JOURNAL OF PURE AND APPLIED MICROBIOLOGY, March 2013. Vol. **7**(1), p. 85-91

Biodegradation of Fenitrothion and Butachlor using Liquid Culture Microorganisms

Sherif H. Abd-Alrahman[1,2] and Mounir M. Salem-Bekhit[3,4]

[1]Biomarkers Research Program, Biochemistry Department, College of Science, King Saud University, PO Box, 2455, Riyadh, 11451, Saudi Arabia.
[2]Pesticides Residue and Environmental Pollution Dept., Central Agricultural Pesticide Laboratory, Agricultural Research Center, Giza 12618, Egypt.
[3]Kayalli Chair for Pharmaceutical Industries, Department of Pharmaceutics, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh, Saudi Arabia.
[4]Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

(Received: 29 January 2013; accepted: 05 March 2013)

Fenitrothion and butachlor are widely used pesticides in pest control. These pesticides are released into the environment presenting a potential hazard risk. Only limited data are available on the microbial biodegradation of fenitrothion butachlor. Biodegradation of fenitrothion and butachlor by different six microorganisms were investigated. These strains were isolated from an agricultural soil and found to actively utilized butachlor, as a sole source of carbon and energy. Based on their morphological and biochemical categorization, the six microorganisms isolates were identified as *Pseudomonas alcaligens*, *Bacillus licheniformis*, *Bacillus megaterium*, *Trichoderma viride*, *Rhizobium huakuii* and *Bradyrhizobium japonicum*. Results showed that the *Trichoderma viride* and *Pseudomonas alcaligens* presented an average value of degradation of 98% and 75% respectively in a medium containing 50 mg/kg of butachlor after 15 and 21 days. According to these results, both organisms revealed considerable potential for application in bioremediation of contaminated water with fenitrothion butachlor residues.

Key words: Pesticides, Fenitrothion, Butachlor, Biodegradation, Microorganisms.

The extensive use of pesticides in agriculture has led to their widespread release into environment. Environmental contamination with pesticide residues increasing the accumulation of these pesticides in environment resources and different food chain (Purkait *et al.,* 2009). Microbial degradation is an important process affecting the fate and behavior of pesticides. The bioremediation techniques have become a popular alternative to chemical or physical remediation because of their relatively low cost and minimal impact on the environment (Akiner & Caglar 2012, Diez 2010). Techniques using microorganisms to degrade contaminates in polluted sites have been commercially available since the 1970s (Newcombe & Crowley 1999).

Fenitrothion and butachlor are model xenobiotic pollutants in environment for biodegradation studies, because it is one of the most widely use in agriculture for pest control. They have been detected in different environmental sources in several monitoring programs. The ability of different microorganisms to degrade the fenitrothion and butachlor for

* To whom all correspondence should be addressed.
Tel: +966 561776615;

environment has been studied in this investigation. Fenitrothion [O,O-dimethyl-(3-methyl-4-nitro phenyl) phosphorothioate, an organophosphorus pesticide] is mainly used against spruce bud worms and cotton pests. It is widely used in tropical countries against malaria. Fenitrothion shows low toxicity against mammals (Nishizawa *et al.,* 1961). Butachlor (N-butoxymethyl-2-chloro- 2,6-diethyl acetanilide) is one of the most widely recommended herbicides. It is a preemergence herbicide belonging to chloroacetanilide group used widely in oriental countries for the control of annual grasses for rice cultivation (Jena 1987, Yu *et al.,* 2003). The recent studies, reported that the application of butachlor has adverse environmental impact; butachlor was found to flow out with effluents, causing contamination of river and ground water (Natarajan *et al.,* 1993, Ohyama *et al.,* 1986, Yamagishi &Akiyama 1981), it has toxicity to aquatic organisms. (Ateeq *et al.,* 2002, Ateeq *et al.,* 2006, Lin 1997).

Nowadays, the use of microorganisms to biodegrade this kind of waste is almost imperceptible, as the most popular way of managing with them is thermal utilization (Martínez November 2004). Bacteria, actinomycetales (special group of bacteria), and fungi showed the biggest capability of degrading pesticides. The species of bacteria and fungi distinguishing the strongest activity in degrading pesticides are: bacteria of the genera Arthobacter, Bacillus, Corynebacterium, Flavobacterium, and Pseudomonas; actinomycetales of the genera Nocardia and Streptomyces; and fungi Penicillium, Aspergillus, Fusarium, and Trichoderma (Feakin 1995, Kucharski 2009, Omar 2001, Rousseaux *et al.,* 2001). (Koliæ 2007, Topp 2001, Vibber *et al.,* 2007) point out that in their research, the biggest biodegradation potential concerned bacteria *Pseudomonas* sp. and *Nocardioides* sp.

The aim of this study is to determine the ability of six different microorganisms to degrade fenitrothion and butachlor to investigate the biodegradation rate provide basic information for developing regulations regarding environmental contamination by fenitrothion and butachlor and their potential public health effects.

MATERIALS AND METHODS

Search for biotopes consisting of butachlor-degrading microorganisms

Six different microorganism strains were obtained from from the microbiology laboratory, Department of life science, faculty of science, King Saud University. All purified microorganisms were tested for their abilities to grow in the presence of pesticides used butachlor in nutrient agar medium (Sigma, USA): Beef extract 3.0gm, Peptone 5.0gm, Agar-agar 20.0 gm and Distilled water to make 1 litter (H2O) 1.0L at pH 7.2 (Jacobs 1960). The resulting colonies were repeatedly subcultured in M9 medium containing 10 ppm butachlor to confirm their butachlor-catabolising ability. Inoculated plates were incubated at (30°C ±2) for 7days. The growth of microorganisms used for standing the toxicity of pesticide was determined and recorded as growth or inhibition. Identification and characterization of the isolates were carried out on the basis of the colony morphology, biochemical characteristics (Singh 2009).

Microorganisms, which were able to utilize Butachlor as a sole carbon and/or nitrogen source, were tested for their abilities to degrade these pesticides. Tested microorganism was incubated into liquid aqueous (basal medium): KH_2PO_4 1.0gm, K_2HPO_4 1.0gm, NH_4NO_3 1.0gm, $MgSO_4.7H_2O$ 0.2 gm, $CaCl_2$ 0.02gm, $Fe(SO_4)_3$ 0.01gm and Distilled water to make 1 liter (H_2O)1.0 L at pH 7.0 (Miles 1996), with pesticide used for 21 day at (30°C ±2). Samples were taken from treatments and control at intervals of 0, 1, 3, 6, 9, 12 and 15 days for determination. Each culture was filtered through whatman No.1 filter paper. Pesticide residues were extracted from filtrate using the method adopted by Letizia (Letizia 1992).

Standard calibration curves

Analyses of butachlor and fenitrothion were performed using certified analytical standard of butachlor and fenitrothion (Fig. 1). The Stock solution of fenitrothion and butachlor was prepared by dissolving 50 mg of the analyte (accurate weight) in 50 mL n-hexane to obtain concentration 1 mg mL^{-1}. Working standard solutions of 0.05, 0.1, 0.25, 0.5 and 1.0 µg mL^{-1} were prepared by appropriately diluting the stock solution with n-hexane. Stock solution was stored at -20 ± 2 °C, and working standard solutions were stored in d”

4 °C when not in use. Calibration curves were generated by plotting peak area versus concentration.

Chromatographic analysis

Agilent 6890 (USA) gas chromatography coupled with electron capture detector (GC-ECD) was used for determination of butachlor residues, for fenitrothion residues GC-with nitrogen phosphorus detector (GC-NPD) was used. Separation was performed using capillary column HP-5 (30m × 0.25mm × 0.25µm). Nitrogen was used as the carrier gas at a flow rate 2ml/min. The following temperature program was employed: initial temperature of 180 ºC held for 1 min; increased at 25 ºC min^{-1} to 220, held for 2 minutes; yet another increase at 3 ºC min^{-1} to reach 245 ºC. The injector temperature was 220 ºC. The injection volume was 1 µl for all standard and samples. Data analysis was performed using Chemistation software.

Statistical analysis

Data were statistically evaluated by one-way analysis of variance (ANOVA). Determination of the differences among means were carried out by using the least significant differences (LSD) test. All statistical analyses were done using the Statistical Package for social sciences (SPSS 16.0) program.

RESULTS AND DISCUSSION

This experiment aimed to clarifying the effect of fenitrothion and butachlor on the growing of different microbial strains to determine capability of these bacteria and fungi strains for biodegradation of these two tested pesticides. Data in (Table 1) showed the inhibition of microbial strains growth by pesticide residues on nutrient agar medium. Five strains of these microbes can be growing without any inhibition with fenitrothion and butachlor pesticides, while other strains showed different trends. The second experiment had studied the biodegradation of both tested pesticides, butachlor and fenitrothion by selected microbial strains into a basal liquid media lacking a carbon source for 15 days incubation time at 30°C ±2. Results showed the microbial degradation of Butachlor as the sole carbon and energy source within 15 days incubation time at 30°C ±2. The obtained results indicated that strains of *Bacillus megatherium, Trichoderma viride, Brady rhizobium sp., E. coli,* and *Pseudomonas sp.* clearly degraded Butachlor from 0.0 % to 28.4, 75.1, 41.7, 43.6 and 71.4% loss, respectively (Table 2 and fig 1). The highest degradation rate was observed with Trichoderma viride and Pseudomonas sp., it's reached to 75.1 and 71.4 % loss respectively. These results are in agreement with that obtained by (Anwar *et al.,* 2009, Mandelbaum *et al.,* 1995), who isolated also a *pseudomonas sp.* capable of degrading a chlorinated herbicide atrazine at conc. 1000 ppm. The organism was isolated from a nutrient agar solid medium and didn't show inhibition zone that indicated that the resistance of pseudomonas to atrazine. A biotic degradation was also noticed in the same table via control treatment, Butachlor degraded from (0.0 to 19.9 loss %). This could be related to physicochemical degradation. The effect of both a biotic and biotic degradation was clearly illustrated via half-life

Table 1. Pesticide-tolerance of microorganisms.

Microorganism	Pesticides were treated at conc. (5 ppm)	
	Fenitrothion	Butachlor
Pseudomonas sp.	-	-
Bacillus megatherium	-	-
Trichoderma viride	-	-
Rhizopus sp.	+	-
Rhizobium sp.	+	+
Brady Rhizobium sp.	-	-
E. coli	-	-

(+): growth was inhibited (-): no inhibition

values was reached to 73.72, 40.57, 11.61, 18.78, 22.17 and 8.51 days for control, *Bacillus megatherium, Trichoderma viride, Brady Rhizobium*, *E. coli*, and *Pseudomonas sp*., respectively, which were determined according to a kinetic model approach for determination of biodegradation half-lives (Matthies *et al.,* 2008). Results of the biodegradation of fenitrothion as a sole carbon and energy source indicated that fenitrothion was microbially degraded from 0.0 % to 44, 48.5, 45.9, 77.9 and 52.1% loss for *Bacillus megatherium, Trichoderma viride, Brady rhizobium sp., E. coli*, and *Pseudomonas sp.,* respectively. The half-life values were reached to 32.77, 28.1, 16.05, 17.11, 6.51 and 13.07 days for control, *Bacillus megatherium, Trichoderma viride, Brady rhizobium sp., E. coli*, and *Pseudomonas sp.,* respectively (Table 3 and fig 2). Our results are in agreement with the results of (Baczynski *et al.,* 2010, Kadian *et al.,* 2008, Singh *et al.,* 2003), who examined the role of microorganisms in the degradation of organophosphorus pesticides in soil. Results showed that chlorpyrifos as organophosphorus pesticide like fenitrothion, was biodegradable by the co metabolic activities of soil microorganisms. Also interestingly results indicated the important role of nitrogen fixing such as *Brady rhizobium sp*. in the degradation of fenitrothion as the sole carbon source. From the above-mentioned results, it could be conclude that both Butachlor and Fenitrothion are biodegradable pesticides. The different microorganisms used could have dioxygenases enzymes, which could initiate alpha or Beta cleavage and subsequent mineralization of both pesticides. This could be noticed with strains *Trichoderma viride, E. coli,* and *Pseudomonas sp*.

Table 2. Biodegradation of Butachlor by different microorganisms

Time	Control		M 1		M 2		M 3		M 4		M 5	
in days	(ug)	% loss	(ug)	% loss	(ug)	% loss	(ug)	% loss	(ug)	% loss	(ug)	% loss
0	100	0	100	0	100	0	100	0	100	0	100	0
1	100	0	100	0	97.6	2.4	98.1	1.9	98.2	1.8	89.9	10.1
3	98.5	1.5	93.5	6.5	87.2	12.8	86.5	13.5	90.1	9.9	76.2	23.8
6	93.9	6.1	89.3	10.7	71.7	28.3	77.4	22.6	85.6	14.4	65.3	34.7
9	89.3	10.7	84.8	15.2	56	44	70.8	29.2	74.8	25.2	54.7	45.3
12	84.7	15.3	78.1	21.9	40.5	59.5	64.3	35.7	63.2	36.8	43.1	56.9
15	80.1	19.9	71.6	28.4	24.9	75.1	58.3	41.7	56.4	43.6	28.6	71.4
T 0.5	73.27	40.57	11.61	18.78	22.17	8.51						

(M1): *Bacillus megatherium,* (M2): *Trichoderma viride*, (M3): *Brady Rhizobium sp.*,
(M4): *E. coli*, (M5): *Pseudomonas sp.*

Table 3. Biodegradation of fenitrothion by different microorganisms

Time	Control		M 1		M 2		M 3		M 4		M 5	
in days	(ug)	% loss	(ug)	% loss	(ug)	% loss	(ug)	% loss	(ug)	% loss	(ug)	% loss
0	100	0	100	0	100	0	100	0	100	0	100	0
1	99.3	0.7	100	0	95	5	95.8	4.2	84.8	15.2	91.5	8.5
3	95.6	4.4	94.3	5.7	89.1	10.9	89.8	10.2	71.9	28.1	88.6	11.4
6	88.2	11.8	86.9	13.1	79.4	20.6	80.9	19.1	59.3	40.7	78.7	21.3
9	79.8	20.2	75.2	24.8	69.2	30.8	69	31	46.8	53.2	64.8	35.2
12	70.1	29.9	66.0	34	57.9	42.1	57.1	42.9	33.5	66.5	53.9	46.1
15	64.5	35.3	56.0	44	51.5	48.5	54.1	45.9	22.1	77.9	47.9	52.1
T 0.5	32.77		28.1		16.05		17.11		6.51		13.07	

(M1): *Bacillus megatherium,* (M2): *Trichoderma viride*, (M3): *Brady Rhizobium sp.*,
(M4): *E. coli*, (M5): *Pseudomonas sp.*

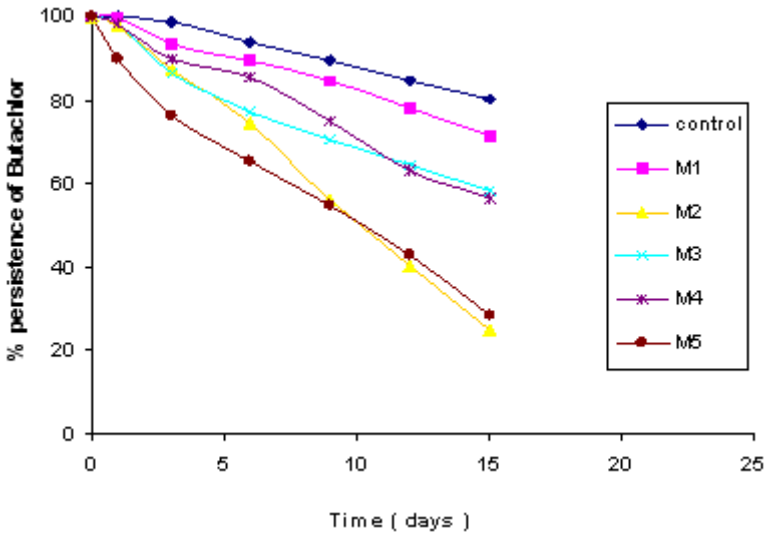

Fig. 1. Biodegradation of Butachlor by different microorganisms.

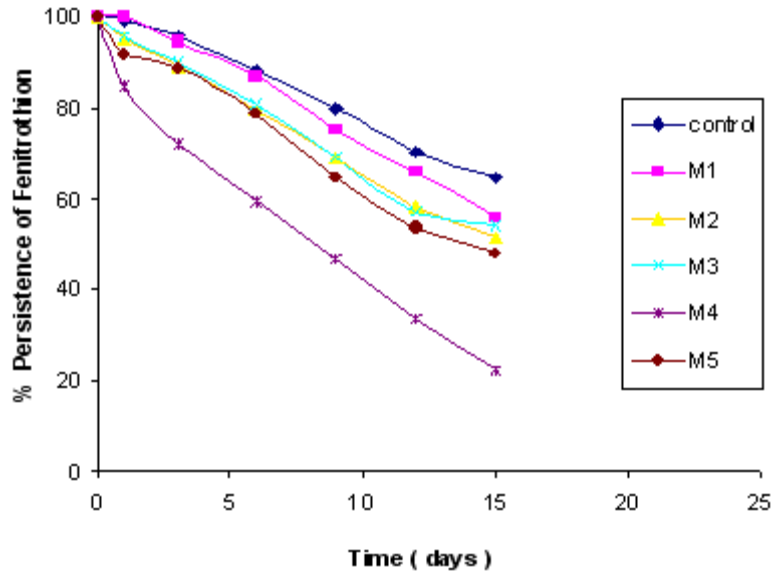

Fig. 2. Biodegradation of Fenitrothion by different microorganisms

CONCLUSIONS

Six different microorganisms' strains were tested to degrade butachlor. Results showed growth inhibition of two microorganisms and four of these tested microorganisms had different degradation rate of butachlor. According the data *Trichoderma viride* has the highest degradation rate followed by *Pseudomonas sp.* 97.6 % and 94.7 % butachlor reduction in 15 and 21 days, respectively. These results presented that both of these two organisms can be used to degrade the butachlor formulation. These results provide basic information for developing regulations regarding the safe disposal of butachlor using eco-friendly method. We suggest that further studies use different microorganisms in the degradation of different pesticides, to protect the environment and public health.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group no: RGP- VPP-202.

REFERENCES

1. Akiner MM, Caglar SS., Monitoring of five different insecticide resistance status in Turkish house fly Musca domestica L. (Diptera: Muscidae) populations and the relationship between resistance and insecticide usage profile. *Turkiye Parazitol Derg* 2012; **36**: 87-91
2. Anwar S, Liaquat F, Khan QM, Khalid ZM, Iqbal S., Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by Bacillus pumilus strain C2A1. *J Hazard Mater* 2009; **168**: 400-5
3. Ateeq B, Abul farah M, Niamat Ali M, Ahmad W., Induction of micronuclei and erythrocyte alterations in the catfish Clarias batrachus by 2,4-dichlorophenoxyacetic acid and butachlor. *Mutat Res* 2002; **518**: 135-44
4. Ateeq B, Farah MA, Ahmad W., Evidence of apoptotic effects of 2,4-D and butachlor on walking catfish, Clarias batrachus, by transmission electron microscopy and DNA degradation studies. *Life Sci* 2006; **78**: 977-86
5. Baczynski TP, Pleissner D, Grotenhuis T., Anaerobic biodegradation of organochlorine pesticides in contaminated soil - significance of temperature and availability. *Chemosphere* 2010; **78**: 22-8
6. Diez MC., Biological Aspects Involved in the Degradation of Organic Pollutants. *J. soil. sci. plant. nutr*. 2010; **10**: 244 - 267
7. Feakin SJ, Blackburn, E., & Burns, R. G.., Inoculation of granular activated carbon in a fixed bed with s-triazinedegrading bacteria as a water treatment process. *Water Research* 1995; **29**: 819-825.
8. Jacobs MBaG, M.d. (Editor), Handbook of microbiology. . D. Van Nostrance Co., Inc., Newyork.: 1960 ; 139-207. pp
9. Jena PK, Adhya, T.K., Rao, V.R., Influence of carbaryl on nitrogenase activity and combination of butachlor and carbofuran on nitrogen-fixing microorganisms in paddy soils. *Pestic. Sci*. 1987; **19**: 179–184.
10. Kadian N, Gupta A, Satya S, Mehta RK, Malik A., Biodegradation of herbicide (atrazine) in contaminated soil using various bioprocessed materials. *Bioresour Technol* 2008; **99**: 4642-7
11. Koliæ NU, Hrasak, D., Begonia, A. K., Petriæ, I., Stipicevic, S., Soulas, G., *et al.,* Combined metabolic activity within an atrazine-mineralizing community enriched from agrochemical factory soil. *International Biodeterioration and Biodegradation* 2007; **60**: 299–307.
12. Kucharski J, Baæmaga, M., & Wyszkowska, J. Effect of soil contamination with herbicydes on the nitrification process. *Ecologocal Chemistry and Engineering* A 2009; **16**: 947–952.
13. Letizia MDMBLPaML., Evaluation of the membrane approach to solid-phase extraction of pesticide residues. *Pesticide Science* 1992; **35**: 63-67.
14. Lin KH, Yen, J.H., Wang, Y.S., Accumulation and elimination kinetics of herbicides butachlor, thiobencarb and chlomethoxyfen by Aristichthys nobilis. . *Pesticide Science* 1997; **49**: 178e184.
15. Mandelbaum RT, Allan DL, Wackett LP., Isolation and Characterization of a Pseudomonas sp. That Mineralizes the s-Triazine Herbicide Atrazine. *Appl Environ Microbiol* 1995; **61**: 1451-7
16. Martínez J., Practical guideline on environmentally sound management of obsolete pesticides. In: Basel convention coordinating centre for Latin America and the Caribbean M, Uruguay, (Hrsg.), 2004.
17. Matthies M, Witt J, Klasmeier J., Determination of soil biodegradation half-lives from simulation testing under aerobic laboratory conditions: a kinetic model approach. *Environ Pollut* 2008; **156**: 99-105
18. Miles JRWCMTaH, C.R, Metabolism of heptachlor and its degradation products by soil microorganisms. *Journal of Economic entomology* 1996; **262**: 1334-1338.
19. Natarajan AT, Darroudi F, Jha AN, Meijers M, Zdzienicka MZ., Ionizing radiation induced DNA lesions which lead to chromosomal aberrations. *Mutat Res* 1993; **299**: 297-303
20. Newcombe DA, Crowley DE., Bioremediation of atrazine-contaminated soil by repeated applications of atrazine-degrading bacteria. *Appl Microbiol Biotechnol* 1999; **51**: 877-82
21. Nishizawa N, Konishike J, Moroki N, Okada J, Takayama Y, Watanabe M, Oda K., [Drug-resistance of tubercle bacilli in patients with pulmonary tuberculosis at the time of entering hospital]. *Iryo* 1961; **15**: 212-6
22. Ohyama T, Jin K, Katoh Y, Chiba Y, Inoue K., 1,3,5-Trichloro-2-(4-nitrophenoxy)benzene (CNP) in water, sediments, and shellfish of the

Ishikari River. *Bull Environ Contam Toxicol* 1986; **37**: 344-9

23. Omar SA, & Abdel-Sater, M. A.., Microbial populations and enzyme activities in soil treated with pesticides. *Water, Air, and Soil Pollution* 2001; **127**: 49–63.
24. Purkait S, Ganguly M, Aktar W, Sengupta D, Chowdhury A., Impact assessment of various parameters polluting Ganga water in Kolkata Region: a study for quality evaluation and environmental implication. *Environ Monit Assess* 2009; **155**: 443-54
25. Rousseaux S, Hartmann A, Soulas G., Isolation and characterisation of new Gram-negative and Gram-positive atrazine degrading bacteria from different French soils. *FEMS Microbiol Ecol* 2001; **36**: 211-222
26. Singh BK, Walker A, Morgan JA, Wright DJ., Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. *Appl Environ Microbiol* 2003; **69**: 5198-206
27. Singh BR, Aminuddin, Abdulaziz A. Al-Khedhairy, Fahad Al-Qurainy and Javed Musarrat. Molecular diagnostics and phylogenetic analysis of 'Candidatus phytoplasma asteris' (16SrI- Aster yellow group) infecting banana (Musa spp.) *African Journal of Biotechnology* 2009; **8**: 5819-5824
28. Topp E., A comparison on three atrazine-degrading bacteria for soil bioremediation. . *Biology and Fertility of Silos* 2001; **33**: 529–534.
29. Vibber LL, Pressler MJ, Colores GM., Isolation and characterization of novel atrazine-degrading microorganisms from an agricultural soil. *Appl Microbiol Biotechnol* 2007; **75**: 921-8
30. Yamagishi T, Akiyama K., 1,3,5-Trichloro-2-(4-nitrophenoxy)benzene in fish, shellfish, and seawater in Tokyo Bay, 1977-1979. *Arch Environ Contam Toxicol* 1981; **10**: 627-35
31. Yu YL, Chen YX, Luo YM, Pan XD, He YF, Wong MH., Rapid degradation of butachlor in wheat rhizosphere soil. *Chemosphere* 2003; **50**: 771-4.