Microbial Degradation of High Endosulfan Concentrations in Carbon Free Media

Osama El Gialani Elsaid [1], Azhari Omer Abdelbagi [2], and Elsiddig Ahmed Elmustafa Elsheikhc [3]

***Abstract*—**Microbial degradation at elevated concentration (500 mg/l) of endosulfan in carbon free by four types of soil microorganisms isolated from highly polluted soil by selective media was studied and the results indicated the rate of reduction in half lives ranges between 64.4 - 72.9% for α- endosulfan and 55.5 - 71.3% for β-endosulfan. Results indicated that high concentration of β-endosulfan caused reduction in the microbial capability of degrading this chemical. The higher reduction occurred in the Organic nitrogen bacteria activity while the lowest reduction was noting in the activity of Fungi. Mixing the various groups of microorganism together died not caused much improvement in their activity on the other hand the results showed that there were no significant differences in the reduction of half-lives between high (500 mg/l) and low (100 mg/l) concentration

***Keywords*—** High, Endosulfan, Biodegradation Sudan

I. Introduction

THE chlorinated cyclic sulfite diester endosulfan is a cyclodien insecticide possessing a relatively board spectrum of activity. Technical-grade is a mixture of two stereo isomers, α and β-endosulfan, in a ratio of 7:3. It is used extensively throughout the world as a contact and stomach insecticide and an acaricide on field crops, vegetables and fruit crops. Because of its abundant usage and potential transport, endosulfan contamination is frequently found in the environment at considerable distances from the point of its original applications (Mansingh and Wilson, 1995; Miles and Pfeuffer, 1997). Endosulfan has been detected in the atmosphere, soils, sediments, surface and rain water, and food stuffs. It is extremely toxic to fish and aquatic invertebrates (Sunderam Horne, M.J. Lacey, R.L. Harcourt, R.J. Russel, and J.G. Oakeshott., 2000) and has been implicated in mammalian gonadal toxicity (Shetty, P.K., J. Mitra, N.B.K. Murthy, K.K.Namitha, K.N. Sovitha, and K.Raghu. 2000), genotoxicity and neurotoxicity (Pual and Balasubramaniam, 1997). These health and environmental concerns have led to an interest in detoxification of endosulfan in the environment.

[1]Faculty of Agricultural Technology and Fish Sciences, Al-Neelain University, Khartoum, Sudan

[2,3]Faculty of Agriculture, University of Khartoum, Khartoum, Sudan

Detoxification of pesticides through biological means is receiving serious attention as an alternative to existing methods, such as incineration and landfill. A preliminary step in the investigation of enzymatic technologies for endosulfan detoxification is the definitive identification of a biological source of endosufandegrading activity. Microorganisms have increasingly been investigated as a source of xenobiotic-degrading enzymes (Chen and Mulchandani, 1998). Several studies have reported the isolation of bacteria co-culture (Awasthi Manickam and A. Kumar 1997) and mixed culture (Sutherland, T.D., I. Horne, M.J. Lacey, R.L. Harcourt, R.J. Russel, and J.G. Oakeshott., 2000) capable of degrading endosulfan. Mukherjee and Gopal (1994) reported the degradation of β-endosulfan by *Aspergillus niger*. Although Tirchoderma harzianum (Katayama and Matsumura, 1993), Phanerochataete chrysosporium (Kullman and Matsumura, 1996) and *Mucor thermohylospora* MTCC 1384 (Shetty, P.K., J. Mitra, N.B.K. Murthy, K.K.Namitha, K.N. Sovitha, and K.Raghu. 2000) have been examined for endosulfan degradation, these fungi were isolated for other degradative activities. In a bioremediation process, heterotrophic microorganisms break down substrates (hazardous compounds) to obtain chemical energy, hence organic pollutants can serve as carbon, energy, and nutrient sources for microbial growth and a poor biological energy source when used as a sole carbon Sutherland, T.D., I. Horne, M.J. Lacey, R.L. Harcourt, R.J. Russel, and J.G. Oakeshott., 2000) selected microorganisms for their ability to release the sulfite group from endosulfan and to use this insecticide as a source of sulfur for bacterial growth. Awasthi, N.N., Manickam and A. Kumar, 1997) isolated a bacterial co culture using endosufan as a sole carbon source. In this study, microorganisms were isolated through enrichment on endosulfan as a carbon sources. The purpose of this experiment to study the microbial degradation at elevated concentration (500 mg/l) of endosulfan in carbon free.

II. Material And Methods

Chemical and Reagents

Analytical grade endosulfan (99.5 pure) was obtained from the Agricultural Research Corporation, (Wed Medani). This grade is a mixture of two di-astereoisomers; α-endosulfan and β-endosulfan (7:3 respectively). Acetone (99.8 pure), Hexane

(99.8 pure), Ethanol (99.8 pure) and other solvents were obtained from Fischer, company.

Soils Sample

Surface soil samples were randomly collected from pesticides polluted storage soil in Hasahesa, (Gezeira scheme) using a soil agar of 10 cm length and 5 cm diameter. Five augers were taken and mixed thoroughly to make the composite sample (one kg). The collected sample was placed in a paper bag, labeled and immediately transported to the pesticides laboratory, Crop Protection Department, Faculty of Agriculture, University of Khartoum.

Preparation of media

Four types of selective media were prepared in four conical flasks (1500 ml) following the method of Tepper, *et al*., (1994) these include:

a) Starch agar (SAA)

This medium was used for isolate inorganic nitrogen bacteria and actinomycetes. The medium was prepared by adding 10 g starch, 2 g $(NH_4)_2SO_4$, 1 g K_2HPO_4, 1 g $MgSO_4$, 1 g NaCl, 1 g $FeSO_4$, 1 g $CaCO_3$ and 25 g agar to one liter distilled water .

b) Nitrate agar (NA)

This medium was used for isolate bacteria and actinomycetes which live in poor media such as, *Mycobacterium*, *Arthrobacterium*, *Micromonospora*, and *Nocardia.* The media was prepared by adding 0.2 g $NaNO_2$, 1 g $NaNO_3$, 0.2 g $FeSO_4$, 1 g $Na2CO_3$, 0.5 g K_2HPO_4, 0.3 g NaCl and 25 g agar to one liter distilled water.

c) Meat Peptone Agar (MPA)

This medium was used for isolate organic nitrogen bacteria. The medium was prepared by adding 7.5 g of peptone, 5 g NaCl and 15 g agar to one liter meat extract.

d) Chabecks media (CHA)

This media was used for isolate fungi. The media was prepared by adding 0.5 g KCl, 0.5 g $MgSO_4$, 1 g K_2HPO_4, 0.01 g $FeSO_4$, 2 g $NaNO_3$, 20 g glucose and 30 g agar to one liter distilled water then 4 ml of lactic acid were added. The flasks containing these media were autoclaved for 20 minutes at 121° C, allowed to cool at room temperature and kept in the refrigerator as stock media at 5° C.

e) Carbon Free Media (CFM):

CFM was prepared following the method described by Tepper, et al., (1994). One g K2HPO4, 0.5 g MgSO4 .7 H2O, 0.5 g NaCl, 0.001 g FeSO4.7 H2O, 0.01g MnSO4.4 H2O, 0.05g CaCO3 were added to a conical flask (1500 ml) and then, the volume was completed to one liter by adding distilled water. The media were autoclaved for 20 minutes, at 121°C then allowed to cool at room temperature and kept in refrigerator at 5 ºC for further use.

Isolated of the microbial inoculums

The microbial inoculums (inorganic nitrogen bacteria and actinomycetes, bacteria and actinomycetes which live in poor media, organic nitrogen bacteria and fungi) were isolated from the soil sample using selective media as following

Four quantities of the media (Starch agar (SAA), Nitrate agar (NA), Meat Peptone Agar (MPA), Chabecks media (CHA)), 200 ml each, were taken and each was placed separately in different 250 ml conical flask. Each flask was inoculated with 10 grams of the soil samples. Inoculated flasks were then closed with sterilized cotton and kept in an incubator (thermostatic cabinet) at 25 °C for 24 hrs for use in biodegradation experiment.

Microbial degradation of high endosulfan concentrations in carbon free media

The aim of this experiment is to evaluate the capability of the isolated soil microorganism in degrading high endosulfan concentrations in carbon free media.

A total of 18 clean test tubes were sterilized in an oven for three hours at 180°C. Ten ml of CFM media was taken from the stock flasks into each test tube. About one ml of inoculums was added to each test tube as following.

i. Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml fungi.
ii. Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml organic nitrogen bacteria.
iii. Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml inorganic nitrogen bacteria and actinomycetes
iv. Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml Bacteria and actinomycetes which live in poor media.
v. Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml mixed microorganisms (i.e. including all of the previous microorganisms).
vi. Three test tubes each containing 10 ml media treated with endosulfan (500mg/l media) and inoculated with one ml Distilled water as control.

Test tubes were arranged in a completely randomized design with three replicates. All test tubes were incubated for a total of 30 days and contents of endosulfan (α and β) and endosulfan sulphate analyzed every 10 days. About two milliliters were taken from each test tube every 10 days for a 30 period. Samples were extracted and analyzed by GLC for α, β and endosulfan sulphate.

III. Results

Tables 1 and 2 show the half lives of endosulfan α and β-incubated for a total of 30 days in carbon free media treated with elevated endosulfan concentration (500 mg/l). The rate of reduction in half lives ranges between 64.4 - 72.9% for α- endosulfan and 55.5 - 71.3% for β-endosulfan. Results (Tables 1 and 2) indicated that high concentration of β-endosulfan caused reduction in the microbial capability of degrading this chemical. The higher reduction occurred in the Organic nitrogen bacteria activity while the lowest reduction was noting in the activity of in Fungi. Mixing the various groups of microorganism together died not cause much improvement in their activity.

The generation of sulphate (Figs 1- 5) was monitored for 30 days. Sulphate was slowly generated from the microbial treatments reaching maximum after 20 days (0.2 m Ml/l), thereafter the Sulphate level slowly decline and became non-detectable after thirty days. On the other hand the Sulphate level in the control gradually increased at but faster rate and apparently did not decline even after 30 days (when the experiments was terminated). The higher level of sulphate generated was 0.2 m Ml/l.

IV. Discussion

Microbial degradation at elevated concentration of endosulfan in media free from carbon sources was studied. The rate of reduction in half lives ranges between 64.4 - 72.9% for α- endosulfan and 55.5 - 71.3% for β-endosulfan.The results showed that there were no significant differences in the reduction of half lives between high (500 mg/l) and low (100 mg/l) concentration (Elsaid,O. G.; Abdelbagi, A. O. and Elsheikh, E. A. E. (2010b 2009; 2010a; 2010b).

Table 1.
Half live (days) and percentage reduction in half live of A-endosulfan incubated with isolated soil microorganisms in Carbon-free media containing endosulfan (500 mg/l)

Microorganisms	R^2	Slope	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %
Fungi	0.9484	2.4027	20.8	64.7
Inorganic nitrogen Actinomycetes and Bacteria	0.8162	2.3128	17.3	70.7
Actinomycetes and Bacteria which lives in poor media	0.8742	2.2362	17.6	70.2
Organic nitrogen bacteria	0.8672	2.4364	15.9	72.9
Mixture	0.9443	2.2989	20.9	64.4
Controls	0.9851	0.8257	58.9	00.0

R^2 = Determination coefficient
$\tau_{½}$ = Half lives

Although there is no significance in half lives but looking the cures in figures 1-5 could conclude that clear but delayed affects were noticeable. Such delayed effects can not easily be observed by examining half lives, since half lives were computed assuming a first order rate. This delayed effect can be explained by the assumption that microorganism slowly adapted themselves to live in such higher level and after the adaptation periods they became highly capable of degrading the pesticides, evident by the delayed sharp drop in degradation rate.

Table 2. Half live (days) and percentage reduction in half live of B-endosulfan incubated with isolated soil microorganisms in Carbon-free media containing endosulfan (500 mg/l)

Microorganisms	R^2	Slope	$\tau_{½}$(days)	Reduction in $\tau_{½}$ %
Fungi	0.9992	3.2821	15.1	55.5
Inorganic nitrogen Actinomycetes and Bacteria	0.7324	2.5339	11.9	64.9
Actinomycetes and Bacteria which lives in poor media	0.8604	2.8620	12.1	63.5
Organic nitrogen bacteria	0.8292	3.2839	09.8	71.3
Mixture	0.9893	3.4039	14.5	57.4
Controls	0.9786	1.3445	33.9	00.0

R^2 = Determination coefficient
$\tau_{½}$ = Half li

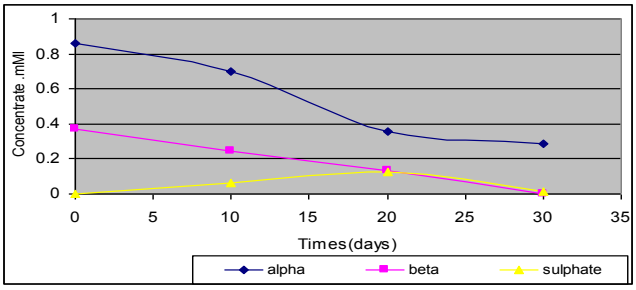


Fig. 1.Degradation of α & β-endosulfan and sulphate generation by soil fungi exposed to endosulfan (500 mg/l) in carbon-free media.

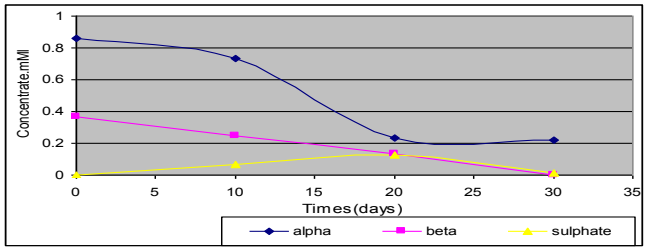


Fig. 2.Degradation of α & β-endosulfan and Sulphate generation by soil inorganic nitrogen `actinomycetes and bacteria. exposed to endosulfan (500 mg/l) in carbon-free media

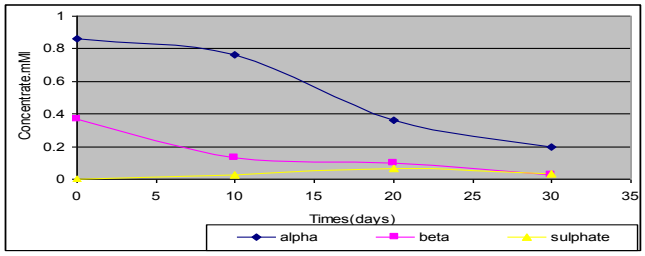
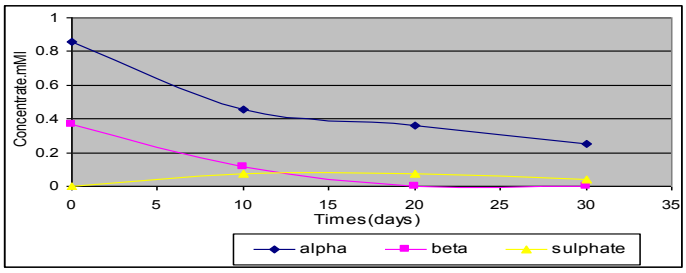


Fig. 3.Degradation of α & β-endosulfan and Sulphate generation by soil bacteria and Actinomycetes which lives in poor media. Exposed to endosulfan (500 mg/l) in carbon-free media



Fig, 4.Degradation of α & β-endosulfan and sulphate generation by soil organic nitrogen Bacteria. exposed to endosulfan (500 mg/l) in carbon-free media.

.

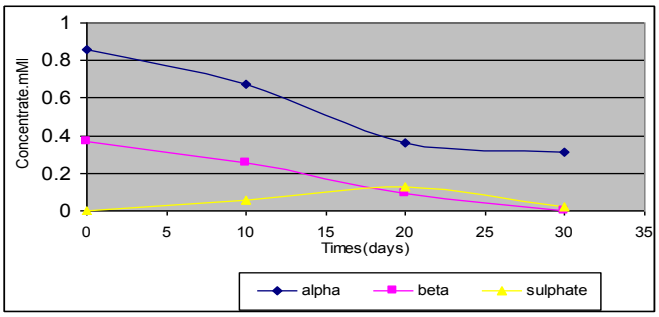


Fig. 5.Degradation of α & β-endosulfan and sulphate generation by soil Mixture Microorganism exposed to endosulfan (500 mg/l) in carbon- free media

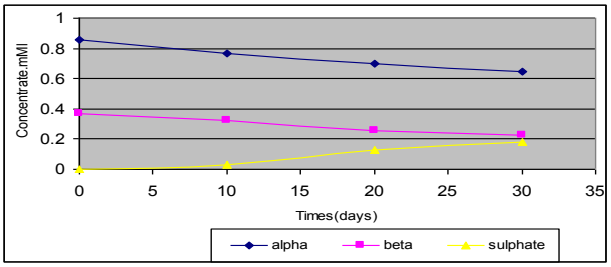


Fig. 6. Degradation of α & β-endosulfan and sulphate generation in sterilized treated soil (control)

ACKNOWLEDGMENT

Thanks are extended to those who contributed in one way or another to this work. They include Prof. Osman Ibrahim Gameel, Prof. Gafar Zorgani, Dr. Awad Galal, Dr. Imad Ali, Ismael Siddeg, Tarig Elsir and Triza My strong appreciation is extended to Lift Engineering Company for financing this work

REFERENCES

[1] Abdelbagi, A.O., M.A. Elmahi and D.G. Osman, 2000. Chlorinated hydrocarbon insecticide residues in the Sudanese soils of limited or no pesticide use. *Arab* Journal of plant Protection., 18: 35-39.

[2] Abdelbagi, A.O., M.A. Elmahi and Osman, 2003. Organochlorine insecticides residues in Sudanese soil of intensive pesticide use and in surface soil of Qurashi pesticide store. U. of K.J, of Agric, Sci., 11: 59-6.

[3] Alexander, M., 1977. Introduction to soil microbiology. 2nd Ed. John Wiley & Sons Inc. New York. ISBN 0-471-02179-2.

[4] Al-Hassan, R.M., I.I. Bashour and N.S. Kawar., 2004. Biodegradation of alpha and beta endosulfan in soil as influenced by application of different organic materials. J. Environ Sci. Health, B. 39: 757-764 . http://dx.doi.org/10.1081/PFC-200030850

[5] Awasthi, N.N., Manickam and A. Kumar, 1997. Biodegradation of endosulfan by a bacteria co culture. Bull. Enveron. Contam. Toxicol., 59: 928-934. http://dx.doi.org/10.1007/s001289900571

[6] Casida, J.E., 1993. Insecticide action at the GABA-gated chloride channel: recognition, progress, and prospects. Arch. Insect Biochem. Physiol., 22: 13. http://dx.doi.org/10.1002/arch.940220104

[7] Elsaid,O. G.; Abdelbagi, A. O. and Elsheikh, E. A. E. (2010b). Microbial degradation of endosulfan in carbon free media and selective media. *Journal of agriculture and biological sciences 6(3), 257-562.*

[8] Elsaid. O. G.; Abdelbagi, A. O. and Elsheikh, E. A. E. (2010c). Survey of naturally occurring microorganisms in different soil from Sudan. *International journal of applied agricultural research 5(3), 301-308.*

[9] El Zorgani, G.A. and M.E.H. Omer, 1974. metabolism of Endosulran Isomers by *Aspergillus niger*-bull. Environ, Contam, Toxical.

[10] El Zorgani, 1976. Residues of DDT in cotton seed after spraying with DDT. Bull. Environ. Contam. Toxically, 16: 15.

[11] El Zorgani, G.A., M.E. Abdalla and E.T. Ali, 1979. Residues of organochlorine insecticide in fishes in Lake Nubia, Bullet. Enviro. Toxicol., 22: 44-48.

[12] Guerin, T.F. and Kennedy, 1999. The Anaerobic Degradation of Endosulfan by indigenous microorganisms from Low oxygen soils and Sediments. Envirn Pollution., 63: 689-697.

[13] Lee, N., J.H. Skerritt and D.P. McAdam, 1995. Hapten synthesis and development of ELISAs for the detection of endosulfan in water and soil, J. Agric. Food Chem., 43: 1730-1739. http://dx.doi.org/10.1021/jf00054a060

[14] Marten, R., 1976. Degradation of (8.9-14) Endosulfan by soil microorganisms. Applied Environmental microbiology, 31: 853-859.

[15] Shetty, P.K., J. Mitra, N.B.K. Murthy, K.K.Namitha, K.N. Sovitha, and K.Raghu. 2000. Biodegradation of cyclodiene insecticide endosulfan by Mucor thermo – hyalospora MTCC 1384. Curr. Sci., 79: 1381-1383.

[16] Shivaramaiah, H.M. and I.R. Kennedy, 2006. Biodegradation of Endosulfan by soil bacteria. J. Enviorn. Sci. Health, B. 41: 895- 905.

[17] Sutherland, T.D., I. Horne, M.J. Lacey, R.L. Harcourt, R.J. Russel, and J.G. Oakeshott., 2000. Enrichment of an endosulfan- degrading Mixed bacterial culture. Appl. Environ. Microbiol., 66: 2822-2828. http://dx.doi.org/10.1128/AEM.66.7.2822-2828.2000

[18] Siddique, T., B.C. Okeke, M. Arshad and W.T. Frankenberger, 2003. Enrichmend and Isolation of Endosulfan-Degrading Microorganisms. J E.Q., 32: 47-54.

[19] Tepper, E.Z., U.K. shilinkova, perver, G.E. Zeva, 1994. Manual of microbiology, Mosco, kolas, 4th Edition.

[20] Van Woerden, H.F., 1963. Organic sulfites. Chem. Rev., 63: 57-571. http://dx.doi.org/10.1021/cr60226a001