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Vermiremediation of Pesticide Contaminated Soil Using *Eudrilus euginae* and *Lumbricus terrestris*

NJOKU, Kelechi Longinus, OGWARA, Chigoze Alexandria, ADESUYI, Adeola Alex*, and AKINOLA, Modupe Olatunde

Environmental Biology Research Unit, Cell Biology and Genetics Department, University of Lagos, Akoka, Lagos, Nigeria

*Corresponding Author: biologistalex@gmail.com, knjoku@unilag.edu.ng

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Abstract

We employed vermiremediation technique in this pilot study to bioremediate dichlorvos pesticide (2, 2-dichlorovinyldimethylphosphate) contaminated soil using *Lumbricus terrestris* and *Eudrilus euginae.* The contaminated soils were analysed for DDVP contents at initial day and final day in three concentrations of 10% v/w, 20% v/w and 30% v/w using gas chromatography and mass spectrometry technique. Soil pH, moisture content and total organic matter content were also determined. The initial and the final levels of DDVP in the soil samples increased with increase in the amount of pesticide added to the soil. The activities of the earthworms resulted in decrease in pesticide level, pH, moisture content and total organic matter content. The treatment with *E. euginae* had the highest reduction of pesticide with 66.44% for 10% pesticide contamination, 72.11% for 20% contamination, and 78.34% for 30% followed by the treatment with the combination of both species with 60.12% for 10%, 66.73% for 20% and 71.96% for 30%, and the least degradation by the treatment with *L. terrestris* 52.96% for 10%, 55.82% for 20%, and 53.91% for 30% DDVP contamination. The reduction across the different concentrations of pesticide for *L. terrestris*, *E. euginae* and the combination of both earthworms treated soils were all significant ($p<0.05$). The initial and the final pH levels were not statistically different ($p>0.05$) and there was no significant difference in the moisture content of the control against the treatments ($p>0.05$) but significant at the 20 and 30% DDVP contaminated soil treated with *E. euginae* only. There was significant difference between the initial and the organic matter contents ($p<0.05$) for the all concentrations treated with *E. euginae* and in the combination of both *L. terrestris* and *E. euginae*. The results suggest that *E. euginae* has greater ability to remediate DDVP than *L. terrestris* while combining *L. terrestris* with *E. euginae* can help to improve their ability to remediate soils contaminated with DDVP.

Keyword: Vermiremediation; Pesticide; Earthworm; *E. euginae*; *L. terrestris*; Dichlorvos; DDVP

1. Introduction

Environmental pollution has become one of the world's major concerns because of the rate and extent of impairment of the ecosystems (Adesuyi *et al.*, 2015a). A great number of toxic compounds, originating mostly from industrial and agricultural activities are being released in to our environment constantly and continuously (Adesuyi *et al.*, 2015b; Njoku *et al.*, 2016a). The use of chemicals in modern agriculture has significantly increased productivity. In view of the world's limited croplands and growing population it is necessary to take all measures to increase crop production in order to ensure food safety and security (Zhang *et al.*, 2007). But it has also significantly increased the concentration of pesticides and other associated chemicals in food and in our environment, with associated negative risks and effects on human health (Anderson *et al.*, 2014; Adesuyi *et al.*, 2015b; Adesuyi *et al.*, 2016). Annually there are dozens of million cases of pesticide poisonings worldwide (Richter, 2002; Anderson *et al.*, 2014). It is now better understood that pesticides have significant chronic health effects, including cancer, neurological effects, diabetes, respiratory diseases, foetal diseases, and genetic disorders. These health effects are different depending on the degree, and the type of exposure (Greenburg *et al.*, 2008; Beseler *et al.*, 2008; Montgomery *et al.*, 2008; Slager *et al.*, 2009; Anderson *et al.*, 2014).

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest (insects, mites, nematodes, weeds, rats, etc.), including insecticide, herbicide, fungicide, and various other substances used to control pests (U.S EPA, 2007; Adesuyi *et al.*, 2018). Globally 4.6 million tons of chemical pesticides are annually sprayed into the environment. There are currently about 500 pesticides with mass applications, of which organochlorined pesticides, some herbicides and the pesticides containing mercury, arsenic and lead are highly poisonous to the environment (Zhang *et al.*, 2011). Generally, only 1% of the sprayed pesticides are effective while 99% of pesticides applied are released to non-target soils, water bodies and atmosphere, and finally absorbed by almost every organism (Zhang *et al.*, 2011). According to a report from the EPA of the United States, many of rural wells in the nation contain at least one of 127 pesticides (Wang *et al.*, 2008; Zhang *et al.*, 2011).

Dichlorvos also known as DDVP (O.-O-dimethyl-O-2, 2-dichloro-vinyl phosphate) is an organophosphate pesticide and has been applied in Nigeria as mosquito insecticides over the decades (Foll and Pant, 1966; U.S. EPA, 2007). Dichlorvos is sold under many trade names including Vapona, Atgard, Sniper, Ota pia-pia and Nuvan (ATSDR, 1997; Chedi & Aliyu, 2010). It is one of the commonly used pesticides in Nigeria. Pure dichlorvos is a dense colourless liquid that evaporates easily into the air and dissolves slightly in water (ATSDR, 1997). Dichlorvos has a sweetish smell and readily reacts with water. The dichlorvos used in pest control is diluted with other liquids and used as a spray (ATSDR, 1997). Dichlorvos is quite acutely toxic by either the oral or the dermal route, indicating good skin absorption (Gupta *et al.*, 1991). Like all organophosphates, dichlorvos exerts its toxic effects, in part, by inactivating a critical nervous system enzyme, acetylcholine esterase (AchE) (Gupta *et al.*, 1991). Acute symptoms of poisoning include: headaches, stomach cramps, excessive salivation, and muscle twitching (Gupta *et al.,* 1991; Montgomery *et al.,* 2008; Slager *et al.,* 2009).

With overwhelming evidences pesticide pose a potential risk to humans and other life forms, and unwanted side effects to the environment (Igbedioh, 1991) No segment of the population is completely protected against exposure to pesticides and the potentially serious health effects, though a disproportionate burden is shouldered by the people of developing countries and by high risk groups in each country (IARC, 1991). Insecticides are generally the most acutely toxic class of pesticides, but herbicides can also pose risks to non-target organisms (Crisp *et al.,* 1998). The global scientific community today need a remediation technology which can be 'economically viable', 'environmentally sustainable' and 'socially acceptable'; and the vermiculture technologies combines all these virtues together (Sinha *et al.,* 2010).

Vermiremediation is the use of earthworms to clean up contaminants from the soil environment (Njoku *et al.,* 2016b). It is a cheap and easy to apply technique. Earthworms are easy to obtain, they have short life cycle and are globally distributed in many types of soil (Dada *et al.,* 2016). Quite a lot of researches have been carried out on earthworms, positive roles in agro-ecosystems, environmental monitoring and sustainability (Maenpaa *et al.,* 2002; Dada *et al.,* 2016). Earthworms have been found to biodegrade contaminants like industrial, municipal and agro wastes (Datar *et al.,* 1997; Edwards, 1998; Fraser-Quick, 2002), heavy metals (Hartenstein *et al.,* 1980; Contreras-Ramos *et al.,* 2006; Dada *et al.,* 2016), organochlorine and organophosphate pesticides (Haimi *et al.,* 1992; Gevao *et al.,* 2001), petroleum and crude oil hydrocarbons (Tomoko *et al.,* 2005; Schaefer, 2005; Martin-Gil *et al.,* 2007; Njoku *et al.,* 2016b), polycyclic aromatic hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) residues in the medium in which they live (Ma *et al.,* 1995; Singer *et al.,* 2001; Sinha *et al.,* 2008).

According to Sinha *et al.* (2010), earthworms' mechanisms of action is through uptake of chemicals from the soil through passive absorption of the dissolved fraction through the moist body wall in the interstitial water and also by mouth and intestinal uptake while the soil passes through the gut. Earthworms apparently possess a number of mechanisms for uptake, immobilization and excretion of heavy metals and other chemicals. They either bio-transform or biodegrade the chemical contaminants rendering them harmless in their bodies (Sinha *et al.,* 2010). Some metals are bound by a protein called "metallothioneins" found in earthworms which has very high capacity to bind metals. The chloragogen cells in earthworms appear to mainly accumulate heavy metals absorbed by the gut and their immobilization in the small spheroidal chloragosomes and debris vesicles that the cells contain (Sinha *et al.,* 2010).

With the aforementioned issues associated with pesticides pollution, the aim of this study was to investigate the potential and effectiveness of two earthworm species, *Lumbricus terristris* and *Eudrilus euginae* in the remediation of Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) contaminated agricultural soil

2 Materials and methods

2.1 Sources of Materials

Top soil sample used for this experiment was collected within 1 to 10 cm depth from the Botanical Garden, University of Lagos, Akoka, Lagos State, Nigeria. Thereafter, the soil was sieved using a 5mm plastic mesh filter according to the ISO (1992) to remove debris and large stones. The earthworms were collected from the Botanical Garden, University of Lagos main campus, at Akoka, Lagos. The worms were kept in a holding container. Earthworms for the study were identified using methods described by Owa (1992) as *Lumbricus terrestris* and *Eudrilus euginae.* Sniper® (active ingredient: 2, 3- dichlorovinyl dimethyl phosphate, 1000g/L. manufactured by Hubei Sanonda Co. Ltd, China) used for the research was purchased from Bariga Market, Lagos state.

2.2 Experimental Set Up

The experiment was conducted inside the Environmental Biology Laboratory. 60 transparent, plastic containers (pots) with perforated lids were filled with 100 grams of soil. The containers were divided into 4 groups of 15 containers each. Each group was subdivided into 3 subgroups and each subgroup represented a three replicate of each concentrations. In order to achieve DDVP contamination of 10%, 20%, and 30% v/w, 100 g of the moistened sample soil was measured into each pot and treated with 10 ml, 20 ml, and 30 ml of DDVP pesticides. The earthworms were introduced 2 days after contamination of the soil with the pesticides. Ten (10) *Lumbricus terrestris* were introduced into each pots (15 pots) of soil contaminated with pesticides; 15 different pots were similarly introduced with same number of *Eudrilus euginae* (10); 15 different pots were inoculated with both species of *L. terrestris* and *E. euginae* (5 *Lumbricus terrestris* + 5 *Eudrilus euginae*); while another 15 pots were without the earthworms (used as control).

plate 1: *Eudrilus euginae*

plate 2: *Lumbricus terrestris*

2.3 Extraction and Determination of Dichlorvos (DDVP) in Soil

The procedure for the extraction of pesticide from soil was as described by Anastassiades *et al.* (2003). 10 g of the contaminated soil sample was weighed into an amber bottle and 20 ml of dichloromethane was added to it. The mixture were shook vigorously for 30 minutes using a mechanical shaker. The suspension was vortexed at maximum speed for 10 min. The solvent extract was recovered by centrifugation at 4000 rpm for 15 min to separate the liquid from the soil, the procedure was repeated twice. The liquid was filtered through filter paper (grade No.1, Whatman, England) using vacuum pump (VP115,Ultimate Vacuum 5pa) and then passed through 0.45 μm PTFE syringe filter before analysis by GC.

The pesticide residues in the soil samples were analyzed by Agilent GC MS 6890/5975. The instrument was equipped with MSD (Electron Impact) detector and column type of (35% phenyl methyl siloxane for MS; length 30 m; 0.32 mm, I.D. & 0,25 m film thickness; temperature was programmed at 150 °C for 1 min to 250 °C at 8 °C/min to 290 °C at 3 °C/min Isothermal 5,00 min. Transfer line temperature 280 °C & Total run time is 34 min. The carrier (Helium) gas flow rate of 2 ml/min were applied. Sample analysis was carried out by injecting (Splitless) 1 μl sample size into the GC (Ntow *et al.*, 2006). Then the peak area of the test sample is compared to that of the standard with respect to the concentration of the standard to get the concentration of the test sample (Hernandez *et al.*, 2005).

2.4 Calculation of Concentration of Test Sample

Sum peak areas of two known values are used to get the unknown concentration of test sample putting the standard concentration into the equation (Smalling and Kuivila, 2008).

$$\text{Conc.of test sample} = \frac{\text{Sum of peak area of sample} \times \text{concentration of standard}}{\text{Sum of peak area of standard}}$$

(Where standard concentration = 99.91 mg/kg, Standard peak area = 8431.7818)

2.5 Percentage of DDVP lost/ degraded in the soil

The percentage of DDVP lost/ degraded in the soil was determined using the following formula:

$$\text{Percentage loss} = \frac{\text{Initial DDVP} - \text{Final DDVP}}{\text{Initial DDVP}} \times 100\%$$

2.6 Analysis of soil physicochemical parameters

The pH of the soil samples were determined following standard methods described by Eneje and Ebomotei (2011). The total organic matter content was determined using Walkley-Black method (1934). The moisture content was determined using a method described by Skoog (2008).

2.7 Statistical Analysis

The results of from the laboratory analysis were subjected to descriptive statistics (mean and standard error of mean) and analysis of variance (two-way ANOVA) at 95% confidence level using GraphPad prism 6.0.

3 Results

3.1 Earthworm survival and their activity on the amount of DDVP in the soil (mg/kg)

There was survival and optimal physical activities (burrowing actions) of the earthworms in all the pesticide experimentally contaminated soils during this study. The total amount of pesticide in the soil were higher in day 0 (initial level) than in the final day (day 28). The initial level of DDVP in the soil samples increased with increase in the level of pesticides added to the soil. Similar trend was observed in the final levels of DDVP in soils with earthworms and control.

The treatment with *Eudrilus euginae* had the highest degradation of pesticide with 66.44 % for 10% contamination, 72.11% for 20% contamination, and 78.34% for 30% contamination followed by the treatment with the combination of both species with 60.12% for 10% contamination, 66.73% for 20% contamination and 71.96% for 30% contamination, and the least degradation by the treatment with *Lumbricus terrestris* 52.96% for 10% contamination, 55.82 % for 20% contamination, and 38.28 ± 0.07 for 30% DDVP contamination. The percentage loss of DDVP due to the activity of the earthworms decreased with the increase in quantity of pesticides added to the soil. The loss across the different concentrations of pesticides for *L. terrestris, E. euginae* and the combination of both earthworms treated soils were all significant (p<0.05).

Table 1: Impact of earthworm activity on DDVP in contaminated soils (values in bracket show the percentage losses from the initial values)

DDVP	10% Contamination	20% Contamination	30% Contamination
Initial DDVP level in soil (day 1)	67.58±0.02 mg/kg	76.41±0.03 mg/kg	83.05±0.04 mg/kg
Final DDVP level in soil without earthworm (day 28)	62.28 ± 0.05 mg/kg (7.84 %)	68.36± 0.10 mg/kg (10.53 %)	71.10± 0.03 mg/kg (14.39 %)
Final DDVP level in soil with *Lumbricus terrestris* (day 28)	31.79 ± 0.04* mg/kg (52.96 %)	33.76 ± 0.04 mg/kg (55.82 %)	38.28 ± 0.07* mg/kg (53.91 %)
Final DDVP level in soil with *Eudrilus euginae* (day 28)	22.68 ± 0.04* mg/kg (66.44 %)	21.31 ± 0.09* mg/kg (72.11 %)	17.99 ± 0.05* mg/kg (78.34 %)
Final DDVP level in soil with *Lumbricus terrestris* and *Eudrilus euginae* (day 28)	26.95 ± 0.10* mg/kg (60.12 %)	25.42 ± 0.12 mg/kg (66.73 %)	23.29 ± 0.03* mg/kg (71.96 %)

in bracket show the percentage losses from the initial values)

*significant at p<0.05

3.2 Earthworm activity on the pH of DDVP contaminated soil

The pH values in the different soil treatments are shown in Table 2. Generally, the pH at day 1 for all the treatments was higher than the final pH on day 28 for the experimental setup. For 10%, 20% and 30% DDVP contamination in soils on day 1 (without earthworms), the pH values ranged from 7.10 ± 0.04 to 7.14 ± 0.03 while on day 28, pH values ranged between 6.85 ± 0.08 and 6.93 ± 0.03 indicating a considerable decrease in the pH level of the soil. The pH of the soil with Eudrilus euginae reduced drastically (6.36 ± 0.05 - 6.47 ± 0.06) when compared with *Lumbricus terrestris* (6.72 ± 0.03 - 6.83 ± 0.02) or both organisms combined (6.49 ± 0.02 - 6.65 ± 0.04) in all treatment of contamination. The observable difference in pH was not statistically significant ($p>0.05$) in initial and final for all treatment of contaminations.

3.3 Earthworm activity on the soil moisture contents

The moisture content of the soil was observed to decrease gradually for all the contaminated soil. For all the treatment, the moisture contents in the initial were higher than the final with or without the earthworms. On the final day, treatment with *E. euginae* had the least moisture content present in the soil with 6.15 ± 0.04, 6.00 ± 0.16, 5.85 ± 0.08 for 10%, 20% and 30% pesticides contamination when compared to treatments with the combination of both species with 6.48 ± 0.04, 6.42 ± 0.02 and 6.32 ± 0.07% and *L. terrestris* with 6.62 ± 0.03, 6.57±0.04 and 6.50 ± 0.04%. Generally, the moisture content at day 0 for all the treatments was higher than the final (day 28) for all the amounts of DDVP. There was no significant difference in the moisture content of the control against the treatments ($p>0.05$) except at 20% and 30% DDVP soil contamination treated with *E. euginae* only ($p<0.05$).

Table 2: Impact of earthworm activity on pH of DDVP contaminated soils (Values in bracket show percentage changes from the initial values)

pH	10% Contamination	20% Contamination	30% Contamination
Initial pH level in soil (day 1)	7.10 ± 0.04	7.10 ± 0.08	7.14 ± 0.03
Final pH level in soil without earthworm (day 28)	6.93 ± 0.03 (2.39 %)	6.90 ± 0.08 (2.82 %)	6.85 ± 0.08 (4.06 %)
Final pH level in soil with *Lumbricus terrestris* (day 28)	6.83 ± 0.02 (3.80 %)	6.80 ± 0.04 (4.24 %)	6.72 ± 0.03 (5.88 %)
Final pH level in soil with *Eudrilus euginae* (day 28)	6.47 ± 0.06 (8.10 %)	6.44 ± 0.05 (9.30 %)	6.36 ± 0.05 (10.92 %)
Final pH level in soil with *Lumbricus terrestris* and *Eudrilus euginae* (day 28)	6.65 ± 0.04 (6.34 %)	6.62 ± 0.03 (6.76 %)	6.49 ± 0.02 (9.10 %)

Table 3: Impact of earthworm activity on moisture content of DDVP contaminated soils (values in bracket show percentage changes from the initial values)

Moisture content	10% Contamination	20% Contamination	30% Contamination
Initial moisture content in soil (day 1)	6.85 ± 0.04	6.88 ± 0.04	6.98 ± 0.03
Final moisture content in soil without earthworm (day 28)	6.77 ± 0.02 (1.17 %)	6.76 ± 0.03 (1.74 %)	6.73 ± 0.04 (3.58 %)
Final moisture content in soil with *Lumbricus terrestris* (day 28)	6.62 ± 0.03 (3.36 %)	6.57 ± 0.04 (4.51 %)	6.50 ± 0.04 (6.88 %)
Final moisture content in soil with *Eudrilus euginae* (day 28)	6.15 ± 0.04 (10.22 %)	6.00 ± 0.16* (12.79 %)	5.85 ± 0.08* (16.19 %)
Final moisture content in soil with *Lumbricus terrestris* and *Eudrilus euginae* (day 28)	6.48 ± 0.04 (5.40 %)	6.42 ± 0.02 (6.69 %)	6.32 ± 0.07 (9.46 %)

3.4 *Earthworm activity on total organic matter content (TOC) (mg/kg) in the soil*

The initial organic matter content of the soil decreased with increase in the concentration of pesticides added to the soil. The highest impact of earthworm on the organic matter content of the contaminated soil was by *Eudrilus euginae* for all the pesticides level (35.58 – 45.93%), followed by the combination of both *Lumbricus terrestris* and *Eudrilus euginae* (27.02 – 33.01%) while the least was by *Lumbricus terrestris* (16.23 - 19.13%). There was significant difference in the organic matter content ($p<0.05$) at all concentrations of the DDVP polluted soil treated with *E. euginae* and in the combination of both *Lumbricus terrestris* and *Eudrilus euginae.*

Table 4: Impact of earthworm activity on organic matter content of DDVP contaminated soils (values in bracket show percentage changes from initial values) (±SEM)

Organic matter content	10% Contamination	20% Contamination	30% Contamination
Initial organic matter content in soil (day 1)	86.52±0.02	86.48±0.04	86.41±0.32
Final organic matter content in soil without earthworm (day 28)	85.74±0.13 (0.90 %)	84.55±0.04 2.23 (2.23 %)	82.45±0.04 (4.58 %)
Final organic matter content in soil with *Lumbricus terrestris* (day 28)	72.48±0.07 (16.23 %)	70.14±0.11 (18.58 %)	69.88±0.08 (19.13 %)
Final organic matter content in soil with *Eudrilus euginae* (day 28)	55.74±0.11* (35.58 %)	53.09±0.07* (38.61 %)	46.72±0.07* (45.93 %)
Final organic matter content in soil with *Lumbricus terrestris* and *Eudrilus euginae* (day 28)	63.14±0.11* (27.02 %)	60.02±0.02* (30.60 %)	57.89±0.09* (33.01 %)

4. Discussion

The higher reduction of the dichlorvos pesticide from the soil with earthworms as compared to the soils without any earthworm indicates that ability of the earthworms to remove such from soil. The higher reduction of pesticide in the contaminated soils by *E. euginae* than *L. terretris* and a combination of both *L. terretris* and *E. euginae* than those soils without earthworms suggests that earthworms are capable of remediating soils contaminated with DDVP. Earthworms use different mechanism for soil remediation. Earthworms combine mechanical activity upon soil through abiotic system and biotic processes (burrowing, ingestion, grinding digestion and subsequent promotion of activities of microorganisms to benefit remediation processes (Njoku *et al.*, 2016b). Hence, earthworms can be directly employed within bioremediation strategies to promote biodegradation of organic contaminants because of their ability to bioturbate soils and improve their nutritional status and fertility, which are variables known to limit bioremediation (Hickman & Reid, 2008; Njoku *et al.*, 2016b).

The species of earthworms used in this study showed differential pesticide remediation capacities. This is in conformity with Sinha *et al.*, (2010) who reported that certain species of earthworms have different capabilities of degrading pesticides in soils depending on their tolerance level and bioaccumulating abilities. The higher remediation of DDVP in soil with *E. euginae* than in soil with *L. terrestris* may suggest that *E. euginae* was a better DDVP pesticide degrader than *L. terrestris*. This corroborates the observation of Sinha *et al.* (2002) in the study of the degradation and composting abilities of three species of earthworms on cattle dung, raw food wastes and garden wastes and found that the worm *Eudrilus euginae* was a better waste degrader followed by *Eisenia fetida* and *Perionyx excavates*.

The results of this study is also similar to Gevao *et al.*, (2001) who applied earthworms (*Aporrectodea longa*) at 5 worms per 2 kg of soil contaminated with non-extractable pesticides (C^{14} - isoproturon, C^{14} – dicamba and C^{14} – atrazine) residues in soil for 28 days. They found that due to earthworm physical activities (burrowing actions) a greater degree of previously bound pesticides residues in soil was released as compared to those without worms. When the study was applied to freshly added pesticides in soil, it was noted that the non-extractable residues of C^{14} - isoproturon, C^{14} – dicamba and C^{14} – atrazine were higher by factors 2, 2 and 4 respectively in the soil without worms. Thus, not only did the earthworms restrict the formation of bound fraction of pesticides, but they also enhanced the release and mineralization of bound pesticides residues.

Soil pH affects pesticide adsorption during abiotic and biotic degradation processes (Burns, 1975). In the biodegradation process, soil pH has been known to play a predominant role by influencing the sorptive behaviour of pesticide molecules on clay and organic surfaces and thus, the chemical speciation, mobility and bioavailability (Hicks *et al.*, 1990; Njoku *et al.*, 2014). The effect of soil pH on degradation of a given pesticide depends greatly on whether a compound is susceptible to alkaline or acid catalysed hydrolysis (Racke *et al.*, 1997). Dichlorvos breakdown is most rapid in moist soils with low acidity (ATSDR, 1997). However, the result of this study shows that more DDVP were lost in soil with *E. euginae* which also had the lowest pH suggesting that other physico-chemical factors apart from pH can influence the degradation or removal DDVP from soils.

Similarly, the total organic matter content and the moisture content of the soil decreased due to activities of the earthworms. Water acts as solvent for pesticides movement and diffusion and is essential for microbial functioning. Pesticide degradation is slow in dry soils. The rate of pesticide transformation generally increases with water content (Pal *et al.*, 2006). Pesticides with low water solubility tend to be more resistant to microbial degradation than compounds of higher water solubility. Microorganisms can use only the dissolved fraction of the compound in soil solution. Therefore, the rate of dissolution of pesticides would govern the rate of their biodegradation (Cork and Krueger, 1991).

Earthworms may contribute to decomposition of organic matter and nitrogen mineralization directly, by affecting the growth rates of other populations of soil organisms through grazing (e.g. negatively through reduction of the prey number or positively by reducing growth limiting factors for the soil organisms), by influencing soil moisture and aeration through soil structure, by fragmenting and redistribution of plant material and excreting nutrient rich faeces; the indirect contribution are difficult to separate (Marinissen and de Ruiter, 1993).

Soil organic matter can either decrease the microbially mediated pesticide degradation by stimulating pesticide adsorption processes or enhance microbial activity (Perucci *et al.,* 2000) by cometabolism (Nair & Schnoor, 1994; Thom *et al.,* 1997). A certain minimum level of organic matter (probably greater than 1.0%) is essential to ensure the presence of an active autochthonous microbial population that can degrade pesticides (Burns, 1975). The species diversity arising from such situation may increase the presence of sufficient number of enzyme systems that are able to attack pesticide molecules (Butcher *et al.,* 1969; Pal *et al.,* 2006).

4.1 Conclusion

Significantly, vermiremediation can lead to total improvement in the quality of agricultural soil Earthworms significantly contribute as soil conditioner by improving the physical, chemical as well as the biological properties of the soil and its nutritive value. Going by the result obtained from this study *E. euginae* and *L. terrestris* has the potential to facilitate the clean-up of Dichlorvos pesticide contaminated soil with *E. euginae* being a better remediator.

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Appendix

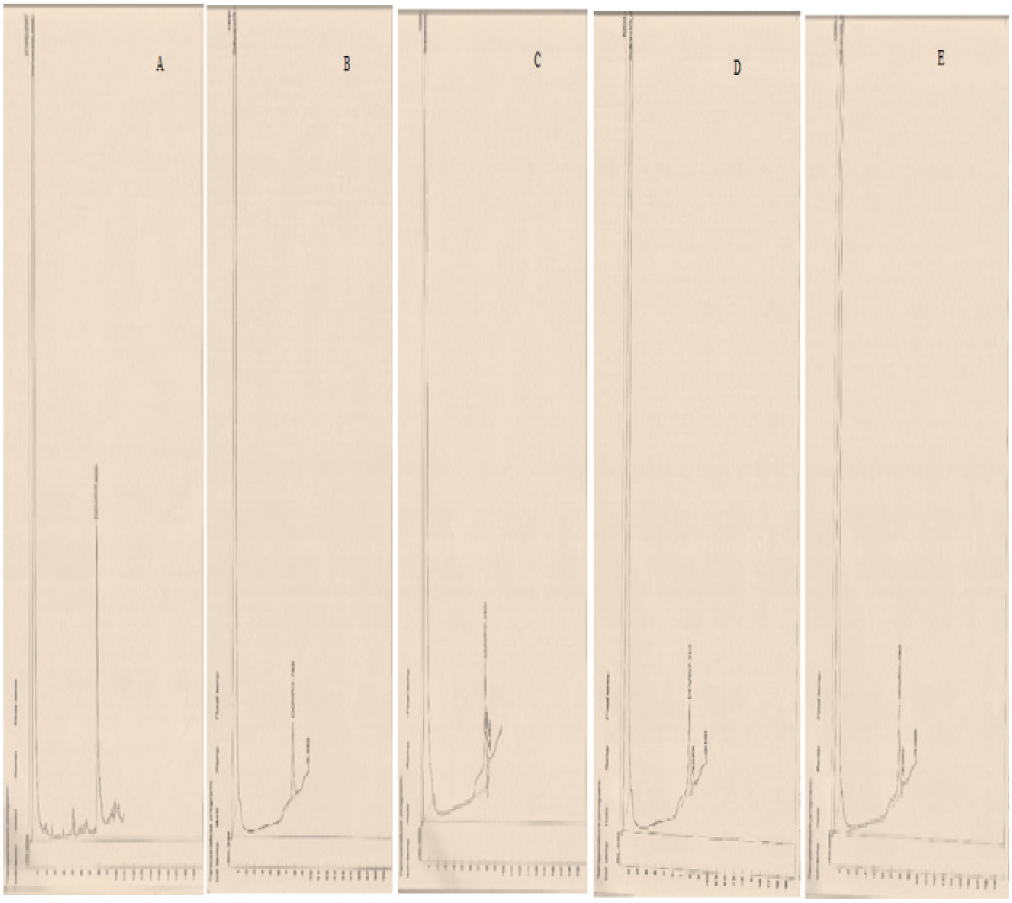

Figure a: Gas chromatogram of 20% DDVP contaminated soil: A=Initial, B= Final with *E. euginae* after 28days, C= Final with *L. terrestris,* D= Final with *L. terrestris* + *E. euginae*, E= Final without earthwworm