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Soil Micro Arthropods Recovery Rates from 0-5 cm Depth Within 5 Months Period Following Endosulfan (Organochlorine Pesticide) Treatment in Designated Plots in Benin City, Nigeria

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Abstract: The monthly soil arthropods recovery rate was monitored for five months (April to August) 2007 to ascertain whether the application of Endosulfan (an organochlorine pesticide) in varying application levels would adversely affect the rate of sampling soil arthropod groups within a 0-5 cm depth. Berlese Tullgren Extraction method, sorting and identification were adopted and some soil physiochemical parameters (soil pH, temperature, moisture and hydrocarbon content) were measured. Insects from eight different orders were consistently sampled and included members of Collembola, Coleoptera, Acarina and Isoptera. Others include Hymenoptera, Myriapoda, Crustaeca and Arachnida There was a general initial decrease in the mean number of sampled soil arthropods in the treated plots from April to May but increased from June to August. Members of Collembola, Coleoptera and Acarina showed highest fauna abundance while species from Isoptera, Hymenoptera, Myriapoda, Crustaeca and Arachnida were least in abundance. Members of Acarina (mites) exhibited the highest recovery rate while Crustacean species were least. The result revealed that, the mean number of sampled soil arthropods were significantly different ($P<0.05$) on the bases of pesticide concentration compared with the control. Except for soil pH and moisture content which showed positive correlation, soil temperature and hydrocarbon content exhibited negative correlation with mean numbers of soil arthropod sampled. The implication of this survey is that, soil micro arthropod abundance in the farm is dependent among others on the concentration of pesticide applied and where application is not indiscriminate, soil micro arthropods have high recovery rate which could enhance high productivity from the farm in the long run.

Key words: Endosulfan · Organochlorine pesticide · Soil · Microarthropod · Recovery rate · Benin City

INTRODUCTION

The soil can be referred to as a world of its own life and biodiversity, consisting of various forms of life in an endless series of interlinked caves with lots of food and stable environmental conditions like a rainforest [1]. It is a natural body, comprised of solids, liquids and gases that occur on the land surface, occupies space and is characterized by one or both of the following; horizons, or layers that are distinguishable from the initial materials as a result of additions, losses, transfer and transformations of energy and matter or the ability to support rooted plant in a natural environment [2].

Soil invertebrates have been exposed to various pesticides meant to combat pest activities in their habitats while others have been directly affected or exposed by deposition of restricted spray which in their real course missed their target organisms [3]. Reed [3] also highlighted the effect of high and low concentration of sprayed organochlorine pesticides on non target organisms i.e. soil and its invertebrates, their effect, recovery rate, level of absorption in the soil and to soil arthropods in general. The soil environment provides, a habitable place for three groups of soil organisms: water film dwellers (Protozoans, Rotifers, Targigrades), soil pore dwellers (Micro arthropods and other micro fauna species) and real soil dwellers (Earthworms and Macro arthropods) [4]. Generally, soil organisms have been classified into five major groupings widely accepted based on body size, time spent in the soil, location in the

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soil profile, feeding strategies and method of locomotion [5]. On the bases of this classification, soil fauna are generally regarded as small animals with appendages and are divided into three groups, which are micro fauna, mesofauna and macrofauna. The microfauna range in size from 0.0002-0.002 cm and of Protozoa (ciliates and testacea). They live in burrow with diameter less than 100 µm. They also live in water film on soil particle and feed on bacteria and yeast. Mesofauna ranges slightly more than 0.002-1.0 cm and includes mites (Acarina), springtail (Collembola), rotifers (Rotifera), pseudoscorpion (Arachnida), targigrades (Targigrada), insect larva (Diptera and Coleoptera), isopods and enchytraeidae. They occur predominantly in the larger pore space of diameter more than 100 µm. They feed on the micro flora and fungi. Macro fauna are at least 1cm and above. They include earthworm (Oligochaetes), some arachnids such as centipedes (class: Chilopoda), millipedes (Myriapoda), snails (Mollusca) and cicada nymphs (order: Homoptera). They occur and live in burrow with diameter more than 2-20mm. they also occur in foot canals of plants, existing cracks in the soil and degrade organic matter [5]. Hugie and Passey [6], identified the special features of soil that are fashioned by soil arthropods to include the thumb sized blocky soil pits shaped by cicada nymphs while tunneling through the soil horizon. They stated that, among the most abundant arthropods are the micro arthropods, which include the mites (Acarina), springtails (Collembola) followed by some families of insecta and then arachnids.

Soil arthropods are a vital link in the food chain as decomposer and without these organisms, nature would have no way of recycling organic material on its own [7]. The process of decomposition are controlled largely by soil arthropods in conjunction with some soil invertebrates like protozoa and worms which also contribute to the soil community by mixing, loosening and aerating the soil [8]. Arthropods also serve as the largest prey base for small predators, thus sustaining other arthropods. Without arthropods most terrestrial ecosystems would rapidly collapse. Arthropods have been able to fill every niche available in the ecosystem. They inhabit different protocols required to survey arthropods in different niches. The Berlese Tullgreen funnel instrument is one of the most frequently used in the assessment of micro soil dwelling arthropods. Hopkins [9] stated that, it is best for extracting soil microarthropods with efficiency of about 90%. The direct ecological effects of these minute arthropods include the reduction in the mass of organic matter and microbial tissue as a result of their ingestion and assimilation of such materials, their respiration and excretion which is important in influencing oxygen-carbon dioxide ratio of the soil and nutrient made available from the breakdown of faecal pellets [10]. Therefore, their secondary production turnover is very fundamental because that is the basis on which the organisms, way up the food chain are dependent upon.

The flow of energy and nutrient through the soil may be accelerated by micro arthropods grazing on micro flora, causing increased rates and biomass turnover. The grazing of the microbial flora of the soil by these soil arthropods is no longer in doubt, as well as control of their numerical strength by grazing in them, on that, the ecosystem balance is deficiently maintained within optimal limits [11]. The investigation of the role of micro arthropods in decomposing forest litter was conducted and it was found that 69% of total decomposition was as a result of micro arthropods activity [12]. Set and Bruns [13] found that soil fauna increased significantly, the levels of nitrogen and phosphorus available in the long term. Soil micro arthropods-microbial community relationship is essential in maintaining soil fertility. It becomes important when these microbes are essentially responsible for the biodegradable and remediation of heavy compound used in the manufacture of fertilizer (synthetic) containing soil pollutant. Besides, soil inhabiting microarthropods remain the primary method of spore dispersal in below ground habitat and for this reason could play important role in the distribution of fungi and bacteria i.e. microbial inoculation. Propaules may either be transported on the outside of these minute animals or are brushed off at a tract before being passed out with the faeces [14].

Many studies have found that community structure, abundance and diversity of soil micro arthropods are influenced by the availability of organic matter, substrate quality, concentrations of macro and micro nutrients and age and biodiversity of the rehabilitating habitat [15]. Environmental fate and behavior of source component (e.g. mobility, volatility and biodegradability) is affected by time and edaphic factors (e.g. soil organic matter content, moisture, temperature and pH) and biological activities and management such as tillage, nutrient addition, moisture or thermal manipulations all interact to make possible predictions of toxic concentration from gross parameters [16].

Many workers have researched into the significance of micro arthropods in the ecosystem. A number of them have also studied the effect of either insecticides or pesticides on various ecosystems. Collembolans are among abundant soil arthropods and play an important

role in decomposing grasses [17]. Frampton [18] has shown that Collembolans are vulnerable to insecticides. Chlorphyrifos is among the pesticides that have the best availability of field data for effects on soil invertebrates [19]. Soil mites (Acarina) constitute the greatest percentage of the world's arthropods living in the soil. They are truly ubiquitous and have successfully colonized nearly every known terrestrial environment [7]. Isopods generally respond quickly to environmental contamination and impact, with increase in mortality, loss of biomass and a decrease in the number of species resulting from heavy levels of population drop [20]. As a result of their relative largeness, conspicuous and their ease of alertness, Jones and Hopkin [20] stated that, they are well suited and act as indicators of heavy metal contamination in saprophagous food chain. On this basis, Janssen *et al*. [19] added that, they have been convincingly useful for monitoring of heavy metal pollution in industrialized and urbanized areas. Though, there are enormous gaps in the knowledge of soil animals, some of the soil micro arthropods have the potential of being excellent indicators of heavy metal pollution because of their relative history and limited tolerance to changes in environmental conditions [21]. Frouz explained that, the toxicity of insecticides affect soil micro arthropods by directly influencing the soil conditions, soil mixture and input of dead organic matter and indirectly influencing plant species composition. Elsevier [22] pointed out that, insecticides are lethal to collembolans and treatment with insecticide resulted in a strong decline in the density of total collembolans. Komal *et al*. [23], found from the toxicity of Endosulfan and Quinalphos that, Acarina was more sensitive to the applied insecticides compared to Collembola.

Badejo [24], has pointed that, there was an increase in the density of micro arthropods as soil moisture content increases and more mites (acarina) are generally supported in the upper layers of fermentation and litter. Accordingly, the 5 cm topsoil provided a more conducive microenvironment for orbited mites [25]. Soil mites are important component of the soil biota whose role in decomposition process is no longer a subject of controversy. The detritivorous forms which constitute a large majority of their population [26] fragment plant litter and render them readily accessible to microbial populations which subsequently release nutrients to enhance soil fertility [27]. It is in view of the numerous benefits accruing from the continual presence of soil micro arthropods to the field of Agriculture and ecosystem balance, together with the use of pesticides (organochlorine) on soil in farmland management that has prompted this investigation on the rate of recovery of soil micro arthropods within 0-5 cm depth, following treatment with organochlorine pesticide.

MATERIALS AND METHODS

Study Area: This study was carried out at the Research Field of Animal and Environmental Biology Department of the University of Benin, Ugbowo- main Campus, Benin City. It is situated on the Southern part of Nigeria (6°19'N°, 6°36' E), located in the rain forest zone of humid tropic. Benin City is characterized by both rainy and dry seasons, with rainy season and dry season lasting March to October and November to March respectively.

Sampling Sites: The investigated area is an expanse of land measuring about 10m x 10m of the study area. The study area was delineated into four stations numbered 1, 2, 3 and 4. Each station was further divided into three sub-stations marked as A, B and C thus giving a total sampling units of twelve (12). Sub-stations A, B and C represented field areas treated with 0.75L of organochlorine (Endosulfan) pesticide in 20L of water, 0.25L of organochlorine pesticide in 20L of water and 20L of water respectively. By these formulations, Sub-stations A, B and C represented field areas with high concentration of organochlorine, low concentration of organochlorine and Control respectively. The sub-stations were well delineated and marked out as presented in Fig. 1 to avoid any form of interference.

Collection and Extraction: Samples from the stations were collected with a split core sampler (5×5.7 cm). Collection of soil samples was done on fortnight basis from April to August (months). The split core sampler was first pushed into the soil by the vertical application of pressure which was used to turn the split core sampler until it reached the 5 cm mark. The obtained soil samples from the different stations and sub-stations were placed in separate black cellophanes and labeled accordingly. This was followed by their movement to the Laboratory where the multifaceted extractor (Berlese Tullgren Funnel) was adopted for the extraction process. Extraction methods were designed to suit, behaviors and body structures of the organisms [26]. A volume of 128cc of soil sample was placed on the sieve mesh size of (1mm) at the top of each funnel and the organisms collected in containers with 70% alcohol within 3 days.

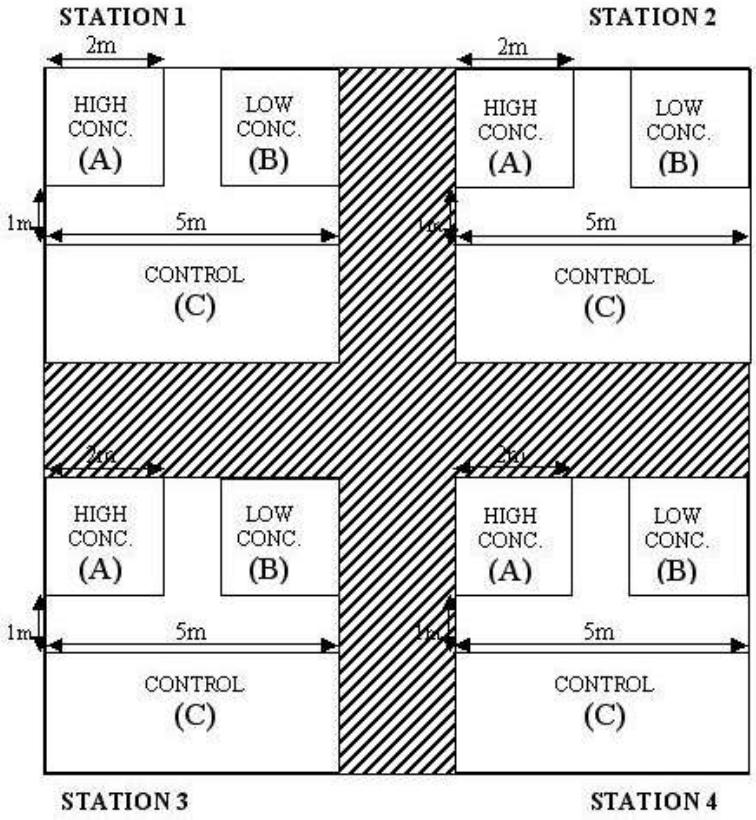

Fig. 1: Description of the experimental layout adopted

Sampling was done fortnightly between the hours of 10am-11am and 12 samples were collected at each sampling period from all stations.

Sorting and Preservation: After the organisms were extracted and collected, they were immediately sorted under a binocular dissecting microscope where individuals were removed from the lot by using a sucking pipette. Individual species were then placed in separate specimen bottles with 70% alcohol for preservation and were later mounted and used for identification.

Preparation of Slide: As result of the small sizes of organisms involved, it was necessary to mount them on slides for examination. The method of making permanent slide described by [28] was adopted to mount the organisms in Canada balsam.

Identification of Collected Soil Micro Arthropods Species: Species identification was carried out at the International Institute for Tropical Agriculture, Entomology Unit, Ibadan, Nigeria and the Prof. A.B.M. Egborge Museum of the Department of Animal and Environmental Biology, University of Benin, Benin City, Nigeria.

Measurement of Physiochemical Parameters of the Soil: Soil pH, soil temperature and soil moisture content were the parameters monitored and measured.

Soil pH: The method described by Bate [29] was adopted. 20g of air dried soil collected within the 5 cm from each station was put in a 50 ml beaker and 20 ml of distilled water was added and allowed to stand for 30 minutes. The mixture was stirred occasionally with a glass rode. The electrode of each pH meter was then inserted into partly settled suspension from each station and reading recorded. The pH meter was calibrated to 7.0, pH 4.0 before use with soil pH readings taken fortnightly.

Soil Moisture Content: 50g of soil sample each taken from within the 5 cm of the stations were weighed and placed in the oven for 24hrs till constant weights were obtained.

Initial weight of samples recorded
Final weight of sample recorded
Loss in weight =initial weight-final weight

$$\text{Soil moisture content in \%} = \frac{\text{Loss in weight}}{\text{Oven dried}} \times 100$$

The soil moisture content was also taken fortnightly along with the sampling time of other parameters.

Soil Temperature: Temperature readings were collected between 8am-9am in the morning and 5-6pm during the evening hours. Temperature reading was achieved by digging a small 5 cm deep hole and followed by tightly fitting the thermometer to the circumference of the hole before covering it. Reading on the thermometer was obtained after 2 minutes. This was repeated thrice and average value taken for both the morning and evening sampling periods.

Soil Total Hydrocarbon: The soil total hydrocarbon was determined using a spectrophotometer, pipette and 250ml separating glass funnel, mechanical shaker and n-hexane. A 5g weight of soil sampled from within the upper 0-5 cm from each site was dried and kept in bottle containers. To each bottle container was added 25 ml of n-hexane to extract the soil total hydrocarbon from the soil. These were placed on the mechanical shaker and shaken for 10 minutes to ensure thorough mixing and thereafter left to stand. A standard of n-hexane was prepared and used to standardize the spectrophotometer before introducing the THC from the soil into the spectrophotometer for the absorbance reading. The soil total hydrocarbon content (THC) concentration in part per million for each was then calculated as follows;

Soil total hydrocarbon content (ppm) =
Instrument Reading × Reciprocal of slope × 25ml/5g

Where, Instrument reading (IR) was from the spectrophotometer,

The reciprocal of slope was calculated for each based on spectrophotometer reading,

Volume of extraction reagent was 25ml,

Weight of each soil sample used was 5g.

RESULTS AND DISCUSSION

Table 1 shows the mean number of soil micro arthropod groups sampled at the different concentrations. The results obtained from this investigation revealed that different soil micro arthropods showed varying sensitivity

Table 1: Mean number of soil micro arthropod groups sampled at the different concentrations (±S.D)

Soil microarthropod groups	Conc. (pesticide vol. per 20L of water)	Mean numbers of arthropod sampled (±S.D)
Collembola	0.00	24.8±2.59[a]
	0.25	18.2±6.18[b]
	0.75	15.2±13.90[c]
Coleoptera	0.00	30.2±2.77[a]
	0.25	17.2±10.64[b]
	0.75	14.2±13.90[c]
Isoptera	0.00	10.6±3.36[a]
	0.25	5.0±2.45[b]
	0.75	4.2±2.39[c]
Hymenoptera	0.00	16.8±1.92[a]
	0.25	5.4±4.22[b]
	0.75	8.4±8.20[c]
Acarina	0.00	24.6±7.13[a]
	0.25	14.0±10.42[b]
	0.75	18.8±18.57[c]
Myriapoda	0.00	19.20±5.17[a]
	0.25	7.60±2.30[b]
	0.75	6.40±3.65[c]
Crustacea	0.00	8.8±2.49[a]
	0.25	4.4±1.67[b]
	0.75	2.4±2.07[c]
Arachnida	0.00	8.0±2.12[a]
	0.25	4.2±2.68[b]
	0.75	5.0±2.65[c]

Each value is the mean of four replicates. Means followed by the same letter are not significantly different (P>0.05) from each other, using New Duncan's Multiple Range Test

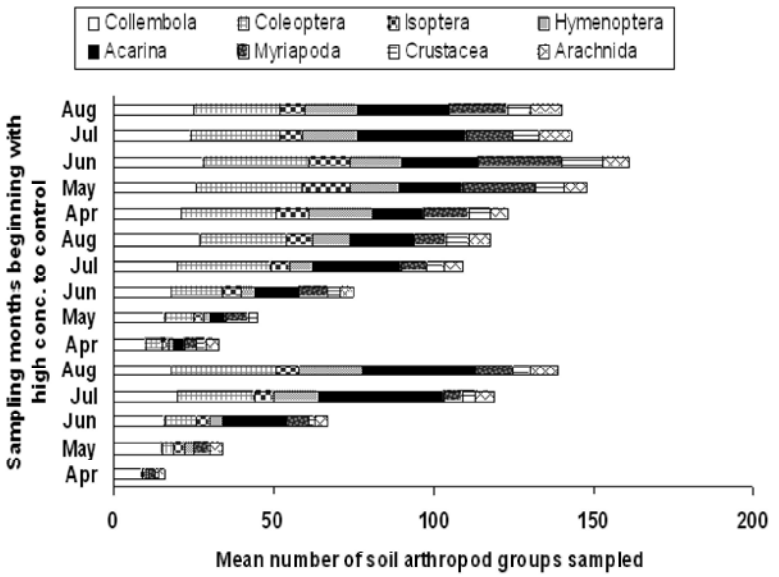

Fig. 2: Monthly mean number of soil arthropod groups sampled progressively from High concentrated stations to controlled ones

to pesticide application and the ability to recolonise differ. Irrespective of levels of contamination, arthropods species from Collembola, Coleoptera, Arachnida, Isoptera, Acarina, Crustacea and Myriapoda were sampled. Groups such as Isoptera, Hymenoptera, Acarina and Crustacea were absent during April period on station treated with 0.75 L of pesticide while station treated with 0.25 L though had members of these groups present, but in fewer numbers compared with the control station as shown in Fig. 2 and Table 1. However, it is remarkable enough from Fig. 2 that, all groups that were initially absent became present after about a month and the population of sampled soil fauna increased thereafter. This suggests that, pesticide application to an area can only temporarily affect the presence and overall population of soil micro arthropods. It also indicates that, if application is not indiscriminate, soil micro arthropods have a high potential of recovery and recolonising such an area, thus ensuring that soil fertility and ecosystem balance is not grossly affected in the long run. This initial decrease in soil arthropod number from April to June in treated stations, compared to the observed increase in the control station for the same period, may be attributable to the harsh micro environment provided by the application of endosulfan pesticide to the environment. Similar observations were made by Frouz [25], [3], [18] and Jones and Hopkin [20]. Though they were not particular on the monthly decrease or increase, they observed that, the application of pesticide affects the environmental condition, thus affecting the number of micro arthropods present in such treated areas. The number of soil arthropod present in treated areas could decrease as observed due mainly to the downward migration or death from contamination caused by harsh conditions occasioned by the application of Endosulfan.

It was also observed that, the different soil fauna encountered showed differences in abundance. Significantly ($P<0.05$), the quantity of Endosulfan pesticide contributed greatly to the abundance of soil fauna sampled. The station treated with 0.75L of the pesticide (endosulfan) recorded the least soil fauna while the control station had the highest mean number sampled as indicated in Table 1. On general fauna abundance, Collembola, Coleoptera and Acarina showed the greatest abundance while Acarina exhibited the highest recovery tendency and Crustacea showing least recovery ability as clearly shown on Fig. 2 and Table 1. These corroborated previous observations made by Trombetti and Williams [7] as well as Brown and Gange [17].

The monthly mean number of sampled soil micro arthropods from the different stations treated with varying concentrations revealed an increase in the mean number of arthropod sampled as months of sampling progressed from April to August as shown in Fig. 2 and Table 2. Though the increase was observed across the stations, including control, there is an indication that stations treated with the pesticide (endosulfan) showed quick recovery ability. This implies that, the micro arthropod shows a tendency of re-colonising an area which was previously uninhabited occasioned by pesticide

Table 2: Monthly Mean number of soil micro arthropods sampled at the different concentrations (±S.D).

Sampling Months	Conc. (pesticide vol. per 20L of water)	Mean numbers of arthropod sampled (±S.D)
April	0.00	15.38±8.25[a]
	0.25	4.13±2.59[b]
	0.75	1.04±2.93[c]
May	0.00	18.50±8.75[a]
	0.25	5.63±5.07[b]
	0.75	4.25±4.71[b]
June	0.00	20.13±8.81[a]
	0.25	9.38±5.83[b]
	0.75	8.38±6.50[b]
July	0.00	17.88±9.91[a]
	0.25	13.63±10.35[b]
	0.75	14.88±12.23[b]
August	0.00	17.50±8.77[a]
	0.25	14.75±8.65[b]
	0.75	17.38±11.48[a]

Each value is the mean of four replicates. Means followed by the same letter are not significantly different (P>0.05) from each other, using New Duncan's Multiple Range Test

application. From Table 2, it took about three months for the population of the soil micro arthropods sampled from treated areas to come to a near level term with the control station as is evident in the months of July to August while in April to June, soil micro arthropod population built-up gradually. This tendency to re-colonise after a period of decline in population following treatment with pesticide could be attributable to the phenomenon of successional order.

This survival and re-colonisation ability of individual insect groups differs. It seems that, members of the Hymenopteran, Collembolan and Isopteran groups exhibited the weakest ability in being able to withstand the application as they were observed to have drastically reduced between May and June but resurfaced strongly in July. This drastic reduction in these groups of soil fauna could be as a result of their soft body which possibly offered least protection against the toxicity of the pesticide. The soft-bodied morphology contrast those of Coleoptera, Myriapoda and Crustacea which enjoy protection based on morphological toughness and fast movement away from areas of contamination. Though the Hymenopteran, Collembolan and Isopteran groups showed least ability among others to withstand the application, they exhibited a great tendency to re-colonise with Acarina group showing the greatest tendency of re-colonisation of the treated areas while Crustacean was least as shown in Fig. 2. This fast re-colonisation may have been facilitated by two factors. It might be that, either the pesticide (endosulfan) affected the parasites that parasitise on these groups of soil fauna or the toxicity of the pesticide (endosulfan) reduced considerably as rainfall increases, thus leading to an initial rapid increase in their numbers from July to August as shown in Fig. 2.

The responses of soil micro arthropods to investigated physiochemical parameters can be deduced from Fig. 2 and 3 and Table 3. The soil temperature showed a negative correlation with all micro arthropod groups sampled throughout the investigated stations. Though, a slight temperature increase was observed from April to May, a steady decrease was thereafter recorded from June to July. This was probably due to seasonal changes from dry season to wet season. This seasonal change perhaps accounted for the increase in soil moisture observed as a result of increased rainfall. Soil moisture values and mean numbers of arthropod sampled showed positive correlation implying that sampled arthropods increased as soil moisture increased. This observation agreed with the summation of Badejo [28], who observed that the density of micro arthropods increased with increase in the soil moisture. However, when soil moisture is in excess, it could lead to the death of soil insects, thus reducing the overall population. The total hydrocarbon content obtained did not follow a regular pattern. Though the obtained values correlated negatively with the mean number of soil arthropods sampled, the stations treated with high concentration of pesticides (Endosulfan) yielded the least mean numbers of micro arthropods while the stations that served as

Table 3: Mean values of investigated parameters at the respective concentrations With their correlation values

Parameters investigated	Conc. (pesticide vol. per 20L of water)	Mean value of parameter (±S.D)	Correlation coefficient value (r)
Soil pH	0.00	6.49±0.09[a]	
	0.25	6.56±0.01[a]	0.02
	0.75	6.89±0.21[b]	0.63
Soil Temperature (°C)	0.00	27.74±1.39 NS	
	0.25	27.68±1.12NS	-0.75
	0.75	27.61±1.12 NS	-0.02
Soil Moisture Content	0.00	7.49±0.21 NS	
	0.25	7.55±0.19 NS	0.04
	0.75	7.45±0.19 NS	0.05
Total Hydrocarbon Content (THC) in (ppm)	0.00	0.03±0.01[a]	
	0.25	1.42±0.02[b]	-0.07
	0.75	0.51±.0.02[c]	-0.99

Each value is the mean of four replicates. Means followed by the same letter are not significantly different (P>0.05) from each other, using New Duncan's Multiple Range Test. *NS- Means are not significantly different (P>0.05) from one another

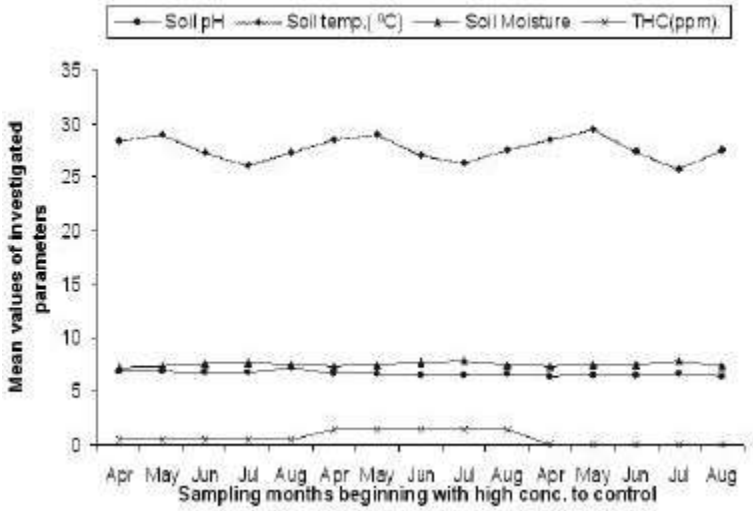

Fig. 3: Mean values of investigated physiochemical parameters at the different concentrations

control recorded the highest mean numbers of micro arthropods. The THC values recorded within the 5 cm depth seem very low and how these accounted for increase or decrease of soil fauna sampled was not quite understood. These low values of THC could be as a result of excessive leaching of the top soil occasioned by series of rainfall experienced in the area.

CONCLUSION

The application of pesticide in the control of insects and other arthropods in the soil ecosystem has been a long practice. In order to have maximum benefit from pesticide application, the chemistry of the chosen pesticide must be understood and application must follow the manufacturers' instruction, so as to avoid the incidence of indiscriminate application which could lead to soil ecosystem imbalances.

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