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Growth and phytoremediative capacity of *Eleusine indica* in a typical farmland soil under previous exposure to organochlorine pesticide

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Abstract. This study investigated the growth and phytoremediative response of *Eleusine indica* in a typical farmland soil that had been exposed to organochlorine pesticides. Different soil treatments were obtained by mixing pesticide polluted soil (P) with control soil (C) in ratios, 1P:99C, 5P:95C, 50P:50C, 25P:75C, 75P:25C, 100P and 100C. Three-leaf tillers of the test plant were sown in all the treatments for 3 months. The results revealed that there were no significant differences in all plant morphological parameters measured between plants in P impacted soils and C-soil. All the pH values were acidic, although an increase in pH and decrease in conductivity was observed with the introduction of the test plant. There was reduction in total pesticide residual (TPR) contents in the soil as a result of the plant activities. Significant reduction in q – BHC, α – chlordane and ϒ – chlordane was observed in the P1:C99 soil mix; a 90% remediation efficiency (1.663 mg kg^{-1}) was registered in the all treatments. This study thus presents *E. indica* as a potential concentration-dependent phytoremediator of pesticide, with no significant morphological changes.

Keywords: *Eleusine indica*, organochlorine Persistent organic compound.

Introduction

The pesticide industry is a major cause of environmental pollution globally, particularly in agricultural production systems. Pesticides, especially the Persistent Organic Pollutants (POPs) are inert and have been documented to leave residues in soils. They are widely studied due to their persistence, high toxicity, low biodegradability, and bio solubility in fat tissue (UNEP, 2007). The persistence and toxic nature of these pesticides has posed a serious environmental and human health risks resulting from pesticides residues discoverable in agricultural products grown using such chemicals (Olawale *et al.,* 2011). Agricultural crops have also been reported to accumulate these pesticides and relay them into food chains and water bodies (Obida *et al.,* 2012), which may likely cause harm to non-target organisms and thereby affect food security. Pesticides have also threatened the survival of less tolerance plant and animal species (Helfrich *et al.,* 2009) causing necrosis and chlorosis of leaf structures (Musa *et al.,* 2019) and human health complications (Lah, 2011).

A number of POPs such as organochlorine pesticides (OCPs) are used to control crop pests abd disease causing human pests (Lal and Saxena, 1982; UNEP, 2003; Arslan *et al.* 2015). As a result of chlorines" affinity for dispersal, transportation over long distances, bioaccumulation in the food chain and long half-life, organochlorine pesticides have been considered of great risk to the global environment and may compromise human health (Laws, 2000). They are majorly five groups of such compounds these include: Dichlorodiphenyltrichloroethane (DDT) and its analogues e.g., the Hexachlorocyclohexane (HCH) and its isomers α-HCH, β-HCH, γ-HCH, and δ-HCH; the Cyclodienes e.g., endosulfan, aldrin, endrin, dieldrin, chlordane, the Chlordecones, Murex, Kelevan and the Toxaphenes (Pope *et al.,* 1994). Its release and persistence in the environment make it to bioaccumulate and biomagnified in the body fat, liver and other organs resulting in serious complications and even death (Dewailly *et al.* 1999). Similarly, the presence of pesticides and pesticide residues in soils can cause abiotic stress symptoms in plants, such as changes in amino acid, carbohydrates and amine metabolic pathways. These changes are in most cases manifested morphologically through symptoms such as necrosis, chlorosis and low plant yield. Such responses may vary and this phenomenon may be important in selecting pesticide stress-tolerant plants (Barrett, 1998). Anoliefo *et al.* (2006, 2008) suggested that the abundance of a particular plant in a contaminated area indicates that such a plant may show tolerance to that particular contaminant, and therefore may be a likely candidate for phytoremediation of that contaminant. Phytoremediation is the biotechnological use of organisms such as plants, animal or microorganisms to take up or breakdown contaminants from the environment (McCutcheon and Schnoor 2003; Yanyu *et al.,* 2010; Khan *et al*., 2014). Previous studies by Sandra *et al.* (2015) have evaluated the effectiveness of *Ricinus communis* to reduce organochlorine pesticides residues in previously exposed soils. Some studies have also shown the use of crops and wetland plants synergistic role with bacteria in the uptake and transformation of contaminants (Abhilash *et al.,* 2011; Becerra-Castro *et al*., 2013; Miguel *et al.,* 2013).

This study aimed to as investigate growth and phytoremediative efficacy of *Eleusine indica* in farmland soil initially exposed to organochlorine pesticides. *E. indica* is regarded as an environmental tolerant plant and can withstand most biotic and abiotic stress, this justified the choice of using it as test plant. Also, the plant has been discussed by previous studies to have capabilities for remediation of hydrocarbon-polluted soils (Anoliefo *et al.,* 2006; Anoliefo *et al.,* 2008; Ikhajiagbe and Anoliefo, 2012). This research thus proposed to test its response to organochlorine. The objective of this study was to investigate growth and phytoremediative response of *Eleusine indica* in a typical farmland soil, previously exposed to organochlorine pesticides.

Materials and Methods

Study Area

A farmland, owned and managed collectively by peasant farmers, located near Ukhun village, en route Ekpoma, Edo State in Nigeria, with history of long-term organochlorine pesticide usage (Ikhajiagbe *et al.,* 2016), was selected for the study (Figure 1). Top soil (0 – 10 cm) was obtained randomly from within a marked out 50 m x 50 m plot within the farm, and transported in polybags to the Screen house at the Department of Plant Biology and Biotechnology, University of Benin, Nigeria, for study.

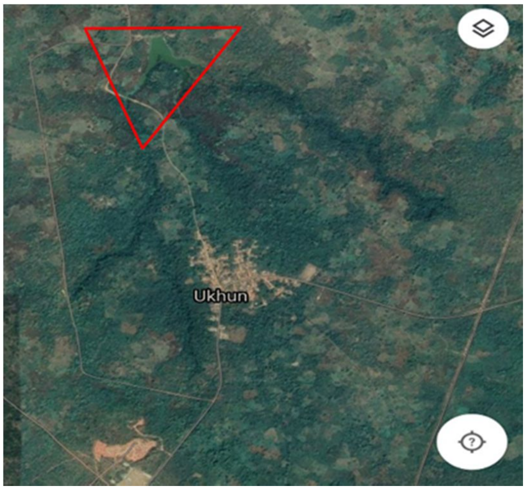


Figure 1. Study Area.

Collection and Processing of Materials

The organochlorine pesticide polluted farmland soils (P) were sun-dried to constant weight 10 kg each were put in wide experimental bowls with no perforations made at the bottom. This was deliberate so as not to lose the pesticide residues to drainage and downward infiltration. The soil was prepared in 7 treatments based on polluted soil-control soil mix. Control soil (C) was collected from the Departmental Botanic Garden, where pesticides had not been used more than 20 years based on information provided by the Chief Laboratory Technologist. Soil was mixed in the following ratios 1P:99C, 5P:95C, 50P:50C, 25P:75C and 75P:25C. 100P as well as 100C were used as both negative controls and positive controls, respectively. These treatments were made in 3 replicates in a completely randomized block design. The set was immediately sown with 4-leaf tillers of *Eleusine indica* that were originally raised in a control nursery soil. The nursery soil used as the control was a humus soil obtained at the Botanic Garden of two weeks old. The general characteristics of the tillers used is presented in Table 1.

The experiment was observed at the postgraduate research farm for 3 months with regular wetting to maintain adequate soil moisture content, relying on the Soil Moisture Feel Test proposed by USDA (1998).

Plant Analysis

Plant morphological parameters considered in this study were plant height, flag leave blade length, peduncle length, number of leaves, total number of primary roots branches, length of main root, number of tillers per plant, number of spikes per plant and length of longest spike were as proposed by Ikhajiagbe *et al.* (2019) using a transparent meter rule and counting.

Soil Sampling and Analysis

Soil samples were taken before establishing the study to assess baseline conditions; and during the study at 3 months to assess changes in residual pesticide residue concentrations. Soil pH was determined using the glass electrode procedure. Measurement was done using a pH meter (Hanna Model). Soil conductivity was determined using conductivity meter (Hanna Model) and soil temperature was obtained by using thermometer. Levels of organochlorine pesticide usage was therefore necessary for analysis of organochlorine pesticide residues in the soil before and after the test plant has been introduced. These were assessed using standard methods following ASTM D6160 – 98 (2013).

Data Analysis

Data obtained were presented in means and standard errors of three replicates. Data were analyzed following two-way analysis of variance using GENSTAT (8th edition). Where significant p-values were obtained, differences between means were separated using Student Newman Keuls test following (Ogbeibu, 2005).

Results and Discussions

Morphology of *Eleusine indica* Tillers

The average morphological parameters of tillers before transplanting (nursery stage) is presented in Table 1. Plants used for this study were within a height of13.1±5.2 cm before exposure. After exposure to different organochlorine pesticide polluted-soil mixtures, results showed no significant differences (p>0.05) in all morphological parameters of *E. indica* plants after 3 months of study (Table 2). Plant height ranged between 42.9 and 59.5 cm (p = 0.203). Likewise, plants exposed to organochlorine pesticide polluted-soil treatments showed no significant differences in peduncle length (5.3 – 7.4 cm; p = 0.632). Similarly, no significant changes in root parameters were registered (p>0.05). From this, it is clear that organochlorine pesticide has no effect on the morphology of *E. indica* and therefore, the crop can tolerate pesticide stress. For a plant to be tested for phytoremediation, it must exhibit the ability to tolerate the chemical (Sarvajeet *et al.,* 2016). Results from this study are in line with those reported by Ikhajiagbe and Anoliefo (2012), who suggested *E. indica* as a potential species for remediation of hydrocarbon pollution. However, the absence of significant changes in plant morphology even when exposed to organochlorine pesticide polluted soil disagrees with work presented by Ikhajiagbe *et al.,* 2016 and Musa *et al.,* 2019 who worked with phosphate pesticide and heavy metals respectively.

Table 1. Morphological parameters of *Eleusine indica* tillers before transplanting.

Plant parameter	Value
Plant Height (cm)	13.1±5.2
Flag leave blade length (cm)	12.6±5.1
Peduncle length	0
No of leaves	6±2
Total No of primary root branches	7±3
Length of main root	11.3±4.6
No. of tillers per plant	1
No of spike/plant	0
Length of longest spike (cm)	0

Table 2. Morphological parameters of *Eleusine indica* plants exposed to pesticide-polluted soils at 3 months.

	100C	1P:99C	5P:95C	50P:50C	25P:75C	75P:25C	100P	p-value
Plant height (cm)	59.5^{a}	49.3^{a}	48.2^{a}	42.9^{a}	51.4^{a}	48.4^{a}	51.5^{a}	0.203
Flag leave blade length (cm)	27.5^{a}	18.4^{ab}	16.4^{b}	22.5^{a}	18.5^{ab}	21.5^{a}	20.6^{ab}	0.249
Peduncle length	6.8^{b}	5.3^{b}	6.7^{b}	7.4^{b}	6.2^{b}	6.6^{b}	5.9^{b}	0.632
No of leaves	54^{b}	45^{ab}	53^{a}	49^{ab}	51^{a}	41^{b}	48^{ab}	0.391
No. of tillers per plant	9^{a}	7^{a}	8^{a}	6^{a}	6^{a}	8^{a}	7^{a}	0.258
No of spike per plant	21^{a}	16^{ab}	18^{ab}	14^{b}	18^{ab}	19^{ab}	19^{ab}	0.428
Length of longest spike (cm)	4.3^{a}	4.2^{a}	4.2^{a}	3.8^{a}	4.3^{a}	4.6^{a}	4.3^{a}	0.582
Total number of primary root branches	32^{a}	24^{a}	21^{a}	28^{a}	27^{a}	21^{a}	29^{a}	0.639
Length of main root	48.6^{a}	46.8^{a}	53.2^{a}	49.6^{a}	47.9^{a}	52.0^{a}	53.4^{a}	0.513

*Means with similar alphabetic superscripts on the same row do not differ from each other (p>0.05). P polluted farm soil; C control soil.

Pesticide Residual Concentrations

After 3 months of study, changes in pesticide residues in the soil sown with *Eleusine indica* plants were evaluated against baseline values (Table 3, Figure 3a-f). There was significant reductions in Aldrin concentrations from 0.099 to 0.004 mg kg^{-1} and total removal of d-BCH, q-BCH and decachlorobiphenyl by *Eleusine indica* across the treatments. This may be as a result of phytoaccumulation, phytotransformation and rhizoremediation which are the strategies plants use in the uptake of organochlorine pesticide (Tanvi and Dileep, 2017). The 100P soil mixture had the highest total pesticide residual content, while the 1P:99C recorded least or no pesticide residual content. The high total pesticide residual content observed in the 100P mixture may have been caused by the high POPs found in the 100P treatment affected the phytoremediative strength of the test plant. On the other hand, the low pesticide residual content observed in the 1P:99C treatment could indicate that *Eleusine indica* showed perfect premeditative activity (>90% remediation efficiency) in soil with the lowest concentration of pesticide (1P:99C). This implies that the performance of the test plant as a phytoremediator may have been concentration-dependent.

The organochlorine pesticides are taken up by plant by penetrating the roots through simple diffusion by the cell wall; and then subsequently translocated through xylem to other parts (Schroll *et al.,* 1994; Trapp and Matthies 1997). Furthermore, differences in the removal and uptake of the pesticide contents were observed across the treatments. This may be attributed to the roots of the test plant in agreement with a study be Agbeve *et al.* (2013) suggesting that organochlorine pesticide contents such as β-HCH, γ-HCH, δ-HCH, aldrin, heptachlor, α-endosulfan, β-endosulfan, γ-chlordane, dieldrin and endrin have been determined in the root tissues of *Cryptolepis sanguinolenta*.

Figure 2 shows the correspondence analyses showing relationship between treatments and pesticide composition of soil samples. The result showed that majority of the pesticide were associated with T6 (the 100% polluted farm soil), including α – chlordane, ϒ – chlordane, p, p DDD, p, p DDE, endosulfan II and Heptachlor.

Table 3. Pesticide residual composition of soils sown with *Eleusine indica* plants after 3 months.

	Baseline, NF	100C	1P:99C	5P:95C	50P:50C	25P:75C	75P:25C	100P
Aldrin	0.099	0.004	0.006	0.009	0.01	0.017	0.013	0.004
a – BHC	0.029	0.003	0.002	0.004	0.003	0.003	0.005	0.011
b – BHC	0.216	0.011	0.002	0.002	0	0	0	0.003
d – BHC	0.016	0	0.004	0.005	0	0.004	0.006	0.01
q – BHC	0.008	0	0	0.008	0.009	0.007	0.021	0.017
α – chlordane	0.014	0.012	0	0	0.006	0.01	0.004	0.01
ϒ – chlordane	0.036	0.007	0	0	0	0	0	0.018
p, p DDD	0.049	0.017	0	0	0	0	0	0.071
p, p DDE	0.101	0	0	0	0.013	0	0.007	0.048
p, p DDT	0.296	0	0	0	0	0.011	0	0.011
Dieldrin	0.061	0	0	0	0	0	0	0.039
endosulfan I	0.052	0.012	0.01	0.009	0.017	0	0	0.024
endosulfan II	0.039	0	0	0	0.006	0.017	0.013	0.074
endosulfan sulfate	0.216	0.011	0.004	0.005	0.009	0.004	0.006	0.016
Endrin	0.021	0.005	0.01	0.005	0.007	0.009	0.006	0.044
endrin aldehyde	0.039	0.008	0	0.004	0.009	0.009	0.007	0.017
Heptachlor	0.040	0.005	0.006	0.007	0.011	0.007	0.011	0.037
heptachlor epoxide	0.009	0.008	0.006	0.007	0.008	0.006	0.008	0.012
Methoxychlor	0.072	0.008	0.007	0.008	0.014	0.01	0.011	0.067
TCMX	0.006	0.007	0.005	0.007	0.024	0.195	0.022	0.005
decachlorobiphenyl	0.307	0.008	0	0	0.011	0.177	0.011	0
Total (mg/kg)	1.726	0.128	0.063	0.079	0.157	0.485	0.151	**0.570**

*P polluted farm soil; C control soil

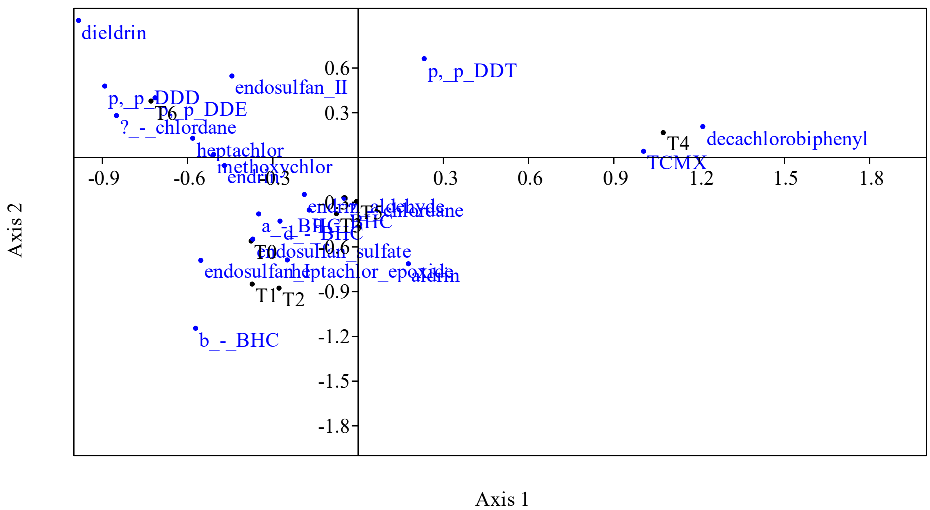
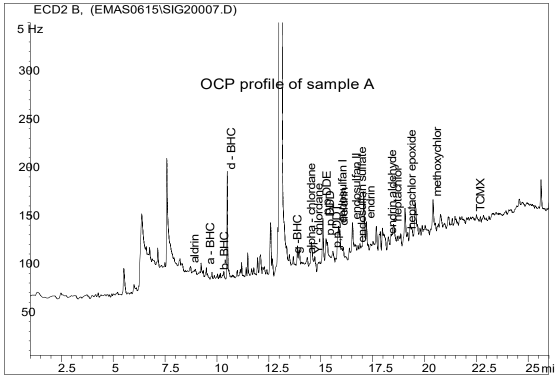
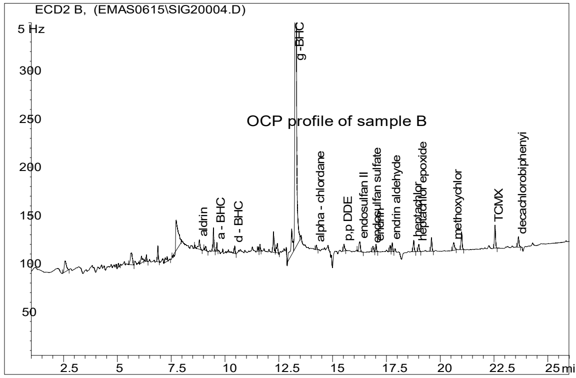


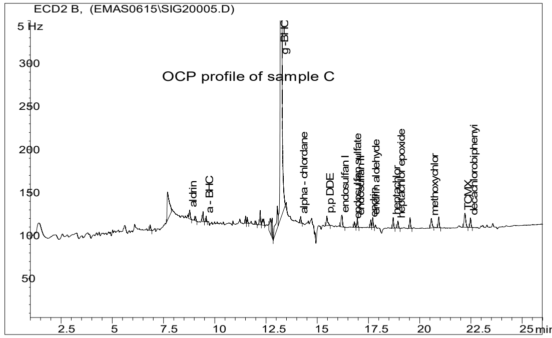
Figure 2. Correspondence analyses showing relationship between treatments and pesticide composition of soil samples.



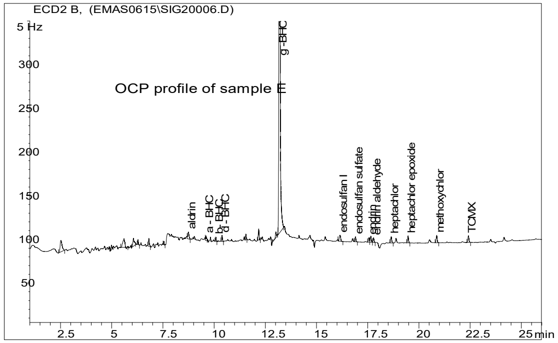
a. Chromatogram profile of 100% polluted farm soil at 3 months after exposure



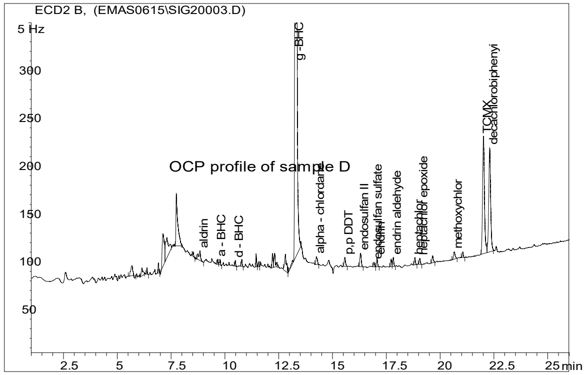
b. Chromatogram profile of polluted farm and control soil mix in the ratio of 75:25 at 3 months after exposure



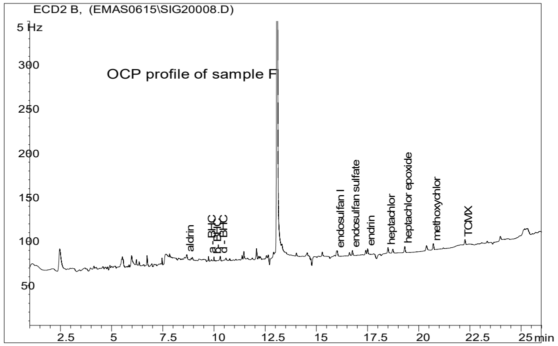
c. Chromatogram profile of 100% control soil at 3 months after exposure



d. Chromatogram profile of polluted farm and control soil mix in the ratio of 5:95 at 3 months after exposure



e. Chromatogram profile of polluted farm and control soil mix in the ratio of 75:25 at 3 months after exposure



f. Chromatogram profile of polluted farm and control soil mix in the ratio of 1:99 at 3 months after exposure

Figure 3

Soil pH and Electrical Conductivity

Figure 4 presents soil pH values recorded a day before transplanting (1DBT), 1 week after transplanting (1WAT) and 11 weeks after transplanting (11WAT). All soil mixtures were acidic, though a minimal increase in pH was observed at 1WAT of the test plant and a further increase was observed at the 11WAT in all treatments. The control soil showed the highest pH increase at 11 WAT (6.23), followed by the soil with least pesticide (1P:99C -5.92). The soil with the highest pesticide showed lowest pH (100P-4.16), showing the high acidic nature of the pesticide impacted soil (Fig. 5). Changes in soil pH have been documented by Marschner (2013) to significantly disturb plant growth and development. This may be the reason why the test plants in pesticide impacted soil, exhibited lower plant heights, peduncle length and number of leaves, compared to the control (Table 2). In present study, a pH range of 4.0-6.23 was observed, which confirms the acidic nature of the soil. pH of this range has been reported by Thompson *et al.* (2001) to impair growth of rice. This is the likely the reason why the control soil flourished better than all treatments (Figure 2).

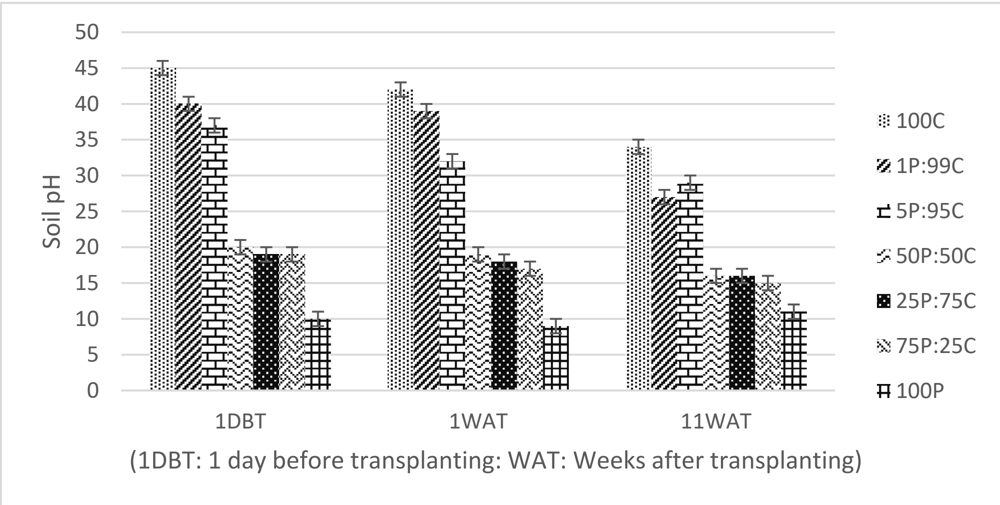


Figure 4. Changes in soil pH of the experimental treatments with the introduction of *E. Indica* seedlings.

The conductivity of the experimental soils ranged between 9-45 µscm^{-1} (Figure 5). The control soil showed greater conductivity, which reduces with the application of the test plant. This may be linked to the work of Corwin and Yemoto (2017), showing increase in pH results decrease conductivity. For an acidic soil, the lower the pH, the greater the conductivity (Ikhajiagbe *et al.,* 2019. The treatment with the least pesticide (P1:C99) showed minimum conductivity (40-27 µscm^{-1}). This may suggest the reason why the (P1:C99) treatment it have the least pesticide residue after 3 months of the experiment. This assumption agrees with the work of Abdul-Ghany *et al.* (2003).

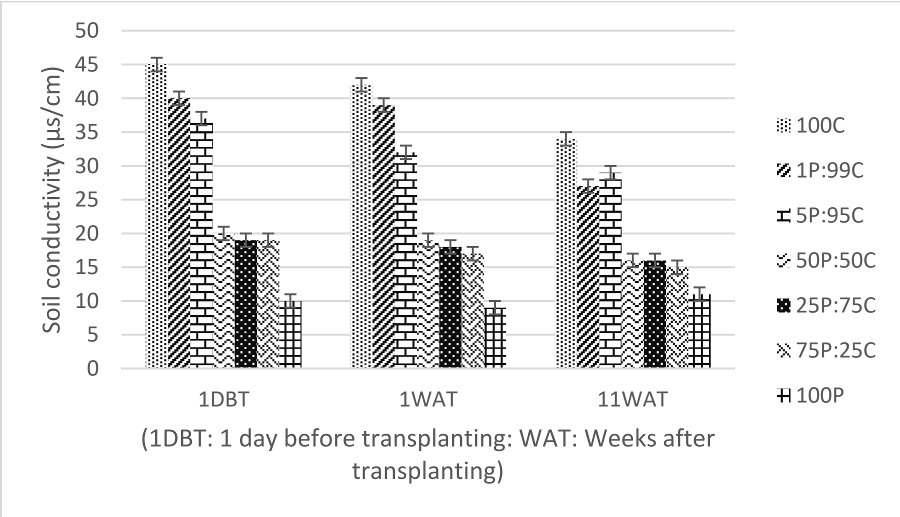


Figure 5. Changes in conductivity of pesticide-contaminated soil after the introduction of *E. indica* seedlings.

Conclusion

Pesticide impacted soils are more acidic than the control, though the pH reduces with the introduction of the test seedlings. The conductivity also reduces with increase in soil pH, although minimal conductivity is observed in P1:C99 treatment. The pesticide impacted soil does not show significant morphological response compared to the control. The soil with the least concentration of pesticide in soil showed the highest phytoremediative efficacy. This research suggests that *E. indica* has potential plant for phytoremediation of organochlorine pesticide polluted soil, though it proved to be concentration-dependent.

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