Vol. 19(1), pp. 26-32, January, 2020
DOI: 10.5897/AJB2019.16962
Article Number: B8961CC62662
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Bacterial and vermi-remediation of soil contaminated with chlorpyrifos insecticide

Nagla Abdelmoniem Mohamed Ahmed[1], Sulieman Hammad Nasser Ali[2], Ahmed Mohammed Ali Hammad[3], Abd Elaziz Sulieman Ahmed Ishag[3*] and Azhari Omer Abdelbagi[3]

[1]Soil and Water Resources Department, Environment and Natural Resources and Desertification Research Institute, National Center for Research, Khartoum, Sudan.
[2]Soil and Environment Sciences Department, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan.
[3]Crop Protection Department, Faculty of Agriculture, University of Khartoum, Khartoum, Sudan.

Received 2 September 2019; Accepted 10 December 2019

This study aimed at investigating the effect of consortia of three types of indigenous bacteria (*Corynebacterium* sp., *Sphingobacterium gobiense* and *Kocuria flava*) and earthworm (at 5 and 10 earthworms/kg soil) and their combination on the percentage removal of chlorpyrifos from chlorpyrifos contaminated soil in Sudan. Silt soil (Gerf soil) samples were mixed with known concentration of chlorpyrifos (450 mgkg^{-1}) and incubated for various exposure periods (3, 7, 15 and 45 days) with the bacterial consortia alone, low and high densities of earth worm and their combinations under laboratory conditions. Remaining chlorpyrifos residues were measured by gas chromatograph equipped with flame ionization detector. Degradation rates and half-lives were found to follow biphasic model with an initial fast rate of disappearance followed by a second phase of slow disappearance. All treatments caused significant ($P<0.05$) effect on the degradation rates of chlorpyrifos compared to the control. The bacterial consortia alone induced the highest effect (73.83%) on the percentage removal of chlorpyrifos, followed by the bacterial consortia plus high density of earth worm (71.22%). Earth worm alone induced the least effect on the rate degradation of chlorpyrifos (64.27 and 66.49% for the high and low concentrations respectively). Based on this finding indigenous bacterial consortia represent a promising bioremediation agent for treatment of chlorpyrifos contaminated soil and therefore may deserve further investigation under different experimental conditions as well as validation of results under real contaminated soil conditions.

Key words: Sudan, earthworm, indigenous bacteria, chlorpyrifos.

INTRODUCTION

Pesticides used in Sudan started in the late 1940s; the irrigated cotton and sugarcane schemes are the major sectors that use pesticides in Sudan. The annual consumption of pesticides in Sudan had changed over time from an average amount of 5000 Metric Ton (MT) before the 1990s to an average of 2000 to 3245 MT after that for many reasons; changes in agriculture policy, reduction in total area of production of cotton, the

*Corresponding author. E-mail: a.aziz30@gmail.com. Tel: +249915378530.

adoption of Integrated Pest Management (IPM), and other reasons (Abdelbagi et al., 2000, 2003). The poor or substandard storage facilities and management practices of pesticide stores led huge amounts of the stored pesticides to become obsolete. According to the last inventory, the total amount of the obsolete pesticides in Sudan was estimated at 666 tons. In addition there is also about 6459 cubic meters of heavily contaminated storage soil scattered over 43 major and minor sites in the country (Butrous, 1999). Horizontal and vertical movements of contaminants have been reported (Babiker, 1998; Abubaker, 2008). Until now there is no update of the previous inventory (inventory carried out in 1999) of pesticides obsolete stocks in the Sudan. However the concerned governmental departments and national experts estimated the current amounts at 1000 tons in addition to 10,000 tons of pesticides heavily contaminated storage soils. FAO with other stakeholders are currently engaged in formulating a project for the disposal of the obsolete stocks in the country (PIF, Project Identification Phase, unpublished). If such project was launched it will most likely focus on disposing of the obsolete stocks and the empty containers leaving the contaminated storage soil behind. Therefore remediation of these sites seems to be the only feasible and attractive method available. Remediation can be done by various methods: chemical, biological and photochemical. Investigations of the potential bioremediation and photodegradation methods using indigenous microbes and sunlight photolysis are underway with promising results (Elsaid et al., 2010; Elsaid and Abdelbagi, 2010; Shaer et al., 2013; Abdurruhman et al., 2015; Ishag et al., 2016, 2017; Abdelbagi et al., 2018; Ishag et al., 2019). However, it might be of relevant importance to assess other biological remediation methods (include the earthworms and bacteria) of the affected sites. Therefore, bioremediation using earthworms and consortia of bacteria should be evaluated.

One of the bioremediation technologies is the use of earthworms (Vermi-remediation). Earthworms represent a major part of the soil fauna and biomass. They live in the certain soil types and contribute fundamentally to the nutrient cycling in the soil ecosystems. Furthermore, earthworms feed on different organic materials and minerals by diffusion from interstitial water throughout the membrane followed by digestion of soil particles and organic materials in the gut and finally come up in form of casts (Shang et al., 2013). Earthworm contributes in the fate of organic pollutants in soil, improves soil aeration and water infiltration, increase microorganisms density and diversity, and enhances the nutrition status of soil (Hickman and Reid, 2008a; Lemtiri et al., 2014). The approaches for earthworm assisted bioremediation could include, direct application of earthworms into contaminated soils (Schaefer et al., 2005), co-application of earthworms to contaminated soil with other organic media such as compost (Ceccanti et al., 2006), application of contaminated media to earthworms as feeding system (Getliff et al., 2002) and indirect use of earthworms through the application of vermidigested materials (Alvarez-Bernal et al., 2006).

Based on the promising results of removal of soil contaminants by earthworms reported elsewhere (Hickman and Reid, 2008b; Gevao et al., 2001; Njoku et al., 2018) and promising results obtained with indigenous bacterial (native bacteria) biodegradation of pesticides in Sudan (Elsaid et al., 2010; Elsaid and Abdelbagi, 2010; Shaer et al., 2013; Abdurruhman et al., 2015; Ishag et al., 2016; 2017; Abdelbagi et al., 2018), it might be of interest to evaluate the combined effect of earthworms and consortia of bacteria in removal of some of the commonly reported pesticide contaminants in storage soils and hot spots in Sudan. One of the frequently reported pesticide contaminants, chlorpyrifos, was selected for the current study. Chlorpyrifos has also been registered under different trade names in Sudan with annual imports exceeding 50 tons representing 17-25% of the total annual import of pesticides in Sudan (PPD, report 2006-2010). Chlorpyrifos was reported as a major contributor to soil contamination in storage soil and hot spots in Sudan (Ishag et al., 2016, 2019). Based on the above, the current study was initiated to investigate the potential of vermi-remediation combined with consortia of indigenous bacteria in removal of chlorpyrifos from contaminated storage soils and hot spots in Sudan.

MATERIALS AND METHODS

Study area

Laboratory experiments were conducted at the Faculty of Agriculture, University of Khartoum, to study the degradation of soil contaminated with chlorpyrifos insecticide by two densities of earthworms (*Lurbicus teristris* L.) and consortia of three types of bacteria.

Chemicals and reagents

Chlorpyrifos insecticide Tricel (SL 48%) and analytical standard of chlorpyrifos (96.5% pure) obtained from Agricultural Research Corporation, Sudan were used. For extraction and GC analysis, the *n*-hexane (95% pure) and acetone (99.9% pure) obtained from Fisher company, U.K.; sodium sulfates anhydrous (99%) and sodium chloride (99.5%) purchased from Lab. Line company, Sudan were used.

Soil sampling

Soil samples were collected from the River Nile first terrace (Gerf) in Shambat area (15.6587° N, 32.5302° E), Khartoum North, Sudan. The samples were air-dried at room temperature, cleaned from debris (to avoid any interaction during chemical analysis), gently crushed, passed through a 2 mm sieve and then sterilized in an oven at 180°C for 3 h according to Ishag et al. (2019). One kilogram of sterile soil was mixed thoroughly with the chlorpyrifos as Tricel SL 48%. The properties of the native soil are illustrated in

Table 1. Some chemical and physical properties of Gerf soil.

Parameter	Value
pH	7.3
Electrical conductivity (dS m^{-1})	0.58
N (%)	0.11
P (%) as P_2O_5	0.43
K (%) as K_2O	0.13
Total organic carbon (%)	0.18
Organic matter (%)	0.31
Clay (%)	31.7
Silt (%)	53.6
Sand (%)	14.7

Table 1. The study was conducted during the period of 2018 to 2019.

Preparation of bacterial growth media

Nutrient Agar (NA) and Meat Peptone Agar (MPA) media were prepared according to the methods described by Tepper et al., (1993).

Biological materials

One liter of MPA was prepared and three quantities (250 each) were taken into three flasks, and each flask was inoculated with one strain of bacteria under study (see below) using sterilized loop. Flasks were tightly closed, shaked using a reciprocating shaker machine for 15 min at 150 rpm, and placed in an incubator at 25°C for 48 h. About 15 ml of inoculums were mixed with 30 ml of distilled water and added to the soil of each container which was pre-treated with bacteria. Earthworms were collected from along the White Nile, Jabal Awlia area (15.2286°N, 32.465260°E), Khartoum, Sudan and were kept in soil rich in organic matter for the experiment.

Bacteria used in this experiment were consortia of *Sphingobacterium gobiense, Kocuria flava,* and *Corynebacterium* sp. which were previously isolated from contaminated soils with pesticides and identified by Osman (2019) at the Pesticide Research Centre of the Department of Crop Protection, Faculty of Agriculture, University of Khartoum. Bacteria were cultured separately in a nutrient agar media and were allowed to grow in an incubator. Then a mixture of the three types of bacteria was prepared. Pre-experiment was conducted to select a concentration of chlorpyrifos not harmful for earthworm existence. Two different earthworm densities were used namely low density (5 individuals per kg soil) and high density (10 individuals per kg soil).

Experimental design

Six different treatments were designed namely: untreated control soil, consortia of native bacteria alone (mixture of *Sphingobacterium gobiense, Kocuria flava* and *Corynebacterium* sp.), earthworm low density (5 individuals per kg soil), earthworm high density (10 individuals per kg soil), mixture of bacteria and low density earthworm, and mixture of bacteria and high density earthworm. Polyethylene containers capacities of 3 kg (17 × 18 cm id) were used as containers for each treatment. One kg of silty clay loam soil artificially contaminated with chlorpyrifos was added to each container. Chlorpyrifos commercial product Tricel (SL 48%) (Equivalent to 450 mg chlorpyrifos per kilogram soil) was diluted with distilled water and thoroughly mixed with soil of each treatment. The container with their contents were wrapped with aluminum foil, and left for three days before adding the bacterial consortia and/or earthworms to protect the organisms from lethal direct effects of the insecticide. The application volume was adjusted so that it did not exceed the soil field capacity. Each treatment was replicated three times and the experimental units were arranged in completely randomized design. Soil samples were taken after 3, 7, 15 and 45 days of incubation (in thermostatic incubator, Austria at 25°C) to determine the remaining concentration of chlorpyrifos and consequently the rate of degradation.

Extraction of chlorpyrifos from the treated soil

At each sampling time, residual chlorpyrifos were determined according to the method described in AOAC-International (1996). Hundred grams of soil were taken from each treatment, placed in clean bottles (capacity 250 ml) and wetted with 30 ml of distilled water. 100 ml of solvent mixture (n-hexane: acetone mixture, 1:1 v/v) was added to each bottle. Each bottle was tightly closed and placed firmly in a rotary shaker for 3 h and then were left to stand for a while to allow the soil particles to settle down and then filtered through 24 cm WHATMAN filter paper No. 1 (Qualitative A1) in round bottom flask (capacity 500 ml). The round bottle flask and its content were placed in a rotary evaporator under vacuum at 40°C to reduce the filtrate volume to 80 ml. The content of each treatment was then transferred to separatory funnel (capacity 1000 ml) then 300 ml distilled water and 10 ml of saturated sodium chloride solution were added and shaken for one min (the cork was opened several times to release pressure) (Ishag et al., 2019). The upper organic phase layer was transferred to another 500 ml separation funnel. The aqueous phase was re-extracted with 30 ml n-hexane, and the n-hexane fraction was transferred to the 500 ml separation funnel. The combined extracts were washed twice with 100 ml of saturated sodium chloride solution (5%) which was then discarded. The organic phase was filtered through glass filter covered with 25 g of anhydrous sodium sulfate, and the solvent was evaporated by a rotary evaporator (Buchi, Postfach, Switzerland) at 40°C until dryness. The residues were reconstituted in 10 mL of n-hexane and kept in 10 ml vials, tightly closed with Teflon lined screw caps and stored in the refrigerator at 4°C for GC analysis (Ishag et al., 2019).

GC- analysis

Gas liquid chromatographic analysis was done according to Ishag

Table 2. Average concentration of chlorpyrifos (mg/kg) after incubations of soil with bacterial. consortia and earth worms.

Treatment	Incubation time (days)			
	3	7	15	45
Untreated control	430.6± 0.03^{A}	426.8± 0.08^{A}	425.5± 0.05^{A}	424.5± 0.45^{A}
Consortia of bacteria	421.8± 0.40^{B}	373.4± 1.26^{B}	342.6± 0.17^{B}	110.1± 0.06^{F}
Earthworm low density	415.5± 0.03^{C}	384.2± 0.7^{B}	252.8± 0.26^{C}	152.0± 0.1 ^{B}
Earthworm high density	374.2± 0.02^{D}	312.6± 0.26^{C}	245.6± 0.16^{D}	148.8± 0.03^{C}
Consortia of bacteria and earthworm low density	334.0± 0.20^{E}	285.6± 0.06^{D}	230.4± 0.39^{E}	147.9± 0.02^{D}
Consortia of bacteria and earthworm high density	322.3± 0.029^{F}	251.1± 0.07^{E}	229.0± 0.55^{E}	129.8± 0.81^{E}
LSD	**2.9223**	**0.3328**	**2.4654**	**0.7513**

Means followed with the same letter (s) in the same column are not significantly different (P<0.05) according to LSD= Least Significant Difference. Means± SD=Standard Deviation.

et al. (2016, 2017). The analysis was done by gas chromatography (GC) (SHIMADZU, Kyoto, Japan model GC-2010) equipped with Flame Ionization Detector (FID) and DB-5 fused silica capillary column of 30 m and 0.25 µm id. The stationary phase is 5% phenyl methyl polysiloxane, 0.25 mm thickness. The detector and injector temperatures were 300 and 280°C, respectively. Nitrogen was used as carrier gas at a flow rate of 1.13 ml minute^{-1}. The oven temperature was programmed as follows: initial temperature was 50°C and increased at 5°C minute^{-1} until 75°C, increased again at 10°C minute^{-1} until 160°C, increased by 5°C minute^{-1} until 180°C and finely increased by 3°C minute^{-1} until the final temperature of 240°C at which it was held for 2 min. Flow rates of the makeup (Nitrogen), Hydrogen and Air were 30, 40 and 400 ml minute^{-1}, respectively. Samples were analyzed in triplicates at an injection volume of 1 µl with spilt injection mode (Spilt Ratio was 2). Four concentrations 5, 50, 100 and 200 ppm of the analytical standard of chlorpyrifos (99.9% pure) were injected under the same conditions and the response was used for the construction of the standard curve. The limit of detection (LOD) was 45.278*10^{-2} mg kg^{-1}. The recovery of the method ranged from 84 to 98%.

Analysis of variance (ANOVA) was used to test if there are significant differences between treatments, and means were separated by Least Significant Difference (LSD) using SAS 9.0 for windows (SAS Institute, 2004) software. The constant rate of chlorpyrifos degradation was calculated by exponential regression analysis. A biphasic model was assumed to calculate the half-lives according to the following equation describe by Ishag et al. (2019):

$$R= A_0e^{-\alpha t}+B_0e^{-\beta t}$$

Where R = the amount of the chlorpyrifos at t days, A_0 and B_0 are concentrations of chlorpyrifos at t=0, α and β are the disappearance rate constant for first and second phase models, respectively.

The half-life of the exponential decay was calculated according to equation:

$$t_{1/2} = (2.303 \log 2)/ \text{constant rate}.$$

RESULTS AND DISCUSSION

Generally, all the treatments showed significant differences from the untreated control (Table 2). Results showed that the degradation rate increases with increasing the incubation periods. A similar finding was reported by Njoku et al. (2018) on their study of vermiremediation of dichlorvos insecticide using *Lumbricus terrestris* and *Eudrilus euginae* species of earthworm. They found that the activities of the earthworms caused a decrease in pesticide level and the total amount of pesticide in the soil was higher in the initial day than that in the final days. Further, Gevao et al. (2001) applied earthworms to the soil contaminated with non-extractable pesticides residues. They found that the physical activities of earthworms (burrowing actions) caused a release of bound pesticides residues compared to those without worms. This may be explained by the increase in microbial activity which may lead to the increase in microbial biomass (Meharg, 1996). Earthworms may enhance the interaction of soil microorganisms with burrow linings through earthworm intestine (Brown and Doube, 2004), mucus, urine, glucose, cast deposition on burrow walls, and other organic carbon sources transferred through the burrow systems. All these may promote the increase and distribution of microorganisms in earthworm burrows (Farenhorst et al., 2001) and subsequently increases the microbial biomass (Scheu, 1987). Further Meharg (1996) mentioned that increases in microbial biomass is linked to increased microbial catabolic activity. Moreover, it was reported that such increased activity, is linked with potential increases in bioavailability due to earthworm actions upon the soil within earthworm guts which could potentially increase compound losses via microbial mineralization (Gevao et al., 2001; Barois et al., 1993). Earthworms were reported by several researchers to accelerate the removal of contaminants from soil, and also facilitate and increase the contact between contaminant and soil microorganism (Hickman and Reid, 2008b).

The degradation rates by earthworm high density, Consortia of bacteria and earthworm low density and Consortia of bacteria and earthworm high density followed a biphasic model with an initial faster rate in the first phase of degradation followed by a second phase of slower rate (Table 3), while the biphasic model did not properly describe on the other two treatments (Consortia

Table 3. The half-lives of chlorpyrifos after incubation with the consortia of bacteria and earthworms (low and high densities) in soil.

Biological material\days	Half-lives (days)			
	3	7	15	45
Control	47.187^{B}	91.66^{A}	185.722^{A}	534.692^{A}
Consortia of bacteria	32.131^{C}	26.003^{C}	38.129^{B}	22.155^{F}
Earthworm low density	26.069^{D}	30.693^{B}	18.030^{C}	28.738^{B}
Earthworm high density	11.273^{E}	13.3179^{D}	17.17^{D}	28.1857^{C}
Consortia of bacteria and earthworm low density	6.975^{F}	10.672^{E}	15.531^{E}	28.032^{D}
Consortia of bacteria and earthworm high density	6.230^{A}	8.317^{F}	15.391^{F}	25.088^{E}
LSD	**0.0028**	**0.0219**	**0.0263**	**0.0043**

Means followed with the same letter (s) in the same columns are not significantly different at p = 0.05 according to LSD= Least Significant Difference.

Table 4. Mean lifetime and decay constant of chlorpyrifos after incubation with the consortia of bacteria and earthworms (low and high densities) in soil.

Biological material\Days	Mean lifetime (days)				Decay constant (d^{-1})			
	3	7	15	45	3	7	15	45
B	46.356	37.514	55.009	31.963	0.022	0.0267	0.018	0.031
W1	37.61	44.280	26.012	41.461	0.027	0.023	0.038	0.024
W2	16.264	19.214	24.771	40.664	0.061	0.052	0.040	0.024
BW1	10.064	15.396	22.407	40.442	0.099	0.065	0.0446	0.025
BW2	8.988	11.999	22.205	36.195	0.111	0.083	0.045	0.028
Control	68.077	132.244	267.94	771.398	0.015	0.007	0.003	0.001

Where; C: Untreated control; B: Consortia of bacteria; W1: Earthworm low density; W2: Earthworm high density; BW1: Consortia of bacteria and earthworm low density; BW2: Consortia of bacteria and earthworm high density.

of bacteria and Earthworm low density). This is clearly reflected in the half-life values obtained (Table 4).

This phenomenon of biphasic biodegradation in soil is common in many pesticides (Shaer et al., 2013; Abdurruhman et al., 2015; Ishag et al., 2016, 2017, 2019; Abdelbagi et al., 2018). The relative importance of the phases depends upon the availability of the pollutants,hydrophobicity, and affinity for organic matter (Rigas et al., 2007). After 45 day the removal of chlorpyrifos from the soil treated with consortia of bacteria alone exceeded 73.83%, whereas the corresponding value for mixtures of earthworms low density and/or high density with the consortia of bacteria did not exceed 71.22%. On the other hand the percentage of removal of chlorpyrifos by earthworms alone (whether high or low density) did not exceeded 66.49% (Figure 1).

Reduction in the levels of chlorpyrifos was substantial and increased with the increase in the length of incubation period. The half-lives, mean lifetimes and decay constants have shown decreased with the time approaching that is clearly manifested in the treatments of earthworm high density; Consortia of bacteria and earthworm low density; Consortia of bacteria and earthworm high density (Tables 3 and 4). Pesticides were reported to be degraded in soil by many microorganisms including *Pseudomonas aeruginosa* (Geetha and Fulekar, 2008; Shaer et al., 2013), *Sphingomonas* sp. (Li et al. 2007), Kocuria sp. (Nagavardhanam and Vihnuvardhan, 2012; Neti and Zakkula, 2013) and Corynebacterium sp. (Dhanya, 2014), B. safensis strain FO-36bT, *B. subtilis* subsp. inaquosorum strain KCTC 13429T and B. cereus strain ATCC14579T (Ishag et al., 2016; 2017). The biodegradation of chlorpyrifos by bacteria was confirmed by (Nagavardhanam and Vihnuvardhan 2012) who reported that the bacteria *Sphingomonas* sp., and *Kocuria flava* and the the bacteria *B. safensis* strain FO-36bT, *B. subtilis subsp. inaquosorum* strain KCTC 13429T and *B. cereus* strain ATCC14579T (Ishag et al., 2016, 2017) can degrade these compounds and have the ability to utilize the chlorpyrifos as source of carbon. Results indicate that treatments with bacteria have the highest degradation after 45 days of incubation compared with other treatments and this may be explained by the possible inhibitory effect of chlorpyrifos on the soil microbial population caused by the transformation of chlorpyrifos into 3, 5, 6-trichloro-2-pyridinol (TCP) which is known to have antimicrobial properties (Chu et al., 2008; Sasikala et al., 2012; Ishag et al., 2016, 2019).

Earth worms were reported to degrade or help in degradation of many soil pollutants (Hickman and Reid,

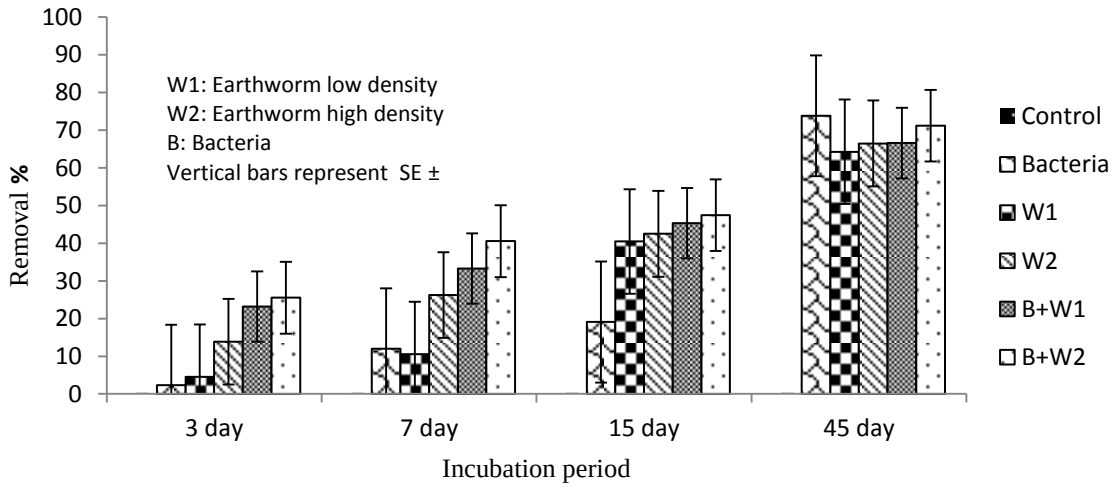

Figure 1. Removal (%) of chlorpyrifos after 45 days of incubation period over all treatments.

2008a; Lemtiri et al., 2014; Njoku et al., 2018). Earthworms can tolerate many chemical contaminants like heavy metals and organic pollutants in soil and also can accumulate them in their tissues. Earthworm species of *Eisenia fetida*, *Eisenia tetraedra*, *L. terrestris*, *L. rubellus* and *Allobophora chlorotica* were reported to remove heavy metals, pesticides and lipophilic organic micropollutants such as Polycyclic aromatic hydrocarbons from soil (Sinha et al., 2008). Microorganisms were the main cause of the biochemical degradation of organic matters by earthworm which play a critical role in this process through fragmentation of the substrates, increase in the surface area for growth of microorganisms and aeration (Aira and Dominguez, 2011). Further, partial bioremediation of polychlorinated biphenyl (PCB)-contaminated soils using bio-augmentation with PCB degrading bacteria and earthworms was reported by Luepromchai et al., (2002) who found that earthworms could facilitate PCB bioremediation by providing environmental conditions favorable for the growth and activity of indigenous PCB-degrading bacteria as well as accelerating the dispersal of PCB-degrading bacteria in bio-augmented columns (Luepromchai et al., 2002).

Kersante et al. (2006) assessed the impact of earthworm on atrazine mineralization in representative soil microsites of earthworm activities. Generally, earthworms enhance the degradation of organic compounds to about 30%, through a mechanism not clearly understood (Blouin et al., 2013).

Conclusion

(i) Both earthworms and bacteria have shown promising potential in enhancing the degradation rate of chlorpyrifos in contaminated soils and therefore may deserve further investigation and validation of results under real contaminated soil conditions.
(ii) Degradation rates and half-lives were found to follow biphasic model in three treatments; earthworm high density, Consortia of bacteria and earthworm low density and Consortia of bacteria and earthworm high density.
(iii) The bacterial consortia alone induced the highest effect on the percentage removal of chlorpyrifos, followed by the bacterial consortia plus high density of earthworms while earthworms alone induced the least effect on the rate degradation of chlorpyrifos.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the members of Shambat Central Lab for their help in GC analysis. The financial support made available by Ministry of Higher Education and Scientific Research, Sudan is highly appreciated.

REFERENCES

Abdelbagi A, Wady A, Ishag A, Hammad A, Abdalla A, Hur JH (2018). Biodegradation of fenthion and temphos in liquid media by Bacillus safensis isolated from pesticides polluted soil in the Sudan. African Journal of Biotechnology17(12):396-404. https ://doi.org/10.5897/AJB20 17.16255

Abdelbagi AO, Elmahi MA, Osman DG (2000). Chlorinated hydrocarbon insecticide residues in the Sudanese soils of limited or no pesticide use. Arab Journal of Plant Protection 18:35-39.

Abdelbagi AO, Elmahi MA, Osman DG (2003). Organochlorine insecticides residues in Sudanese soil of intensive pesticides use and in surface soil of Qurashi pesticide store. University of Khartoum Journal of Agricultural Science 11: 59-68.

Abdurruhman A, Abdelbagi A, Ahmed A (2015). Biodegradation of

pendimethalin and atrazine by *Pseudomonas pickettii* isolated from pesticides polluted soil under laboratory conditions. Journal of Biotechnology and Science Research 2(3):94-102.
Aira M, Dominguez J (2011). Earthworm Effects without Earthworms: Inoculation of Raw Organic Matter with Worm-Worked Substrates Alters Microbial Community Functioning. PLoS ONE 6(1):e16354
Alvarez-Bernal D, Garcia-Diaz EL, Contreras-Ramos SM, Dendooven L (2006). Dissipation of polycyclic aromatic hydrocarbons from soil added
with manure or vermicompost. Chemosphere Journal 65(9):1642-1651.
AOAC-International (1996). Association of Official Analytical Chemists. Official methods of analysis of the ed. Anonymous, Washington, USA.
Babiker M (1998). Levels and movement of some pesticides in Qurashi store area. Hessahisa province, central Sudan, Dissertation, University of Khartoum.
Barois I, Villemin G, Lavelle P, Toutain F (1993). Transformation of the soil structure through *Pontoscolex corethrurus* (Oligochaeta) intestinal tract. Geoderma 56:57-66
Bloui M, Hodson ME, Delgado EA, Baker G, Brussaurd L, Butt, KR, Dai J, Dendooven L, Peres G, Tondoh JE, Cluzeau D, Brun JJ (2013). A review of earthworm impact on soil function and ecosystem services. European Journal of Soil Science 64:161-182.
Butrous MG (1999). Evaluation and assessment of obsolete and banned pesticides in five agricultural schemes. Report, pp. 83-89, Sudan.
Ceccanti B, Masciandaro G, Garcia C, Macci C, Doni S (2006). Soil bioremediation: combination of earthworms and compost for the ecological remediation of a hydrocarbon polluted soil. Water Air and Soil Pollution Journal 177:383-397.
Chu X, Fang H, Pan X, Wang X, Shan M, Feng B, Yu Y (2008). Degradation of chlorpyrifos alone and in combination with chlorothalonil and their effects on soil microbial populations. Journal of Environmental Science 20(4):464-469.
Dhanya MS (2014). Advances in microbial biodegradation of chlorpyrifos. Journal of Environmental Resources and Development 9(1):232-240
Elsaid OG, Abdelbagi AO (2010). Comparative biodegradation of endosulfan by mutant and their native microorganisms. Research Journal of Agricultural Biological Science 6(6): 953-961.
Elsaid OG, Abdelbagi AO, Elmustafa EA (2010). Microbial Degradation of Endosulfan in Carbon Free Media and Selective Media. Research Journal of Agricultural Biological Science 6(3):257-562.
Farenhorst A, Topp E, Bowman BT, Tomlin AD, Bryan RB (2001). Sorption of atrazine and metolachlor by burrow linings developed in soils with different crop residues at the surface. Journal of Environmental Science and Health, Part B 36 (4):389-396.
Geetha M, Fulekar H (2008). Bioremediation of chlorpyrifos by *Pseudomonas aeruginosa* using scale up technique. Journal of Applied Bioscience 12:657-660.
Getliff J, Mc Ewan G, Ross S, Richards R, Norman M (2002). Drilling fluid design and the use of vermiculture for the remediation of drill cuttings. AADE 2002 Technology Conference “Drilling Fluids and Waste Management” 2002.
Gevao B, Mordaunt C, Semple KT, Piearce TG, Jones KC (2001). Bioavailability of Nonextractable (Bound) Pesticide Residues to Earthworms. Environmental Science and Technology 35(3):501-507.
Hickman ZA, Reid BJ (2008a). Earthworm assisted bioremediation of organic contaminants. Environment International 34(7):1072-1081.
Hickman ZA, Reid BJ (2008b). Increased microbial catabolic activity in diesel contaminated soil following addition of earthworms (*Dendrobaena veneta*) and compost. Soil Biology and Biochemistry Journal 40(12):2970-2976.
Ishag A, Abdelbagi A, Hammad A, Elsheikh A, Elsaid O, Hur J (2017). Biodegradation of endosulfan and pendimethalin by three strains of bacteria isolated from pesticides-polluted soils in the Sudan. Applied Biological Chemistry 60(3):287-297.
Ishag A, Abdelbagi A, Hammad A, Elsheikh A, Elsaid O, Hur J, Laing M (2016). Biodegradation of Chlorpyrifos, Malathion, and Dimethoate by Three Strains of Bacteria Isolated from Pesticide-Polluted Soils in Sudan. Journal of Agriculture and Food Chemistry *64*(45):8491-8498.
Ishag A, Abdelbagi A, Hammad A, Elsheikh E, Elsaid O, Hur JH (2019). Photodegradation of Chlorpyrifos, Malathion, and Dimethoate by Sunlight in the Sudan. Environmental Earth Sciences 78(3:1-14
Lemtiri A, Colinet G, Alabi T, Cluzeau D, Zirbes L, Haubruge É, Francis F (2014). Impacts of earthworms on soil components and dynamics. Biotechnology, Agronomy, Society and Environment 18(1):121-133.
Li XH, He J, Li SP (2007). Isolation of a chlorpyrifos-degrading bacterium, *Sphingomonas* sp. strain Dsp-2 and cloning of the mpd gene. Research in Microbiology 158(2):143-149.
Luepromchai E, Singer AC, Yang CH, Crowley DE (2002). Interactions of earthworms with indigenous and bioaugmented PCB-degrading bacteria. FEMS Microbiology Ecology 41(3):191-197.
Meharg AA (1996). Bioavailability of atrazine to soil microbes in the presence of the earthworm *Lumbricus terrestrius*. Soil Biology and Biochemistry 28(4):555-559.
Nagavardhanam N, Vishnuvardhan Z (2012). Isolation, screening and identification of Organophosphate pesticide degrading Bacterium, Kocuria sp. Asian Journal of Biological and Life Sciences 1:204-207. https://www.ajbls.com/article/2012/1/3/204-207**.**
Neti N, Zakkula V (2013). In silico Structural Analysis and Binding of Organophosphorus hydrolase of Kocuria sp with Chloropyrifos. International Journal of Computer Applications 66(5):8875-8887.
Njoku KL, Ogwara CA, Adesuyi AA, Akinola MO (2018). Vermiremediation of Pesticide Contaminated Soil Using *Eudrilus euginae* and *Lumbricus terrestris*. Environment Asia 11(3):133-147.
Osman AMA (2019). Biodegradation of DDA, Diazinon, Oxyflourfen and Profenofos by Four Indigenous Types of Bacteria Isolated from Pesticides Polluted Storages Soils in the Sudan. M.Sc. Thesis, University of Khartoum.
Plant Protection Directorate (PPD). Annual Report of Pesticides Import Statistics, Khartoum north, Sudan, 2006−2010.
Rigas F, Papadopoulou K, Dritsa V (2007). Bioremediation of a soil contaminated by lindane utilizing the fungus Ganoderma austral via response surface methodology. Journal of Hazardous Materials 140(1-2):325-32.
SAS Institute (2004). System 2000® Software: Product Support Manual Software. Version 1, First Edition. SAS Institute Inc., Cary, NC, USA.
Sasikala C, Jiwal S, Rout P, Ramya M. (2012). Biodegradation of chlorpyrifos by bacterial consortium isolated from agriculture soil. World Journal of Microbiology and Biotechnology 28(3):1301-1308.
Schaefer M, Petersen SO, Filser J (2005). Effects of *Lumbricus terrestris*, *Allolobophora chlorotica* and *Eisenia fetida* on microbial community dynamics in oil-contaminated soil. Soil Biology and Biochemistry 37(11):2065-2076.
Scheu S (1987). Microbial activity and nutrient dynamics in earthworm casts (Lumbricidae). Biology and Fertility of Soils 5:230-234.
Shaer I, Abdelbagi A, Elmustafa A, Ahmed A, Osama G (2013). Biodegradation of pendimethalin by three strains of bacteria isolated from pesticides polluted soils. University of Khartoum Journal of Agricultural Science 21(2):233-252.
Shang H, Wang P, Wang T, Wang Y, Zhang H, Fu J, Ren D, Chen W, Zhang Q,Jiang G (2013). Bioaccumulation of $PCDLF_S$, PCB_S, and $PBDE_S$ by earthworms in field soils of an E-waste dismantling area in China. Environment International 54:50-58.
Sinha RK, Bharambe G, Ryan D (2008). Converting wasteland into wonderland by earthworms-a low-cost nature’s technology for soil remediation: a case study of vermiremediation of PAHs contaminated soil. Springer Science+Business Media.
Tepper EZ, Shilnikova VK, Perverzeva GI (1993). A Manual of Microbiology. Kolas publishers, Moscow P170.