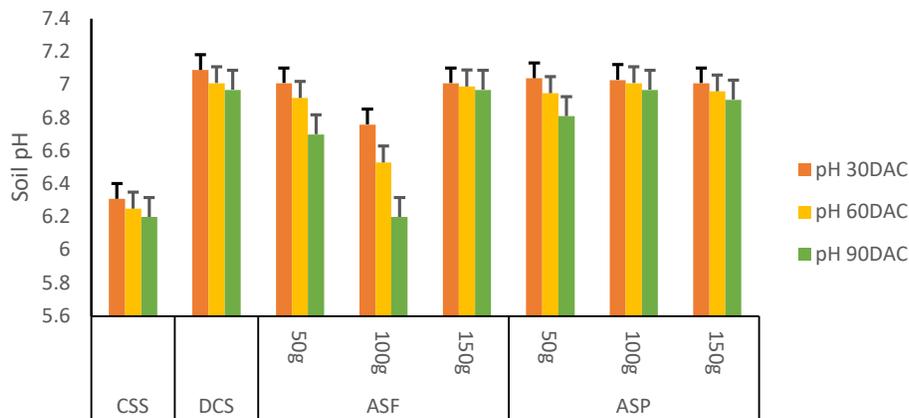
Table 1. Soil physicochemical parameters before bioremediation analysis

Parameters	pH	TOC (%)	TPH	VM (%)	OM (%)
CSS	6.49 ± 0.13 ^a	3.36 ± 0.12 ^a	ND	3.90 ± 0.03 ^a	5.00 ± 0.09 ^a
DCS	7.12 ± 0.08 ^b	77.92 ± 0.23 ^b	49.16 ± 1.2 ^b	96.03 ± 1.2 ^b	0.10 ± 0.03 ^b

CSS = Control Soil, DCS = Diesel Contaminated Soil, TOC = Total Organic Carbon, TPH = Total Petroleum Hydrocarbon content, VM = Volatile Matter, OM = Organic Matter, ND = Not Detected. Alphabet (a and b) denotes significant difference when parameters for both soil samples were compared.

Effect of diesel contamination on soil pH during bioremediation

Soil pH value in DCS was observed to be up to 7.12 and significantly higher than the CSS 6.31 ($p < 0.05$; Figure 1). However, slight decrease in soil pH was witnessed in the CSS with increasing DAC. This suggests natural attenuation consistent with the work of Njoku *et al.* (2009). Furthermore, *E. crassipes* significantly decreased soil pH in DCS with increasing DAC ($p < 0.05$). This is likely due to *E. crassipes* production of low molecular weight organic acids that lower soil pH and improve soil fertility through enhanced nitrification and degradation of pollutants (Tang and Angela, 2019). Our result corroborates the work of Ayotamuno *et al.* (2006) who showed that increased organic matter has lowered the soil pH in a model crude oil contaminated soil. DCS treated with 100 g of fresh *E. crassipes* showed the lowest pH which was nearly equal to that of the CSS. This suggests that fresh *E. crassipes* is likely a strong biostimulant that can be used to decrease the pH of contaminated soils, and improve soil properties.

**Figure 1.** Change in soil pH levels in diesel contaminated soil during bioremediation analysis

Values are means with standard errors. CSS = Control Soil, DCS = Diesel Contaminated Soil, ASF = Amended Soil with Fresh *E. crassipes*, ASP = Amended Soil with Powdered *E. crassipes*, DAC= Days After Contamination.

Effect of diesel contamination on total organic carbon (TOC) during bioremediation

TOC concentration was significantly higher in the DCS than the CSS ($p < 0.05$; Figure 2). After amendment with *E. crassipes*, TOC significantly decreased with increasing DAC. Fresh 100 g of *E. crassipes* at 90 DAC had the least TOC (24.12), which was significantly higher than the CSS at 90 DASC. Comparison of both fresh and powdered *E. crassipes* on TOC showed a significant decrease in concentration for the soils amended with fresh *E. crassipes* above that of the powdered form across all DAC. TOC has been observed to be higher in soils amended with fresh organic matter compared to the processed form (Waleed *et al.*, 2020). However, soil contaminated by derived hydrocarbon substances could lead to high TOC of soils. This result is consistent with the work of Devatha *et al.* (2019) who reported that diesel contamination significantly increased TOC levels due to increase in TPH. Also, Musa *et al.* (2018) suggested that reduction in soil TOC may signify biodegradation of soil hydrocarbons.

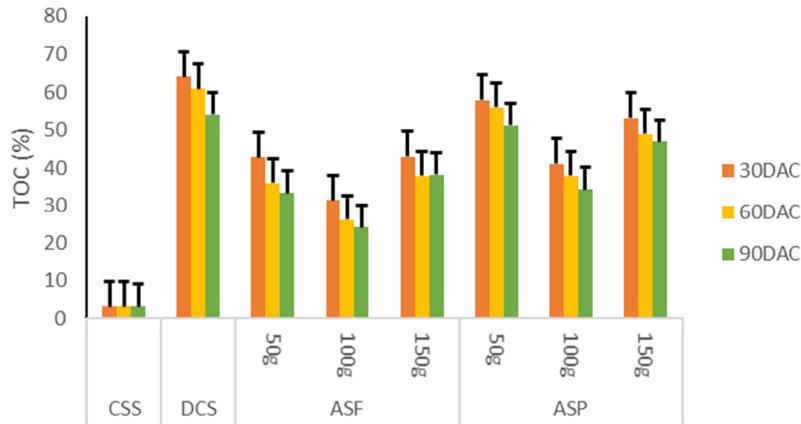


Figure 2. Change in soil TOC levels in diesel contaminated soil during bioremediation analysis. Values are means with standard errors. CSS = Control Soil, DCS = Diesel Contaminated Soil, TOC = Total Organic Carbon, ASF = Amended Soil with Fresh *E. crassipes*, ASP = Amended Soil with Powdered *E. crassipes*, DAC= Days After Contamination.

Percentage TOC loss was significantly different ($p < 0.05$) across all analyzed soils for different amendment concentration (Figure 3). Contaminated soil amended with 100 g of fresh *E. crassipes* at 30 DAC showed the highest TOC loss (59.7%). After this was, the contaminated soil amended with 100 g of powdered *E. crassipes* (47.36%), while the CSS at 60 DAC showed the least TOC (0.91%). This suggests that fresh *E. crassipes* exerts a greater biostimulation effect in TOC in diesel contaminated soils than both the powdered *E. crassipes* and the process of natural attenuation.

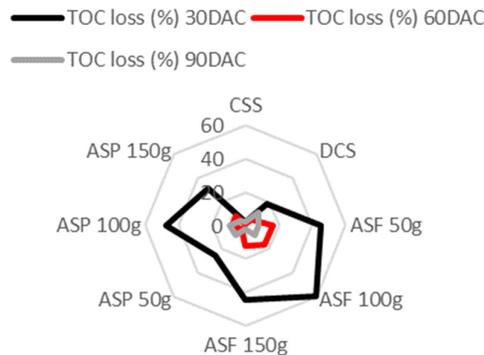


Figure 3. Percentage loss in total organic carbon (% TOC) of *E. crassipes* amended diesel contaminated soil. CSS = Control Soil, DCS = Diesel oil Contaminated Soil, ASF = Amended Soil with Fresh *E. crassipes*, ASP = Amended Soil with Powdered *E. crassipes*, DAC= Days After Contamination.

Effect of diesel contamination on total petroleum hydrocarbon (TPH) during bioremediation

TPH concentration in the DCS for the three assayed DACs was significantly higher than the CSS ($p < 0.05$), However, TPH was not detected in the CSS at 10, 45 and 90 DAC (Figure 4). TPH concentration slightly reduced with increasing DAC. This phenomenon may be due to natural attenuation. Upon soil amendment with *E. crassipes*, TPH significantly decreased with increasing DAC across all treatments. TPH was lowest in soil treated with 100 g of fresh *E. crassipes* at 90 DAC (9.09), whereas it was highest in DSC at 1 DAC (49.02). This result indicates that the use of *E. crassipes* in the treatment of diesel contaminated soils can aid in TPH reduction. Furthermore, TPH significantly decreased in soils treated with fresh *E. crassipes* than

in soils treated with powdered *E. crassipes* across all DACs. This is likely due to the active living matter in the fresh *E. crassipes* which is a direct contrast to the powdered *E. crassipes*. This result is consistent with the work of Udeh *et al.* (2013) who reported that fresh *E. crassipes* is an effective biostimulant in the treatment of hydrocarbon polluted soils.

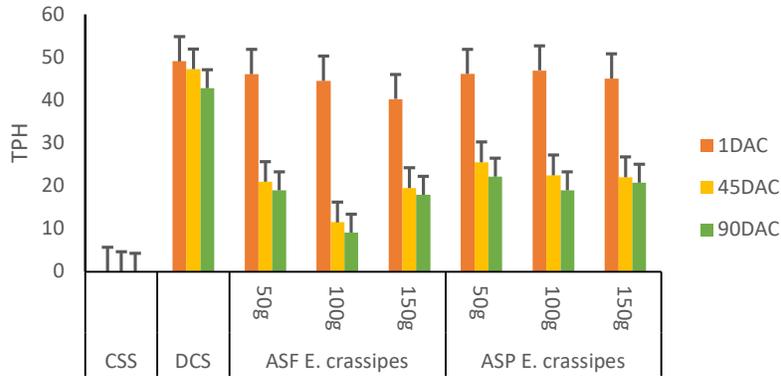


Figure 4. Change in soil TPH levels in diesel contaminated soil during bioremediation analysis. Values are means with standard errors. CSS = Control Soil, DCS = Diesel oil Contaminated Soil, TPH = Total Petroleum Hydrocarbon, ASF = Amended Soil with Fresh *E. crassipes*, ASP = Amended Soil with Powdered *E. crassipes*, DAC= Days After Contamination.

TPH significantly decreased at 1, 45 and 90 DAC ($p < 0.05$; Figure 5). Contaminated soils amended with 100 g of fresh *E. crassipes* had the highest TPH loss (72.24%). Following this were contaminated soils amended with 50 g of fresh *E. crassipes* (54.45%). Compared with other treatments, diesel contaminated soil with no treatment showed the least TPH loss (3.71%). Although soil TPH can be reduced by natural attenuation, it takes a longer time comparison with the use of organic matter as stimulants. This result corroborates the findings of Akpe *et al.* (2015), who demonstrated high TPH loss in hydrocarbon polluted soils within 56 days after biostimulation with plantain peels and guinea corn shaft. TPH was not detected in the CSS. Also, within the amendment types, percentage TPH loss with the fresh *E. crassipes* was significantly higher than in the powdered *E. crassipes*. This is likely due to high utilization of organic carbon in fresh *E. crassipes*. This is also similar to the study of Swamps (2013), who used powdered *E. crassipes* to assess bioremediation in petroleum polluted mangrove swamps in the Niger Delta and observed high TPH loss of up to 75.36 % after 70 days. They suggested that the high TPH loss recorded was due to the presence of nutrients from organic wastes.

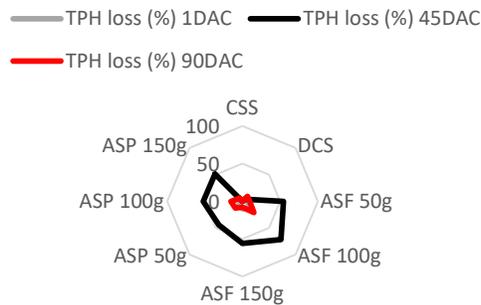


Figure 5. Percentage total petroleum hydrocarbon (% TPH) loss in the *E. crassipes* amended diesel contaminated soil. CSS = Control Soil, DCS = Diesel Contaminated Soil, ASF = Amended Soil with Fresh *E. crassipes*, ASP = Amended Soil with Powdered *E. crassipes*, DAC= Days After Contamination.

Conclusions

This research has demonstrated that fresh *E. crassipes* is likely a more potent biostimulant in the remediation of soils contaminated with petroleum diesel than its powdered variant. This was buttressed by the significant decrease in soil pH, soil total organic carbon (TOC) and total petroleum hydrocarbon (TPH), in concert with high TOC and TPH loss. Because of the timeframe of this study and limitations with the microbial assessment of fresh organic matter, further work is required to isolate the microbes that aid fresh *E. crassipes* in biodegradation for improved remediation strategies.

Authors' Contributions

OFM and CCN designed and conducted the study, including the initial writing and editing of the manuscript. OFM wrote the first draft of the manuscript. MSI conducted the statistical analysis and wrote the final draft of the manuscript.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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