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Original Research Article

Degradation of 2, 2-Dichlorovinyl Dimethyl Phosphate and N-Phosphoromethyl (1) Glycine by Microorganisms Isolated from Pesticide-Polluted Cassava Farm Soils

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Abstract

Microorganisms isolated from pesticide-contaminated soils using pour-plate method were investigated for their ability to degrade dichlorvos and glyphosate pesticides. The initial concentrations of the insecticides in the parent stock were determined by spectrophotometry. The organisms isolated from the contaminated soils included Bacillus sp, Arthrobacter sp, Klebsiella sp, Chromobacterium sp.; Micrococcus sp. And Staphylococcus sp. The fungi were isolated as Aspergillus niger, Trichoderma sp, Penicillium sp. and Rhizopus sp. Of all the isolates, only Bacillus sp, Proteus sp and Aspergillus sp were able to grow on the media incorporated with the pesticide. The pattern of degradation on the dichlorvos was similar for all the microbial treatments with the percentage degradation ranging from 98.87% to and 99.25%. The effects of the different treatments were significantly different. However, the treatments on glyphosate showed varying degrees of degradation ranging from 21% degradation to 81.92%. There was no significant difference in the degradation after 8 weeks and after 12 weeks for the dichlorvos treatment. The treatments on the glyphosate showed a more efficient degradation after the 12th week than after the 8th week hence prolonged time enhanced the degradability. The tested microbial treatments with the isolates except Proteus sp. showed excellent potential for biodegradation of dichlorvos and glyphosate pesticides hence they may be candidates for biodegradation or removal of these pesticides from the soil

Keywords: Pesticides, dichlorvos, glyphosate, degradation, microorganisms, cassava, soil

Introduction

Cassava (*Manihot esculenta*. Crantz) is extensively cultivated as an annual crop in tropical and sub-tropical regions for its edible starchy tuberous root, a major source of carbohydrate, $(CHO)_n$. It is the third largest source of $(CHO)_n$. in the tropics after rice and maize (Adekanye *et al*., 2013). It is a major staple food in the developing world, producing a basic diet for over half a billion people. It is one of most drought-tolerant crops, capable of growing on marginal soils. Nigeria is

the world's largest producer of cassava while Thailand is the largest exporter of dried cassava. In Africa, the cassava mealybug (*Phenacoccus manihot*) and cassava green mills (*Mononydiellus tanajoa*) can cause up to 80% crop loss which is extremely detrimental to the subsistence farmers. (Adekanye *et al*., 2013)

Cassava mosaic virus causes the leaves to wither thereby limiting the growth of the root. It is spread by the whitefly and by the transplanting of the diseased plants into new fields. Brown steak disease has been identified as a major threat to cassava cultivation worldwide. A wide range of plant nematodes have also been associated with cassava worldwide.

Nigeria is noted to be one of the largest producers of cassava in the world with an estimated 45 million tons produced as at 2009 (Adekanye *et al.*, 2013). To ensure optimal crop yield and reduce losses during cultivation, various farming inputs are used. One of such inputs is the application of pesticides (Miles *et al*., 1980; Damuralas, 2009). Pesticides reaching the soil in significant quantities or accumulation of residual pesticides even when applied slightly have direct effect on the soil which, in turn, influences plant growth. Some of the most important effects of these pesticides are alterations in the ecological balance of microflora, permanent changes in the soil microflora, adverse effects on soil fertility and crop productivity, and alterations in the nitrogen balance of the soil. Organophosphorus and dichlorvos pesticides take about 1-2 years to degrade normally in the soil. When they accumulate in the soil within this periodic, they have adverse effects on the soil and the inhabitants of the soil, and consequently on crop yield. Sometimes, these pesticides percolate the soil and pollute water bodies thereby affecting aquatic organisms, man and other animals that make use of this water. Recent researches have shown that some soil microorganisms can degrade the pesticides fast in less than a year.

The persistence of pesticides in soils is of great importance in relation to pest management and environmental pollution (Ijah and Ndana, 2003). Persistence of pesticides in the soil for a long period of time is undesirable because accumulation of chemicals in soil to highly toxic levels, may lead to their assimilation by plants, and their presence in the edible products. The may also be eroded with soil particles and may enter into water, streams and finally, lead to soil, water and air pollution, cause changes in the soil microflora and may constitute environmental hazards, and cause public health problems (Gilden *et al*., 2010; Damalas, 2009).

The persistence of pesticides in soils varies from weeks to several years depending upon the structure and properties of the constituents in the pesticides, availability of moisture in the soil, microorganisms present in the soil, temperature, soil pH and nutrient availability (Arias-Estevez and Garcia-Rio, 2008). Organophosphorus and dichlorvos pesticides take about 1-2 years while chlorinated hydrocarbon insecticides are known to persist for at least 4-5 years, and sometimes more than 20 years (Karpouzas and Sing, 2006).

Glyphosate and dichlorvos are used extensively in cassava farming and have been known to be persistent in the soil where they can lead to accumulation of residue in the soil which may result

into the increased absorption of such toxic chemicals by plants to the level at which the composition may prove deleterious/hazardous to human as well as livestock health (Kuzmin *et al*., 2005). Some of these chemicals have entered into the food chain at highly inadmissible levels, leading to food contamination and health hazards (Wauchope *et al*., 1992).

Pesticides reaching the soil are acted upon by several physical, chemical and biological forces. While physical and chemical forces act upon or degrade pesticides to some extent, microorganisms play a major role in the degradation of pesticides (Chen *et al*., 2001; Millioli *et al*., 2009). Many soil microorganisms have the ability to act upon pesticides and convert them into simpler non-toxic forms. Metabolic activities of bacteria, fungi and actinomycetes play significant roles in the degradation of pesticides (Mallick *et al.* (1999). Maila and Cloete (2004) stated that for every naturally occurring compound, there is a microbe or enzyme system capable of its degradation. Some pesticides such as the organophosphates are degraded by microorganisms and used as phosphorus and/or carbon source.

The degradation of pesticides such as organophosphorus, dichlorovinyl dimethyl phosphate, and organochlorines has been attributed to microorganisms such as *Azotobacter sp, Pseudomonas sp, Proteus vulgaris*, *Aspergillus sp., Bacillus sp., Klebsiella sp.,* and *Kurthia sp*.

Not all pesticides reaching the soil are biodegradable. Due to the health hazards and other problems associated with the use of pesticides, early detection and subsequent decomposition and detoxification of the polluted environment is essential.

Pesticides, especially dichlorvos (O, O-dimethyl O, 2-2 chlorovinyl phosphate) and glyphosate (organophosphate), are used for the cultivation of cassava in Nigeria and in some other West African countries. This study therefore was aimed at isolating microorganisms in pesticides-contaminated and non-contaminated soil samples, and investigating their potential in degrading the pesticides.

Materials and Methods

Collection of samples

Soil samples were collected with a sterile trowel from 10-30 cm depth at cassava two farmlands that had been sprayed with glyphosate and dichlorvos. Samples from non-contaminated soils were collected to serve as control. The soil samples were collected in polythene bags and transported to the Microbiology Department of the Delta State University, Abraka, Nigeria for analyses.

Isolation of test organisms

Diluents (0.1ml) from serially diluted (10^{-3} and 10^{-6}) samples were aseptically withdrawn and introduced into Nutrient agar and potato dextrose Agar (PDA) plates using pour plate technique. The Nutrient agar plates were incubated at 37^{o}C for 24 hours for bacterial isolates while the PDA plates were incubated at 28 ±2^{o}C for fungal isolates.

Identification of isolates

Preliminary identification based on observation of colonial morphology (color and gram-staining reactions) was done. Bacterial isolates were further subjected to an array of biochemical tests for proper characteristics and identification. The biochemical tests included oxidase test, catalase test, indole test and triple sugar iron test (Collins and Lyne, 2007).

The identification of the fungal isolates was achieved by placing a drop of Lactophenol blue on a clean slide with the aid of a mounting needle. A small portion of the mycelium from the fungal culture was removed and placed in the drop of Lactophenol. The mycelium was teased out on the slide with the aid of a sterile needle. A coverslip was then mounted and observed under the microscope (Collins and Lyne, 2007).

Test for ability of organisms to grow in the pesticides

Ability of the organisms to survive in different concentrations of the pesticides was determined. About 2ml of each pesticide was dissolved in 1ml of water to give 100% v/v concentration of the pesticide. Further concentrations were constituted to give 50%, 25%, 12.5% and 6.25% v/v concentrations. One ml of each isolate was introduced and observed for the ability to grow.

Preparation of standard inoculum

A loopful of test organism was inoculated into sterilized nutrient broth and potato dextrose broth, and incubated at 37^{o}C and 28 ±2^{o}C for 48 hours for the bacterial and fungal isolates respectively. After incubation, the flasks were kept in a mechanical shaker at 120rpm for 16-18 hours at 28 ±2^{o}C. The culture broth was centrifuged at 100rpm for 20 minutes. The supernatant was decanted and cell suspension prepared using sterile distilled water. The absorption was then read on a spectrophotometer (Shimadzu, Model 660if) at 150nm, and adjusted to 0.50D corresponding to 10^5 cfu/ml which served as the standard inoculum.

Biodegradation ability

This was done by dissolving 3ml of pesticide into 250ml of sterile distilled water and homogenizing. Ten millilitres (10ml) was pipetted from the solution into test tubes, and 0.5ml of each standard inoculum was introduced into each tube of diluted pesticide. Combinations of all bacteria, all fungi and all organisms were also introduced into three tubes respectively. A tube (containing only the pesticide solution (without any treatment)) served as control. The flasks were incubated at 28 ±2^{o}C and observed for 12 weeks for biodegradation by determining the level of the glyphosate and dichlorvos initially and after 12 weeks of biodegradation.

Determination of dichlorvos level of the pesticide

Spectrophotometric determination of dichlorvos was done by diluting 10ml of pesticide-treated samples into 1ml of 0.1ml sodium hydroxide (NaOH) for hydrolysis, after which 1ml of 0.05% of diphenylsemicarbonizile was added. The pH was adjusted to 9.0 with 0.1ml hydrochloric acid after 30 minutes. Wine coloration was observed and pH further adjusted to 9.0 with 0.1ml HCl. Readings were taken at 490nm using Bruker 320-Ms triple quadruple mass spectrophotometer.

Determination of glyphosate

The modified spectrophotometric method of Sherma *et al*. (2012) was used. To 20ml of pesticide-treated samples was added 3ml of ethyldiamine tetra acetic (EDTA), 1ml of carbon disulphide (N-acetonitrile) and 1ml of 2% aqueous sodium bicarbonate. The solution was kept in a microwave oven for 50 seconds. Then 1ml Copper I perchlorate and 1ml perchloric acid (2% in acetonitrile was added and made up to 10ml with distilled water. Absorbance was measured using spectrophotometer at 392nm.

Results

The identities of the organisms isolated are shown in Tables 1 and 2. The bacterial isolates from dichlorvos-contaminated soil were gram negative and gram positive rods (*Arthrobacter sp., Kurthia sp., Bacillus sp, Klebsiella sp., Enterobacter. and Proteus sp*.) while those from glyphosate contaminated soil had gram positive cocci in addition to the rods (*Chromobacterium sp., Micrococcus sp, Staphylococcus aureus* and *Bacillus sp*.).The fungal isolates from the contaminated soil samples are shown in Table 2.

Only *Aspergillus niger* and *Trichoderma* sp were isolated from dichlorvos-contaminated soils, while *Trichoderma sp., Rhizopus sp., and Aspergillus sp*. were isolated from glyphosate-contaminated soils. The dichlorvos-contaminated soils had one or another of the isolates occurring, while *Kurthis* and *Arthrobacter spp*. were not found in the glyphosate-contaminated soils (Table 3).

Table 1: Identification of Bacterial Isolates

Cultural Characteristics	**Morphological Characteristics**	**Motility**	**GS**	**Catalase**	**Indole**	**Citrate**	**Oxidase**	**Glucose**	**H_2S**	**Endospore**	**Cocci**	**Urease**	**Identity**
Creamy colonies with smooth edges	Cocci in clusters	-	+	+	+/-	+/-	+/-	-	+	-	+	-	*Staphylococcus*
Creamy colonies, mucoid flat with smooth edges	Rods	+	+	+	-	+/-	+/-	+	+	+	-	-	*Bacillus*
Cream colonies	Short rods		-	+	+	-	-	+	-	-	-	-	*Escherichia coli*
Flat irregular colonies	Rods		-	+	+	-	-	+	-	-	+	-	*Proteus mirabilis*
Yellowish colonies, raised, irregular with smooth edges	Cocci in clusters	-	+	+	-	+	+/-	+	-		NT	+	*Micrococcus* sp.
Creamy, smooth-edge and convex	Rods	-	-	+	-	-	+	+	-	-	NT	-	*Klebsiella*
Circular, pink and raised	Rods	+	+	+	-	+	-	-	-	+	NT	-	*Kurthia* sp.
Creamy, flat, round, smooth-edged colonies		+	-	+	+	+	-	+	-		NT		*Chromobacterium* sp.

Key: GS= Gram stain; +=positive; - = negative; NT = not tested

Table 2: Characteristics of Fungal Isolates

Characteristics	A	B	C
Cultural	White luxuriant with concentric ring	Brown wooly with profused growth	Black, Wooly with profused growth
Colour of Colonies	White	Brown	Dark
Spore attachment	Bear phialides compacted in mucous balls	Bear phialides at the apex with conidia at the top	Bear phialides at the apex with conichia at the top
Microscopy of hyphae	Septate	Septate	Septate
Conidiospore	Non-septate	Non-septate	Non-septate
Spore Colour	White	Upright Brown	Dark
Conidia	Spherical, finely rough conidia formed in slightly swollen divergent phialides	Present, 1-2 glo bose on dry basipetal claims	Present, one-cell globose in dry bisipetal claims
Tentative organism	*Trichoderma sp.*	*Rhizopus sp.*	*Aspergillus sp.*
Stolon	Absent	Absent	Absent
Rhizoid	Absent	Absent	Absent

Aspergillus niger, Bacillus sp. and Proteus sp were the only organisms able to grow when they were introduced respectively into the medium of growth (Table 4). There was efficient degradation when the indigenous isolates were used for the degradation of dichlovros. The degradative ability was similar for both single and consortia treatments. Table 6 presents the ability of the isolates to degrade glyphosate. The degradation of glyphosate was not as efficient as that of dichlorvos especially after 4 weeks. *Proteus sp*. was able to bring about a very slight reduction even after 12 weeks (21.17%). The best results were got with treatments with *Bacillus* sp. (89.92%). Treatment with all organisms gave a moderate degradation but was enhanced after 8 weeks.

Table 3: Occurrence of the Isolates in the Soil Samples

Isolates	DS_1	DS_2	DS_3	DS_4	DS_5	GS_1	GS_2	GS_3	GS_4	GS_5	US
Trichoderma sp	-	-	+	-	-	+	+	-	-	+	-
Rhizopus sp	-	-	-	-	-	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+
Bacillus sp	+	+	+	+	+	+	+	+	+	+	+
Kurthia sp	+	+	+	-	+	-	-	-	-	-	-
Arthrobacter sp	-	+	-	+	+	-	-	-	-	-	-
Enterobacter sp	+	+	+	+	-	-	+	-	-	-	+
Klebsiella sp	+	+	-	+	-	-	-	+	-	-	-
Proteus sp	+	+	+	-	+	+	+	+	+	-	-
Micrococcus sp	-	-	-	+	-	+	+	+	-	+	-
Chromobacterium sp	+	+	-	-	-	-	+	+	+	+	-
Staphylococcus sp	+	-	-	-	-	+	+	+	+	+	+

Key: DS= Dichlorvos contaminated soil: GS= Glyphosate contaminated soil; US= Control

Aspergillus niger, Bacillus sp. and Proteus were able to grow when both pesticides were introduced into the medium of growth, and also were the only organisms that survived in dichlorvos-incorporated medium (Table 4).

Table 4: Growth of Isolates in the Pesticide Incorporated Medium

	Pesticide	
Isolate	Glyphosate	Dichlorvos
Trichoderma sp	+	-
Rhizopus sp	-	-
Aspergillus sp	+	+
Bacillus sp	+	+
Kurthia sp	+	-
Arthrobacter sp	-	-
Enterobacter sp	-	-
Klebsiella sp	-	-
Proteus sp	+	+
Micrococcus sp.	+	-
Chromobacterium sp	+	-
Staphylococcus sp	-	-

There was better degradation when the indigenous isolates were used for the degradation. There was significant degradation of glyphosate when *Chromobacterium*, *Micrococcus* and *Aspergillus* were applied singly. The best degradation ability was achieved when the bacteria were applied as a consortium. The fungal combination also produced good degradation potential but not as significant as the consortium of bacteria (Table 5). *Proteus sp* and *Kurthia sp* gave a slight reduction.

Table 6 presents the ability of the isolates to degrade Dichlorovos, *Chromobacterium sp, Micrococcus sp and Trichoderma sp* were able to reduce the pesticide very slightly but was not significant. Application of *Aspergillus sp, Proteus sp Kurthia sp and Bacillus* sp singly gave a very significant reduction of the pesticides after 12 weeks however the combination of the bacterial, fungal isolates and all isolates respectively were more effective. Treatment with combination of the fungal isolates (*Trichoderma sp and Aspergillus sp*) gave the best results.

Table 5: Bio-degradative Ability of the Isolates on the Glyphosate

Isolate	**Concentration of dichlorvos (mg/ml)**		**Degradation (%)**	
	8 weeks	12 weeks	8 weeks	12 weeks
Aspergillus sp.	10.41 ±0.27	7.48 ±1.12	98.96	99.25
Proteus sp.	13.37 ±2.96	11.09 ±2.79	98.66	98.89
Bacillus sp.	13.53 ±2.94	11.26 ±2.84	98.65	98.87
B + P	11.89 ±1.48	9.22 ±2.08	98.81	99.08
A + B + P	12.23 ±2.50	8.96 ±0.01	98.78	99.1
Nut Broth + Pesticide	898.45 ±24.26	890.04 ±27.37	10.16	10.99
PDB + Pesticide	925.20 ±2.50	838.88 ±68.07	7.48	16.11
Initial concentration of dichlorvos = 1000mg/ml				

Key: Initial concentration of glyphosate was 50.11mg/ml;
A – *Aspergillus* sp; B – *Bacillus*; P –*Proteus*

Table 6: Bio-degradative Ability of Isolates on Dichlorvos

Isolate	Concentration of dichlorvos (mg/ml)		Degradation (%)	
	8 weeks	12 weeks	8 weeks	12 weeks
Aspergillus sp.	26.37 ±5.85	12.24 ±1.07	47.38	75.57
Proteus sp.	41.58 ±6.68	39.50 ±647	17.04	21.17
Bacillus sp.	17.46 ±0.76	5.76 ±0.12	65.16	88.51
B + P	13.79 ±2.80	5.05 ±0.49	72.48	89.52
A + B + P	35.24 ±1.35	24.62 ±1.06	29.67	50.87
Initial concentration of glyphosate = 50.11mg/ml				

Key: Initial Concentration of Dichlorvos was 100omg/ml;
P – *Proteus*; B – *Bacillus*; A - *Aspergillus*

Discussion

The presence of the isolates in the soil samples may be attributed to either ability to degrade the pesticides (dichlorvos and glyphosates) or their ability to tolerate the toxicity. Isolation of the isolates in one and not on the others may be due to resistance of those that grew to the chemical components of the pesticides or the inhibition of those that were not able to grow. This conforms with the study of Arias-Estevez and Garcia-Rio (2008) who isolated similar organisms (*Pseudomonas, Micrococcus, Bacillus, Proteus,* and *Penicillium*) from pesticide-polluted soils.

Foster *et al*. (2004) reported the significant roles of *Proteus sp., Enterobacter sp., Aspergillus sp., Penicillium sp., Bacillus sp., and Klebsiella sp*. in the biodegradation of pesticides. The organisms may possess the ability to utilize both pesticides as sources of energy. Not all the organisms isolated were able to grow on the pesticide-incorporated medium. Others might not have grown suggesting that some of them were just transient microorganisms. The organisms that survived may have acquired a variety of mechanisms for adaptation to the presence of these toxic constituents (Taint-Selor, 2012)**.** These organisms have developed capabilities to protect themselves from chemical constituents by various mechanisms such as absorption, uptake, methylation, oxidation and reduction (Zahoo and Rehma, 2008). There was a more efficient degradation of dichlorvos (98.87%-99.25%) than glyphosate (21.17%-89.92%). All the test trials reduced dichlorvos effectively while there was variation in the effectiveness of glyphosate, with *Proteus sp*. having very low degradative potential, and consortium of organisms having a moderate degradative potential of 50.87% after 12 weeks. The ability of the isolates to degrade the pesticides may be attributed to their resistance to some of the chemicals constituting the pesticides, and also to the ability to produce relevant enzymes capable of degrading these pesticides. *Proteus sp*. has

been reported by Gupta *et al*. (2016) to degrade all sorts of hydrocarbons and pesticides in contaminated soils.

Proteus sp., Enterobacter sp., Klebsiella, Kurthia sp., Bacillus, and Aspergillus have been reported to be good degraders of organophosphorus and other pesticides. Microorganisms such as *Pseudomonas putida*, *Acinetobacter rhizophaeza* have been implicated in the microbial degradation of fenamiphos (Chanda *et al*., 2006; Maila *et al*., 2006).

A combination of all isolates, bacteria and fungi, presented a low degradative potential which may be due to competition among the organisms for the available nutrient source. The organisms might have inhibited the growth of one or another thereby reducing the overall utilization of the glyphosate. Prolonged exposure to all isolates resulted in an increased percentage degradation which may be due to the organisms having acclimatized to the environment or that the "fittest" which are able to effectively utilize the environment are able to efficiently degrade the pesticides.

Most of the degradative protocols with the isolates produced positive results within 12 weeks. The treatment of dichlorvos-contaminated soil with single and combination of isolates used in this study is recommended. The recommendation for the treatment of glyphosate-contaminated soil is slightly different, in that *Proteus sp*. did not give a promising result, but all the other protocols are recommended.

References

Adekanye, T.A., Ogunijinu, S.I. and Ajala, A.O. (2013). An assessment of cassava processing plants in Irepodun Local Government Area, Kwara State, Nigeria. *World Journal of Agricultural Research* 1 (11): 14 – 17

Arias-Estevez, J.C. and Garcia-Rio, L. (2008). The Mobility and Degradation of Pesticides in Soil and the pollution of Groundwater Resources. *Agricultural Ecosystem Environment* 132: 247 – 260

Chanda, A., Khelan,S.K. and Banajee, D. (2006). Total degradation of fenitothrion and other organophosphorus pesticides. *Journal of American Chemical Society* 128(37): 12058-12059

Chen, S.K., Subler, S. and Edward, C.A. (2001). Effect of fungicide, benonyl captan on soil microbial activity and nitrogen dynamics. *Soil Biology and Biochemistry* 33: 1971 – 1980

Collins, E. and Lyne, P. (2007). Microbiological Methods (5^{th} ed.) Butterworth Publishing Company London 92p.

Damalas, C.A. (2009). Understanding benefits and risks of pesticide use. *Scientific Research and Essay* 4(10): 945 – 949

Foster, L.J.R., Kwan, B.H. and Vancou, T. (2004). Microbial degradation of the organophosphate pesticide. *FEMS Microbiology Letter* 240 (1): 49-53

Gilden, R.C., Hufing, K. and Saltler, B. (2010). Pesticides and health risks. *Journal of Obsterics, Gynaecology and Neonatal Nursing* 39(1): 3 – 10

Gupta, G., Kumar, V. and Pal, A.K. (2016). Biodegradation of polycyclic aromatic hydrocarbons by microbial consortium; a distinctive approach for decontamination of soil. *Soil Sediment Contaminants* 25: 597-623.

Ijah, U. J. J. and Ndana, N. (2003). Biodegradation of crude oil in soil and amended with periwinkle shell. *Environmentalist* 23(3): 249 – 254

Karpouzas, G.G. and Sing, B.K. (2006). Microbial degradation of organophosphate xenobiotics. *Environmental Health Perspective* 102(1): 18-38

Kuzmin, S., Slezak, G., Elie, C., de Rcyke, Y., Boitewe, S. and Sage, E. (2005). UV radiation in highly mutagens cells unable to repair 7, 8, dihydro-8-oxoguanis *Saccharomyces cerevisae*. *Proceedings of the National Academy of Sciences* 102(38): 13538 – 13543

Maila, M.P. and Cloete, T.E. (2004). The Use of Biology Activities to monitor the removal of contaminants. Perspective of monitoring hydrocarbons contamination. A review. *International Biodeterioration and Biodegradation* 55:1-8

Maila, M.P., Randius, P., Droner, K. and Cloete T.E. (2006). Soil microbial communities influence geographic location and hydrocarbon pollutants. *Soil Biological Biochemistry* 38: 303 – 310.

Mallick, K., Bharati, K., Banerji, A.S. and Sethunathan, N. (1999). Bacterial degradation of chlorpyrifos in pure cultures in soil. *Bulletin of Environmental Contaminants and Toxicology*. 62:48-54

Millioli, V.S., Servulol, E.L.C., Sobrald, L.G.S. and De-Carvallio, D.D. (2009). Bioremediation addition to soil toxiciy and crude oil degradation efficiency. *Global Nest Journal* 11: 187 – 188

Oritz-Hernandez, M.I., Sanchez-Salinas, E., Godinez, M.L.C., Gonzalez, E.D. and Ursino, E.C.P.(2013). Mechanisms and strategy for pesticide biodegradation of waste, soils and water. *Revisia International de Contaminacion Ambiental* 29: 85-104.

Petra, L., Iva, P., Blanka, V. (2013) Organochlorinated pesticides degrading microorganisms isolated from contaminated soils. *Journal of Biotechnology* 31 (1): 26 – 31.

Taint-Selor, L. (2012). Biosorption and transformation of chromium by *Serratia* sp. from tannery effluent. *Environmental Toxicology* 33(1): 113-122.

Wauchope, R.J, Buttle, T.M, Hornsby, A.G, Augustyn Beckers, P.W.N and Burt, J.P. (1992). Pesticide Properties database for environment decision making. *Review of Environmental Contamination and Toxicology* 123(10 - 12): 157

Zahoo, A. and Rehman, A. (2018). Isolation of bacteria from toxic industrial effluents and their potential in bioremediation. *Journal of Environmental Sciences* 2: 814-820.