Effects of some environmental factors on microbial growth and biodegradability of a pesticide mixture by soil micro organisms

Lienou C. TAZIEBOU, Francois-Xavier ETOA*., J. J. ESSIA NGANG

Faculty of Science, University of Yaoundé I

ABSTRACT

The effects of some environmental factors on microbial growth and biodegradability of butachlor [N-(butoxymethyl-2-chloro-2', 6'-diethyl acetanilide)] and oxyfluorfen [2-chloro-1- (3-ethoxy-4-nitrophenoxy) 4-(trifluoromethyl) benzene] mixture, applied in a water submerged soil were investigated. To determine the effects of pH and temperature, pesticides were inoculated in fresh soil samples and cultured aerobically at different pH ranges from 3 to 9. Different incubation temperatures ranging from 15°C to 45°C were used. Both fresh and pesticide exposed soil samples were used to study soil water content effects at different soil water content ranges of 60 to 90%. The results showed that at 25°C, bacteria and fungi were more active at pH7.2 and pH5 respectively. Bacteria supported high temperature at neutral pH better than they did at acid pH. The maximum degradability of the chemicals was obtained at pH5 for fungi and pH7.2 for bacteria. According to statistical analysis using student t-test of the three parameters studied, only one (water content) did not correlate to the micro-organisms cell count. Values obtained were as follow: p=0.476 for bacteria, p= 0.810 for actinomycetes and p= 0.812 for fungi. Correlation between pH, temperature and soil microbial count were statistically significant (p= 0.002 and p=0.006 for both bacteria and fungi).

Key words: biodegradability, pesticide.

RESUME

Les effets de certains facteurs environnementaux sur la croissance microbienne et la biodégradabilité, par les micro-organismes du sol, d'un mélange de pesticide (butachlor [n-(butoxymethyl-2-chloro-2', 6'-diethyl acetanilide)] et oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenoxy) 4-(trifluoromethyl) benzene]) appliqué à un sol submergé d'eau ont été étudiés. Les cultures en aérobie dans les boîtes de pétri ont été effectuées, avec les échantillons de sols non incubés pour les études de pH variant de 3 à 9 et de température variant de 15°C à 45°C et, les échantillons de sols incubés d'une part et non incubées d' autre part pour les études en teneur en eau variant de 60 à 90% ont été utilisées. Les résultats ont montrés que : Les bactéries et les champignons étaient plus actifs à 25°C aussi bien à pH acide qu'à pH neutre. Les bactéries supportaient mieux l'élévation de température à pH neutre qu'à pH acide. La biodégradabilité maximale des produits utilisés a été enregistrée à pH5 pour les champignons et à pH7,2 pour les bactéries. Les analyses statistiques utilisant le test de student (MINITAB, 1991) montrent que, des trois paramètres étudiés, seul la teneur en eau ne corrélait pas directement avec le nombre de colonies de microorganismes dénombrées. Les valeurs suivantes ont été obtenues: p= 0.476 pour les bactéries, p= 0.810 pour les actinomycètes, p= 0.812 pour les champignons. Le pH et la température ont donnés les valeurs de corrélation positives suivantes: p= 0.002 et p= 0.006 pour les bactéries et les champignons respectivement.

Mots clés: biodégradabilité, pesticide.

***Corresponding author.**

Faculty of Sciences, University of Yaoundé I, Department of Biochemistry. P. O. Box 812 Yaoundé. Email: fxetoa@yahoo.fr

I.INTRODUCTION

It is well known that for a micro-organism to succeed in a given environment, the various physicochemical factors such as temperature, redox potential, and pH must remain within the tolerable range of that organism. The presence of xenobiotics in their natural habitat complicate their main role, which is the bio-geo-chemical cycling of materials. Although several studies (Bollen, 1961; Tu & Bollen, 1968 Khan *et al*, 1975) indicated that these chemicals had little effect on microbial activities related to soil fertility, inhibitions were often observed (Moorman, 1989).

Because of the persistence of these compounds in soil, their accumulation in micro-organisms, surface and ground water; many results in medical problems reported in human beings (Mc Ewen & Stephenson, 1979), it is of importance to investigate if these pesticides have any pronounced influence on soil micro-organisms. The present physiological diversity of life could not exist if the dynamic equilibrium were perturbed.

However the use of chemicals has become an integral and economically essential part of modern agriculture, contributing to increased agricultural productivity; chemicals are being used increasingly in many countries especially where pests and diseases are the major factors limiting food production. Our concern in this work is the concern that, continuous input of pesticide might affect the microflora and so impair the soil fertility. For this reason a study of environmental parameters that can affect soil microflora needs to be done before a close definition of concentration / dosage that environmentally their application. It is in this regard that we chose to study the pH, temperature and soil water content as preliminary indicators of biodegradability of butachlor-oxyfluorfen mixture by soil micro-organisms in a water-logged soil.

II MATERIALS AND METHODS

II.1 Materials

II.1.1 Collection of soil samples

Water-submerged soil samples were collected up to a depth of 15 - 20 cm in a stream running nearby the F.M.B.S (Faculty of Medecine and Biomedical Sciences, University of Yaoundé I) and distributed in duplicates in 250 ml universal bottles. One of the samples was used for soil water content determination while the other was used for microbial analyses. A total of two samples were collected.

II.1.2 Pesticides

Pesticides used were a solution of butachlor (1) 50 %(w/v) and (2) oxyfluorfen powder (79.8 %W/W). Butachlor is sold under the name Machete-lambast-pillarsete (JaIger, 1983) and it is a synthetic herbicide acting as a protein synthesis inhibitor. Oxyfluorfen is sold under the name GOAL or KOOLTAR; it is a synthetic herbicide acting as a protoporphyrinogen oxidase inhibitor (Jaïger, 1983).

II.1.3 Culture media composition

The culture media used were prepared using the following formulations: the medium for total bacterial counts contained beef extract (3g/l), Nacl (3g/l), peptone (5g/l) and agar (20g /l) with pH 7.2-7.4. it is a modification of solid beef peptone medium as described by Min Hang (1999), with 2g/l less for both NaCl and beef extract. A basal salt medium contained $KN0_3$ (1g/l), KHP04 (5g/l); $MgS0_4$. 7H20 (0.5g/l) $FeS0_4$.7H20(0.01g/l) and agar (20g/l) with pH7.4 have been used for total actinomycetes count. The medium for total fungal counts contained chloramphenicol (0.5g/l), agar (20g/l) and 700ml of potato juice from 300g of diced and boiled potato with pH4.5-5.5; a modification of potato dextrose agar

II.2 METHODS

II.2.1 Pesticide solutions

100 ml of 10000 ppm butachlor solution was made by adding 2 ml of the stock solution (500000 ppm/ml) to 98ml of distilled water. For oxyfluorfen, 1.25g of the chemical was weighed and introduced into a conical flask of 100 ml, then diluted with acetone to make a 10000 ppm solution. From this, 1ml was pipetted into 50ml conical flask to make 50 ml of 200 ppm oxyfluorfen solution using diluted acetone and distilled water as solvent (50% v/v).

Previous studies (Taziebou, 2000) show that, at the concentration D1 of pesticide mixture (0.01ppm oxyfluorfen + 0.2 ppm butachlor), the number of bacteria and fungi that grow on media was higher than their number in the same media at the concentration D0 (0.00ppm oxyfluorfen + 0.00ppm butachlor).

II.2.2 Preparation of soil samples

For temperature and pH studies, 5ppm of butachlor (but.) and 0.3ppm of oxyfluorfen (ox.) were added to 25g of fresh soil sample in a 125ml bottle.

For water content studies, 50g of fresh soil sample were each placed in a series of 135 ml bottle and the desired concentration obtained by adding respectively 3.3ml, 8.3ml 13.3 ml and 18.3 ml of sterilised distilled water to make four concentrations (60, 70, 80 and 90%) of water content in the soil. Oxyfluorfen and 6ppm of butachlor were added to each soil sample. This permitted us to achieve the concentration (D1) in the first solution (60% water content).

The soil samples were shaken vigorously and incubated in the green house for 4 weeks. At the end of each week, 5 ml of soil sample were taken and diluted for quantitative determination of micro-organisms.

II.2.3 Quantitative determination of micro organisms

Two different diluted soil samples were used in the preliminary studies (pH and temperature). For temperature study, two pH (7.0-7.2 ranges and 4.5-5.0 ranges) were used for the study on bacteria and fungi at four different incubation temperatures (15°C, 25°C, 37°C and 45°C). For the pH study, 5 solutions of pH values 3, 5, 7.2, 8 and 9 were used for the study on bacteria and fungi, and emphasis was laid on detection of actinomycete. This is because actinomycete is a large group of bacteria in which many possesses both fungal and bacterial properties. In the soil they make up a significant proportion of the bacterial population, (about 10% to 33% (Alexander, 1977)). For the water content study, the pH and temperature were chosen according to preliminary studies(Taziebou, 2000). Four concentrations (60%, 70%, 80% and 90%) of water content in the soil were made.

Enumeration of microbial populations was done by standard plate count/ viable count using serial dilutions of the sample (Madigan *et al.,* 1997). The diluted soil sample, (1ml) was pipeted into a sterile Petri dish and melted agar medium was added. The suspension was carefully mixed, and allowed to set. The petri dishes were inverted subsequently before incubation. Bacteria and fungal colonies were counted after an interval of 3 to 9 days (Taziebou, 2000). The number of cells (N) was calculated using the formula: N = n x d x Ø where n is the number of colonies from a diluted sample d and Ø the percentage of dry soil.

III. RESULTS

The results of the effects of pH on soil microbial growth, presented in fig. 1a, b and c indicate that there very few of micro-organisms grew at pH3. The highest number of micro-organisms was obtained at pH 7.2 and at pH 5 for bacteria and fungi respectively.

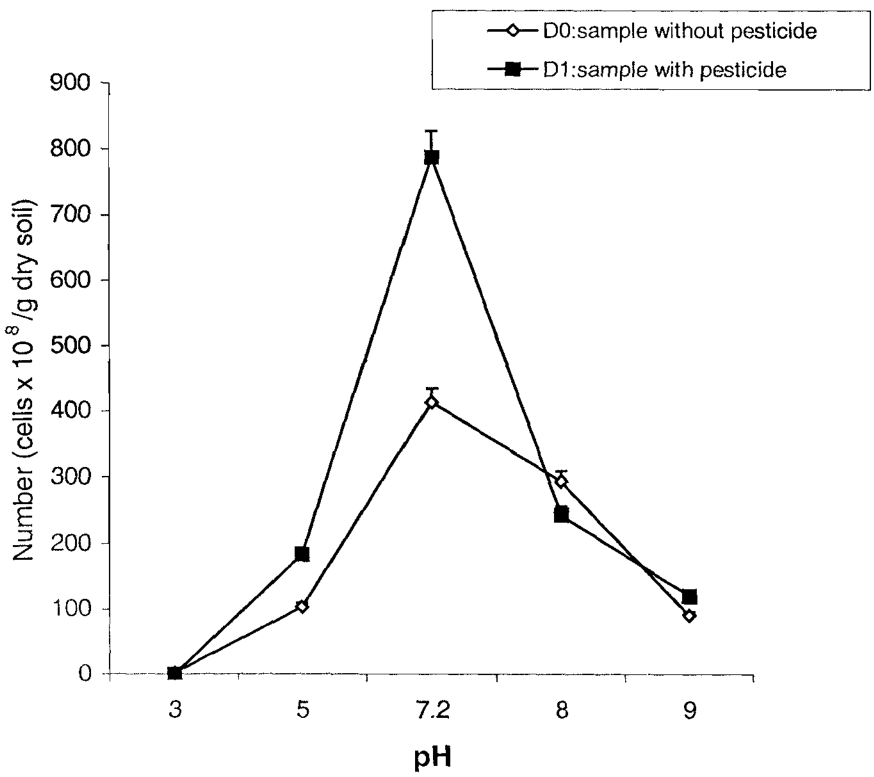

Fig. 1a: Effects of pH on soil bacterial growth from a pesticide exposed soil.

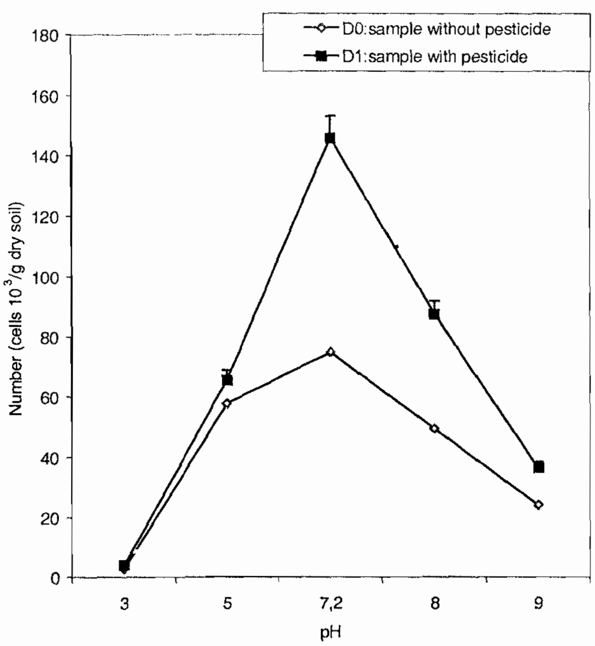

Fig. 1b: Effects of pH on soil actinomycetes growth from a pesticide exposed soil

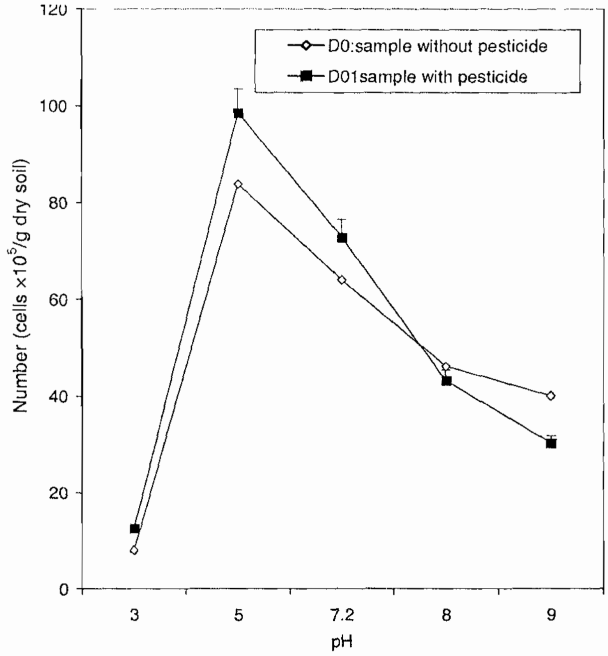

Fig. 1c: Effects of pH on soil fungi growth from a pesticide exposed soil

The results of the investigations on the effects of temperature and pH on microbial growth are presented in fig. 2a and 2b. It shows that, whatever the temperature, bacteria were more active at neutral pH (pH 7.2) compared to fungi that were more active at pH5. At 25°C, bacteria and fungi were more active at neutral pH than they do at acid pH.

The study of the effects of soil water content on soil micro-organisms in pesticide exposed soil, and its correlation with time of incubation (Fig.3) shows that: the fungi content was highest in fresh soil sample and this was found to be decreasing progressively during the 4 weeks incubation (Fig. 3c.). In contrast for bacteria, there was an increase of the population as long as the soil was being incubated (Fig3a). The number

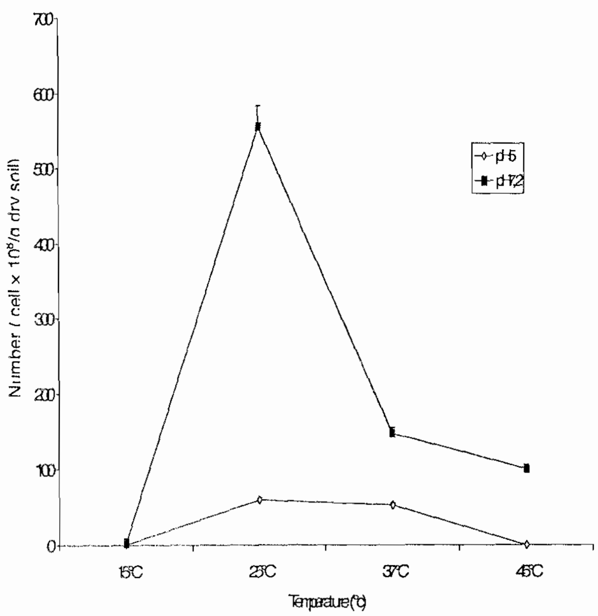

Fig 2a: Effects of temperature and pH on soil bacterial growth from a pesticide exposed soil

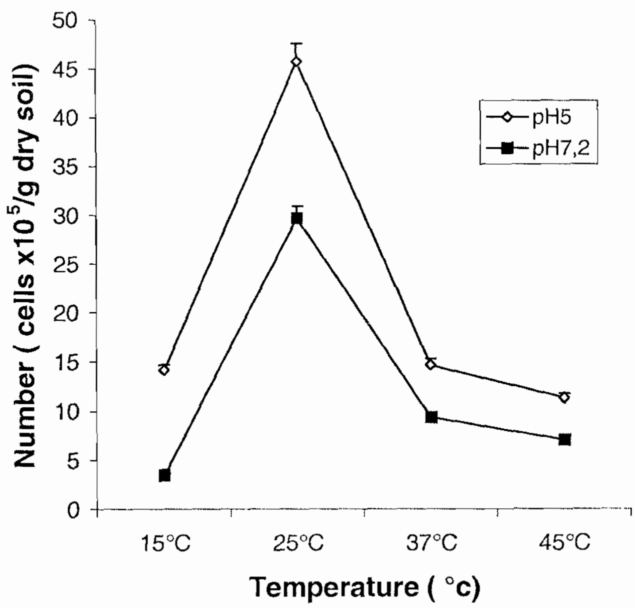

Fig. 2b: Effects of temperature and pH on soil fungi growth from a pesticide exposed soil

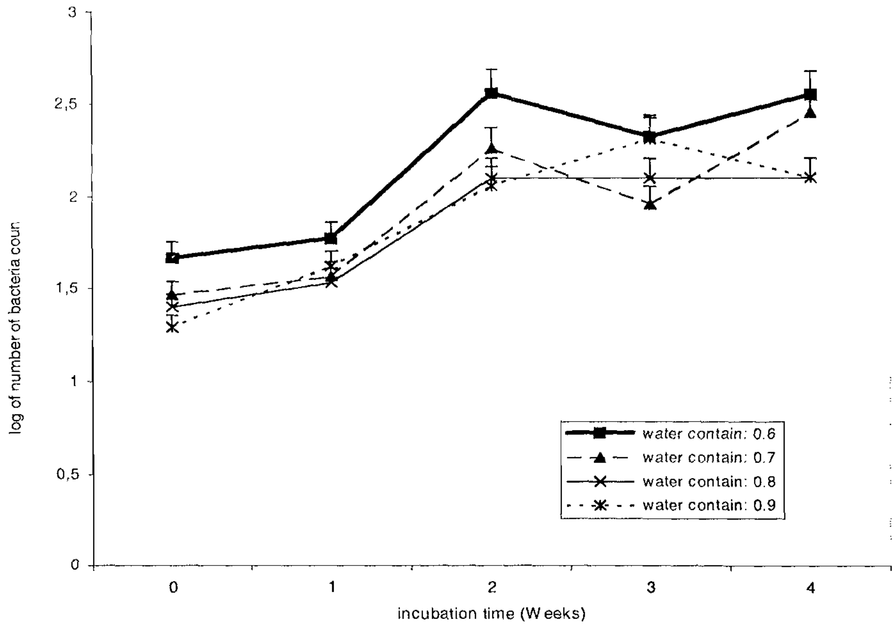

Fig. 3a: Time-dependent effects of soil water content on bacterial growth

of actinomycetes rose to a peak during the second week and decreased steadily till the last week of incubation. The highest number of micro-organisms was registered with the lower water content concentration of the soil, with the result for fungi being more distinct.

Statistical analysis using student test gave the following results: for water content studies relating to microbial growth, p= 0.476 for babteria, p= 0.810 for actinomycetes and p= 0.812 for fungi.
For pH and temperatures studies relating to soil microbial growth, p=0.002 and p=0.006 for both bacteria and fungi respectively.

IV. DISCUSSION

Results of the effects of pH on soil microbial growth indicated that, under conditions of high acidity, biodegradability of butachlor-oxyfluorfen mixture is lower

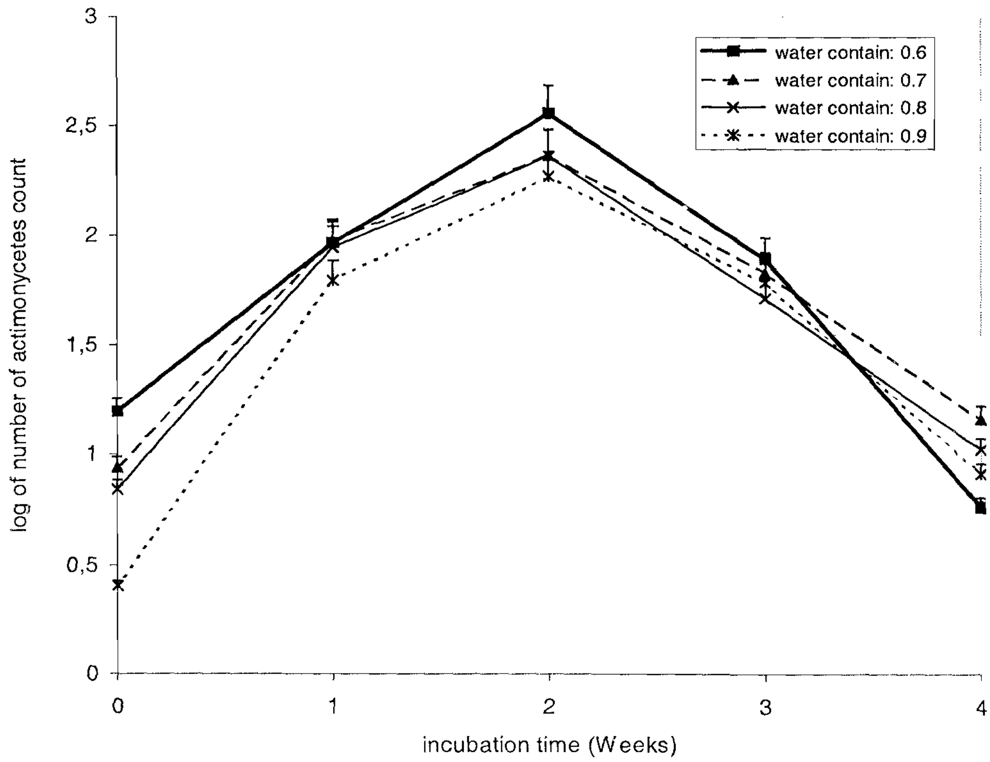

Fig. 3b: Time-dependent effects of soil water content on actinomycetes growth.

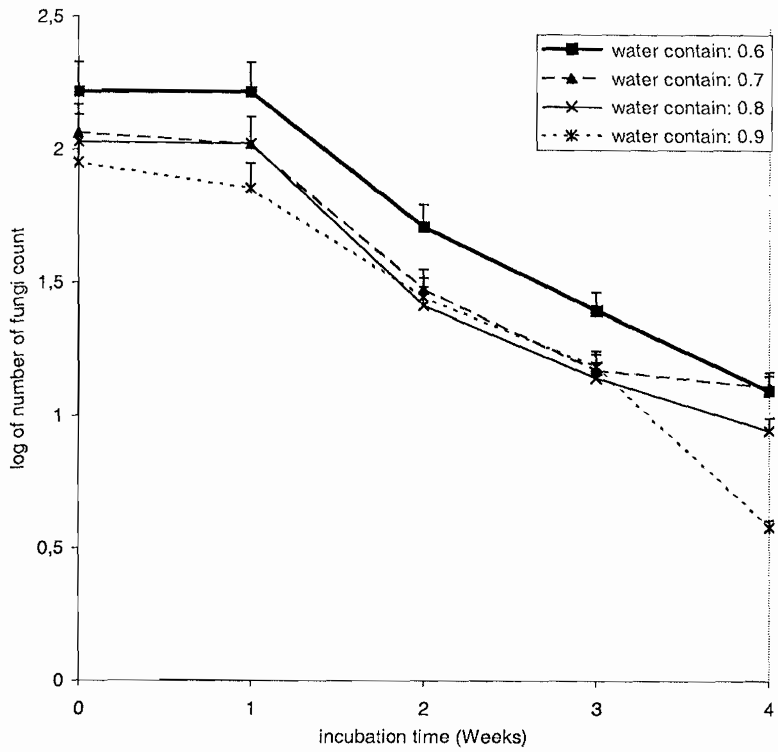

Fig. 3c: Time-dependent effects of soil water content on fungi growth

and slower due to decreased growth of micro-organisms. In a soil with a pH close to neutral, biodegradability of the mixture was more due to the increased activities of several groups of micro-organisms. In 1996, Eric Meller *et al., also* observed that, the right pH, not too acidic nor too alkaline, between 6 and 9 on the pH scale was suitable for bioremediation of xenobiotics. Also, Madigan *et al.*, (1997) noted that bioremediation of contaminated soil can be favoured by adjusting the soil pH to a value between 7 and 8. Biodegradability of butachlor-oxyfluorfen mixture effected more of fungi numbers at pH5. Similar results have been obtained by Okeke *et al.,* in 1996. They noted that some fungi grow optimally at pH 4 in the presence of pentachloramphenol.

Result of the effects of temperature and pH on soil microbial growth showed that biodegradation of the mixture used was more effective at 25°c under acidity close to neutral pH. The study of biotransformation of pentachloramphenol by *Lentinula edodes* and *Phanerochaete chrysosporium* showed that, the highest levels of degradation occurred for both fungi at 25°c (Okeke *et al.*, 1996).

From the results obtained for water content studies, it can be stated that, rainfall might have a negative influence on microbial growth after a field application of pesticide. This is because the saturation of the soil with water decreases oxygen level, thus limiting aerobic microbial activity. As observed by Zeyer *et al.*, (1990), biodegradation of aromatics is definitely slower under anaerobic than aerobic conditions.

Temperature and pH are essential parameters for microbial growth. They have to be well chosen for a better management of bacteria and fungi cultivation.

V. CONCLUSION

Before the use of pesticide, care should be taken to maintain the balance between temperature, pH and soil microbial flora. This will help in the rapid detoxification of the soil. For a given water content, sensitive species may be eliminated and replaced by less sensitive ones.

A study of the effects of environmental factors in relation to microbial activities might be done before a close definition of chemical application rates

RECOMMENDATION

In view of the hazards of misuse of chemicals in our society and environment, we are recommending that the authorities in charge of laws and policy of commercialisation of chemicals should endeavour to scientifically screen chemicals before their widespread use in the environment.

VI. BIBLIOGRAPHY

BOLLEN, W. B., 1961. Interactions between pesticides and soil micro-organisms. Ann. Rev. Microbiol. 15: 69-92.

ERIC MELLOR, PAUL LANDIN, CHRITINE DONOVAN, DAVE CONVOR, 1996. The microbiology of insitu bioremediation. Ground water pollution. Priner CE4594// soil and ground water pollution civil engineering. Dept, Virginia Tech.

JAÏGER G., 1983. Chemistry of pesticide. Buchel K. H. (eds) John Wiley and Son, New York, pp. 322-392.

KHAN, M. A. Q., GASSMAN, M. L. and ASHROFI S. H., 1975. Detoxification of pesticides by biota. In: Environmental dynamics of pesticides, Haque R., and V. H. Freed (eds) Phenum press, New York & London pp. 289-329.

MADIGAN T. M., MARTINKO M. J. and PARKER J., 1997. *Brock* Biology of microorganisms (beb) printice-hall, New York. Pp

MCEWEN F.L. and STEPHENSON G.R., 1979. The use and significance of pesticides in the environment. By John Wiley and sons, I.N.C

MIN H., ZHAO Y., ZHENG P;, WU W;, and CHEN J, 1999. Microbial research techniques. Science press. Beijing.

MINITAB. Inc.1991 MINITAB. Reference manual. Release 8 PC Version. MINITAB statistical software, PA USA.

MOORMAN T.B.,1989. A review of pesticide effect on soil micro-organisms and microbial processes related to soil fertility. *J. Prod. Agric.* 2(1): 14-23.

OKEKE B.C., SMITH JE, PATERSON A., VATSON-CRAIK A., 1996. Influence of environmental parameters on pentachlomphenol biotransformation in soil by Lentinula edode and phenerochaete chrysosporium. PubMed index for MEDLINE* ppl Microbiol Biotechnol.

TAZIEBOU LIENOU C., 2000. In: biodegradation of waste materials: Effects of butachlor-oxyfluorfen mixture on soil micro-organisms. Master Thesis; University of Yaoundé I. Pp. 18-27

TU C. M. & BOLLEN W. B., 1968. Effects of paraquat on microbial activities in soil. Weed Rev. 64: 17-65.

ZEYER J., EICHER E., J. DOLFING and R.P. SCHWARZENBATCH., 1990. Anaerobic degradation of aromatic hydrocarbons. Inc. D. Kamely, A. Chakraborty, and G. S. Omenn (ed); Biotechnology biodegradation. Gulf pub. Co., HOUSTON, Tex., 34-40.

Received: 14/07/2003
Accepted: 23/01/2004