International Journal of Science, Environment and Technology, Vol. 4, No 4, 2015, 1089 – 1097
ISSN 2278-3687 (O)
2277-663X (P)

IMPACT OF MICROBIAL DIVERSITY AND SOIL ENZYMATIC ACTIVITY IN DIMETHOATE AMENDED SOILS SERIES OF TAMIL NADU

S.F. Maleeka Begum[1*] and G. Rajesh[2]
[1]Head, [2]Research fellow
Department of Biotechnology,
Sri Krishna Arts and Science College, Kuniamuthur (P.O)
Coimbatore District, Tamil Nadu, India-641008
E-mail: maleekailliasbiotech@gmail.com (*Corresponding Author*)

Abstract: The evaluation of the adverse effect of pesticide on the microbial diversity and the soil enzyme activity were evidenced in soil amended with Dimethoate. Variations in activity were independent upon the period of incubation. An increasing trend in the activity of microbial population and soil enzyme activity were observed during 30th - 60th day of incubation in both control and treated soil. These fluctuations in activities were in accordance with soil pH and organic matter.
Key words: Amylase, Cellulase, Invertase, Microbial diversity, Pesticide, Urease

INTRODUCTION

Pesticides are widely used against a range of pests infesting agricultural crops. Globally, about 3×10^9 kg of pesticides is applied annually with a purchase price of nearly $40 billion each year (Pan-UK, 2003). The amount of applied pesticides reaching the target organism is about 0.1% while the remaining bulk contaminates the soil environment (Carriger *et al.,* 2006).Pesticides in soil undergo a variety of degradative, transport, and adsorption/desorption processes depending on the chemical nature of the pesticide (Laabs *et al.,* 2007) and soil properties (Weber *et al.,* 2004). Pesticides interact with soil organisms and their metabolic activities and may alter the physiological and biochemical behavior of soil microbes. Microbial biomass is an important indicator of microbial activities and provides direct assessment of the linkage between microbial activities and the nutrient transformations and other ecological processes. Generally, a decrease in soil respiration reflects the reduction in microbial biomass (Klose and Ajwa, 2004) or increase in respiration implies the enhanced growth of bacterial population (Haney *et al.,* 2000).

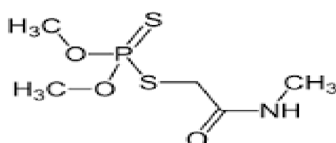
*Received July 10, 2015 * Published Aug 2, 2015 * www.ijset.net*

Some microbial groups are capable of using applied pesticide as a source of energy and nutrients to multiply, whereas the pesticide may be toxic to other organisms. Likewise sometimes, application of pesticides reduces microbial diversity but increases functional diversity of microbial communities even sometimes demonstrate the tendency of reversible stimulatory/inhibitory effects on soil microorganisms (Pampulha and Oliveira, 2006). Pesticides application may also inhibit or kill certain group of microorganisms and outnumber other groups by releasing them from the competition. Hence in the present study an attempt has been made to evaluate the dynamics of micro organism in pesticide (Dimethoate) amended soil further to correlate the percent inhibition of soil enzymes due to pesticide contamination. Further, it is also aimed to set up a strategy to use the indigenous organism for clean up mechanism by the process of Bioremediation. Because, Bioremediation constitutes an alternative to physio- chemical methods of remediation, as it as lees expensive and can selectively achieve complete destruction of organic pollutants.

MATERIAL AND METHODS

Pesticides used

The pesticide selected for the present study is Dimethoate which is basically an organo phosphate (OP) pesticide, chemically termed as O, O - dimethyl - S - methyl carboxyl - methyl phosphorodithioate (Chemical Abstracts Service (CAS) number 60-51-5) widely used in Tamil Nadu (Personal Survey). The commercial grade pesticide Dimethoate (30% E.C. Hygro) was procured from the market is a systemic organo phosphorous insecticide used for control the pests on cotton, tobacco, chilies, paddy, tea, ground nut, citrous fruit, vegetables and other crops. Dimethoate is considered to be highly toxic and carcinogenic substance WHO has categorized it as highly hazardous (HH) class I of compound.



DIMETHOATE

Soil selected for the study

Soil samples were collected from three different District representing the major soil series of Tamil Nadu viz., Achuvayal soil series of Ramnad District (Sample - A), a black clay soil rich in organic carbon. Paddy and chilies are mostly grown in this soil. Germination is more vigorous; yield will be higher in this soil. Pannivayal series - Pattukottai District (Sample - B) is brown clay soil rich in TAP, urea and potassium and paddy is mostly grown in this soil.

Irugur series - Coimbatore District (Sample - C) is a red clay soil. Banana and brinjal are cultivated in this soil. All the above informations are collected from the villagers through personal survey. The soil samples of the above mentioned three series were collected, labeled and packed in sterile bags and brought to the laboratory for further analysis.

Physicochemical properties of soils

The physicochemical properties, viz. soil moisture, water holding capacity, soil pH, and organic carbon were determined following standard methods (APHA)

Bacterial and fungal population in soil

The soil samples were amended with 1% Dimethoate and moisture content was maintained regularly and appropriate samples were withdrawn at regular intervals of 0, 2, 7, 15, 30, 45, 60, 90 and 120 days. The populations of bacterial and fungal isolates in terms of colony forming units (CFUs) were determined using viable plate count technique using N-agar and RBS-agar plates. All the plating were performed in triplicates and represented as mean values.

Enzyme studies

Soil samples were collected from Ramnad, Pattukottai and Coimbatore Districts of Tamil Nadu were dried in air dried, passed through 2 mm sieve and autoclaved at 121^0C, 15 psi for 30 minutes, allowed to cool to room temperature. Soil samples were amended with Pesticide solution (Dimethoate) at a concentration of 1%. Moisture content of the soils were maintained and at regular intervals and samples were withdrawn at regular intervals of 0, 2, 7, 15, 30, 45, 60, 90 and 120 days and analyzed for the activity of Amylase, Invertase, Cellulase and Urease.

Amylase, Invertase and Cellulase activity

Amylase and Invertase activity were assayed at pH 5.2 employing sodium acetate - acetic acid buffer with 0.6% soluble starch and with 4% sucrose as substrates respectively and Cellulase activity was assayed using phosphate buffer (pH 4.8) with 1% carboxymethyl cellulose. The enzyme activities were expressed in mg of glucose released/g of soil on dry weight basis for 24 hours of incubation (Nelson, 1944)

Urease activity

Urease activity in soil was expressed based on ammonia released which was determined spectrophotometrically after the formation of indophenol blue and the amount of ammonia released was calculated following the method of (Hofmann, 1965).

RESULT

Physico-Chemical characterization of soil

The pH of the sample A and C were found to be in a stabilized state throughout the experimental period, ranging between 7.2 - 7.4 and 7.4 - 7.5 respectively. Where as in the case of sample B the pH remained stabilized upto 60 days and on the 90th day the pH was dropped to 7.1 and on 120th day the pH regained to 7.8 (Figure 1).

The organic content of pesticide amended soil varied among the samples when compared with the control soil. The % organic carbon was high on the 60th day of incubation in all the three soils samples (Figure 2).

Viable count of bacterial and fungal population

The bacterial and fungal population in Dimethoate amended soil and control soil were determined in terms of CFUs using viable plate count technique. The results of the study revealed that in the control soil a decline in bacterial population were observed upto 45 days. Later an increase in bacterial population was recorded during 45th and 60th day of incubation. Followed by that a declining trends were recorded until 120 days. Where as in dimethoate amended soils different patterns of trend were recorded. The bacterial populations were high during 30th - 60th day, and 45th - 60th days in sample A and C respectively, however in sample B the highest population were recorded during 2nd - 7th day of incubation and 15th - 45th day of incubation. After 90 days a declining trend were observed in all control and amended soils (Figure 3).

The results of the fungal population revealed that a substantial higher count was recorded on 15th - 45th days of incubation in the control soil and in sample C. In sample A the fungal populations were high during 0th - 7th day and 30th - 60th day of incubation. Where as in sample B, fugal population were high during 0th - 2nd day and on 45th - 60th day of incubation (Figure 4).

Amylase activity

The amylase activities of soil were expressed as mg glucose/g soil dry weight. The amylase activity was found to be elevated on the 60th, 30th and 15th day of incubation in control soil and in sample A, B and C respectively. The maximum activity of amylase were recorded as 0.392 mg glucose/g soil dry weight, in control soil and 0.348 mg glucose/g soil dry weight on 15th day of incubation for sample C. 0.29 and 0.212 mg glucose/g soil dry weight for sample A , B on 60th day of incubation. However in all the samples a declining trend was observed on 120th day of incubation (Figure 5).

Invertase activity

The activity of invertase enzyme in the pesticide amended soil were found to be the highest activity of 0.3276 mg of glucose/g soil dry weight was record on 60th and 45th day of incubation in soil sample A and B respectively. Where as in sample C and control soil, the highest activity was found to be 0.342 and 0.392 mg of glucose/g respectively on 45th day of incubation (Figure 6).

Cellulase activity

The activity of cellulase in Dimethoate amended soils were found to be around 0.36 mg of glucose/g soil dry weight, during the incubation period of 60th day and 45th day of incubation in sample A and B respectively. The maximum cellulase activity of 0.327 mg glucose/g soil dry weight recorded during the incubation period of 45th day for sample C and 0.298 mg of glucose/g soil dry weight on 60th day of incubation in control soil (Figure7).

Urease activity

The activity of urease in Dimethoate amended soils were found to be 14.4, 16.3 and 18.2 µg ammonia/g soil dry weight for sample A, B and C on 60th, 60th and 30th day of incubation respectively and 23.0 mg of ammonia/g soil dry weight on 60th day of incubation in control soil. Lower urease activity of 7.3, 7.1, 9.2 and 12.4 µg ammonia/g soil dry weight was recorded on the 2nd (Sample A) and 120th day of incubation for sample B, C and control soil (Figure 8)

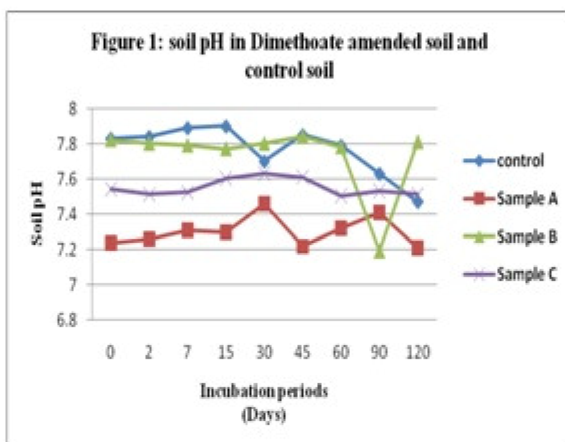
DISCUSSION

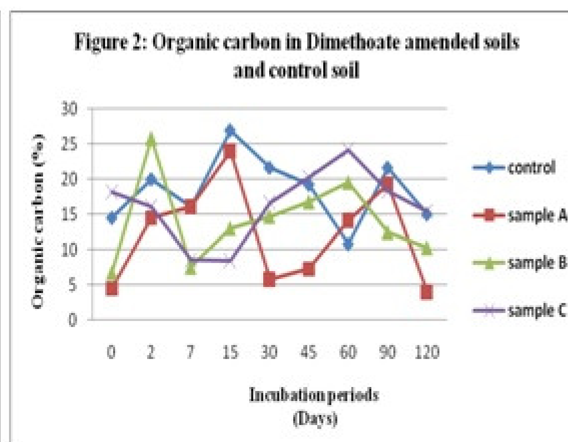
Bacteria because of their small size have a high surface area - to volume ratio with large contact interfaces with their surrounding environment. Soil bacteria thus have high potential as sensitive bioindicators of perturbations of soil quality by pesticide treatments. Toxic effects of pesticides are often evaluated by measuring the functional response of investigated soils, e.g. as overall rates of microbial activity. Thus, measurements of several, relatively specific enzymatic activities (amylase, invertase, cellulase and urease activity), or a combination of them, may contribute to characterize more precisely the response of soil bacterial communities to pesticides. Alkaline phosphatase, protease, urease, amidase, asparaginase and dehydrogenase activities have been used to assess the effect of the fungicide mancozeb (Rasool *et al.,* 2010), and dehydrogenase, phosphatase and urease in the case of the propiconazole and profenofos (Kalam, 2004). Combining such data with complementary measurements helps to assess key functions of the response of soil bacteria to pesticides at a more satisfactory level.

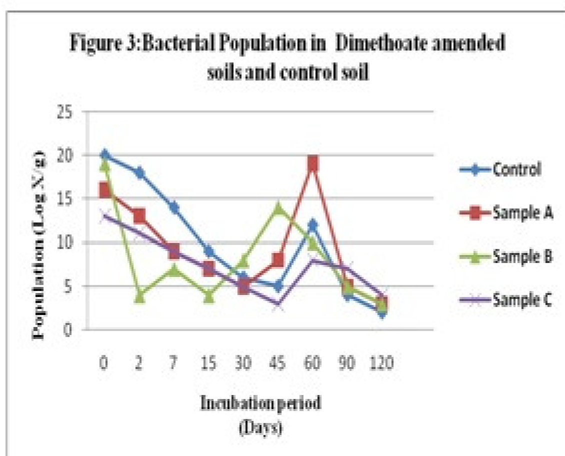
The results obtained by our investigations suggest that recovery of enzymatic activity following pesticide application was enhanced by repeated applications. This response may include pesticide degradation capacities and/or overall community tolerance to toxic bioactive chemicals. If a pesticide-dependent response may often be detected, however, demonstration of its dose-dependence is more complex and often seems to depend on the experimental methods used the characteristics of the investigated compounds, and soil properties. It often remains unclear to what extent such observed changes in enzyme activity result from an adaptative shift of soil communities towards more pesticide-tolerant types of bacteria, rather than from a lower nominal exposure to pesticides owing to dissipation or sorption of bioactive compounds to soil organic matter or minerals through time. The enzymatic response of soil bacteria to pesticides is also influenced by soil physico-chemical characteristics (Gevao *et al.,* 2000) and/or agricultural practice (Alletto *et al.,* 2010). These factors strongly affect the fraction of contaminant that causes an effect on soil micro-organisms. The bioavailable fraction of pesticides is controlled by soil properties, in particular by organic matter content, and by the physico - chemical properties of the pesticide molecule itself (its hydrophobicity in particular). The toxicity of pesticides to soil microorganisms may be markedly reduced in soils containing large amounts of organic matter or amendments. In one key study, dehydrogenase activity was undetectable after application of Dimethoate in both amended and unamended soils, but recovery of activity was observed after eight weeks in amended soils (Dungan, 2003). Such results are in agreement with assumptions that soil organic carbon content is a reliable predictor of soil bacterial biomass, independently of the presence or level of organic contaminants, and that effects of pesticides on soil microorganisms are more pronounced in light-textured soils with low organic content. Nevertheless, the limitations in the interpretation of enzymatic activities in pesticide contaminated soils are more profound.

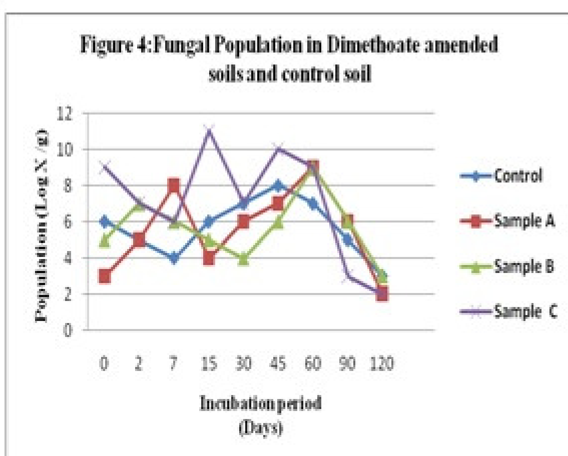
The heterogeneous physiological states of bacterial populations with respect to the effect of pesticides, and the difficulties to measure the bioavailable fraction of pesticides that affect bacterial populations do not permit a precise evaluation of the bacterial response. Secondly, soil enzymes may remain active after bacterial death and cell lysis. Therefore, enzyme activities cannot directly be used as a proxy for microbial biomass. Thirdly, soil organic matter and mineral surfaces may also contribute to enzyme stability. Fourthly, enzyme activities are often measured using convenient model substrates which bear only a passing resemblance with natural substrates in soil, so that measured activities may not adequately

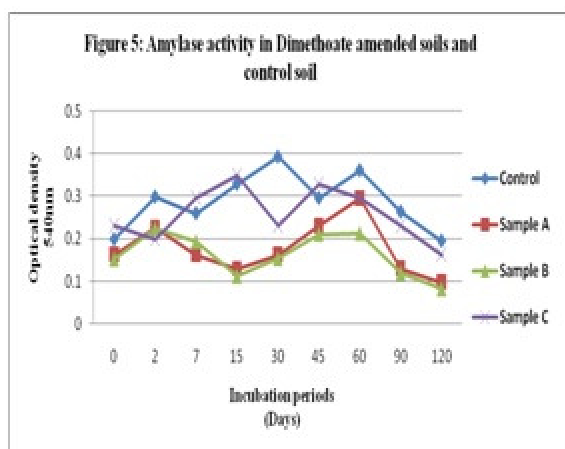
reflect actual microbial activities. Development of bioassays which are both more ecotoxicologically relevant (i.e. more sensitive) and more ecologically relevant (i.e. with a broader spectrum of application) will represent welcome improvements.

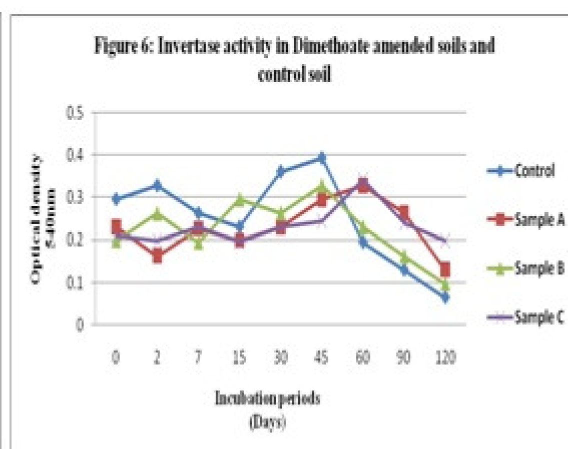


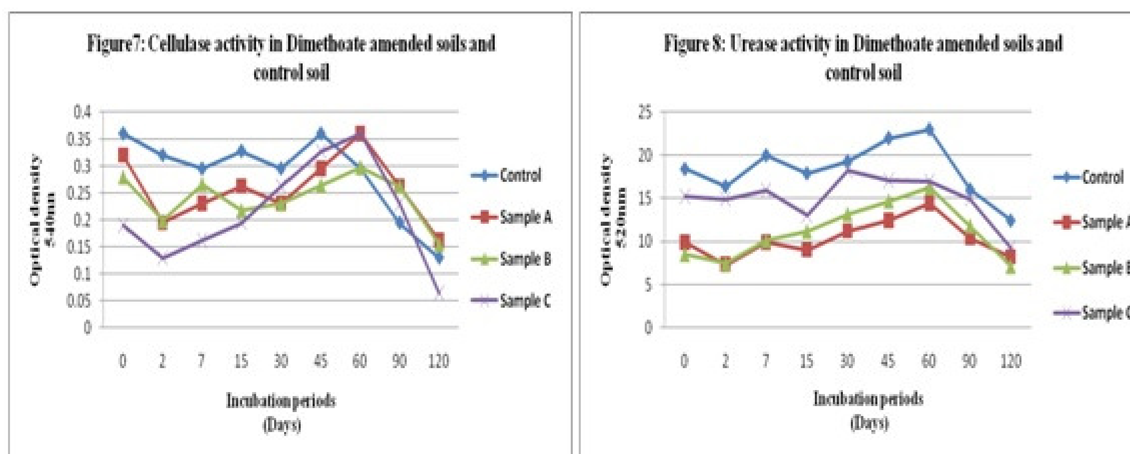












ACKNOWLEDGEMENT

The financial support by the Tamil nadu State Council for Science and Technology (TNSCST) for the conduction of this study is highly acknowledged.

REFERENCES

[1] Alletto, L.Y., Coquet, P. Benoit, D., Heddadj, E. and Barriuso. Tillage management effects on pesticide fate in soils. *A review Agron Sustain Dev.,* 30: 367-400 (2010).

[2] Carriger, J.F., Rand, G.M., Gardinali, P.R., Perry, W.B., Tompkins, M.S. and Fernandez A.M., Pesticides of potential ecological concern in sediment from South Florida Canals: An ecological risk prioritization for aquatic arthropods. *Soil Sed. Contam.,* 15: 21-45 (2006).

[3] Dungan, R.S., Ibekwe, A.M. and Yates, S.R., Effect of propargyl bromide and 1, 3 – dichloropropene on microbial communities in an organically amended soil. *FEMS Microbiol. Ecol.,* 43: 75-87 (2003).

[4] Gevao, B., Semple, K.T. and Jones, K.C., Bound pesticide residues in soils: *A review, Environ. Pollut.,* 108 (2000).

[5] Haney, R.L., Senseman, S.A., Hons, F.M. and Zuberer, D.A., Effect of glyphosate on the soil microbial activity and Biomass. *Weed sci.,* 48: 89-93 (2000).

[6] Hofmann, G. and Teicher, K., Colorimetric determination of urease activity in soils. *Z. Pflanzenernahr. Dung. Bodenk.,* 95: 55-63 (1961).

[7] Kalam, A., Tah, J. and Mukherjee, A.K., Pesticide effects on microbial population and soil enzyme activities during vermin composting of agricultural waste. *J. Environ. Biol.,* 25: 201-208 (2004).

[8] Klose, S. and Ajwa, H.A., Enzymes activities in agricultural soils fumigated with methyl bromide alternatives. *Soil Biology and Biochemistry.,* 36: 1625-1635 (2004).

[9] Laabs, V., Wehrhan, A., Pinto, A., Dores, E. and Amelung, W., Pesticide fate in tropical wet- lands of Brazil. An aquatic microcosm study under semi-field conditions. *Chemosphere.,* 67: 975-989 (2007).

[10] Nelson, N.A., Photometric adaptation of the Somogyi method for the determination of glucose. *J Bio Chem.,* 153: 373-380 (1944).

[11] Pampulla, M.E. and Oliveria, A., Impact of herbicide combination of bromoxynil and prosulfuron on soil microorganisms. *Curr. Microbial.,* 53: 238-243 (2006).

[12] Pan - UK., Current pesticide spectrum, global use and major concerns. (Online) http://www.pan-uk.org/brie.ng/sida_fil/chap1.htm. (14 February 2008) (2003).

[13] Rasool, N. and Reshi, Z.A., Effect of the fungicide Mancozeb at different application rates on enzyme activities in a silt loam soil of the Kashmir Himalaya, India, Trop. *Ecol.,* 51; 199-205 (2010).

[14] Weber, J.B., Wilkerson, G.G. and Reinhardt, C.F., Calculating pesticide sorption coefficients (K sub (d)) using selected soil properties. *Chemosphere.,* 55: 157-166 (2004).