Degradation Kinetics of Jet Fuel A and Bunker C Oil in sandy soils: the effect of plant materials' amendments and nutrients

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Abstract— In this work, the kinetics of the degradation of two petroleum hydrocarbon fraction (Jet fuel A (JFA) and bunker C oil (BCO)) in sandy soil was studied during the winter and spring seasons of 2010. The effect of adding some plant materials with oxidative enzymes content (*A Sativum*, *A Cepa*, *Brassica Rapa* and *Raphanus Sativus*) as bulking agent as well as the addition of N and P nutrients upon the degradation process was also investigated. The results indicated that the kinetics for JFA degradation followed first order equation ($\ln C = \ln C_0 - kt$) for natural attenuation and in the presence of *A Cepa* and *Raphanus Sativus* minced roots. Meanwhile, in the case of the addition of *A Sativum* and *Brassica Rapa* roots and in the application of commercial nitrate and phosphates fertilizers to the contaminated soil mixtures, the zero order rate equation ($C = C_0 - kt$) was obtained. The degradation rate for the heavier fuel oil fraction applying minced *A Cepa* root materials to the soil mixtures was better described by the zero order rate equation ($C = C_0 - kt$). However, the biodegradation kinetics rates for *A Sativum*, *Brassica Rapa* and *Raphanus Sativus* followed the zero order rate equation $C = C_0 - kt^{(0.2)}$ based upon higher R^2 values obtained. Overall, the applied four root materials achieved ≈ 90% reduction in JFA content while a maximum of 32% reduction in BCO content was achieved using *Raphanus Sativus* root with and without nutrients.

Index Terms— Jet Fuel A, Bunker C Oil, degradation kinetics, oxidative enzymes, plant roots, nutrients, sandy soil.

1 INTRODUCTION

Petroleum extraction, refining, transportation, storage, supply and consumption are sources for soil and water contamination with TPH (total petroleum hydrocarbons) and PAH (poly-aromatic hydrocarbons) [1], [2], [3], [4], [5], [6], [7]. This constitutes a hazardous and ever growing environmental problem because crude oil and petroleum products are complex and heterogeneous mixtures of organic compounds - predominantly aliphatic and aromatic hydrocarbons- all of which have different rates of natural attenuation [8]. In addition to that, once spilt on soil, these hydrocarbons may leach to groundwater and their residuals remain bound to soil for years causing significant deterioration in its physical and microbial properties [9]. Moreover, with a global crude oil production of above twelve million metric tons, it was estimated that between 1.7 to 8.8 million metric tons of petroleum hydrocarbon did escape into the soil and world's water bodies annually [9]. Effectively, 90% of this wasted content is caused by deliberate waste disposal practices and other human activities [10].

There are a number of physical, chemical and biological processes that can be applied to treat contamination caused by petroleum and petroleum products [9], [11]. Generally, both physical and chemical methods are considered to be expensive, compared to biological treatments. In this effect, biological methods are seen as more efficient and adequate in the cleanup of soil contaminated with petroleum hydrocarbons as they result in no adverse effects on site. This is may be explained by the fact that the microorganisms utilized in these processes may be directly involved in biogeochemical cycles and thus, have the inherent capacity to either degrade or produce hydrocarbons depending on the presence of certain metabolic pathways, specific to each function in the environmental conditions [8]. However, one disadvantage of these methods is that they are time consuming [12], [13]. As well, they may not be as effective when subjected to severe conditions of extreme pH and temperature, toxins, and high concentrations of pollutants or their products which may damage or metabolically inactivate the microbial cells [12]. Thus, in order to avoid such shortcoming, simple amendments such as nutrients and water, bulking agents, co-substrates to stimulate microbial metabolism, lime to adjust pH, or bacterial inoculations may be introduced during the waste treatment operations [13], [14], [15], [16], [17], [18].

One of those amendments that played an essential role in hydrocarbon treatment procedures has been oxidative enzymes emanating from bacteria, fungi and plants [2], [14], [18], [19], [20], [21], [22], [23]. These enzymes were reported to enhance the bioremediation of recalcitrant wastes and pollutants in soil and water and alleviate the adverse effects incurred [2], [12], [14], [18], [19], [20], [21], [22], [23]. As well, these microbial derived enzymes did exhibit specificity in their reactions with pollutants and did effectively accelerate the chemical reaction rate by lowering its energy of activation. Notably, oxygenase, dehydrogenase and lignolytic enzymes have been identified as main enzymes involved in the degradation of PAHs [23]. As well, the aerobic degradation of both aliphatic and aromatic hydrocarbons utilizing oxygenase enzymes (monooxygenases and dioxygenases) was reported to be fast due to the availability of O_2 as an electron acceptor [8], [12],

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[24]. Lignolytic enzymes, on the other hand, are a group of extracellular enzymes that include lignin peroxidase, laccase, and manganese peroxidase which have the capacity to catalyze radical formation by oxidation to destabilize bonds in organic molecule. However, in order to attain higher degradation efficiencies, it has been recommended that these enzymes be bound to solid supports prior to any application so as to prolong its activity; increase its recoverability and thermo stability [12].

Nonetheless, Durán and Esposito [19], Bollag and Dec [15] and Coniglio et al. [24] reported that that some plant material containing enzymes may be as effective as crude enzymes in some waste treatment procedures. This is mainly because such plant materials did exhibit significant enzyme activity even after crude peroxidase extraction. With no literature present concerning the application of plant materials with peroxidase enzyme content directly in the treatment of petroleum products or residues, the aim of the present work is to evaluate such the potential of some local plant roots in the decontamination of two petroleum hydrocarbon fraction-polluted soils on a laboratory scale. The two petroleum hydrocarbons (PH) fractions selected were Jet fuel A (JFA) and Bunker C fuel oil (BCO), both of which are widely used for energy production, transportation and in industrial power and production operations. The roots selected for this study represent common ingredients within the Egyptian diet and they are onions *(A Cepa)*, garlic (*A Sativum*), turnip (*Brassica Rapa)* and radish (*Raphanus Sativus*). During the lab scale experiments, the rate of hydrocarbon mineralization in the presence of specific weights of plant material added to soil mixtures was studied relative to control soil with PH samples. The impact of using inorganic nutrients in the form of commercial (N and P) fertilizers upon the rate of hydrocarbons mineralization in soil mixtures in the presence of plant materials was also investigated and the results of these treatment trials were compared thereafter. The kinetics of the biostimulated procedures was elucidated and the reaction rate equations and half time were calculated and set forth for each case.

2 Materials and Methods

2.1 Characteristics of the Petroleum Hydrocarbon fractions used:

Jet Fuel A (JFA) and Bunker C Oil (BCO) used in the soil contamination experiments were obtained as products marketed by Misr Petroleum Company, Cairo, Egypt. The specification of both of these petroleum fractions is provided in Table 1 [26], [27]. A detailed survey of the chemical composition of both of these petroleum fractions/ products has been elaborated upon elsewhere [28].

2.2 Hydrocarbon Degradation studies:

According to Law 4/1994 [29], the maximum detection limits for petroleum hydrocarbon in soil per location are 50 ppm (in lab) and 100 ppm (in field). For the purpose of this experimental design, the criteria adopted to define soil contamination with petroleum hydrocarbon content was: 15 g/kg dry soil for 'slightly', 30 g/kg dry soil for 'moderately' and 60 g/kg dry soil for 'highly' polluted soil. For simulation of contaminated soil, sand soil (No. 45/Builder's Sand) was obtained from the construction site at Helwan University for use in batch experiments. Before application, the soil was checked for irregular particles, sieved, dried and weighed portions of sand were put in clean beakers before each experimental run (200g in 300-400 mL glass beakers). Prior to the addition of each petroleum fraction weight to soil, the soil was moistened to 20 % of its water holding capacity with distilled water to ensure proper mixing with the contaminant. The beakers were then labeled according to the weight of JFA and/ or BCO added, amendments applied and date of experimental trial commencement (three replicates for each fraction weight (i.e. 3 x 3 x3) per trial for a total of 27 beakers+ 6 control).

TABLE 1
PRODUCT SPECIFICATIONS FOR KEROSENE MISR AND MAZOTTE MISR (180/240) [26], [27].

Jet Fuel A / Kerosene Misr		Bunker C Oil / Mazotte Misr	
Parameter	*Limit*	*Parameter*	*Limit*
Change in flame length after ignition for 24 hrs (max)	5 mm	Calorific value (min)	10000 cal/g
Change in flame width after ignition for 24 hrs (max)	6 mm	Calorific value (min)	42 M kJ/kg
Corrosion of Cu at 100°C for 3 hrs	1 w%	Flash point (min)	65°C
Distillate at 200°C	20 w%	Fluidity	unknown
End of boiling point (maximum)	300°C	Relative density at 15°C (max)	0.995 g/cc
Flash point (maximum)	38°C	Total sulfur	Unknown
Fuming point (minimum)	22 ml	Viscosity at 50°C (max) summer	240 Stock
Relative density at 15°C (minimum)	0.82 g/cc	Viscosity at 50°C (max) winter	180 Stock
Residue and loss (max)	2 w %	Viscosity by viscometer (max) summer	2000 Stock
Total Sulfur (max)	0.25 w%	Viscosity by viscometer (max) winter	1500 Stock
		Water & sediment content by centrifuge (max)	1 w %

Prior to the commencing of treatment procedures and during the experimental runs, the initial and periodic petroleum hydrocarbon content in the individual soil mixtures (g/kg.dry soil) was determined gravimetrically in triplicates after the soil mixture was homogenized well according to ASTM [30], USEPA [31] and Chiu et al. [32]. Overall, 240 ± 5 samples were analyzed during the above procedures. The results obtained were used to deduce the kinetics of degradation of each petroleum fraction in the experimental mixtures in the absence and presence of amendments such as control sample, plant root weights, and plant roots weights plus commercial fertilizers/ nutrients.

2.3 Preparation of the selected plant roots and their addition to PH mixtures :

After their purchase from the local market, the selected plant roots were washed to remove dirt and were towel dried before dicing and fine mincing prior to storage in 1 l covered glass jars in the fridge ready for use. To each mixture of jfa and bco contaminated soil mixtures (15, 30 and 60 g/kg dry soil), the equivalent weights of (10, 20 and 30g/ kg dry soil) of the finely ground onions *(A Cepa)*, garlic (*A Sativum*), turnip (*Brassica Rapa)* and radish (*Raphanus Sativus*) roots were added accordingly (triplicates of 1 root weight /3 beakers/ each petroleum hydrocarbon soil mix-

ture).

2.4 The Effect of plant roots and N and P fertilizers addition:

The impact of commercial fertilizers, namely, nh4no3 (abu qir fertilizers company, alexandria, egypt) and super phosphate (p2o5) (financial and industrial company, kafr el zayat, gharbiyah, egypt) upon the degradation rate of each pah/ root mixture was also investigated. Prior to their application to the tested mixtures, 1 g of each fertilizer was first dissolved in 1 ml and the individual soil mixtures were mixed thoroughly after addition. Essentially, the dissolution of inorganic fertilizer in water before addition to soil mixture was deemed necessary for better nutrient diffusion and microbial movement and activity [4], [23]. The initial and periodic contaminant content was determined as previously stated in order to evaluate the process degradation rate.

3. Results and Discussion:

3.1 Degradation studies of Jet Fuel A:

Jet Fuel A potential for natural degradation in soil vs. thermal volatility

In general, evaporation was considered the major route for the reduction of crude oils and petroleum hydrocarbons after their accidental release into the environment (water or soil) [4], [16], [34], [35], [36], [37], [38], [39]. On the other hand, petroleum hydrocarbons were reported to have the ability to remain adsorbed onto soil particles and, subsequently, may dissipate or migrate down through soils [1], [4], [7], [16], [36], [37]. Generally, it was reported that the biodegradability of petroleum hydrocarbons in soils was dependent upon the ambient temperatures, the increase of which may also enhance its volatility [17], [18], [40], [41]. Concerning the natural attenuation of JFA fraction in sandy soil, the first order kinetics model given by equation (1) was used to describe the degradation process, based upon the best fit line and R^2 coefficient values over 0.9:

$$C = C_o e^{-kt} \quad \text{.....(1)}$$

this may be rewritten as

$$\ln C = \ln C_o - kt \quad \text{.....(2)}$$

and half life (t ½) was calculated from the above equation (2) as:

$$t_{1/2} = \ln 2/k \quad \text{.....(3)}$$

The results obtained indicated that the rate of kerosene reduction in sandy soil averaged 0.0118 day^{-1} for the first 10 days, which was then followed by a slower rate of 0.0094 day^{-1} for the extended trial period (t ½ = 73.73 days) (approx 200 days). These combined rates provided for an overall 83.2% weight loss of the initial kerosene concentration in the soil mixtures. Similar conclusions regarding the kerosene reduction rate and behavior pattern in soil were also reported by Galin et al. [42], Nocentini et al. [43] and Marin et al. [18]. Kerosene was reported to have an overall natural reduction rate between 0.0184 to 0.0196 day^{-1} in sandy soil [43], [44]. As well, it was reported that, in some soils, 72% reduction of the kerosene (JFA) initial content was achieved within a 5-months period [36]. On the other hand, JFA was reported to have significant lower rate of evaporation from soils (48-51 x 10^{-3} /day) which was incurred during some venting and bioventing experiments [40]. As for the temperature induced volatility of JFA, the data obtained herewith indicated that it averaged 8.77 x 10^{-3} /day at 25°C (200 days). At elevated temperatures (98-100°C), the rate increased to 4.76 /hour to provide for an almost 99% reduction of the initial kerosene content within 2-3 day period.

Essentially, kerosene (JFA) is a blend of relatively non-volatile petroleum fractions (nC_6 –nC_{16}) with 60% of paraffins, 32% of naphthenes, and 7.7% of aromatics, the majority of which are in the C_{10}–C_{14} range [54], [46]. Therefore, upon its release into soil, kerosene was reported to have a moderate evaporation rate [5], [45]. In this respect, the volatility associated with this petroleum hydrocarbon (PH) fraction was mainly attributed to its lower carbon-containing fraction (C_9–C_{11} components) [36], [43], [47], [48], [49]. This was further asserted by Dror et al. [35] during a study concerning the fate of kerosene in sandy soil which demonstrated that the volatilized PH fraction of kerosene was enriched mainly with aliphatic compounds. Subsequently, this selective volatilization did increase the concentration of higher carbon number fraction (C_{13}-C_{15} components) and, thus, resulted in a slower rate during further volatilization process [40], [42], [43], [47]. Another factor that may have contributed to such a delayed rate of removal has been that the higher molecular portion of kerosene has been its ability to readily partition with soil and, thus, remain adsorbed onto sediments [1], [40], [45], [49]. Accordingly, land spreading and mixing of petroleum hydrocarbons with soil was effective in reducing its (C_{10}-$_{16}$) and ($C_{16\text{-}23}$) fractions [2], [46], [50].

Kinetics of degradation of JFA in sandy soil in the presence of enzyme-containing plant materials:

Statistical mean data for the degradation of JFA containing soil mixtures (15g, 30g and 60 g/kg dry soil) including rate constants, treatment time, half life and % removal in the presence of (10g, 20g and 30g) of the selected root plant material are provided in Table 2. A general observation made through data manipulation was that kerosene reduction rates in these soil mixtures mainly depended upon its initial content rather than the weight of plant material added. As well, it was observed that the rate equation obtained for *A Cepa* and *Raphanus Sativus* aided degradation of JFA followed the first order kinetics which is in agreement with previous studies [43]. However, for *A Sativum* and *Brassica Rapa* amended soils; the rate followed the zero order kinetics (equation (4)) based on higher values of R^2 (0.9)

TABLE 2
MEAN AND STATISTICAL RATE CONSTANTS FOR JET FUEL A CONTENT REDUCTION IN SANDY SOIL, TREATMENT TIME (DAYS) AND % REMOVAL IN THE PRESENCE OF SOME PEROXIDASE CONTAINING PLANT ROOTS (*A SATIVUM*, *A CEPA*, *BRASSICA RAPA* AND *RAPHANUS SATIVUS)* USING WEIGHTS FROM (10 – 30G) AND IN THE PRESENCE OF NITRATE AND PHOSPHATE FERTILIZERS – TRIAL PERIOD 150 DAYS

Jet Fuel-A soil mixtures, rate equations and treatment periods	initial PH conc (g/kg dry soil)	Plant materials used, average reaction rates, $t_{½}$ (days) and overall % contaminant reduction			
		Allium		Brassicaceae	
JFA+ plant materials weights (10- 30 g)		**A Cepa**	**A Sativum**	**Brassica Rapa**	**Raphanus Sativus**
Rate: C= C_o – kt for Brassica Rapa and A Sativum	15	2.75 x 10^{-2} /day	2.07 x 10^{-1} g/kg/day	1.12 x10^{-1} (g/kg.day)	1.97 x 10^{-2} /day
	t ½	25.59 day	37.53 day	68.05 day	43.16 day
Rate: ln C = ln C_o – kt for A Cepa and **Raphanus Sativus**.	*% removal*	*95.58*	*95.19*	*97.80*	*87.38*
	30	1.52 x10^{-2} /day	3.65x10^{-1} g/kg/day	1.76x10^{-1} (g/kg.day)	3.02 x10^{-2} /day
total trial period for A Sativum = 90 days	t ½	47.04 day	41.20 day	91.06 day	22.93 day
	% removal	*95.50*	*95.05*	*97.77*	*95.73*
for all the remainder of plant roots: 120 days	60	1.31 x 10^{-2} /day	7.87 x 10^{-1} g/kg/day	4.63x10^{-1} (g/kg.day)	2.02 x 10^{-2} /day
	t ½	55.93 day	38.12 day	69.97 day	34.26 day
	% removal	*94.90*	*94.84*	*95.73*	*95.21*
JFA+ plant materials weights (10- 30 g) + 1 g Nitrate fertilizer	15	3.09x 10^{-1} (g/kg.day)	3.17x10^{-1} (g/kg.day)	2.623x10^{-1} (g/kg.day)	3.43 x10^{-1} (g/kg.day)
	t ½	24.30 day	23.65 day	28.59 day	21.89 day
	% removal	*89.30*	*89.89*	*77.82*	*92.0*
Rate: C = C_o- kt for all mixtures	30	6.02 x 10^{-1} (g/kg.day)	6.13x10^{-1} (g/kg.day)	6.478x10^{-1} (g/kg.day)	6.77 x10^{-1} (g/kg.day)
	t ½	24.90 day	24.47 day	23.18 day	22.30 day
Trial period duration for al soil mixtures = 50 days	*% removal*	*89.2*	*89.19*	*91.13*	*93.43*
	60	1.28 (g/kg.day)	1.277 (g/kg.day)	1.2676 (g/kg.day)	1.31 (g/kg.day)
	t ½	23.48 day	23.65 day	23.63 day	22.86 day
	% removal	*87.37*	*88.53*	*88.91*	*94.53*
JFA+ plant materials weights (10- 30 g)+ 1 g Phosphate fertilizer	15	1.46 x 10^{-1} (g/kg.day)	1.31x10^{-1} (g/kg.day)	1.85 x10^{-1} (g/kg.day)	1.89 x10^{-1} (g/kg.day)
	t ½	51.32 day	54.82 day	39.22 day	39.76 day
	% removal	*94.36*	*93.04*	*95.11*	*94.72*
Rate: C = C_o- kt for all mixtures	30	2.76 x 10^{-1} (g/kg.day)	2.34x10^{-1} (g/kg.day)	3.55 x 10^{-1} (g/kg.day)	3.46 x 10^{-1} (g/kg.day)
	t ½	54.45 day	62.0 day	36.16 day	43.34 day
- A Sat mixture :120 days (15 weeks)	*% removal*	*93.31*	*88.34*	*94.50*	*93.68*
	60	5.93 x 10^{-1} (g/kg.day)	4.69x10^{-1} (g/kg.day)	6.99 x10^{-1} (g/kg.day)	7.00 x10^{-1} (g/kg.day)
- A Cepa, R. Sativus and B Rapa mixtures: 90 days	t ½	50.65 day	62.47 day	42.26 day	42.86 day
	% removal	*93.0*	*88.41*	*94.28*	*89.8*

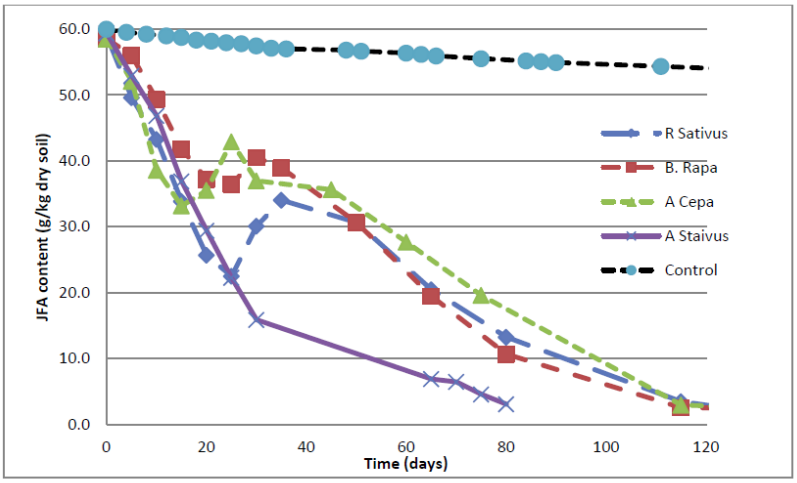

Fig. 1. Variation in the reduction in JFA content (60 g/kg dry soil) with time in soil mixtures amended with (10-30 g) weight of plant materials of *A Cepa*, *A Sativum*, *Brassica Rapa* and *Raphanus Sativus* relative to control sample (treatment period 120 days).

$$C = C_o - kt \quad \text{.....(4)}$$

From this equation, the half life time (t ½) was calculated as:

$$t(1/2) = C_o / 2k \text{.....(5)}$$

Overall, the treatment period of the plant amended soils was shorter than the control samples 200 days period as well as JFA reduction in the presence of garlic (*A Sativum*) added to soil mixtures reached a maximum reduction of 95% within a 90 day period. It was reported that the use of plant material with peroxidase activity [15], [51], or its hairy roots [25] yielded high PH contaminant removal efficiencies (90-95%). This was mainly attributed to the fact that decreasing the size of root materials through mincing may have maximized the enzymes' contact area with contaminants; an observation confirmed comparing the control and the treatment data in this study.

Moreover, the current results indicated that there was an initial period of a rapid decrease in the initial hydrocarbon concentration during the first 30-40 days, which was then followed by a smaller and continuous decrease for all plant materials applied (Figure 1). Similar patterns of hydrocarbon depletion were previously obtained during some degradation studies of middle-distillate petroleum products [18], [43], [50]. This process was said to be a 2 distinct stages process with the first stage spanning from 1-3 months and the second from 3-11 months. The initial stage was considered more rapid and accounted for the mineralization of 55% of the total hydrocarbon content (mostly the biodegradable fraction). The second stage, on the other hand, provided slower rates of hydrocarbon degradation and, ultimately, did account for another 45% reduction in pollutant content. Effectively, this latter process rate was equal to that of the control sample. However, the deceleration in rate during this final stage was attributed to the fact that the residual hydrocarbon fraction was more complex than the parent fraction and it had a low biodegradability which caused the soil microbial activity to significantly drop. This process was called 'weathering' or 'aging' of kerosene fractions, which is largely slower even under optimal conditions [40], [52].

Another observation made with these kerosene soil mixtures was during the application of *A Cepa*, *Brassica Rapa* and *Raphanus Sativus* plant materials (Figure 1). First, applying these three roots did cause an initial rapid reduction in hydrocarbon content in soil under 40 days which was followed by an increase in the extractable hydrocarbon content from the soil mixture. Such an occurrence was more pronounced as the initial kerosene concentration was increased to 60 g /kg dry soil. This phenomenon may be attributed to the high levels of microbial activity induced through the addition of high hydrocarbon content to soils [53], [54], [55]. Accordingly, and under these stress conditions, the active bacteria may excrete extracellular surfactant–like polymers during their metabolic cycle for contaminant degradation [52], [55], [56], [57]. Subsequently, these surfactant polymers may be capable of increasing the mobility and bioavailability of organic pollutants by modifying the physical and chemical adsorption properties of the soil, with which the current findings is in agreement.

On the other hand, this increase in content was not observed during the application of *A Sativum* plant material to the kerosene-contaminated soils. This may be ascribed to the fact that *A Sativum* contained a primary active ingredient known as allicin, which is produced by the enzyme allinase [58], [59]. Allicin has the property of decreasing lipids in blood (hypolipidaemic) as well as has been proven to successfully penetrate the biofilm (lipoproteins) of most antibiotic resistant bacteria. Consequently, the presence of allicin may have aided the breakdown of the metabolites formed during the initial kerosene reduction in soils as well as it may have increased the dissolution of the formed hydrophobic components to facilitate its further degradation.

Kinetics of JFA plant aided mixture degradation: the effect of N and P nutrients:

Effectively, contaminated soils are considered to be nutrient poor and do lack microbial diversity, which impedes the rate of remediation within [21]. As well, sandy soil was reported to exhibit poor characteristics (low nutrient, low organic matter and low microbial biomass), which decreases its ability to remediate hydrocarbons alone [60]. On the other hand, the presence of a certain amount of TPH in soils may serve as a fertilizer and may stimulate plant and bacterial growth [54]. However, during a treatment procedure induction stage, soils may suffer from an elevated carbon and lower nutrient content [6], [33].

The data for nutrient (N and P) aided decontamination of kerosene (JFA) containing soils in the presence of the four-selected plant roots is provided in Table 2. Overall, the results indicated that ≈ 90% removal efficiency of the initial kerosene was maintained using ammonium nitrate. The reaction followed zero order rate equation with the treatment period decreasing below the period of the control samples. More specifically, the data revealed that for all treatments with nitrate fertilizer, the treatment period was reduced to less than 50 days, while that for phosphates, it remained unchanged relative to the root amended samples. However, in both the case of nitrates and phosphates addition, no increase in the extractable hydrocarbon content from the soil mixture was noticed. As well, it was observed that the reaction rates for PH mineralization obtained using nitrate fertilizer were almost twice that obtained when phosphates were applied. As well, the obtained degradation rates increased significantly as the initial JFA content increased from 15 to 60 g/ kg dry soil using both nutrients.

The current results are in agreement with the findings of Nikolopoulou et al. [61] who indicated that treatments with inorganic or organic nutrients were effective in achieving 97% degradation of C_{12}–C_{35} n-alkanes and 95% of polyaromatic hydrocarbons with two or three rings were within 45 days. The results clearly showed that the addition of nutrients specifically nitrates to contaminated sand significantly enhanced the activity of indigenous microorganisms to aid in the re-

moval of total recoverable petroleum hydrocarbons (TRPH) [54], [61].

As well, in a previous study, Gramss et al. [62] demonstrated that fertilization with mineral salts enhanced the yield in peroxidases even in the case of dying plants. While it has been demonstrated that the addition of inorganic nitrogen and phosphorous resulted in higher efficiency of hydrocarbon degradation, it was reported that maintaining an optimal ratio of soil nutrients C : N : P at 100-200:10:1-3 was required for the effective removal of hydrocarbons from soils [7], [33], [63], [64]. The increased hydrocarbon degradation rate at this ratio was attributed to the increased expression of microbial populations involved in the N, P and C cycles in contaminated soils [33], [60], [65]. As well, such a differential ratio was found to help in maintaining the nutrient ratios for C/N and C/P in soils closer to the bacterial C/N and C/P requirements [66]. This is important because sandy-loam type soils are considered deficient in nutrients, specifically N and C, which render it unfavorable for biodegradation [32], [60]. As well, soils contaminated with hydrocarbons were reported to be deficient in P as a result of its high C content [64].

From another perspective, nitrogen is considered a key building block of proteins and nucleic acids required in soil media for hydrocarbon degradation [5]. Positive effects of nitrogen amendment on petroleum hydrocarbon degradation and microbial activity have been previously demonstrated [9]. As well, the impact of this nutrient (N) was demonstrated to be quantitative in the degradation of kerosene rather than phosphorous [5], an observation noted during the current study. This may be due to the fact that soil microcosms use the inorganic part of the NO_3^- and NH_4^+ available as a nitrogen source will be incorporated into their biomass, which then helps them in the rapid uptake of hydrocarbons [65], [67]. In nutrient rich treatments, an increase in the number of total viable aerobic bacteria, hydrocarbon utilizing bacteria and actinomycetes was indicative of the nutrient nitrogen concentration added, especially in sandy soils [68]. In addition, it was demonstrated that ammonium nitrate was a better nitrogen amendment for use in treatment of Egyptian sandy soil contaminated with petroleum wastes rather than urea, the latter was found to have adverse effects on the degradation process [54]. However, Ramirez et al. [33] reported that the addition of excessive nitrogen to clayey soils negatively influenced the hydrocarbon degradation and total carbon consumption by increasing the levels of soluble salts. As well, they concluded that the addition of higher phosphates content had a more positive impact on hydrocarbon degradation in these soils.

On the other hand, previous studies have indicated that some antioxidant compounds present in the studied roots (namely: phenols, flavonoids, and terpenes) may serve not only as nutrients for microorganisms but also to induce bacterial degradation of PCBs and PAH [21], [69]. These compounds act as bacterial growth substrates to stimulate hydrocarbon degradation. The selected roots were reported to contain several antioxidant compounds, mainly polyphenol, such as flavonoids, and sulfur-containing compounds [67], [70], [71], [72]. In this respect, these phytonutrients may have contributed to the decrease in treatment time for nitrate containing mixtures relative to those in which phosphates were applied.

3.2 Degradation studies of Bunker C Oil:

Bunker C Oil potential for natural degradation in soil vs. thermal volatility

The rate of reduction of BCO content in sandy soil also followed equations (1 and 2). However, the obtained rates were significantly sluggish relative to JFA control samples, providing for an average rate of 7.54×10^{-5} day^{-1} ($t_{½}$ = 9192 days). This rate provided for less than 3% reduction in the initial BCO content during the same experimental period. Comparatively, the rate of volatility of mazotte (BCO) averaged 3.35×10^{-5} /day and $1.0 - 1.39 \times 10^{-2}$ /day at 25°C and 98-100°C, respectively. This achieved a respective reduction average of 0.14% and 3.6% of the initial BCO content. Such a trend in this PH weight loss in soil mixtures may be attributed to the fact that Bunker C fuel oil is a residual petroleum hydrocarbon fraction that contains compounds ranging from C_{20}-C_{25} up to higher than C_{40} [46], [57]. As well, this fuel oil fraction was reported to mainly consist of high concentration of saturates (n-alkanes) and aromatics (PAH) and, to a lesser extent, polars, resins and asphaltenes [57], [63], [73], [74]; as well as non-hydrocarbons (S, N, and O containing molecules) which are difficult to biodegrade [28]. Subsequently, Bunker C fuel oil was reported to have minimal to no volatility as well as it has the ability to transform into persistent solid residue upon release on land (tar balls) [57]. Furthermore, the low biodegradability of this fuel oil did not only depend upon its chemical composition but also upon the crude oil source [74]. In effect, it was reported that during some experimental studies of this heavy oil, the biodegradability rate reached 11 % of the initial content during a treatment period for over 80 days [24], [74]. The degradability of this heavy fuel oil was generally related to saturates and aromatics content within this fraction that had a carbon number < C_{44} [57], [73], [74]. On the other hand, the decrease in saturates content of this fuel oil was counterbalanced by an increase in its asphaltenes. Therefore, the extent of its degradation remained constant during the period of treatment, an observation which is in agreement with the current study observations. On the other hand, the observed volatility of this PH fraction in this study may be attributed to the fact that heavy fuel oils may be blended with lower molecular fraction distillates (such as diesel and kerosene) in order to decrease its viscosity and render it usable [46].

Kinetics of degradation of BCO in sandy soil in the presence of enzyme-containing plant materials:

Table 3 provides the statistical mean data for the degradation of BCO containing soil mixtures (15g, 30g and 60 g/kg dry soil) including rate constants, treatment time, half time and % removal in the presence of (10, 20 and 30g/ kg dry soil) of the selected root plant material as well as N and P nutrients.

TABLE 3

MEAN AND STATISTICAL RATE CONSTANTS FOR BUNKER C OIL CONTENT REDUCTION IN SANDY SOIL, TREATMENT TIME (DAYS) AND % REMOVAL IN THE PRESENCE OF SOME PEROXIDASE CONTAINING PLANT ROOTS (*A SATIVUM*, *A CEPA*, *BRASSICA RAPA* AND *RAPHANUS SATIVUS)* USING WEIGHTS FROM (10 – 30G) AND IN THE PRESENCE OF NITRATE AND PHOSPHATE FERTILIZERS – TRIAL PERIOD 150 DAYS

Bunker C Oil / soil mixtures rate equations and treatment periods	initial PH conc (g/kg dry soil)	Plant materials used, average reaction rates, t ½ and overall % contaminant reduction			
		Allium		Brassicaceae	
		A Cepa	**A Sativum**	**Brassica Rapa**	**Raphanus Sativus**
Bunker C oil + plant materials weights (10, 20 and 30 g) Trial period= 150 days for all plant materials	15	2.56 x10^{-2} (g/kg/day)	9.29 x 10^{-3} (g/kg/ $day^{0.2}$)	5.27x10^{-1} (g/kg /$day^{0.2}$)	4.33x10^{-1} (g/kg/$day^{0.2}$)
	t ½	338.35	416.5	843.61	973.72
Rate : C = Co –k ($t^{0.2}$) for **A Sativum, Brassica Rapa** and **Raphanus Sativus**	*% removal*	*14.64*	*9.11*	*7.0*	*9.25*
	30	3.20 x 10^{-2} (g/kg /day)	2.38 x 10^{-2} (g/kg / $day^{0.2}$)	5.54 x10^{-1} (g/kg /$day^{0.2}$)	1.84 (g/kg /$day^{0.2}$)
Rate : C = Co –k t for A Cepa	t ½	499.84	1251.9	1473.17	507.50
	% removal	*5.29*	*3.48*	*11.05*	*11.34*
	60	1.04 x 10^{-1} (g/kg /day)	6.17 x10^{-3} (g/kg / $day^{0.2}$)	2.34 (g/kg /$day^{0.2}$)	4.06 (g/kg /$day^{0.2}$)
	t ½	437.25	3095	817.28	445.09
	% removal	*8.05*	*1.56*	*12.27*	*31.48*
Bunker C Oil soil mixtures + plant material + 1 g Nitrate fertilizer Trial period= 150 days for all plant materials **Rate: C = Co –k ($t^{0.2}$)** for **A Sativum, Brassica Rapa** and **Raphanus Sativus** **Rate : C = Co –kt** for A Cepa	15	3.08 x 10^{-2} (g/kg /day)	2.83 x 10^{-2} (g/kg/ $day^{0.2}$)	9.37 x 10^{-1} (g/kg/$day^{0.2}$)	1.09 (g/kg /$day^{0.2}$)
	t ½	250.83	270.87	425.10	336.73
	% removal	*19.8*	*11.2*	*8.63*	*9.61*
	30	2.78 x 10^{-2} (g/kg /day)	2.49 x 10^{-2} (g/kg/ $day^{0.2}$)	2.12 (g/kg/$day^{0.2}$)	1.58 (g/kg /$day^{0.2}$)
	t ½	571.08	636.03	411.37	463.11
	% removal	*8.1*	*4.3*	*15.94*	*10.77*
	60	5.97 x 10^{-2} (g/kg /day)	1.51 x10^{-2} (g/kg / $day^{0.2}$)	5.76 (g/kg/$day^{0.2}$)	7.81 (g/kg /$day^{0.2}$)
	t ½	589.05	1998.52	295.94	186.50
	% removal	*10.9*	*1.7*	*23.85*	*31.82*
Bunker C oil soil mixtures + plant material + 1 g Phosphate fertilizer Trial period= 150 days for all plant materials **Rate : C = Co –k (t)** for A Cepa, **rate = C= C_0 -k t ($^{0.2}$) for A Sativum, Brassica Rapa** and **Raphanus Sativus**	15	2.67 x 10^{-2} (g/kg /day)	2.68 x 10^{-2} (g/kg / $day^{0.2}$)	8.71 x 10^{-1} (g/kg/$day^{0.2}$)	1.02 (g/kg /$day^{0.2}$)
	t ½	301.66	288.63	447.77	356.95
	% removal	*16.0*	*9.6*	*7.74*	*8.29*
	30	2.46 x 10^{-2} (g/kg /day)	2.38 x 10^{-2} (g/kg/ $day^{0.2}$)	1.82 x 10^{-1} (g/kg/$day^{0.2}$)	1.28 (g/kg /$day^{0.2}$)
	t ½	664.67	673.70	464.78	572.48
	% removal	*6.7*	*3.8*	*13.10*	*7.98*
	60	5.64x 10^{-2} (g/kg /day)	2.34x10^{-2} (g/kg / $day^{0.2}$)	4.05 (g/kg/$day^{0.2}$)	7.37 (g/kg/ $day^{0.2}$)
	t ½	627.11	1635.62	459.76	197.45
	% removal	*10.2*	*3.5*	*16.13*	*29.8*

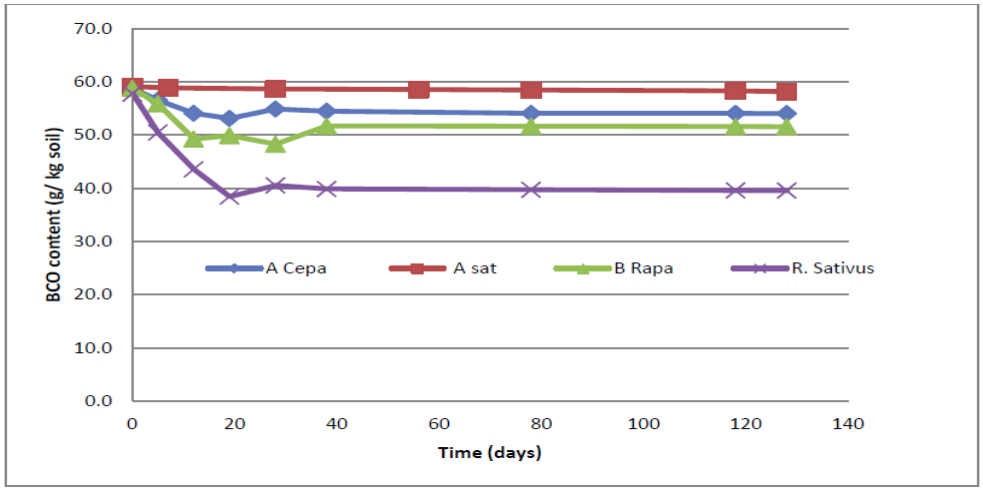

Fig. 2. Variation in the reduction in BCO content (60 g/kg dry soil) with time in soil mixtures amended with (10-30 g) weight of plant materials of *A Cepa*, *A Sativum*, *Brassica Rapa* and *Raphanus Sativus* (treatment period 150 days).

. Generally, it was observed that the rate equation for garlic and onions followed a zero order rate [equation (4)] while the process with turnip and radish, followed a zero order equation with $t^{(0.2)}$ [equation (6)]:

$$C = C_o - kt^{0.2} \quad \text{.....(6)}$$

Overall, the treatment period did not extend over the 150 days mark as no significant change in the remaining BCO content could be measured accordingly. However, a general trend observed during these treatment trials that using *A Sativum* and *A Cepa* was that the % reduction decreased as the BCO content increased from 15 to 60g/ kg dry soil, which is contrary to that observed using turnip and radish roots. Overall, a maximum reduction in BCO (32%) was obtained for mixtures containing 60 g/ kg BCO and the different weights of radish root (Figure 2). This in accordance with the data presented by Ali et al. [75] who reported that the maximum reduction in the heavy fuel oil fraction containing asphaltenes may range between 35-46%. As well, the increase in biodegradation of this resin and asphaltenes containing heavy fuel oil may be due to the microbial attack on the polysulfide linkages, which leads to biodepolymerization of the asphaltene fraction.

Nonetheless, the noted slow down in BCO content reduction using these 4 roots may be attributed to the depletion of degradable fraction of this fuel oil, a fact previously indicated by Jézéquel et al. [76]. This reduction was reported to contribute to the increase in the amount of resins as a result of the partially degraded hydrocarbons or adsorbed biomass, all of which were notably hydrophobic. However, heavy fuel oil was also reported to contain non-hydrocarbons (S, N, and O containing molecules) [28]. In this respect, the noted content reduction using *R Sativus* and *B Rapa* may be explained as follows. As members of the crusifera family, both turnip and radish roots were reported to contain glucosinolates- amino acid-derived secondary metabolites containing nitrogen and sulfur [77], [78], [79]. These compounds are hydrolyzed upon cellular disruption into various bioactive breakdown products by the endogenous enzyme myrosinase (thioglucohydrolase; E.C. 3.2.1.147) [77], [78], [79], a fact that may have contributed to the significant mazotte reduction using these roots. These compounds also have several functions within the plant, one of which has been to activate the sulfur and nitrogen metabolism if the plant was injured or in defense against stress [77].

Another possible explanation for such a slowdown in PH reduction in mazotte containing soil mixtures was also provided by Gramss et al. [62], Macek et al. [69] and Labud et al. [60]. This was mainly attributed to the fact that soil microbial population may have exhibited a stage of activity inhibition upon the immediate exposure to this petroleum hydrocarbon content. Subsequently, to compensate for this situation, the inherent bacteria depended on extracting more peroxidase enzymes from the root material to stimulate both microbial and biochemical activity and improve its performance [53], [62], [69]. Consequently, as the soil microorganisms recover, they begin to consume the available hydrocarbons as a source of carbon and, thus, their metabolic activity and metabolite production increase accordingly [60]. Nevertheless, this metabolite effect was not evident in the case where *A Sativum* was applied, an observation similar to the case of kerosene reduction treatments.

Furthermore, previous field and lab investigations of petroleum hydrocarbons fraction degradability indicated that the straight-chain alkanes were more readily degraded than the branched alkanes [2], [38], [43], [56], [57], [63], [73], [74]. The (C_{16} -C_{50}) fraction of this residual fuel oil was considered hydrophobic and more resistant to microbial degradation, a fact that may decrease its further reduction. On the other hand, it was reported that the biodegradation of the saturate fraction of this residual fuel oil contributed to an increase of its asphaltenes content due to the production of cross-linked, high molecular weight polymers during the biotransformation of lower molecular weight components [57], [73]. More specifically, the degradation of the residual oil lighter fraction was not attributable to the total mineralization of the depleted products but to the formation of partially oxidized compounds, a fact affirmed by Jézéquel et al. [76] and Fernández-Alvarez et al. [74]. They ascertained that the oxygen content of asphaltenes increased due to the accumulation of products coming from the partial oxidation of TPHs. Nonetheless, this weight loss in BCO content was reported to reduce its inherent toxicity [57]. As well, it was demonstrated that after the degradation of the most labile hydrocarbons, the enzymatic activities in soils may be diminished [18], which is in agreement with the current findings.

Kinetics of BCO plant aided mixture degradation: the effect of N and P nutrients:

As for the treatment trials with BCO and nutrients, the results in Table 4 indicated that there was relatively no change concerning the % removal efficiencies in the attested mixtures. Generally, a slight increase in the reduction of BCO content was obtained applying nitrates to soil mixtures compared to that obtained with phosphate application. However, the most significant reduction for this residual fuel oil was maintained in the case *R Sativus* and *B Rapa*. This may be attributed to the fact that *Brassica* were reported to contain high nitrate levels [80]. However, the maximum percentage of mazotte mineralization (Bunker C Oil) obtained in this study was significantly higher than that obtained using chitin amended mixtures (8%) [57] and that for the natural attenuation of spilt fuel oil in France (11%) [74].

In the case of BCO, Gallego et al. [63] demonstrated that the absence of N and P contents in soils was not the only limiting parameter that impeded its biodegradation. In this respect, the other limiting factor was the high content of heavy non-toxic oil fractions present within this oil, namely: resins and asphaltenes [75]. Bento et al. [66] demonstrated that nutrient addition to fuel oil contaminated beach soils promoted a significant degradation of the light fraction of diesel oil during 6 weeks of incubation. As well, the best bioaugmentation with

nutrients may increase the microorganisms' activity and its abundance in soils. On the other hand, with the decrease in the labile carbon sources, nutrients were found to be most likely limited in supporting microbial growth. In other words, the degradation of higher molecular weight hydrocarbons might have produced toxic intermediates that inhibited hydrocarbon-degrading microorganisms. As well, it was confirmed that while the initial addition of nitrogen and phosphorus stimulated the activity of indigenous microbial population up to six weeks, the slow rate of degradation that followed was related to a small decrease in dehydrogenase–related activity. This dehydrogenase was reported to be involved in the electron transport system to remove the oxidative substrates, an activity that was correlated with the oxygen uptake and organic substance removal in aerobic system [36].

On the other hand, some researchers reported that neither bioaugmentation formulations nor biostimulation products accelerated the degradation of residual fuel oil in sand relative to its natural attenuation [74]. As well, it was demonstrated that the depletion rate of residual oil in sand remained constant and that neither microorganism nor nutrients stimulated its further degradation, a fact that agrees with the results obtained in this study. On the other hand, Bento et al. [50] concluded that biostimulation of microorganisms with the addition of N and P resulted in the least degradation of diesel oil (kerosene like fraction) in sandy beach soils. However, in a more recent study, they reported that phosphorus had a significant impact upon the rate of hydrocarbon degradation of such petroleum product [66], an effect which was observed in the case of A Cepa and A Sativum amended mixtures. They also concluded that while some sources may supply enough P to restore the C/P ratio in soils, P might become unavailable thereafter due its low solubility. From another perspective, previous studies have indicated that some antioxidant compounds present in these roots (such as flavonoids) may have increased the hydrocarbon degrading capacity in the soil mixtures in the case of nutrient addition to mazotte containing soils, thus, producing slightly higher hydrocarbon degradation rates [21], [69]; an observation recorded for both B Rapa and R Sativus.

3.3 An overlook on the enzyme content of the 4 selected plant roots:

Generally, it was reported that oxidative enzymes played an important role in the mineralization of recalcitrant pollutants in soil and water either through land farming of contaminated soils or through phytoremediation /rhizoremediation [15], [19], [21], [25]. Three principal enzyme groups that were identified to degrade and mineralize a large variety of recalcitrant compounds are the versatile peroxidases, Mn-dependant peroxidases and phenol oxidase [81], 82]. While peroxidase is known to catalyze the conversion of some xenbiotics, phenol oxidase was reported to catalyze the oxidation of phenolics, aromatic amines, indole and sulfonates by hydrogen peroxide and related oxidants [72]. As well, glutathione-S-transferase aided the nucleophilic attack on the sulfur atom of glutathione-electrophilic group in a variety of hydrophobic xenbiotics substances. Finally, the increase in superoxide dismutase was regarded as the key enzymatic antioxidant that produced the H_2O_2 needed for the activity of all other enzymes [83].

Overall, all of the selected roots are rich in peroxidase activity [84], [85], [86], [87]. As well, they contain other enzymes that as equally important such dehydrogenase and manganese peroxidase [19], [20], [50], [88], [36], [18], [81], [72], [23], [82]; and glutathione S-transferase [[69], [72]. *Allium Sativum* and *Allium Cepa* were reported to contain significant amounts of superoxide dismutase, catalase, guaiacol peroxidase, glutathione reductase, glutathione S-transferase [87], [89]. Moreover, turnip roots were reported to contain significant amounts of dehydrogenase [90], [91], [92]; phloroglucinol oxidase – a Mn dependant oxidase [93]; polyphenol oxidase, ascorbate and glutathione [94]. Radishes also were reported to contain significant amounts of superoxide dismutase and catalase [94]. Moreover, a study of the impact of drought treatment upon plants containing oxidative enzymes revealed that the enhancement of its superoxide dismutase, glutathione reductase and guaiacol peroxidase activity [87]. The increase in superoxide dismutase was considered essential for the production H_2O_2 required to recover the peroxidase activity [96].

4 Conclusion:

In the current study, the effectivity of the use of four roots namely: onions *(A Cepa)*, garlic (*A Sativum*), turnip (*Brassica Rapa)* and radish (*Raphanus Sativus*) in the decontamination of JFA and BCO polluted sandy soil was investigated. The results showed that the four roots were effective in reducing the decontamination period of JFA polluted soil while achieving over 90% in a period of 120 days. Amending the JFA mixtures with nitrate and phosphates fertilizer further increased the reaction rates. However, nitrates were more effective in reducing the overall treatment period. On the other hand, the degradation rate of BCO in sandy soil was rather sluggish compared to that of JFA and notably did vary slightly with the addition of N and P nutrients. Overall, the results affirmed that while all roots afforded higher JFA % removal, the addiition of Raphanus Sativus achieved with and without nutrients afforded a maximum removal efficacy of 32% of the initial BCO content.

References

[1]. T Balba, "Bioremediation of Oil-Contaminated Soils: Case Studies involving light and Heavy Petroleum Hydrocarbons, http://www.remtech2008.com/remtech/2003/pdf/_Balba.pdf. 2003. (accessed 2012)

[2]. X-D. Huang, Y. El-Alawi, J. Gurska, B. R. Glick and B. M. Greenberg, "A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils", Microchemical Journal, 81, pp. 139– 147, 2005.

[3]. B. Mrayyan and M.N. Battikhi, "Biodegradation of total organic carbons (TOC) in Jordanian petroleum sludge", J of Haz Mat, B120, pp.127–134, 2005.

[4]. A. Serrano, M. Gallego, J. L. González and M. Tejada, "Natural Attenuation of diesel aliphatic hydrocarbons in contaminated agricultural soil", Environmental Pollution, 151: pp. 494- 502, 2008.

[5]. G. Shabir, M. Afzal, F. Anwar, R. Tahseen and Z. M. Khalid, "Biodegradation of kerosene in soil by mixed bacterial culture under different nutrient conditions", International Biodeterioration & Biodegradation, 61, pp. 161-166, 2008.

[6]. I. Kisic, S. Mesic, F. Basic, V. Brkic, M. Mesic, G. Durn, Z. Zgorelec and L. Bertovic, "The effect of drilling fluids and crude oil on some chemical characteristics of soil and crops", Geoderma 149, pp. 209–216, 2009.

[7]. D. Wolicka, A. Suszek, A. Borkowski and A. Bielecka, "Application of aerobic microorganisms in bioremediation *in situ* of soil contaminated with petroleum products", Bioresource Technology, 100, pp. 3221-3227, 2009.

[8]. R. S. Peixoto, A. B. Vermelho and A. S. Rosado, "Petroleum-Degrading Enzymes: Bioremediation and New Prospects", Enzyme Research, Volume 2011, Article ID 475193, 7 pages, doi:10.4061/2011/475193.

[9]. P. Agamuthu and A Dadrasnia, "Potential Of Biowastes To Remediate Diesel Fuel Contaminated Soil", Global NEST Journal, Vol 15, No 4, pp 474-484, 2013.

[10]. A. Dadrasnia and P. Agamuthu, "Dynamics of diesel fuel degradation in contaminated soil using organic wastes", Int. J. Environ. Sci. Technol. 10, pp. 769–778, 2013.

[11]. S. Sihag and H. Pathak, "Factors Affecting the Rate of Biodegradation of Polyaromatic Hydrocarbons", Int. J. Pure App. Biosci. 2 (3), pp. 185-202, 2014.

[12]. C-Y. Fan and S. Krishnamurthy, "Enzymes for Enhancing Bioremediation of Petroleum-Contaminated Soils: A Brief Review", Air & Waste Manage. Assoc. 45, pp. 453-460, 1995.

[13]. A. O. Vincent, E. Felix, M. O. Weltime, O. K. Ize-iyamu and E. E. Daniel, "Microbial Degradation and its Kinetics on Crude Oil Polluted Soil", Research Journal of Chemical Sciences Vol. 1(6), pp. 8-14, Sept. 2011.

[14]. M. Vidali, "Bioremediation. An Overview", Pure Appl. Chem., vol. 73, no. 7, pp. 1163-1172, 2001.

[15]. J-M. Bollag and J. Dec, "Use of plant materials to decontaminate water and soil", Proc. of the 17th World Congress of Soil Science, Bangkok, Thailand, 14-20 August 2002; http://natres.psu.ac.th/Link/SoilConress/bdd/ Sym38/ 986-t.pdf.

[16]. R. F. Hejazi, T. Husain and F. I. Khan, "Landfarming operation of oily sludge in arid region—human health risk assessment", J of Haz Mat, B99, pp. 287–302, 2003.

[17]. K. McCarthy, L. Walker, L Vigoren and J Bartel, "Remediation of spilled petroleum hydrocarbons by in situ landfarming at an arctic site", Cold Regions Science and Technology, 40, pp. 31– 39, 2004.

[18]. J. A. Marin, T Hernandez and C. Garcia, "Bioremediation of oil refinery sludge by landfarming in semiarid condition: Influence on soil microbial activity", Environmental Research, 98, pp. 185-195, 2005.

[19]. N. Durán and E. Esposito, "Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review", Applied Catalysis B: Environmental, 28, pp. 83–99, 2000.

[20]. L. Chroma, M. Mackova, P. Kucerova, C. In Der Wiesche, J. Burkhard and T. Macek, "Enzymes in Plant Metabolism of PCBs and PAHs", Acta Biotechnol. 22 (1-2), pp. 35-41, 2002.

[21]. K.E. Gerhardt, X-D. Huang, B.R. Glick and B.M. Greenberg, "Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges", Plant Science, 176, pp. 20-30, 2009.

[22]. M. Hamid and K-Ur- Rehman, "Potential application of peroxidases", Food Chemistry, 115, pp. 1177-1186, 2009.

[23]. A.K. Haritash and C.P. Kaushik, "Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review", J of Haz Mat, 169, pp. 1–15, 2009.

[24]. P.K. Jain, V. K . Gupta, R. K. Gaur, M. Lowry, D. P. Jaroli and U. K. Chauhan, "Bioremediation of Petroleum oil Contaminated Soil and Water", Research Journal of Environmental Toxicology 5(1): pp. 1-26, 2011.

[25]. M.S. Coniglio, V.D. Busto, P.S. Gonzalez, M.I. Medina, S. Milard and E. Agostini, "Application of *Brassica napus* hairy root cultures for phenol removal from aqueous solutions", Chemosphere, 72, pp. 1035-1042, 2008.

[26]. Misr Petroleum Company, "Specifications for Kerosene Misr", Misr Petroleum Company, Ghamara, Cairo, Egypt, 1993a.

[27]. Misr Petroleum Company, "Specifications for Mazotte Misr (180/240)", Misr Petroleum Company, Ghamara, Cairo, Egypt, 1993b.

[28]. Total Petroleum Hydrocarbon (TPH), "Criteria Working Group Volume 2: Composition of Petroleum Mixtures"; Wade Weisman (ed), Amherst Scientific Publishers, MA., 1998b.

[29]. Egyptian Environmental Affairs Agency (EEAA), "Environmental Promulgation Law (Law 4/ 1994)", Egyptian Environmental Affairs Agency, Cabinet of Ministers, Egypt, 1994.

[30]. American Standard Testing Methods (ASTM), "Standard test methods for gravimetric determination of total solvent extractable content (TSEC) of solid waste samples", D5368-93 (R06), 1993.

[31]. USEPA, "Method 1664, Revision A: n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated/ n-Hexane Extractable Material (SGTHEM; Non-polar Material) by Extraction and Gravimetry", Office of Water, USEPA, EPA-821-R-98-002, 1999.

[32]. S-W. Chiu, T. Gao, C. S-S. Chan and C. K-M Ho, "Removal of spilled petroleum in industrial soils by spent compost of mushroom *Pleurotus pulmonarius"*, Chemosphere, 75, pp. 837-842, 2009.

[33]. M.E. Ramírez, B. Zapién, H.G. Zegarra, N.G. Rojas and L.C. Fernández, "Assessment of hydrocarbon biodegradability in clayed and weathered polluted soils", International Biodeterioration & Biodegradation, 63, pp. 347-353, 2009.

[34]. M. R. Riazi and M. Edalat, "Prediction of the rate of oil removal from seawater by evaporation and dissolution", Journal of Petroleum Science and Engineering, 16, pp. 291-300, 1996.

[35]. I. Dror, Z Gerstl and B Yaron, "Temporal changes in kerosene content and composition in field soil as a result of leaching", Journal of Contaminant Hydrology, 48, pp. 305-323, 2001.

[36]. M. R. Mehrasbi, B. Hahighi, M. Shariatm S. Naseri, and K. Naddafi, "Biodegradation of Petroleum Hydrocarbons in Soil", Iranian J of Public Health, 32 (3), pp. 28-32, 2003.

[37]. M. P. Maila and T. E. Cloete, "Bioremediation of petroleum hydrocarbon through landfarming: Are simplicity and cost-effectiveness the only advantages?", Reviews in Environmental Science & Bio/Technology, 3, pp. 349–360, 2004.

[38]. F. Coulon, E. Pelletier, L. Gourhant and D. Delille, "Effects of nutrient and temperature on degradation of petroleum hydrocarbons in contaminated sub-Antarctic soil. Chemosphere, 58, pp. 1439-1448, 2005.

[39]. V. Schifano, C. MacLeod, N. Hadloo and R. Dudeney, "Evaluation of quicklime mixing for the remediation of petroleum contaminated soils", J of Haz Mat, 141, pp. 395-409, 2007.

[40]. G. Malina and J.T.C. Grotenhuis, "The role of biodegradation during bioventing of soil contaminated with Jet fuel", Applied Biochemistry and Biotechnology, 88, pp. 59-76, 2000.

[41]. S. J. Baptista, M.C. Cammarota, and D. D. de C. Friere, "Production of CO_2 in crude oil Bioremediation in Clay Soil", Brazilian Archives of Biology and Technology, vol. 48, Special n., pp. 249-255, 2005.

[42]. Ts. Galin, Z Gerstl and B Yaron, "Soil pollution by petroleum products, III. Kerosene stability in soil columns as affected by volatilization", Journal of Contaminant Hydrology, 5 (4), pp. 375-385, 1990.

[43]. M. Nocentini, D. Pinelli, and F. Fava, "Bioremediation of a soil contaminated by hydrocarbon mixtures: the residual concentration problem", Chemosphere, 41, pp. 1115-1123, 2000.

[44]. S. E. Agarry, C. N. Owabor and R. O. Yusuf, "Studies on Biodegradation of Kerosene in Soil under Different Bioremediation Strategies", Bioremediation Journal, 14: 3, pp. 135 – 141, 2010; doi: 10.1080/10889868.2010.495364.

[45]. N. Benazon, P. Lafrance, R.R. Simard and J-P. Villeneuve, "The effect of residual kerosene on the transport of ammonium and nitrate ions in sandy soil", Journal of Contaminant Hydrology, 20, pp. 111-126, 1995.

[46]. Total Petroleum Hydrocarbon (TPH) Criteria Working Group, "Volume 1: Analysis of Petroleum Hydrocarbons in Environmental Media", Wade Weisman (ed), Amherst Scientific Publishers, MA., 1998a.

[47]. J. Jarsjö, G Destouni and B Yaron, "Retention and volatilisation of kerosene: Laboratory experiments on glacial and post-glacial soils", Journal of Contaminant Hydrology, 17(2), pp. 167-185, 1994.

[48]. P. Fine, E.R. Garber and B Yaron, "Soil interactions with petroleum hydrocarbons: Abiotic processes", Soil Technology, 10, pp. 133-153, 1997.

[49]. Exxon Mobile, "Kerosene Safety Data Sheet", http://www.sweeneyoil.com/ uploads/MSDSSheetEssokerol.pdf., 2007.

[50]. F. M. Bento, F.A de O. Camargo, B. Okeke and W.T. Frankenberger-Júnior, "Bioremediation of Soil Contaminated by Diesel Oil", Brazilian Journal of Microbiology, 34 (Suppl. 1), pp. 65-68, 2003.

[51]. M. Tonegawa, J. Dec and J-M. Bollag, "Use Of Additives To Enhance Removal Of Phenols From Water Treated With Horseradish And Hydrogen Peroxide", J. Environ. Qual. 32, pp. 1222-1227, 2003.

[52]. H-H. Liste and I. Prutz, "Plant performance, dioxygenase-expressing rhizospheres bacteria and biodegradation of weathered hydrocarbons in contaminated soils", Chemosphere, 62, pp. 1411-1420, 2006.

[53]. J-J. Ma, H-S. Ko, J-S. Hwang, M-J. Jung, S. Choi and K-J Kim, "The Pilot-scale Treatability Studies of Co-Composting for the Remediation of Diesel Contaminated Soil during the Winter", Journal of KoSES, 4(2), pp. 193-201, 1999. (article in Korean with English Abstract).

[54]. S. Jin, P.H. Fallgren and M. Li, "Limitations of urea as the nutrient source in bioremediation of Petroleum Wastes", SPE Annual Technical Conference and Exhibition, 9-12 October 2005, Dallas, Texas; Paper Number: 96924-MS, 2005.

[55]. D. B. Vance, "On-Site Bioremediation of Oil & Grease Contaminated Soils", 2 The 4 Technology Solutions, http://2the4.net/biocell.htm, 2008.

[56]. J.P. Del'Arco, and F.P. de França, "Biodegradation of crude oil in sandy sediment", International Biodeterioration & Biodegradation, 44, pp. 87-92, 1999.

[57]. S. A.; Richmond, J.E. Lindstrom and J.F. Braddock, "Effects of Chitin on Microbial Emulsification, Mineralization Potential, and Toxicity of Bunker C Fuel Oil", Marine Pollution Bulletin, 42 (9), pp. 773-779, 2001.

[58]. J-H. Choi and D-S. Byun, "Studies on anti-aging action of Garlic, Allium Sativum L, (I) Comparative Study of Garlic and Ginseng Components on Anti-aging Action", Korean Biochem J., vol. 19, no. 2, pp.140-146, 2007.

[59]. L. Loughry, "Allicin-"Beyond Immunity": today Infectious Disease ranks as the #1 Killer of Humans, World Wide!", www.astrologyzine.com/allicin-beyond-immunity.shtml, 2006.

[60]. V. Labud, C. Garcia, and T. Hernandez, "Effect of hydrocarbon pollution on microbial properties of a sandy and a clay soil", Chemosphere, 66, pp. 1863-1871, 2007.

[61]. M. Nikolopoulou, N. Pasadakis, H. Norf, N. Kalogerakis, "Enhanced ex situ bioremediation of crude oil contaminated beach sand by supplementation with nutrients and rhamnolipids", Marine Pollution Bulletin 77, pp. 37–44, 2013.

[62]. G. Gramss, K.-D. Voigt and B. Kirsche, "Oxidoreductase Enzymes Liberated By Plant Roots And Their Effects On Soil Humic Material", Chemosphere, vol. 38, No. 7, pp. 1481-1494, 1999.

[63]. J. R. Gallego, E. González-Rojas, A.I. Peláez, J. Sánchez, M.J. García-Martínez, J.E. Ortiz; T. Torres and J.F. Llamas, "Natural attenuation and bioremediation of Prestige fuel oil along the Atlantic coast of Galicia (Spain)", Organic Geochemistry, 37, pp. 1869–1884, 2006.

[64]. A. Roldán-Martín, G. Calva-Calva, N. Rojas-Avelzapa, Ma. D. Díaz-Cervantes and R. Rodríguez-Vázquez, "Solid culture amended with small amounts of raw coffee beans for the removal of petroleum hydrocarbon from weathered contaminated soil", International Bioremediation & Biodegradation, 60, pp. 35-39, 2007.

[65]. J. Sabaté, M. Viñas and A.M. Solanas, "Laboratory–scale bioremediation experiments on hydrocarbon–contaminated soils", International Biodeterioration & Biodegradation, 54, pp. 19-25, 2004.

[66]. F. M. Bento, F. A O. Camargo, B. C. Okeke, and W.T. Frankenberger, "Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation", Bioresource Technology, 96, pp.1049-1055, 2005.

[67]. J. Dou, X. Liu and A. Ding, "Anaerobic degradation of naphthalene by mixed bacteria under nitrate reducing conditions", J of Haz Mat, 165, pp. 325-331, 2009.

[68]. A. Yateem, M.T. Balba and N Al-Awadhi, "White rot Fungi and their role in remediating oil-contaminated soil", Environmental International, vol. 24 (1/2), pp.181-187, 1997.

[69]. T. Macek, M. Macková, and J. Káš, "Exploitation of plants for the removal of organics in environmental remediation", Biotechnology Advances, 18, pp. 23–34, 2000.

[70]. J. Isayenkova, V. Wary, M. Nimtz, D. Strack and T. Vogt, "Cloning and functional characterization of two regioselective flavonoid glucosyltransferases from *Beta vulgaris*", Phytochemistry, 67, pp. 1598-1612, 2006.

[71]. F. J. Moreno, M. Corzo-Martínez, M. D. del Castillo and M. Villamiel, "Changes in antioxidant activity of dehydrated onion and garlic during storage", Food Research International, 39, pp. 891–897, 2006.

[72]. S. Eapen, S. Singh and S. F. D'Souza, "Advances in Development of transgenic plants for remediation of xenbiotics pollutants", Biotechnology Advances, 25, pp. 442-451, 2007.

[73]. S.J.T. Pollard, M. Whittaker, and G.C. Risden, "The fate of heavy oil wastes in soil microcosms I: a performance assessment of biotransformation indices", The Science of the Total Environment, 226, pp. 1-22, 1999.

[74]. P. Fernández-Álvarez, J. Vila, J.M. Garrido-Fernández, M. Grifoll and J.M. Lema, "Trials of bioremediation on beach affected by the heavy oil spill of Prestige", J of Haz Mat, B137, pp. 1523-1533, 2006.

[75]. H. R. Ali, N. Sh. El-Gendy, Y. M. Moustafa, M. I. Roushdy and A. I. Hashem, "Degradation of Asphaltenic Fraction by Locally Isolated Halotolerant Bacterial Strains", International Scholarly Research Network ISRN Soil Science Volume 2012, Article ID 435485, 11 pages; doi:10.5402/2012/435485.

[76]. R. Jézéquel, L. Menot, F-X. Merlin and R.C. Prince, "Natural cleanup of heavy fuel oil on rocks: an in situ experiment", Marine Pollution Bulletin, 46, pp. 983-990, 2003.

[77]. I. Blažević, and J Mastelić, "Glucosinolate degradation products and other bound and free volatiles in the leaves and roots of radish (*Raphanus Sativus* L.)", Food Chemistry, 113, pp. 96–102, 2009.

[78]. M. Francisco, D.A. Moreno, M.E. Carteaa, F. Ferreres, C García-Viguera and P. Velascoa, "Simultaneous identification of glucosinolates and phenolic compounds in a representative collection of vegetable *Brassica rapa"*, Journal of Chromatography A, 1216, pp. 6611–6619, 2009.

[79]. T. Janowitz, I. Trompetter and M. Piotrowski, "Evolution of nitrilases in glucosinolate-containing plants", Phytochemistry, doi: 10.1016/j.phytochem. 2009.07.028, 2009.

[80]. J. Jost, "Brassicas", Kansas Rural Center Sustainable Agriculture Management Guides, MG4C.1, http://www.kansasruralcenter.org/publications/brassicas.pdf, 1998.

[81]. E. Rodríguez, O. Nuero, F. Guillén, A.T Martínez and M.J. Martínez, "Degradation of phenolic and non-phenolic aromatic pollutants by four Pleurotus species: the role of laccase and versatile peroxidase", Soil Biology and Biochemistry, 36, pp. 909-916, 2004.

[82]. A. Mohammadi and B. Nasernejad, "Enzymatic degradation of anthracene by the white rot fungus Phanerochaete chrysosporium immobilized on sugarcane bagasse", J of Haz Mat, 161, pp. 534-537, 2009.

[83]. B. K. Singh, S. R. Sharama and B. Singh, "Combining ability of superoxide dismutase, peroxidase and catalase enzymes in cabbage head (Brassica oleracea var. capitata L.)", Scientia Horticulturae, 122, pp. 195-199, 2009.

[84]. J. M. Conroy, D. C. Borzelleca and L.A. McDonell, "Homology of Plant Peroxidases: An Immunochemical Approach", Plant Physiol. 69, pp. 28-31, 1982.

[85]. M. Y Lee and S.S. Kim, "Essential Sulfhydryl residue in Radish Peroxidase Isozyme", Korean Biochem. J., vol. 26 (2), pp. 151-156, 1993.

[86]. S.A. Ziai, S.S. Eshraghi, M. Taghizadeh, and F. Vahabzadeh, "Garden Radish (*Raphanus sativus L.*) Peroxidase Eliminates Alachlor and Butachlor Herbicides as Well as Hazardous Aromatic Pollutants", *Proc. for Biology In Asia International Conference, Singapore, 7-10 December, 2004;* http://www.dbs.nus.edu.sg/ sibiolconference/program(all).pdf., 2004.

[87]. J. Csiszár, E. Lantos, I. Tari, E. Madoşă, B. Wodala, Á. Vashegyi, F. Horváth, A. Pécsváradi, M. Szabó, B. Bartha, Á. Gallé, A. Lazăr, G. Coradini, M. Staicu, S. Postelnicu, S. Mihacea, G. Nedelea, and L. Erdei, "Antioxidant enzyme activities in *Allium* species and their cultivars under water stress", Plant Soil Environ, 53 (12), pp. 517–523, 2007.

[88]. K. L. Lau, Y.Y Tsang, and S.W. Chiu, "Use of spent mushroom compost to bioremediate PAH-contaminated samples", Chemosphere, 52, pp.1539-1546, 2003.

[89]. R. A. Fatima, and M. Ahmad, "Certain enzymes of Allium cepa as biomarkers for the detection of toxic heavy metals in wastewater", Science of the Total Environment, 346, pp. 256-273, 2005.

[90]. M. Saigusa, K. Ohira and A. Fujiwara, "Glutamate Dehydrogenase in higher plants. V. Purification and Properties of the enzyme for Turnip Roots", Tohoku Journal of Agricultural Research, vol. 25 (2), pp. 86-95, 1974.

[91]. P. O'Carra, and P. Mulcahy, "Lactate Dehydrogenase in Plants: Distribution and Function", Phytochemistry, Vol. 42 (3), pp. 581-587, 1996.

[92]. P. Mulcahy and P. O'Carra, "Purification and Substrate Kinetics of Plant Lactate Dehydrogenase", Phytochemistry, Vol. 45 (5), pp. 889-896, 1997.

[93]. S. Fujita and T. Tono, "Activating Effect of Manganese on Phloroglucinol Oxidase of Turnip", Bull. Fac. Agr., Saga. Uni.; 58, pp. 45-50, 1985. (English Abstract)

[94]. S. Ueno, M. Hayashi, T Shigematsu and T. Fum, "Formation of Green-blue compunds in Brassica Rapa root by high pressure processing and subsequent storage", Biosci. Biotechnol. Biochem., 73 (4), pp. 943-945, 2009.

[95]. A. Noreen and M. Ashraf, "Changes in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.)", Environ. Exp. Bot., doi:10.1016/j.envexpbot.2009.05.011, 2009.

[96]. C. Novotný, K. Svobodová, P. Erbanová, T. Cajthaml, A. Kasinath, E. Lang and V. Šašek, "Ligninolytic fungi in bioremediation: extracellular enzyme production and degradation rate", Soil Biology & Biotechnology, 36, pp. 1545-1551, 2004.