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Utilisation of Pesticides by Soil Microorganisms

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Abstract
While pesticides are considered somewhat essential in modern agriculture, their indiscriminate use has been linked to loss of biodiversity and ecosystem function as well as accumulation in food produce and the poisoning of groundwater. The utilisation of selected pesticides by microorganisms isolated from soil samples was observed. The pesticides tested were the organophosphorus insecticide – Diazinon and Herbicides – Primextra 500FW and Vetox 85. Detection of a zone of clearing was used to identify the Vetox 85-utilising microorganisms. Utilisers of Diazinon and Primextra 500FW were isolated by enriching the soil with mineral salt broth and providing Diazinon and Primextra as sole carbon and energy sources. This method was equally used for the *in vitro* degradation of the pesticides. Degradation was monitored using total viable cell numbers, pH and optical density. Generation times and growth rates of selected utilisers were determined. The Vetox 85-utilisers were found to be 0.32% of the total aerobic heterotrophic counts. The pesticide-degraders isolated were *Vibrio, Acinetobacter, Pseudomonas, Arthrobacter, Flavobacterium, Bacillus, Aeromonas, Rhizopus* and *Penicillium* species. *Vibrio, Acinetobacter, Pseudomonas, Arthrobacter* and *Rhizopus* were selected for degradation studies. *Vibrio* sp. showed the greatest pesticide utilisation capacity unexpectedly surpassing the mixed cultures, however, mixed cultures generally showed better degradative capacities than single cultures. *Vibrio* sp. had the highest growth rate while *Rhizopus* sp. had the lowest; *Rhizopus* sp. consequently showed the highest generation time alongside the mixed culture of *Vibrio* sp. and *Acinetobacter* sp. while *Vibrio* had the lowest generation time. The results showed that while these pesticides are relatively biodegradable *in vitro*, they are only utilisable by a limited number of indigenous soil microorganisms.
Keywords: Biodegradation, Herbicide, Organophosphorus Insecticide, Pesticide, Soil.

INTRODUCTION

Pesticides are widely used in modern agricultural practice. They are used extensively in the control of agricultural pests that normally cause decline in the size, yield and quality of crops often through the spread of disease (Sharma *et al.,* 2013; Aziz *et al*., 2014). Approximately 2 – 3 million tonnes of pesticides are consumed each year across the globe with the highest usage attributed to the Europe (45%) and the USA (24%). China, Korea, Japan and India are found to be the highest consumers amongst the Asian countries (Hussain *et al*., 2009; Abhilash and Singh, 2009; Rani and Dhania, 2014). Zhang *et al.* (2011) state that worldwide pesticides use on average is 47% for herbicides; 79% for insecticides and 19% fungicides. De *et al.* (2014) however placed the values at 47.5% for herbicides and 29.5% for insecticides.

Applied pesticides can be a big concern as they tend to travel quite far, often migrating downwards into groundwater. These pesticides and their toxic intermediates get into ground water, surface water and the atmosphere, commonly via run-off and evaporation, where they are harmful to many non-target species including man (Parte *et al*., 2017; Sharma *et al*., 2013). Typically, only 0.1% of the applied pesticide reaches the target organism (Hussain *et al*., 2009; Carriger *et al*., 2006). Javaid *et al*. (2016) placed this figure at 5% or less. Excessive and indiscriminate use of pesticides may lead to their accumulation in food crops (Tayade *et al.,* 2013). Readily biodegradable pesticides too pose a risk as relatively unsafe concentrations of their by-products and residues may persist in humans, animals and the environment (Tayade *et al.,* 2013).

The WHO has estimated that there are 3 million cases of pesticide poisoning annually which result in approximately 200,000 human deaths (Aziz *et al*., 2014). Many recent studies highlight that certain concentrations of pesticides may disrupt the natural biotic balance in edaphic systems, leading to loss of biodiversity, suppression of bio-control agents and significant harm to aquatic fauna and flora (Pampulha and Oliveira, 2006; Chen *et al*., 2001; Wang *et al.,* 2006; Rajendram *et al*., 2007; Yue *et al.,* 2007; Rani and Dhania, 2014; Sharma *et al.,* 2013). Laschi *et al*. (2007) maintain that pesticides contribute significantly to cancer mortality. They have further been implicated in long-term neurological effects, skin disorders, miscarriages and foetal deformities (Bag, 2000). Most synthetic organophosphate pesticides are toxic and inhibit acetylcholinesterase, a vital enzyme in neurotransmission (Bakry *et a*l., 2006; Oritz-Hernandez and Sanchez-Saliñas, 2010). The use of pesticides has been linked to the presence of heavy metals in the soil which significantly inhibit microbial activity (Su *et al*., 2014; Zhang *et al.,* 2011). Inhibition of ATP synthesis, cathode pathways, nitrogen fixation and even mutation induction has also been reported amongst microbial populations (Brooks, 1977).

The action of microorganisms is the principal means of pesticide breakdown in the environment (Surekha *et al*., 2008; Aislabie and Lloyd-Jones, 1995). Johnsen *et al.* (2001) illustrated that several microbial groups are able to utilise pesticides as a source of nutrients and energy. The rate at which different pesticides are biodegraded varies widely depending on both biotic and abiotic factors. Several have been shown to be recalcitrant remaining in the environment and accumulating in the food chain long after their application (Aberdeen, 1993; Kannan *et al*., 1994). DDT (1,1,1-Trichloro-2,2-bis-(p-chlorophenyl)ethane) and Dieldrin are known recalcitrant pesticides (Aberdeen, 1993; Kannan *et al*., 1994). Carbofuran, Atrazine and Sumazine while not recalcitrant are biodegraded very slowly providing a higher possibility of being leached into ground water (Aislabie and Lloyd-Jones, 1995). In spite of the harmful effects observed, some researchers still debate the extent of the impact of these pesticides arguing that microbial communities are remarkably resilient to most stressors (Valentine *et al*., 2013). With the growing use of these chemicals, an understanding of their possible fate in the environment is essential. In addition to the general environmental and health importance, the decontamination of pesticide contaminated environments can be quite costly and sometimes difficult. Microorganisms are considered the best option as they adapt relatively quickly and are able to develop effective alternative pathways for the breakdown of these compounds.

This study was designed to investigate the possible persistence of commonly used pesticides, to determine the soil microorganisms capable of breaking down the pesticides and to evaluate the time lag for the degradation of the selected pesticides by these soil microorganisms using total viable count, growth rate and generation time as parameters for positive degradation.

MATERIALS AND METHODS

Sample Collection

The pesticides used for the study were bought from Port Harcourt town market. The pesticides tested were the organophosphorus insecticide – Diazinon (also Diazide or Diethoxy- [(2-isopropyl-6-methyl-4-pyrimidinyl) oxy]-thioxophosphorane) and Herbicides – Primextra 500FW (acetanilide and triazine combination) and Vetox 85 (1-naphthylmethylcarbamate). The degrading microorganisms were environmental isolates obtained from soil samples collected from the botanical gardens of the University of Port Harcourt, Nigeria. Samples were collected from five different locations within the garden. The upper 15cm of the soil was collected. Analysis was done within 25 minutes of collection.

Enumeration of Total Heterotrophic Microorganisms

Ten grams of soil samples collected from each of the five sample locations were suspended in 90ml of sterile physiological saline. This was homogenised and a 10 fold serial dilution was done. A 0.1 ml aliquot of the serially diluted samples were plated out in triplicate on oxoid nutrient agar and incubated at 37^{o}C for 24 hours. Fungi were isolated using Potato Dextrose Agar acidified with 0.1% lactic acid (BDH) and incubated at 30^{o}C for 72 hours. Reported plate counts were those that had 30 – 300 cfu/g. Representative isolates were characterised as described by Holt (1982).

Enumeration of Pesticide-Degrading Microorganisms and *In Vitro* Degradation of Pesticides

Mineral salt agar (MSA) modified using the overlay method described by Okpokwasili and Nwosu (1990) was used for the powdered pesticide – Vetox 85 while the enrichment method described by APHA (1985) was adopted for the isolation from Diazinon and Primextra 500FW degraders. The *in-vitro* method of assaying the degradation of the pesticide was carried out by preparing sterile mineral salt broth containing 100mg/ml of the test pesticide dispensed in a 250ml flask. The isolates were then inoculated in single and mixed cultures and incubated at room temperature in a shaker. Sample cultures were collected from the incubated samples in the shaker for pesticide degradation analysis involving pH, total viable count (TVC) and optical density (O.D.) at 540nm.

RESULTS

As shown in Figure 1, total heterotrophic counts across the five sites range from 1.2 x 10^5 – 9.5 x 10^5 cfu/g with a mean count of 5.49 x 10^5 $\pm$ 4.2 cfu/g. A mean count of 1.73 x 10^3 $\pm$ 0.3 cfu/g was obtained for Vetox 85 utilising bacteria which is about 0.32% of the mean total count across the sites.

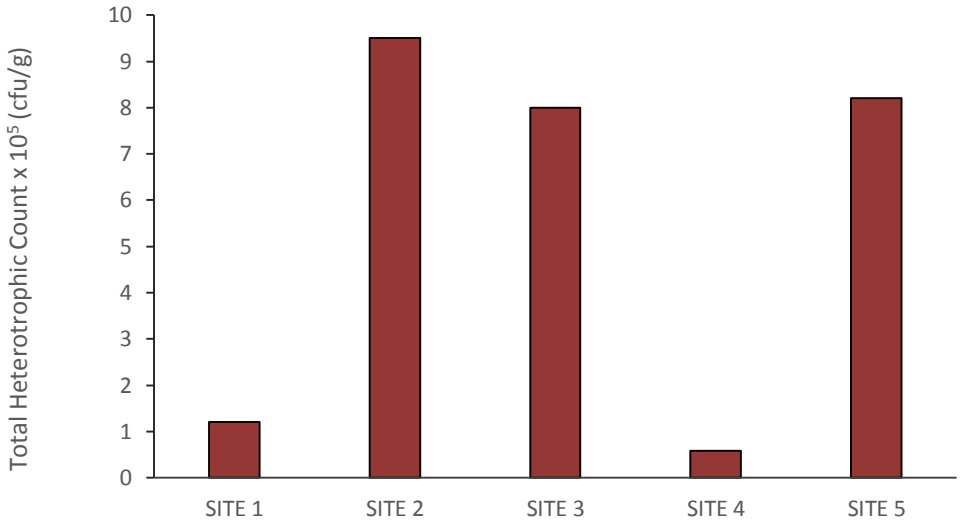

Fig. 1. Site Specific Mean Total Heterotrophic Count

The characterisation of pesticide-degraders obtained from the different soil samples revealed seventeen isolates from nine genera – *Vibrio* (3), *Acinetobacter* (4), *Arthrobacter* (2), *Pseudomonas* (2), *Flavobacterium*, *Aeromonas*, *Bacillus*, *Rhizopus* and *Penicillium*. *Rhizopus* was the only fungus isolated. The isolates *Vibrio, Acinetobacter, Pseudomonas, Arthrobacter* and *Rhizopus* were selected for further degradative studies based on their ability to utilise the pesticides. *Vibrio* and *Acinetobacter* grew best on Primextra 500W; *Arthrobacter* and *Rhizopus* grew best on Vetox 85 while *Pseudomonas* and *Arthrobacter* grew best on Diazinon so further testing was done accordingly. Changes in optical density, pH and viable cell count were used to evaluate the extent of degradation of pesticides. The results are depicted in Figures 2 – 4. The growth profile of the isolates on mineral salt agar supplemented with pesticides as sole carbon and energy source are as shown in Figures 5 – 7.

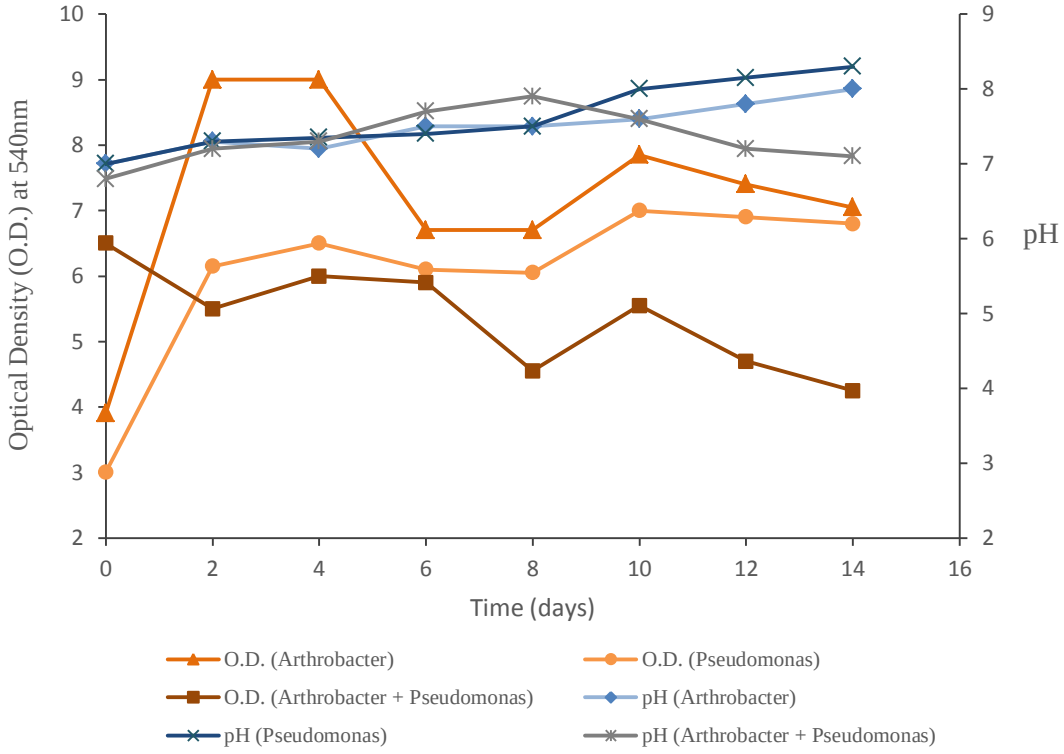

Fig. 2. Growth Kinetics of Single and Mixed Cultures of Isolates on Diazinon

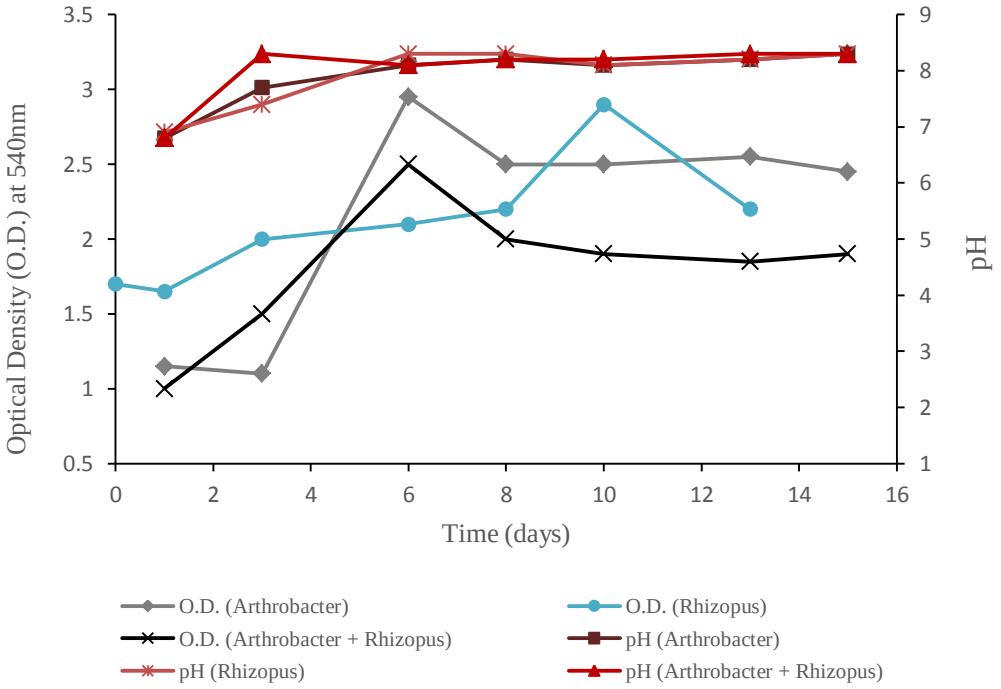

Fig. 3. Growth Kinetics of Single and Mixed Cultures of Isolates on Vetox 85

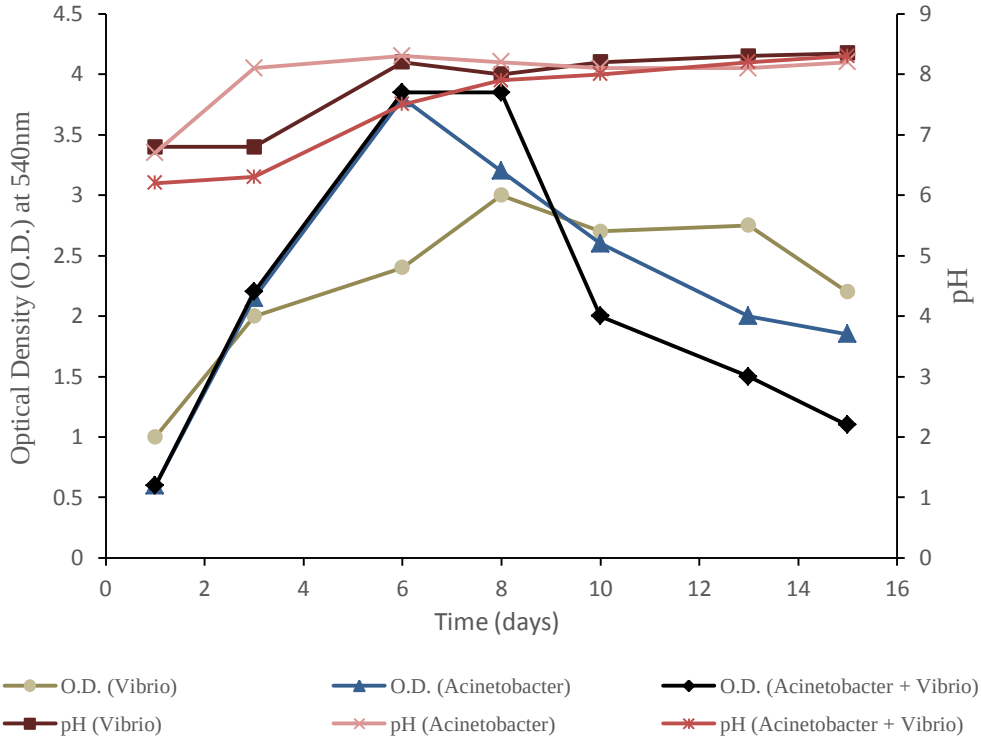

Fig. 4. Growth Kinetics of Single and Mixed Cultures of Isolates on Primextra 500FW

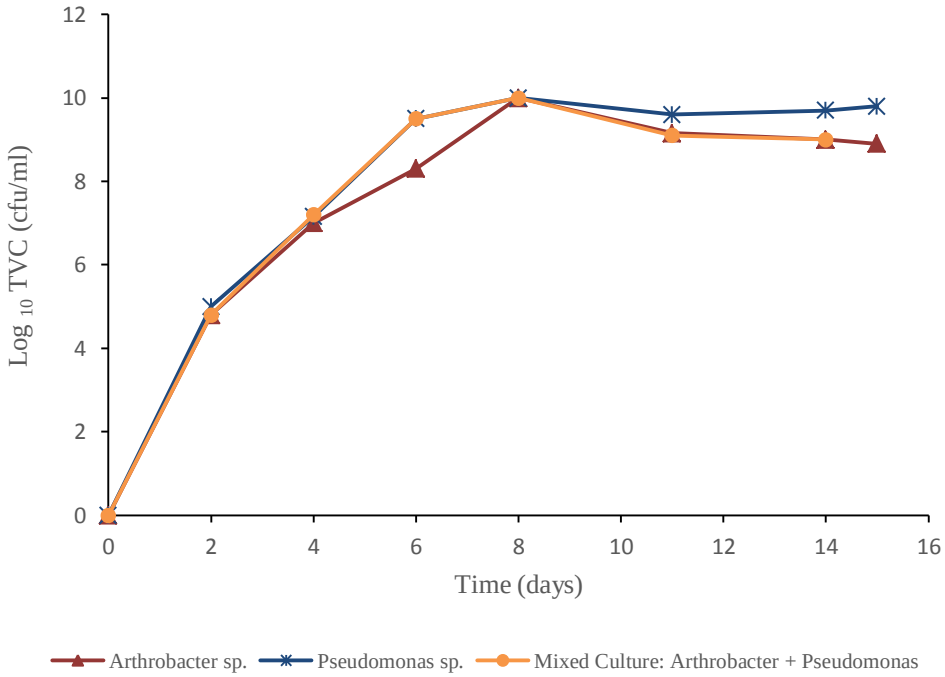

Fig. 5. Growth Curves of Single and Mixed Cultures of Isolates on Diazinon over time

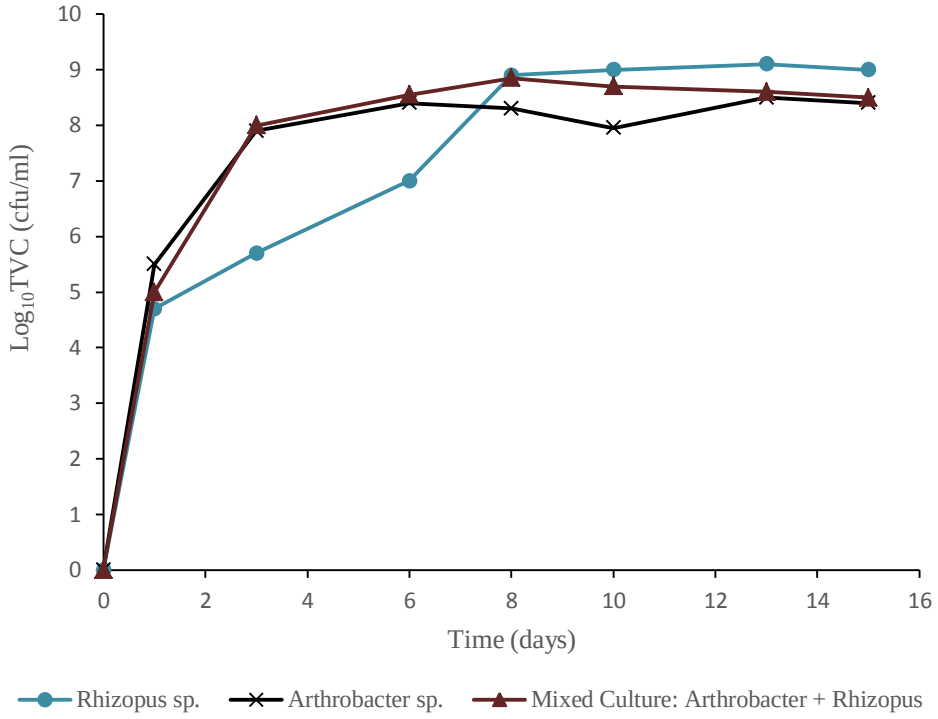

Fig. 6. Growth Curves of Single and Mixed Cultures of Isolates on Vetox 85

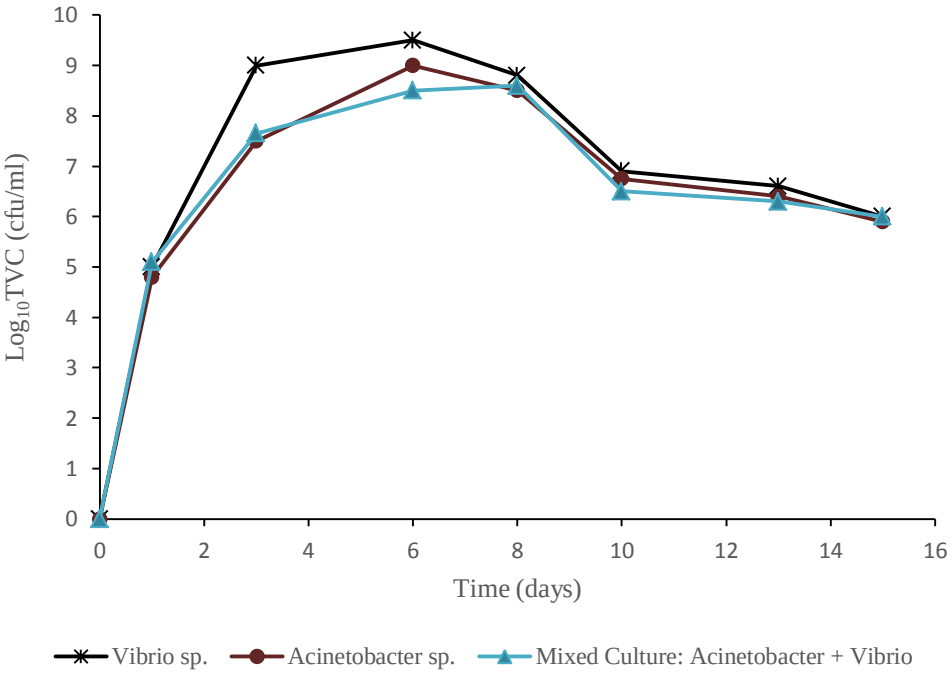

Fig. 7. Growth Curves of Single and Mixed Cultures of Isolates on Primextra 500FW

The growth rates and generation times of the different isolated pesticide degraders in mineral salt broth containing a test pesticide as the sole carbon and energy source are outlined in Figures 8 and 9 respectively. The least growth rate of 0.05 h^{-1} was recorded for *Rhizopus* sp. (VD1) growing on Vetox 85, while the highest was recorded for the Vibrio sp. (PD1) growing on Primextra 500FW. The generation time for the *Rhizopus* sp. (VD1) and *Vibrio* (PD1) were 12.3 h^{-1} and 3.8 h^{-1} respectively. There was no marked change in growth rates of the mixed cultures. *Arthrobacter* sp. had similar growth rates and generation times on both Vetox 85 and Diazinon.

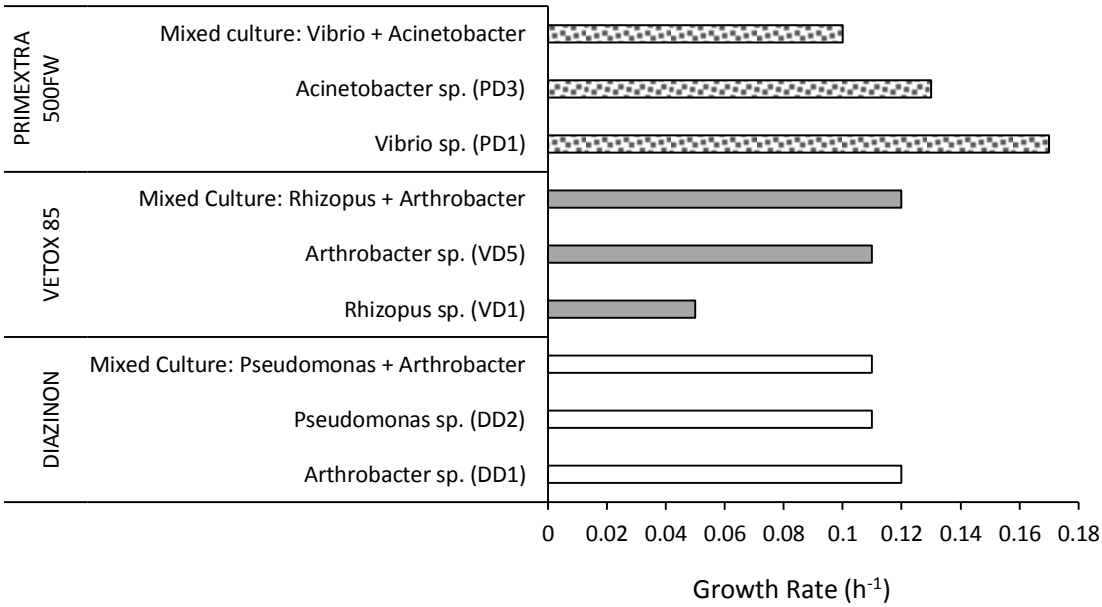

Fig. 8. Growth Rates of the Selected Isolates on the Different Pesticides

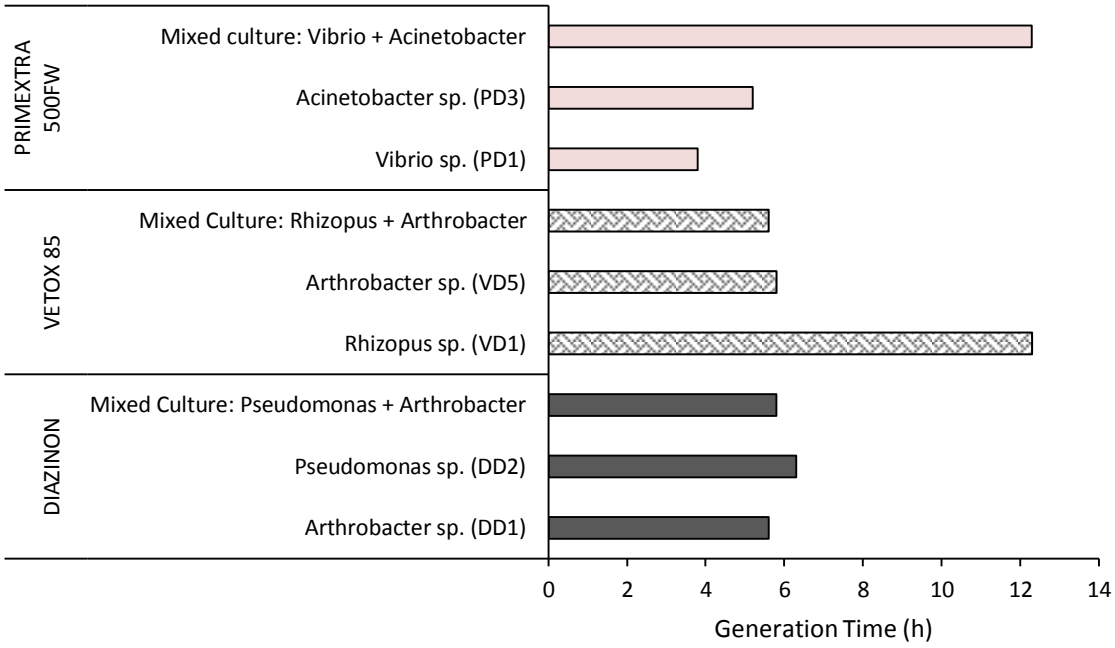

Fig. 9. Generation Times of the Selected Isolates on Different Pesticides

DISCUSSION

The study revealed that the percentage of the microbial population able to utilise the pesticides was relatively low at only 0.32% on average. Such low levels of microbial utilisation are not surprising as the organisms may not have been previously exposed to the pesticide. Previous studies found degradation of pesticides by microorganisms on initial contact to be low (Loos *et al.,* 1979; Nelson, 1982). This can be associated with the limited number of utilisers and the absence of a more rugged metabolism of the compound due to unspecialised or delayed induction of the necessary enzymes for degradation. This may be particularly true with these isolates which were obtained from soil samples that had not been previously subjected to pesticide treatment.

Although several bacterial and fungal genera are capable of degrading pesticides, some groups including *Flavobacterium, Pseudomonas, Bacillus* and *Arthrobacter* as found in this study have been consistently reported (Parte *et al*., 2017; Jamaluddin and Pandey, 2017). Rani and Dhania (2014) list *Flavobacterium, Arthrobacter, Azotobacter, Burkholderia* and *Pseudomonas* as degraders of pesticides. They further stated that *Pseudomonas* possesses hydrolytic enzymes capable of effectively breaking down a number of pesticide groups. Mulbry and Kearney (1991) found that *Pseudomonas* and *Alcaligenes* were able to degrade the herbicide, 2,4–D(2,4-dichlorophenoxy)acetic acid while Aislabie and Lloyd-Jones (1995) identified *Alcaligenes, Flavobacterium, Pseudomonas* and *Rhodococcus* as being able to metabolise selected pesticides. Aziz *et al*. (2014) isolated *B. subtilis* and *P. aeruginosa* isolated from the organophosphate insecticide – Malathion. Studies by Kanekar *et al*. (2004) found *Pseudomonas diminuta, Flavobacterium, Penicillium corrylophylum* and *Escherichia coli* to be implicated in pesticide degradation in the soil due to their possession of the required enzymes. Similarly, Mulbry (2000) and Serdar *et al*. (1989) also identified *Pseudomonas diminuta* and *Flavobacterium* as degraders of pesticides in soil. *Acinetobacter calcoaceticus* and *Streptomyces* sp. have been found to degrade the pesticides Bifenthrin and Chlorpyrifos respectively (Javaid *et al*., 2016) while *Pseudomonas* was considered effective in the degradation of endosulfan (Wyss *et al*., 2006; Bhalerao and Puranik, 2007).

Changes in optical density, pH and viable cell count were used to evaluate the extent of degradation of pesticides. The results of *in-vitro* pesticide degradation by the isolates showed a steady increase in pH, optical density and total viable count as the test organisms proliferated until the 15th day when a sharp decrease was observed. This indicates obvious utilisation of the test pesticide by the test isolates. The progressive increase in pH reveals the accumulation of metabolites concomitant with metabolism of the test pesticide. The observed increase in viable cell count connotes biodegradation of the pesticide in question. Counts however also declined with time. This decrease is indicative of depletion of nutrients in the system and possible accumulation of toxic metabolites.

The growth profiles highlighted that the biodegradation of the pesticides proceeded first with a lag phase (acclimatisation period) during which no significant decomposition was observed. This is the time taken for the pesticide-degrading microbial population to increase to such a level that enhanced degradation occurs (Aislabie and Lloyd-Jones, 1995). Robertson and Alexander (1994) observed that with the application of 2,4-D mineralisation rates were mutually slow becoming more rapid over time with concomitant increase in abundance of relevant degraders. Acclimatisation periods may be lengthened by unfavourable environmental conditions. In other instances specific genes need to be activated or enzymes synthesized often via the adaptation of an already existing gene that could then become part of the community genome with sustained exposure to the pesticide (Aislabie and Lloyd-Jones, 1995; Rani and Dhania, 2014). Aziz *et al.* (2014) postulated that enzymes were secreted by bacterial cells in response to pollutant exposure due to observations that the rate of biodegradation increased with increase in the concentration of the pesticide.

The degradation of the test pesticides were relatively rapid, occurring within 14 days of incubation. Some cultures showed a continuous rise after initial decline. This observation could be from second phase utilisation of either the metabolites or other active ingredients from the test pesticide. This result is corroborated by the findings of Nelson (1982) and Okpokwasili and Nwosu (1990). The ability of a pure culture of *Arthrobacter* and a mixed culture of *Arthrobacter* and *Pseudomonas* to utilise Diazinon was more than that of a pure culture of *Pseudomonas* alone which indicates that *Pseudomonas* is not a good degrader of Diazinon but can effectively contribute to the degradation process through interaction with other micro-organisms. *Vibrio* showed a better ability to utilise Primextra 500FW than a mixed culture of *Vibrio* and *Acinetobacter*. This is unusual as studies show that mixed cultures would normally breakdown compounds better than single cultures but Oritz-Hernandez and Sanchez-Saliñas (2010) found that *Vibrio metschinkouii* showed 49% pesticide decomposition higher than other test organisms. This value increased to 98% in the presence of an additional carbon source. They further reported that other test organisms as single strains made no significant impact. Unexpectedly, *Arthrobacter* sp. showed better utilisation ability on the organophosphate insecticide – Vetox 85 than the fungus *Rhizopus* but a mixed culture of *Rhizopus* and *Arthrobacter* showed the best utilisation. Most of the organisms seem to

have entered the death phase by between Day 8 and Day 14.

There is an established inverse relationship between generation times and growth rates; generally the faster an organism's growth rate, the shorter its generation time. Consequently, *Rhizopus* grown on the Mineral salt broth containing Vetox 85 had the lowest growth rate and the highest generation time. Whereas the *Vibrio* sp. using Primextra 500FW as the sole carbon source had the highest growth and the lowest generation time. With Diazinon, the growth rates of the mixed isolates were very similar to those observed with the pure cultures of *Arthrobacter* and *Pseudomonas* probably as a result of the competition between the isolates in the mixed culture which then had a stronger levelling effect on the observed generation time. The somewhat unusual decreased growth rate in the mixed isolates on Vetox 85 may be attributed to incompatibility of the isolates following negative interaction. Such negative interactions are corroborated by the report of Deng and Wang (2016) and have been associated with impaired degradation of compounds by microorganisms in the environment. The poor growth rate observed for *Rhizopus* may be indicative of the harmful effect of pesticides on fungi (Lo, 2010; El-Ghany *et al*., 2015; Tkaczuk *et al*., 2015) compared to bacteria bearing in mind that only two genera of fungi were isolated in this study. Organophosphorus pesticides have been reported to reduce the abundance of soil fungi by about 26% - 56% with diazinon recording a reduction of 51% in fungal populations (El-Ghany and Masmali, 2016). The microorganisms involved in the degradation of the selected pesticides were predominantly Gram negative. Several earlier reports confirm that biodegradation of organopollutants is normally mediated by Gram negative bacteria (Campbell, 1977; Lal, 1982). *Acinetobacter* and *Vibrio* spp. demonstrated a high capacity to utilise the different pesticides studied.

The limited percentage of utilisers present highlights the possibility of persistence where these pesticides are employed regularly and in considerably large quantity. Persistence quite often translates to bio-accumulation in plants and animals as well as run-off into surrounding water bodies. The subsequent consideration is that the presence of microorganisms with the ability to degrade pesticides underlines the likelihood that these chemicals, when used indiscriminately would ultimately upset the balance of the soil ecosystem encouraging the rapid proliferation of these pesticide utilisers at the expense of non-utilisers including some of the microorganisms vital to biogeochemical cycling and other ecosystem services. Furthermore, biodegradation though desirable, may result in the production of toxic intermediates or recalcitrant complexes which are detrimental to the environment.

CONCLUSION

With growing populations across the globe, it is inevitable that the demand for food crops will rise and thus agricultural activities, including pesticide use, have to match this demand. This study indicates that a limited number of naturally-occurring soil microbiota including *Pseudomonas, Vibrio, Arthrobacter, Acinetobacter* and *Rhizopus* spp. exhibit strong potential for utilisation of pesticides so with measured use, these compounds are unlikely to accumulate in the natural environment in the long term. There may however, be impacts on the abundance and diversity of soil microbiota. It should be noted that both biotic and abiotic factors play a role in the fate of pesticides in the environment. Regular monitoring of pesticide usage is important because of risks posed by pesticides on human, animal and plant health and on the environment. Pesticides which are more readily biodegraded and less toxic to the environment are recommended. The impact of farming practice on the biodegradation of pesticides must be taken into consideration – proper irrigation of soils is generally found to enhance pesticide biodegradation. While many researchers suggest the use of bio-insecticides, it is essential to take into cognizance the possible impact of introducing foreign species into any ecosystem. These organisms introduced as anti-pest measures could impact on the prevailing microbial community structure and function. Further research is encouraged to better appreciate the long term impact of pesticide use on microbial diversity and abundance and the community genome.

CONFLICT OF INTEREST

The Authors declare that no competing interests exist.

REFERENCES

Aberdeen, I., 1993. Litigation over dieldrin in Victoria. Agric. Sci., 5: 42 – 45.

Abhilash, P. C. and Singh, N., 2009. Pesticide use and application, an Indian scenario. J. Hazard. Mater.,165(1-3):1 – 12. https://doi.org/10.1016/j.jhazmat.2008.10.061

Aislabie, J. and Lloyd-Jones, G., 1995. A review of bacterial degradation of pesticides. Aust. J. Soil Res., 33: 925 – 942. https://doi.org/10.1071/SR9950925

APHA, 1985. Standard Methods for the Examination of Water and Wastewater. 16th ed. American Public Health Association, Washington D.C.

Aziz, M. W., Sabit, H. and Tawakkol, W., 2014. Biodegradation of Malathion by *Pseudomonas* spp. and *Bacillus* spp. isolated from polluted sites in Egypt.

Amer-Eurasian J. Agri. Environ. Sci., 14 (9): 855 – 862. https://doi.org/10.58299/idosi.aejaes.2014.14.09.867

Bag, D., 2000. Pesticides and Health Risks. Econ. Politic. Wkly, 6 (16): 20 – 21. https://doi.org/10.2307/4409739.

Bakry, N. M., El-Rashidy, A. H., Eldefrawi, A. T. and Eldefrawi, M. E., 1988. Direct actions of organophosphate anticholinesterases on nicotinic and muscarine acetylcholinic receptors. J. Biochem. Toxic., 3: 235 – 259.

Bhalerao, T. S. and Puranik, P. R., 2007. Biodegrdation of organochlorine pesticide, endosulfan, by fungal soil isolate, *Aspergillus niger*. Int. Biodeter. Biodegradation, 59 (4): 315 – 321. https://doi.org/10.1016/j.ibiod.2006.09.002

Brooks, G. T., 1977. Chlorinated insecticides: retrospect and prospect. In: Plimmer, J. R.,, Kearney, P. C., Kohn, G. K., Menn, J. J. and Ries, S., eds. ACS Symposium Series, vol. 37. American Chemical Society, Washington, DC, USA.

Campbell, R., 1977. Microbial Ecology. Blackwell Scientific Publications, Oxford, United Kingdom. pp148.

Carriger, J. F., Rand, G. M., Gardinali, P. R., Perry, W. B., Tompkins, M. S. and Fernandez, A. M., 2006. Pesticides of potential ecological concern in sediment from South Florida canals, An ecological risk prioritisation for aquatic arthropods. Soil Sediment Contam., 15 (1): 21 – 45. https://doi.org/10.1080/15320380500363095

Chen, S. K., Edwards, C. A. and Subler, S., 2001. A microcosm approach for evaluating the effects of fungicides benomyl and captan on soil ecological processes and plant growth. Appl. Soil Ecol., 18 (1): 69 – 82. https://doi.org/10.1016/S0929-1393,01.00135-4

De, A., Bose, R., Kumar, A. and Mozumdar, S., 2014. Worldwide Pesticide Use In: De, A., Bose, R., Kumar, A. and Mozumdar, S., eds. Targeted Delivery of Pesticides Using Biodegradable Polymeric Nanoparticles. Springer Briefs in Molecular Science. Springer, New Delhi.

Deng, Y. and Wang, S. Y., 2016. Synergistic growth in bacteria depends on substrate complexity. J. Microbiol., 54 (1): 23 – 30. https://doi.org/10.1007/S12275-016-5461-9

El-Ghany, T. M. A., Masmali, I. A. and Negm, M. Pesticide-fungi interaction and their impact on fungal population in agricultural soil. Int. J. Microbiol. Allied Sci., 2015, 1 (4): 18 – 22.

El-Ghany, T. M. A. and Masmali, I. A. Fungal biodegradation of organophosphorus insecticides and their impact on soil microbial population. J. Plant Pathol. Microb., 2016, 7: 349.

Holt, J. G., 1982. The Shorter Bergey's Manual of Determinative Bacteriology. 8th Ed. The Williams and Wilkins Company, Baltimore, USA.

Hussain, S., Siddique, T., Saleem, M., Arshad, M. and Khalid, A., 2009. Impact of pesticides on soil microbial diversity, enzymes and biochemical reactions. Chapter 5 In: Sparks, D. L., ed. Advances in Agronomy, Vol. 102. Elsevier Inc., Netherlands.

Jamaluddin, M. A. and Pandey, A. K., 2017. A review on microbial degradation of organophosphorus pesticide: Methyl Parathion. Austin J. Biotechnol. Bioeng., 4(1): 1074

Javaid, M. K., Ashiq, M. and Tahir, M., 2016. Potential of biological agents in decontamination of agricultural soils. Scientifica, Article ID: 1598325. https://doi.org/10.1155/2016/1598325

Johnsen, K., Jacobsen, C. S. and Torsvik, V., 2001. Pesticides effects on bacterial diversity in agricultural soils - A review. Biol. Fertil. Soils 33: 443 – 453. https://doi.org/10.1007/S003740100351

Kanekar, P. P., Bhadbhade, B. J., Deshpande, N. M. and Sarnaik, S. S., 2004. Biodegradation of Organophosphorus Pesticides. Proc. Indian Natn Sci. Acad. B, 70 (1): 57 – 70.

Kannan, K., Tanabe, S., Williams, R. J. and Tatsukawa, R., 1994. Persistent organochlorine residues in foodstuffs from Australia, Papua New Guinea and the Solomon Islands, Contamination levels and dietary exposure. Sci. Total Environ., 153: 29 – 49.

Lal, R., 1982. Accumulation, metabolism and effects of organophosphorus insecticides on microorganisms. In: Allen, I. L., ed. Advances in Applied Microbiology. Academic Press, New York. pp149 – 200.

Laschi, S., Ogoncyzk, D., Palchetti, I. and Mascini, M., 2007. Evaluation of pesticide induced acetylcholinesterase inhibition by means of disposable carbon modified electrochemical biosensors. Enzym. Microbiol. Technol., 40: 485 – 489. https://doi.org/10.1016/j.enzmictec.2006.08.004

Lo, C., 2010. Effects of pesticides on soil microbial community. J. Environ. Sci. Health B, 45: 348 – 359.

Loos, M. A., Schlosser, I. F. and Maphan, W. R., 1979. Phenoxy-herbicide degradation in soils. Quantitative studies of 2,4 –D– and MCPA degrading microbial populations. Soil Biol. Biochem., 11 (4): 377 – 385. https://doi.org/10.1016/0038-0717,79.90051-8.

Mulbry, W., 2000. Characterisation of a novel organophosphorus hydrolase from Nocardiodes simplex NRRL B-24074. Microbiol. Res., 154 (4): 285 – 288. https://doi.org/10.1016/S0944-5013,00.80001-4

Mulbry, W. and Kearney, P. C., 1991. Degradation of pesticides by microorganisms and potential for genetic manipulation. Crop Prot., 10 (5): 334 – 346. https://doi.org/10.1016/S0261-2194,06.80021-9

Nelson, L. M., 1982. Biologically induced hydrolysis of parathion in soil isolation of hydrolysing bacteria. Soil Biol. Biochem., 14 (3): 219 – 222. https://doi.org/10.1016/0038-0717,82.90028-1

Okpokwasili, G.C. and Nwosu, A. I., 1990. Degradation of aldin by bacterial isolates. Nig. J. Technol. Res., 2:1 – 6.

Oritz-Hernandez, M. L. and Sanchez-Salinas, E., 2010. Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in Mexico. Rev. Int. Contam. Ambient., 26 (1): 27 – 38.

Pampulha, M. E. and Oliveira, A., 2006. Impact of an herbicide combination of bromoxynil and prosulfron on soil microorganisms. Curr. Microbiol., 53 (3): 238 – 243. https://doi.org/10.1007/S00284-006-0116-4.

Parte, S. G., Mohekar, A. D., Kharat, A. S., 2017. Microbial degradation of pesticide: a review. Afr. J. Microbiol. Res., 11(24): 992 – 1012.

Porto, A. L. M., Melgar, G. Z., Kasemodel, M. C. and Nitschke, M., 2011. Biodegradation of Pesticides. Chapter 20 In: Stoycheva, M., ed. Pesticides in the Modern World – Pesticides Use and Management. InTech Open, Croatia.

Rajendram, U. M., Kathrive, E. and Narayanaswamy, A., 2007. Effects of a fungicide, an insecticide and a bio-pesticide on *Tolypothrix scytonemoides*. Pestic. Biochem. Physiol., 87: 164 – 171. https://doi.org/10.1016/j.pestbp.2006.07.006

Rani, K. and Dhania, G., 2014. Bioremediation and biodegradation of pesticide from contaminated soil and water – A novel approach. Int. J. Curr. Microbiol. App. Sci., 3 (10): 23 – 33.

Robertson, B. K. and Alexander, M., 1994. Growth linked and co-metabolic biodegradation, possible reason for occurrence or absence of accelerated pesticide biodegradation. Pest Manag. Sci., 41 (4): 311 – 318. https://doi.org/10.1002/ps.2780410405

Serdar, C., Murdock, D. and Rohde, M., 1989. Parathion hydrolase gene from *Pseudomonas diminuta* MG, sub-cloning, complete nucleotide sequence and expression of the mature portion of the enzyme in *Escherichia coli*. Nat. Biotechnol., 7: 1151 – 1155. https://doi.org/10.1038/nbt1189-1151

Sharma, P., Sharma, S. K., Sharma, A., Sharma, A. and Paraher, P., 2013. Biodegradation of endosulfan using microbial culture. Int. J. Rec. Res. Rev., 6 (2):18 – 22.

Su, C., Jiang, L. and Zhang, W., 2014. A review on heavy metal contamination in soil worldwide, situation, impact and remediation techniques. Environ. Skep. Crit., 3 (2): 24 – 38.

Surekha, R. M., Lakshmi, P. K. L. and Suvarnalatha, D., 2008. Isolation and characterisation of a chlorpyrifos-degrading bacterium from agricultural soil and its growth response. Afr. J. Microbiol., 2 (2): 26 – 31.

Tayade, S., Patel, Z. P., Mutkule, D. S. and Kakde, A. M., 2013. Pesticide contamination in food, A review. IOSR J. Agric. Vet. Sci., 6 (1): 7 – 11.

Tkaczuk, C., Harasimiuk, M., Król, A. and Bereś, P. K. The effect of selected pesticides on the growth of entomopathogenic fungi *Hirsutella nodulosa* and *Beauveria bassiana*. J. Ecol. Eng., 2015, 16 (3): 177 – 183.

Valentine, N., Nousiainen, A. and Mikkonen, A., 2013. Introduction to organic contaminants in soil, Concepts and risks. Chapter 1 In: Vincent, T., Caminal, G., Eljarrat, E. and Barceló, D., eds. Emerging organic contaminants in sludges, analysis, fate and biological treatment. Volume 24 In: Barceló, D. and Kostianoy. A. G., eds.-in-chief. The Handbook of Environmental Chemistry, Springer-Verlag, Berlin Heidelberg. pp1 - 30.

Wang, M. C., Gong, M., Zang, H. B., Hua, X. M., Yao, J., Pang, Y. J. and Yang, Y. H., 2006. Effect of metamidorphos and urea application on microbial communities in soils as determined by microbial biomass and community level physiological profiles. J Environ Sci Health, 41: 399 – 413. https://doi.org/10.1008/03601230600616155

Wyss, A., Boucher, J., Montero, A. and Marison, I., 2006. Micro-encapsulated organic phase for enhanced bioremediation of hydrophobic organic pollutants. Enzyme Microb Technol 40 (1): 25 – 31. https://doi.org/10.1016/j.enzmictec.2005.10.033

Yue, X., Yu, X., Liu, Y. and Dong, Y., 2007. Effect of bensulfuron–methyl on growth of *Chlorella pyrenoidosa*. Agric Sci. China, 6: 316 – 321. https://doi.org/10.1016/S1671-2927,07.60051-0

Zhang, W., Jiang, F. and Ou, J., 2011. Global pesticide consumption and pollution: with China as a focus. Proc. Int. Acad. Ecol. Environ. Sci., 1 (2): 125 – 144.