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Effect of Oil on Phytoremediation of PCB Co-Contamination in Transformer Oil Using *Chromolaena odorata*

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Abstract

Greenhouse assessment of the effect of oil on *Chromolaena odorata* ability to remove PCB from soil treated with transformer oil co-contaminated with Aroclor 1260 was done.

Method: Plants were transplanted into one kilogram of soil contained in 1L pots differently containing 100, 200, and 500 ml of transformer oil (T/O), co-contaminated with 100 ppm of Aroclor. Treatments were done in two microcosms; direct contamination and soil cultured method. Measured plant growth parameters showed that *C. odorata* growth was affected by the different concentrations of oil. Inhibition of plant growth by oil increased with concentrations.

Results: At the end of six weeks, plant growth was affected in T/O amended soil. Plants size was increased by 1.4, 0.46 and -1.0% in direct treatment and 17.01, 6.09 and 1.08% in soil culture at the 100, 200 and 500 ppm respectively. Untreated control showed a 43.07% increase. Slight PCB recovery was observed in root tissues of *C. odorata* but soil PCB was reduced by 66.6%, 53.2%, 41.5% and 77.3%, 74.7%, 58.8% at both treatments in their respective concentrations of oil. However, unplanted control was reduced by 21.4% and 16.7% in the two treatments at 100 ppm of oil.

Conclusion: This study has shown that with improved agronomic practices, there is a possibility of phytoremediation of soil PCB from PCB contained transformer oil contaminated soil using *Chromolaena odorata*, hence it should be optimized in the field.

Keywords: Phytoremediation • Transformer oil contamination • *Chromolaena odorata* • PCB • Soil remediation • South Africa.

Introduction

Advances in science and technology have enabled man to exploit natural resources largely, generating unprecedented disturbances in global elemental cycles [1]. The relatively recent introduction of man-made toxic chemicals, and the massive relocation of natural materials to different environmental compartments; soil, ground water, and atmosphere, has resulted in severe pressure on the self-cleansing capacity of recipient ecosystems. Various accumulated pollutants are of concern relative to both human and ecosystem exposure and potential impact. There have been efforts by many authorities in different countries to control the release of contaminants [2], and to accelerate the breakdown of existing contaminants by appropriate remediation techniques. Such techniques and technologies are marred by various disadvantages and usually require relatively high capital expenditure and man power as well as long term operating cost. Hence, recent interests are geared towards developing more cost effective approach to treat large volumes of contaminated natural resources such as soil, ground water and wetlands [3].

Bioremediation is the use of plants and the associated rhizospheric microorganisms to remove, transform, or contain toxic chemicals located in soils, sediments, ground water, surface water, and even the atmosphere [4]. This technique is currently used to treat many classes of contaminants including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radionuclides, and landfill leachates [1]. Biological method has been used for hundreds of years to treat human waste, reduce erosion, and protect water quality [5], until about 25 years ago which saw some significant rise in the use of plants known as phytoremediation in the removal of contaminants from the environment [6]. In the present study, *C. odorata* (Siam weed), was grown in Aroclor 1260 amended transformer oil-contaminated soil in order to study the effect of oil on the ability of plants in the remediation of soil-PCB from a transformer oil impacted soil. This is of importance as literatures have only reported on plants remediation of PCB without considering the impact of co-contamination of the oil, considering the fact that PCB has not been used in isolation [7,8].

Polychlorinated biphenyls (PCBs) are a family of anthropogenic organic compounds that is persistent in the environment causing its bioaccumulative phenomenon that enables the contaminant to be found in every part of the environment. PCB is commercially produced by direct chlorination of biphenyls [9]. A good commercial form of PCB is Aroclor 1254 and 1260, although other brand names exist [10]. Various negative health effects in humans as well as the animals are linked to PCB compounds, this call for an urgent action on how the compound can be removed from the environment [11,12]. The physico-chemical properties of PCB depend on the congener composition, but generally they are resistant to acids and bases, resistant to oxidation and hydrolysis, thermally stable, excellent electrical insulators, sparingly soluble in water and have low flammability [13]. These characteristics conforms the usefulness of PCBs in diverse industrial applications, such as liquid components of transformers, capacitors, heat-exchangers, and vacuum pumps. PCB mixtures have also been used in open systems, such as plasticizers, drinking solvents, water-proofing agents, sealing liquids, fire retardants and pesticides [14-17].

Transformer oil also known as insulating oil is a highly refined mineral oil

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that is stable at high temperatures and usually possesses excellent electrical insulating properties. Transformer oil is mostly used in oil-filled transformers, in high voltage capacitors, fluorescent lamp ballast, as well as in some high voltage switches and circuit breakers. The functions of transformer oil to these equipments ranges from insulation, suppression of corona and arcing, and also as a cooling liquid [18]. Properties of transformer oil in transformers require periodic testing to make sure that the basic electrical properties of the oil are intact as it is in operation. This informs the filtration and regeneration activities on transformers [18]. Therefore, once transformer oil is contaminated above its recommended value with PCB, it becomes hazardous and should be discharged. During the process of discharge, the environment becomes the recipient. PCB release to the atmosphere has been through the following means: from uncontrolled landfills and hazardous waste sites; incineration of PCB containing wastes; leakage from older electrical equipments in use and improper disposal of spills [19,20].

Various traditional remediation measures for example chemical (treatment with solvents); mechanical (soil excavation), thermal (incineration), and biological (use of microorganisms), have been used for the elimination of PCBs and other organics from the environment [21]. These remediation techniques have been successful in the remediation of organic contaminants, but are marred by various disadvantages [22]. These include the fact that the processes are expensive, some of the processes are slow hence targets only the low chlorinated biphenyls, others live the finger print of other more toxic compounds at the end as a result are not environmentally friendly and lacks general public acceptability [23,24]. It is therefore imperative to develop a more cost effective technique for the treatment of complex PCB as contained in transformer oil.

Plants are known to take up large amount of water with nutrient from soil in soluble form in order to increase in biomass. This phenomenon has been investigated to be of benefits in the removal of pollutants from the environment and is referred to as phytoremediation [25]. Phytoremediation is the use of vegetation for in situ treatment of contaminants from soil and water body. It is a promising technique that can be used to manage pollution [26]. Phytoremediation is cost effective and eco-friendly strategy that can complement or replace conventional approaches especially in the remediation of soil contaminated by PCBs [27]. The principle mechanism of phytoremediation is either by stimulation of soil microbial activity and degradation of contaminants or through plant uptake of contaminants or by even their degradation products [28]. As plant-based remediation technology, phytoremediation has its general limitations- tolerance and uptake ability of plants for organics differs widely, pollutant concentrations and the presence of other toxins must also be within the limits of plants tolerance [29]. Several factors are known to affect the effectiveness of any phytoremediation projects; they include Soil properties, Physiochemical properties of organic pollutants, Soil amendments as well as the type of Plant [30].

Chlomolaena odorata (L) R.M. King & H. Robinson (Siam weed) is an invasive bushy shrub of South American origin. The plant is a member of the tribe Eupatoreae in the sunflower family Asteraceae and is been regarded as one of the most notorious invasive alien plant in the plant community [31]. *C. odorata* have been found to possess amongst its strong morphological status, to possess most qualities of a phytoremediation plant. These features are responsible for the plant's success as invasive species in its new environments. These factors therefore present *C. odorata* as a potential plant for phytoremediation of a complex organic system as seen in the co-contamination of oil with Aroclor [32].

The interest in transformer oil arose as a result of the fact that T/O is one of the most widely used organic chemicals and the unscrupulous discharge of the oil calls for concern. However, T/O is linked with PCB contamination and has contributed in the continued proliferation of PCB in the environment. At present, little information is known regarding the treatment of transformer oil contaminated soil using phytoremediation processes [33-35]. Thus this study aimed to investigate the effect of oil on the ability of *Chromolaena odorata* to phytoremediate soil-PCB from transformer oil co-contaminated Aroclor 1260 treated soil under greenhouse conditions.

Material and Methods

Soil

Soil samples were collected from a depth of up to 30 cm, from the main campus of University of South Africa, Pretoria. The soil samples were homogenized with hand to remove pebbles, stones and gravels and, air dried, before it was put in cellophane bags and stored at 4°C before use. Sub-samples of the soil (250 g) each were taken and used for soil characterization at the laboratory. Composite samples from the stored soil were separated as the cultured soil sample. The soil used has a clay features with the following characteristics: clay (72.0%), silt (18.5%), sand (9.5%), pH (6.7), total organic carbon (7.0 ppm), total N (0.03% wt), total P (9.0 ppm), K (15.5 ppm), Ca (83.0 ppm), Mg (1.2 ppm), Fe (58.6 ppm) moisture content (4.8%), thermal conductivity ($0.2Wm^{-2}k^{-1}$), and soil density (1.25 g/cm).

Plants

Chromolaena odorata plants were collected from the Department of Botany University of KwaZulu-Natal Pietermaritzburg and propagated by stem cuttings in the greenhouse at the University of South Africa. Soil samples were mixed with animal manure that was obtained from the Department of Veterinary Science, University of Pretoria, Onderstepoort, at the ratio of 9:1. The carbon, nitrogen and phosphates values (CNP) of the animal manure were analyzed at a private laboratory, the values were C=52.7 ppm; N=81.0% wt and P=50 ppm). The plant cuttings were planted into the prepared soil bed employing the method described by Anyasi and Atagana [30]. Plants rooting hormone "Indole Butyric Acid" IBA, supplied by Plantland Malanseuns in Roslyn-South Africa was applied, this was to aid rooting of the cuttings. The plants in the soil bed were allowed to grow for three months and were then used for subsequent propagation and experimentation. The bed was watered manually using watering can to maintain 70% moisture at field capacity.

PCB and transformer oil

Commercial PCB in form of Aroclor 1260 in surrogate standard concentration of 1000 ppm in hexane was supplied by Sigma Aldreich-Germany, and used Transformer oil (T/O) was provided by City power Johannesburg-South Africa and new oil (Nynas-LYRA X) supplied by Nynas oil- Sweden. Working standard solution was prepared from the surrogate standard using hexane fraction to make out composites of 100 ppm concentration. This means that transformer oil samples were amended to contain 100 ppm of PCB using Aroclor in hexane.

Treatments

- Transformer oil direct treatment samples (T/O_D) and
- Transformer oil culture Suzuki samples (T/O_S).

Control samples

- Soil samples planted without contamination to test the toxicity of the contaminants on plants (C1);
- Soil samples contaminated without plants to test for other possible measures of dissipation of the contaminants (C2);

Experimental design

A 42 set of PVC pot were used for the experiment each filled with 1 kg of soil. The pots were divided into two (21 each for the two transformer oil treatments), each section were further divided into six (3) replicates each for the treatments and controls). Thus, three (3) *C. odorata* plants were tested in two (2) pollutants among six (6) treatments replicated into three (3). A total of one hundred and twenty six plants were used in the study.

Experimental procedures

Five weeks old *C. odorata* plants were used in this study; the plants were transplanted into contaminated soil according to the treatment and were allowed for six weeks. In T/O direct treatments, plants were directly transplanted into the T/OD treated soil samples. In Suzuki (sprout culture adopted from Suzuki

et al. [36] method however, plants were transplanted in a cultured soil which is contained in cellophane bags with holes at the bottom for protrusion of the roots. The bags containing the cultured soil and plants with protruded roots were placed on T/O treated soil contained in the PVC cups. This adopted and amended Suzuki method was designed to avoid the toxic effects of T/O on plants which posed a problem during the preliminary stage of the study. The initial length and number of mature leaves per plants (MLPP) was noted. The experiment was allowed for six weeks at prevailing environmental conditions, watered to maintain moisture at 75% field capacity with manual watering can. Effort was made at ensuring that watering was done in such a way as to only wet the soil at any point in time avoiding much run off, weeds were removed manually at intervals. Measurements were also made at weekly intervals for the plant length, MLPP, leaf colour at different treatments and the root length which was only measured on the day of harvest. There was no application of inorganic manures to the soil mixes, but organic animal compost was used during the preparation of the soil at the ratio 1:9 manure to soil.

Sampling

After six weeks of growth of the plant in the contaminated and control set up, the soil and plants were sampled. The plants were removed carefully from the pots after loosening the soil around the pot using a kitchen knife; the roots were separated from the soil by shaking off the soil leaving the only adhering particles of the soil regarded as the rhizosphere soil. During this process, the entire plants were washed using running tape water, rinsed with distilled water and allowed to air dry, it was weighed afterwards to get the wet biomass and root lengths were measured. The plants were then separated into leaf, stem and roots, and the entire samples weighed using Mettler Toledo balance model PB1502 with maximum capacity of 1510 g. The soil samples were carefully collected also, homogenized and divided into sets together with the plants samples in preparation for subsequent extraction and analysis. All cuttings of the plants were done with a kitchen knife rinsed with acetone between uses to minimize cross contamination. Harvested and prepared plant samples were kept in WhirlpakTM bags; Nasco-South Africa, in the refrigerator until time for analysis. Before the analysis, composite samples of the plant tissues were oven dried for the determination of the change in biomass. This was deduced from the initial (wet weight) at harvest and final weight (dry weight) after the entire plant was oven-dried until constant weight was attained. However any plant matter that was not collected for analysis was left in the green house in airtight containers for later use and appropriate disposal.

Determination of final PCB in soil and PCB recovery in plant tissues after six weeks of treatment with transformer oil co-contaminated with Aroclor 1260

All glass wares were washed with liquid detergent, rinsed with water and then soaked in Dichloromethane (DCM) over night. They were then rinsed with water, followed with distilled water and finally with acetone to remove any adhering organic substances [37]. Soil and plant samples were thoroughly homogenized for analysis and sub-sampled for the determination of wet and dry weight ratio. The samples for biomass determination were dried at 50°C until constant mass using Lancon industrial oven with heating integration of 40-100°C and were measured to obtain the dry mass. The dried plant samples were then ground using commercial blender, sieved at 2 mm and were stored prior to extraction while the soil samples were ground using a commercial mortar and was sieved at 2 mm. Five grams of 2 mm sieved dry soil as well as 5 g of 2 mm sieved plant samples were extracted using soxhlet apparatus for 4 hrs at 4-6 cycles per hour with 50 ml mixture of hexane-acetone (1:1, v/v), after which the extracted solution was concentrated to 2 ml in rotary evaporator (Buchi Rota vaporTM Japan model R-200 with heating bath B-490 and heating intensity of 20-180°C). USEPA Method 3630 B: Silica Gel Cleanup was used, this method have been shown to specifically address Aroclors [37].The extract from soxhlet extraction was dissolved in 10 ml hexane and passed from a glass chromatographic column (i.d 20 mm and 400 mm height) parked with layers of silica gel and anhydrous sodium sulphate and then eluted with 50 ml of hexane. The eluent was finally concentrated with rotary evaporator for the second time to about 1 ml and was analyzed using GC-MS.

Analysis and quantification of total PCB recovery in extracts from soil and plant samples

The method adopted here was the USEPA modified 8089/8081 method for the determination of total PCB. The analysis was conducted using Agilient 7890 GC equipped with 5975 Mass Spectometry and auto injector, an SupelcoWAX SPBTM-1 (30 m × 0.25 mm × 0.25 μm) column was used with N2 as the carrier gas. Prior to analysis of samples, recovery test was carried out using the standard Aroclor samples to ascertain the linearity of the response. One micro liter of the sample extract was injected into the GC. Injector and detector chamber temperatures were 260°C and 300°C, respectively. The oven temperature was initially set at 180°C for 0.5 min, ramped at 30°C per min to 260°C, it was held for 18 min then 15°C per min to 270°C and held for 25 min. PCB congeners were identified by retention time matching to the surrogate standards which was prepared using the commercial stock samples of PCB inform of Aroclor 1260 prepared in concentrations of 1, 5, 10, 20, and 50 μg/ml in hexane. The value of the chromatogram was quantified using peak area integration. The initial content of PCB in transformer oil spiked was measured to be 6.8, 7.1, 6.1, 6.9, 6.3, and 6.7 in T/OD and also 8.9, 7.4, 6.5, 7.3, 7.8, and 6.4 ppm in T/OS.

Statistical analysis of values

Results were analyzed by analysis of variance using three replicates at 95% level of significant difference to determine the mean differences of treatments in the experiment.

Results

Soil and plants growth parameters

The conditions of the soil in which the plants was grown is as presented above, although the soil was slightly acidic, other parameters indicated that *C. odorata* roots including the soil organisms will not have any resistance to thrive. After six weeks of growth in soil treated with Transformer oil co-contaminated with Aroclor 1260, growth inhibition was observed in plants especially the direct treated samples; such inhibition was even lethal to plants in higher concentration of 500 ppm of oil. There was improved growth of *C. odorata* in the soil cultured Suzuki method. Plant growth measured from the difference between the initial and final length of *C. odorata* is presented in Figure 1, while percentage growth rate deduced is as presented in Table 1. Mean percentage growth rate was higher in T/OS at 100 ppm (17.01) than the T/OD (1.40). Untreated control C1 have percentage growth rate of 43.3 which is significantly different from that of the treated samples (p= 0.07). Relative mean Percentage growth rate at 200 ppm were lower than in 100 ppm treatment but were higher than the 500 ppm respectively. Mean percentage growth rate of value less than zero (-1.03), was obtained in T/OD at 500 ppm treatment. Meanwhile, the growth of *C. odorata* was found to be negatively correlated with increase in concentration of soil transformer oil. In all treatments, percentage growth rate between treated and untreated control was significantly different at p= 0.005 (Figure 1).

The number of mature leaf per plant (MLPP) of *C. odorata* at any interval of time was observed to be influenced by the presence of oil in its surrounding. MLPP followed the same trend as seen in growth rate as well as increase in root length. MLPP in T/OD at 100 ppm increased from initial 27 leaves to final 29 leaves, leaving a mean percentage increase to 7.41. Mean percentage increases in MLPP in T/OD at 200 and 500 ppm were zero respectively as the plant leaves were completely dried at the end of the experiment. In T/OS, MLPP values were higher; hence at 100 ppm it was 50.0 but dropped to zero at 200 ppm and remained in zero at 500 ppm (Table 1). Relative mean Percentage change in MLPP at untreated control (C1) was higher than the treated samples; and such values were significantly different (p=0.013) from that of the treated experiments (Figure 2 and Table 2).

Root lengths of *C. odorata* at different concentrations of transformer oil co-contaminated with 10% Aroclor 1260, maintained the same trend as observed in growth rate and MLPP. Percentage change in root length was high in T/

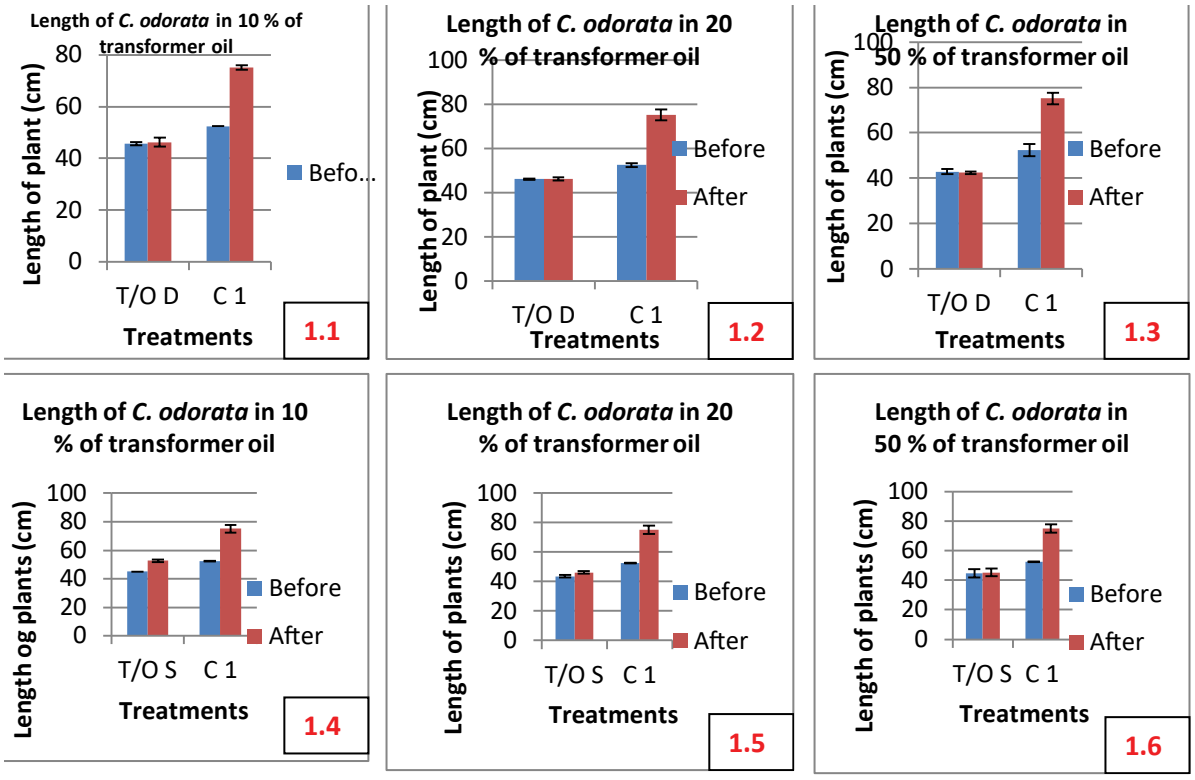

Figure 1. Length of plant at different concentrations of T/O co-contaminated with Aroclor 1260 in soil (Error bars indicate standard error of the mean), Before=Initial length, After=Final length

Table 1. Percentage growth rate of *C. odorata* at different concentrations of T/O co-contaminated with Aroclor 1260 in soil.

Treatments	% growth rates of *C. odorata*		
	100 ppm T/O	200 ppm T/O	500 ppm T/O
T/O_D	1.40[a]	0.46[a]	-1.03[a]
C1	43.30[b]	41.30[b]	42.36[b]
C2	NP	NP	NP
Treatments	**% growth rates of *C. odorata***		
	100 ppm T/O	**200 ppm T/O**	**500 ppm T/O**
T/O_S	17.01[a]	6.09[a]	1.08[a]
C1	43.30[b]	41.30[b]	42.36[b]
C2	NP	NP	NP

Values with the same alphabets in superscript in the same column were not significant at 5% level according to Bonferoni test. T/O_D=Direct Transformer Oil, T/O_S=Suzuki Transformer Oil, C1=Control 1, C2=Control 2, NP=Not Planted

OS at 100 ppm (50.60), which is not significant with that of untreated control (68.98). Root length at this treatment was however reduced considerably at 200 and 500 ppm (20.34 and -0.24) respectively. In T/OD treatment, percentage change in root length was lower at 100 ppm than in T/OS and such effect was increased at higher concentrations. Results of root length of *C. odorata* after six weeks of growth in an Aroclor treated soil is presented in Figure 3 and Table 3.

The leaves of *C. odorata* at different concentration of transformer oil co-contaminated with 100 ppm of Aroclor were marked by significant colour changes few days after contamination. The colour changes were evident with the light green colouration and black spots, an evidence of the inhibitive effect of transformer oil to the growth of plants. Untreated control maintained its green leaf colour throughout the six weeks of the experiment.

Effect of different concentrations of transformer oil co-contaminated with 100 ppm of Aroclor 1260 treatment on the ability of *C. odorata* to retain water in its tissues

As was deduced from the initial (wet weight) at harvest and final weight (dry weight), at 100 ppm, percentage water retention ability was higher in T/OS than in T/OD (49.25 and 27.07) respectively, these values were lower than what was obtained in untreated control (75.12%). As the concentration of the oil is increased, such phenomenon was found to increase, this can be found in 200 and 500 ppm treatments where the corresponding values were 59.84 vs. 34.48% and 62.81 vs. 40.67% for T/OS and T/OD respectively (Tables 4a-4c). In all, water retention ability of treated samples were significantly different from untreated control at p=0.05.

Total PCB recovery from soil and plant tissues

Mean percentage change in soil total PCB concentration at T/OD were 61.6, 53.2, 41.5, 55.9, 16.3, 18.8 for 100, 200 and 500 ppm of transformer oil as well as in control experiments respectively, having reduced to 2.61, 3.32, 3.51, 5.42, 5.27, 5.44 ppm from initial concentration of 6.8, 7.1, 6.1, 6.9, 6.3, 6.7 ppm of PCB among the treatments and treated controls respectively. No PCB detection was observed both at the beginning and at the end of the six weeks experiment in untreated controls. In T/OS however, mean percentage change in total soil PCB at the end of the experiment were 77.3, 74.7, 58.8, 16.7, 25.5, 21.1 for the 100, 200 and 500 ppm of transformer oil as well as treated controls. The initial concentrations of PCB in the soil were 8.9, 7.4,

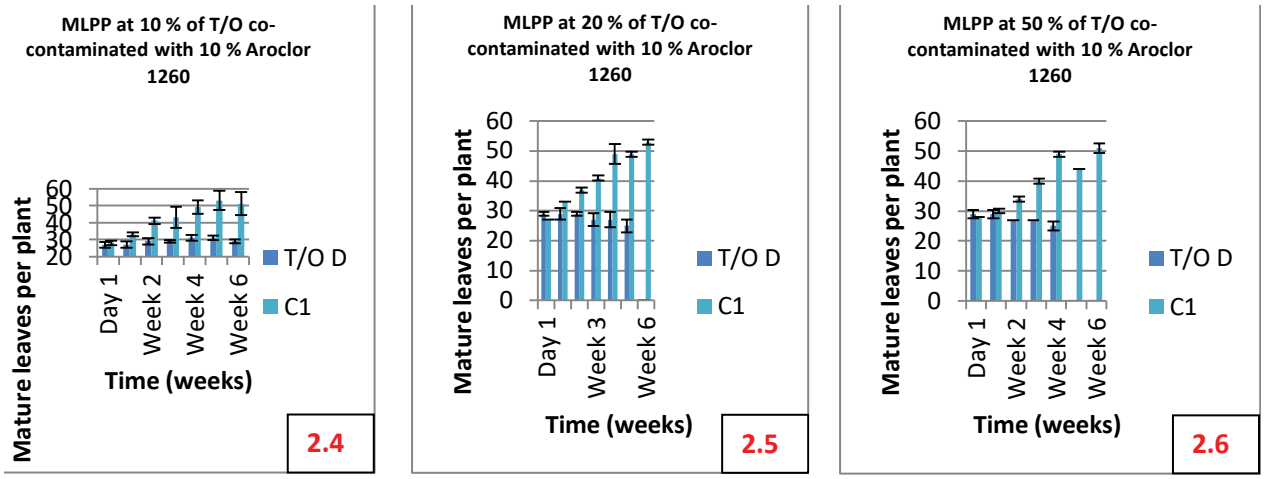

Figure 2. MLPP at different concentrations of T/O co-contaminated with Aroclor 1260 (Error bars indicate standard error of the mean), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, C1=Control 1,

Table 2. Percentage change in mature leaves per plant in different concentrations of Aroclor and T/O treated soil 2.

Treatments	% increase in mature leaves per plant		
	100 ppm T/O soil (cm)	200 ppm T/O soil (cm)	500 ppm T/O soil (cm)
T/O $_D$	7.41^a	0.00^a	0.00^a
C1	82.14^c	96.30^c	82.14^b
C2	NP	NP	NP
Treatments	**% increase in mature leaves per plant**		
	100 ppm T/O soil (cm)	**200 ppm T/O soil (cm)**	**500 ppm T/O soil (cm)**
T/O $_S$	50.00^b	20.69^b	0.00^a
C1	82.14^c	96.30^c	82.14^b
C2	NP	NP	NP

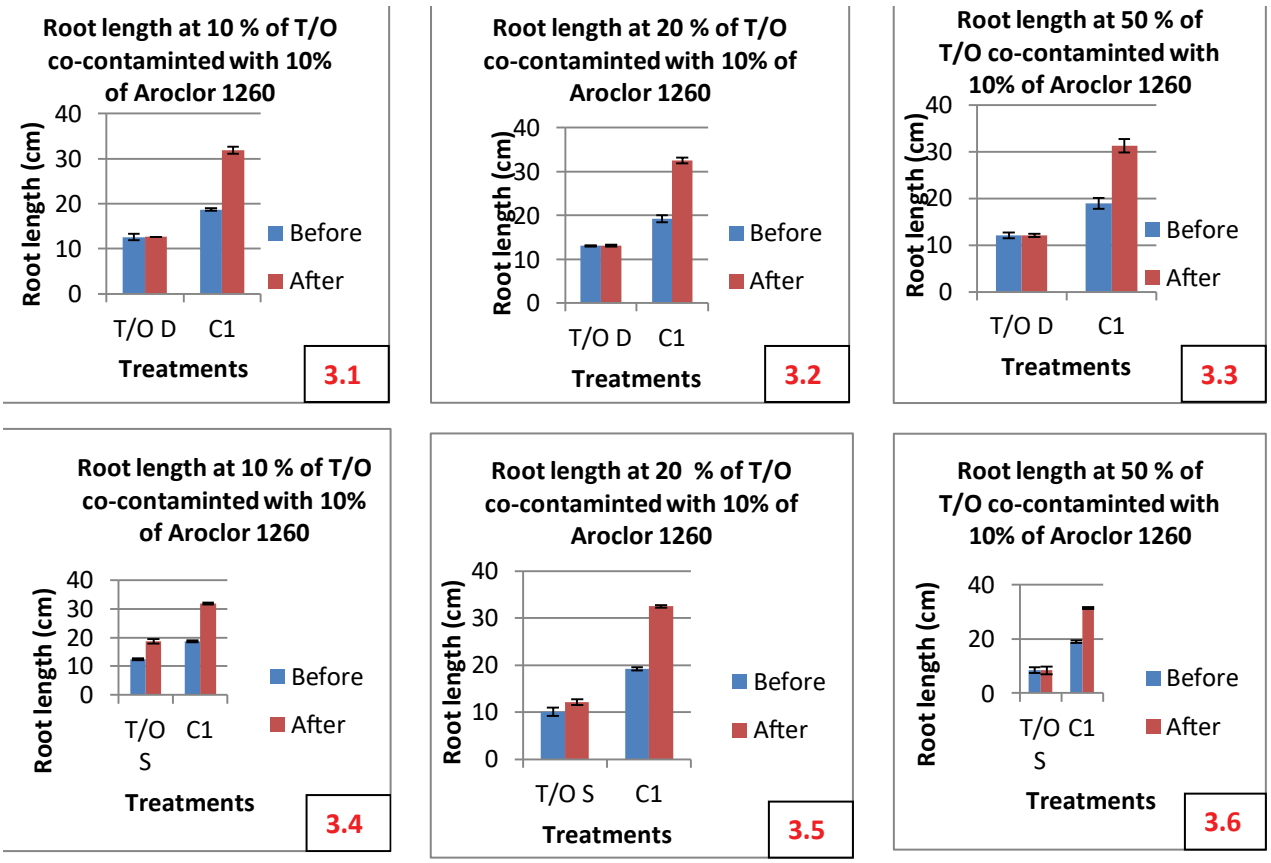

Figure 3. Root length at different concentration of Transformer oil co-contaminated with of Aroclor 1260 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length

Table 3. Percentage change in root length at different concentrations of Transformer oil co-contaminated with Aroclor 1260.

Treatments	Percentage change in root length		
	100 ppm T/O soil (cm)	200 ppm T/O soil (cm)	500 ppm T/O soil (cm)
T/O_D	0.30[a]	0.08[a]	-0.33[a]
C1	69.98[b]	69.13[b]	65.18[b]
C2	NP	NP	NP
Treatments	**Percentage change in root length**		
	100 ppm T/O soil (cm)	**200 ppm T/O soil (cm)**	**500 ppm T/O soil (cm)**
T/O_S	50.60[a]	20.34[a]	-0.24[a]
C1	69.98[b]	69.13[b]	65.18[b]
C2	NP	NP	NP

Values with the same alphabets in superscript in the same column were not significant at 5% level according to Bonferoni test. T/O_D=Direct Transformer Oil, T/O_S=Suzuki Transformer Oil, C1=Control 1, C2=Control 2, NP=Not Planted

6.5, 7.3, 7.8, and 6.4 ppm and the final soil concentrations were 2.02, 1.87, 2.68, 6.08, 5.81and 5.05 ppm for 100, 200 and 500 ppm of transformer oil and treated controls 1, 2 and 3 respectively.

At the end of the phytoremediation experiment on the effect of oil on remediation of PCB co-contaminated in transformer oil using *Chromolaena odorata* for six weeks, there was no detection of PCB in the tissues of the plants in T/OD treatment, although mean final soil PCB concentration in that treatments were lower than the initial treatment with Aroclor 1260 of 100 ppm (Tables 5 and 6). However, in T/OS treatments PCBs were found in the root tissues of plants especially at the 100 and 200 ppm of oil treatments (0.10 and 0.11 ppm) given a total root PCB of 0.62 and 0.52 µg for respective 100 and 200 ppm of oil concentrations respectively. Mean root bioaccumulation factors of 0.001 were recorded in those treatments respectively.

Discussion

Growth of *C. odorata* in transformer oil contaminated soil

Phytoremediation of PCB co-contaminated in transformer oil in form of 100 ppm of Aroclor by *C. odorata* in this study behaved differently at different concentrations of transformer oil in the soil. This could be attributed to the toxicity of transformer/hydrocarbon containing oil to plants. Increase in concentration of oil in soil has been reported to increase the phytoxicity to plants until such concentration that it became lethal to the plants [38-42]. In this study, the growth of *C. odorata* was tremendously affected by the presence of transformer oil in the soil especially when the oil is in direct contact with the plant (T/OD). 500 ppm of transformer oil per kilogram of soil was lethal to *C. odorata* at this treatment. This was shown by the withering of the plant the first week they were transplanted into the contaminated soil, as evidenced by the low percentage growth obtained in T/OD experiment. Such effect has been described as physiological shock experienced by plants when it changes environment [43]. However, growth performance of *C. odorata* were favorably in the T/OS experiments, the reason perhaps being that the plant did not have direct contact with the contaminated soil except through the roots. Hence the plants in this treatment had relatively high percentage growth with values not significant with that of T/OD but significantly different from the values of the untreated control. Meanwhile, various weeds have shown strong adaptability to poor soil condition, therefore it is not out of place that *C. odorata* was able to thrive on T/O treated soil [44,45]. *Chromolaena odorata* being a very resilient plant with such good properties for example the ability to survive in oil contaminated soil and other harsh environment was harnessed in this present study [46]. Furthermore, plants in the higher concentration of transformer oil (500 ppm) of T/OD had percentage growth rate which is less than zero meaning that the plants could not survive the duration of the experiment. This evidently implies that only the high T/O treatments have lethal inhibitive effects to the growth of *C. odorata*. Lethality of *C. odorata* in this experiment may have been caused by the depletion of the nutrient in the soil as a result of the contaminant [12,30].

Mature leaves per plant were increased especially in the lower oil treatments, an indication of growth among the plants growing in that contaminated soil. However, higher oil concentration of 200 and 500 ppm contributed in the reduction of plant growth rate to 1.08% and 6.09% respectively within the Suzuki ammended experiment with a slight increase in MLPP in 200 ppm of oil, but withering of the plants was observed at 500 ppm of both Suzuki method as well as in direct treatment with transformer oil as a result, it could not develop more leaves. Untreated control maintained a high growth percetage increase in MLPP with values significantly different from those of T/OD and T/OS treatments. This result therefore is in aggreement with the report that exposure of plants to a concentration higher than what it can tolerate may cause chlorosis of the leave, plant dehydration, stunted growth and perhaps death [47-49], reported on the inhibition of red bean and corn by poly aromatic hydrocarbon (PAHs) content in crude oil contamination of between 10-1000 ppm in soil. Showing that phytoxicity of oil increases with the increase in the number of aromatic rings [50]. Meanwhile the multiplication of leaves by plants in the T/OS treatment is a good correlation to the fact that *C. odorata* can tolerate high concentration of oil in soil compared to the results of other scholars that have used other plants species [51,52].

In this study, mean leaf colour change was greener in untreated control, and progressively turned pale green with dark spots as the concentration of transformer oil was increased from 100-500 ppm. Leave of *C. odorata* varies in colour when it is growing in an environment that possesses growth supportive enabling nutrients. Leave colour could range from light to middle green colour [53,54]. The greener the colour of the leaf, the more supportive the nutrient are to the growth of the plants in a soil [55].

This study presented an average shoot to root ratio range of 4:1 to 13:1. This ratio is within the range observed by one of the first studies on phytoremediation using a field tobacco plants [36,37]. The study reported that plants with low concentration of organics could still extract a valuable quantity of PCBs with a large shoot biomass. Contrastingly this study could not obey the model reported by Gler [56], but proffers a higher root biomass increase which explains the reason why PCB absorption by the plant was concentrated in the root tissue. This should not be a surprise considering the tremendous improvement in science and technology between 1940 to present. The values of the percentage change in root length of the plants as used in this greenhouse experiment maintained the same trend that was reported in the growth rate and MLPP. Wiltse et al. reported an increased root biomass which was as a result of root length increase. These lead to increased surface area of the root, causing a subsequent increase in rhizosphere volume [57]. This however means that root biomass is also important indicator in organic contaminant remediation process [58]. Reduced root length resulting to low biomass increase of the root could lead to reduced rhizosphere volume and thus will have impact on the root surface of the plant towards the contaminants. Smith et al. [59] agrees that high root biomass enhances contaminant degradation. Increased shoot biomass was however suggested by Ficko et al. for optimization of phyoremediation of organic contaminants which synonymously increased the amount of the contaminant removed by the shoot tissues [9]. Such increased shoot biomass concomitantly lead to an increase in root biomass enabling the adsorption of the contaminants in the root. The progressive reduction in the measured

Table 4a. Percentage change in biomass of *C. odorata* at 100 ppm of transformer oil co-contaminated with Aroclor 1260 treatments.

Treatments/Set-up (ppm)	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T/O_D	5.69[a]	4.15[a]	1.54[a]	27.07[a]
C1	13.06[b]	1.76[a]	11.30[b]	86.52[b]
C2	NP	NP	NP	NP
Treatments/Set-up (ppm)	**Wet weight (g)**	**Dry weight (g)**	**Difference (g)**	**% change**
T/O_S	6.64[a]	3.37[a]	3.27[a]	49.25[a]
C1	13.06[b]	1.76[a]	11.30[b]	86.52[b]
C2	NP	NP	NP	NP

Values with the same alphabets in superscript in the same column were not significant at 5% level according to Bonferoni test. T/O_D=Direct Transformer Oil, T/O_S=Suzuki Transformer Oil, C1=Control 1, C2=Control 2, NP=Not Planted

Table 4b. Percentage change in biomass of *C. odorata* at 200 ppm of transformer oil co-contaminated with Aroclor 1260 treatments.

Treatments	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T/O_D	7.25[a]	4.75[a]	2.50[a]	34.48[a]
C1	92.07[b]	6.99[a]	85.08[b]	92.41[b]
C2	NP	NP	NP	NP
Treatments	**Wet weight (g)**	**Dry weight (g)**	**Difference (g)**	**% change**
T/O_S	13.57[a]	5.45[a]	8.12[a]	59.84[a]
C1	92.07[b]	6.99[a]	85.08[b]	92.41[b]
C2	NP	NP	NP	NP

Values with the same alphabets in superscript in the same column were not significant at 5% level according to Bonferoni test. T/O_D=Direct Transformer Oil, T/O_S=Suzuki Transformer Oil, C1=Control 1, C2=Control 2, NP=Not Planted

Table 4c. Percentage change in biomass of *C. odorata* at 500 ppm of transformer oil co-contaminated with Aroclor 1260 treatments.

Treatments	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T/O_D	8.95[a]	5.31[a]	3.64[a]	40.67[a]
C1	13.06[ab]	1.33[a]	11.73[b]	89.82[b]
C2	NP	NP	NP	NP
Treatments	**Wet weight (g)**	**Dry weight (g)**	**Difference (g)**	**% change**
T/O_S	18.66[a]	6.94[a]	11.72[a]	62.81[a]
C1	13.06[a]	1.33[a]	11.73[a]	89.82[b]
C2	NP	NP	NP	NP

Values with the same alphabets in superscript in the same column were not significant at 5% level according to Bonferoni test. T/O_D=Direct Transformer Oil, T/O_S=Suzuki Transformer Oil, C1=Control 1, C2=Control 2, NP=Not Planted

Table 5. PCB recovery results: Final soil PCB concentration, total PCB concentration, percentage PCB absorbed, percentage change in PCB, and PCB concentration factor

Treatments	Initial soil PCB conc. (ppm)	Final soil PCB conc. (ppm)	Total PCB in plants tissue (ppm)	% PCB absorbed	% change in PCB
T/O_D 100	6.8	2.61	--	--	61.6
C1	BD	BD	BD	BD	0
C2	6.9	5.42	NP	NP	21.4
T/O_D 200	7.1	3.32	--	--	53.2
C1	BD	BD	BD	BD	0
C2	6.3	5.27	NP	NP	16.3
T/O_D 500	6.1	3.57	--	--	41.5
C1	BD	BD	BD	BD	0
C2	6.7	5.44	NP	NP	18.8
T/O_S 100	8.9	2.02	--	--	77.3
C1	BD	BD	BD	BD	0
C2	7.3	6.08	NP	NP	16.7
T/O_S 200	7.4	1.87	--	--	74.7
C1	BD	BD	BD	BD	0
C2	7.8	5.81	NP	NP	25.5
T/O_S 500	6.5	2.68	--	--	58.8
C1	BD	BD	BD	BD	0
C2	6.4	5.05	NP	NP	21.1

Conc.=Concentration, BD=Below Detection, NP=Not Planted, RF=Remediation Factor, T/O_D=Direct Transformer Oil, T/O_S=Suzuki Transformer Oil, C1=Control 1, C2=Control 2

Table 6. PCB concentrations in different plants tissues among different concentrations of Aroclor and T/O soil treatments (values are reported as the mean of three replicates and their subsequent standard deviation).

			Root			Stem			Leaf			Total			
Treatments	Init. Soil PCB (ppm)	Residual Soil PCB (ppm)	Dry weight biom. (g)	Absd. Root PCB (ppm)	Total root PCB (ppm)	Dry weight biom. (g)	Absd. Stem PCB (ppm)	Total stem PCB (ppm)	Dry weight biom. (g)	Absd. Leaf PCB (ppm)	Total leaf PCB (ppm)	Total plant biom. (g)	Total plant PCB (ppm)	TLF	RF / BAF
T/O_D 100	6.8 ±0.24	2.61 ±0.02	1.04 ±0.12	BC	N/A	2.92 ±0.10	BC	N/A	2.30 ±0.13	BC	N/A	6.26 ±0.26	N/A	N/A	N/A
C1	BD	0	1.83 ±0.36	BC	N/A	3.72 ±0.25	BC	N/A	3.64 ±0.06	BC	N/A	9.19 ±0.70	N/A	N/A	N/A
C2	6.9 ±0.24	5.42 ±0.23	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
T/O_D 200	7.1 ±0.49	3.31 ±0.08	0.34 ±0.07	BC	N/A	2.65 ±0.08	BC	N/A	2.02 ±0.19	BC	N/A	5.01 ±0.21	N/A	N/A	N/A
C1	BD	0	1.83 ±0.36	BC	N/A	3.72 ±0.25	BC	N/A	3.64 ±0.06	BC	N/A	9.19 ±0.70	N/A	N/A	N/A
C2	6.3 ±0.00	5.27 ±0.69	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
T/O_D 500	6.1 ±0.16	3.57 ±0.02	0.20 ±0.05	BC	N/A	1.42 ±0.34	BC	N/A	1.12 ±0.25	BC	N/A	2.74 ±0.15	N/A	N/A	N/A
C1	BD	0	1.83 ±0.36	BC	N/A	3.72 ±0.25	BC	N/A	3.64 ±0.06	BC	N/A	9.19 ±0.70	N/A	N/A	N/A
C2	6.7 ±0.16	5.44 ±0.42	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
$T/0_S$ 100	8.9 ±0.33	2.02 ±0.00	6.24 ±1.24	0.10 ±0.07	0.62	3.07 ±0.08	BC	N/A	1.64 ±0.05	BC	N/A	10.95 ±0.21	N/A	N/A	0.001
C1	BD	BC	6.40 ±0.56	BC	N/A	3.83 ±0.31	BC	N/A	1.83 ±0.08	BC	N/A	12.06 ±0.07	N/A	N/A	N/A
C2	7.3 ±0.06	6.08 ±0.02	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
$T/0_S$ 200	7.4 ±0.49	1.87 ±0.06	4.77 ±0.20	0.11 ±0.07	0.52	2.36 ±0.35	BC	N/A	1.27 ±0.20	BC	N/A	8.40 ±0.14	N/A	N/A	0.001
C1	BD	BC	6.40 ±0.56	BC	N/A	3.83 ±0.31	BC	N/A	1.83 ±0.08	BC	N/A	12.06 ±0.07	N/A	N/A	N/A
C2	7.8 ±0.16	5.81 ±0.06	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
$T/0_S$ 500	6.5 ±0.08	2.68 ±0.08	0.98 ±0.10	ND	N/A	1.41 ±0.02	ND	N/A	0.81 ±0.06	ND	N/A	3.20 ±0.00	N/A	N/A	N/A
C1	BD	BC	6.40 ±0.56	BC	N/A	3.83 ±0.31	BC	N/A	1.83 ±0.08	BC	N/A	12.06 ±0.07	N/A	N/A	N/A
C2	6.4 ±0.24	5.05 ±0.65	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP

Conc.=concentration, BD=below detection, NP=not planted, RF=remediation factor, T/O_D=direct transformer oil,
T/O_S=Suzuki transformer oil, C1=control 1, C2=control, N/A=not applicable, TLF=translocation factor, RF/BAF=bioaccumulation factor

parameters of *C. odorata* grown in a soil treated with different concentrations of transformer oil co-contaminated with Aroclor 1260 should be attributed to changes in soil condition as a result of hydrophobicity of the oil which interferes with nutrient and water uptake as well as gaseous exchange [60].

Effect of different concentrations of transformer oil co-contaminated with Aroclor 1260 on the ability of *C. odorata* to retain water.

Percentage change in biomass at T/OD and T/OS was significantly different from each other and an increase in their value was observed as the concentration was increased from 100 to 200 to 500 ppm. This explains the fact that the presence of transformer oil in soil containing *C. odorata* affects transpiration ability of the plants thence affecting its physiological responses. Such increased trend was also significantly different from that of untreated control which recorded all time high of above 89%. This is synonymous with the reports of Minai-Tehrani on a study of the effect of heavy crude–oil contamination on germination and growth of Rough meadow-grass [61]. Presence of water in plants signifies presence of nutrient and these aids plants growth and replenishment [61]. High change in biomass from wet to dry is an indication of high water content in plants, hence an indication of plant grown in a growth supportive environment. Therefore, increased change in biomass is a good indicator for plants phytoremediation ability. *C. odorata* possesses good remediation ability as it has been linked with the ability to travail in oil contaminated environments [51]. Meanwhile, the presence of organic pollutant in soil is known to cause a lot of adversities to plants, a good example being that when a plant is growing in an organic contaminated soil, transpiration pull is reduced by the closure of stomatal walls reducing evaporation of water from the plants [61].

Phytoremediation ability of *C. odorata* to PCB on transformer oil contaminated soil.

In transformer oil amended soil treatments, the concentrations of PCB in different oil treatments were not phytotoxic to *C. odorata* as the plant has been shown to survive 500 ppm of PCB concentration in authors previous study hence was able to complete the growth duration of the experiment in those treatments. That is to say if the plant could manage the inhibition of the transformer oil then there is possibility of phytoremediation of the PCB content as observed in different concentration of transformer oil in the experiment [3]. There was no measurement of concentration of the oil at the end of the experiment but the influence of the oil was observed on the percentage reduction of PCB in the experiment. Percentage reduction of PCB in the experiment was high at the lower transformer oil treatment but continued to decrease as the content of oil was increased. However, percentage reduction of PCB in unplanted control experiment showed a reduced value as compared to that of oil treated and planted experiments. This shows that actual phytoremediation of PCB from the co-contamination of Aroclor in transformer oil was aided by the presence of *C. odorata*. Although *C. odorata* was able to cause reduction of PCB in this experiment, the plant was affected by phytotoxicity of the oil in the 500 ppm concentration of oil in the two treatments. This was shown by the downwards trend of the percentage reduction of PCB concentration in the residual soil as the content of the oil per kilogram of soil was increased and the low concentration of PCB traces in the root tissues of the plants in the

Suzuki treatment at both the 100 and the 200 ppm per kilogram treatment of the oil. PCB contamination between 0-260 µg/g have been reported not to be phytotoxic to various plants tested for its phytoremediation ability, but higher concentration of PCB was seen to cause stress to the plants [62,63]. The severity of transformer oil to growth of *C. odorata* throughout the duration of this experiment at higher concentration of the oil may not have been caused by co-treatment with Aroclor as the plant was found to survive at much higher PCB-concentrated soil (Authors unpublished work). This implies that it could have been caused by oil inhibition and perhaps other factors not measured.

However, total PCB concentrations found in the root tissues of *C. odorata*, ranges from 0.10 to 0.11 ppm, the values could not give any remediation factor for the plants as a result of the fact that such presence of PCB was not found in the above ground tissues of the plant. Therefore *C. odorata* only absorbed PCB at 100 and 200 ppm transformer oil per kilogram of soil in the Suzuki experiment. Such effect was not possible at 500 ppm concentrations as well as in the direct treatment of the soil with T/O. This is in agreement with the study of Pinsker [64], which reported that initial soil PCB has a great effect on the amount of PCB absorbed by plants, its translocation as well as the concentrations of the residual PCB in the soil at the end of a phytoremediation study. There was higher mean percentage reduction of PCB concentration in the entire experiment compared to the mean percentage PCB reductions of other plants species in phytoremediation studies reported in literature per unit time. However PCB reduction was also observed at the unplanted control, the reason behind such observation could be attributed to natural attenuation and perhaps other parameters not measured [65-70].

Different plant species have been reported on their ability to grow in high PCB or oil content in the soil as to be able to phytoremediate the soil containing the contaminants [71,72]. In those reports such plants were found to be able to tolerate the contaminants in soil even at high concentration and were able to grow and remediate such soil [73]. In this study, *C. odorata* was able to grow through the duration of the experiment and contributed in the reduction of soil PCB concentration to about 73% compared to unplanted control that was only reduced to about 25%. This action by the plant to such soil contamination was most obvious when the plants were prevented from direct contact with the oil because it was only at such treatment that the plant grew well except at the high concentration of the oil per kilogram of the soil. The fact that no PCB congeners were recovered at the shoot but only trace quantities at the root tissues of *C. odorata* even when there was appreciable reduction in the concentration of the contaminant is an indication of probable rhizodegradation although the resultant degradation product was not analyzed. This report is in agreement with Epuri and Sorensen that reported a complete mineralization of hexachlorobiphenyl in Aroclor 1260-contaminated soil that was planted with Tall fescue [74]. Such effects have also been elucidated by other studies [75]. However, future studies should involve the analysis of the resultant compound from the degradation of Aroclor 1260 in the presence of transformer oil by the root of *C. odorata* to enable a conclusive result to be drawn [76].

Conclusions and Recommendations

The results of this study showed that the presence of transformer oil in soil at both low and high concentration inhibits *C. odorata* growth parameters, hence reduces the ability of the plant to phytoremediate soil PCB. The presence of *C. odorata* in this experiment contributed in the remediation of the soil at low co-contamination of the oil, but the effects of the plants were negatively impacted at high co-contamination of the oil at direct contact with the plant. At such high concentration, plant growth was hampered by the oil hence reducing phytoremediation ability. *C. odorata* contributed in the reduction of soil PCB concentration at the end of the experiment. The use of soil culture Suzuki method demonstrated that by using appropriate methodology, phytoremediation of soil contaminated by transformer oil co-contaminated with Aroclor could be enhanced. Although natural attenuation was found to also act on such unaided environment, but such effect was not appreciable. Therefore soil culture phytoremediation of soil PCB contained in transformer oil using *Chromolaenana odorata* should be tried in the field. As *C. odorata* has so demonstrated the ability to withstand the inhibition of PCB co-contaminated in transformer oil as it could for other pollutants, it then present the plant as a good candidate for the remediation of PCB contained transformer oil-contaminated soil using appropriate method.

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