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Study of Microbial Degradation of high Endosulfan Concentrations in Carbon Free Medium

Osama El Gialani Elsaid[1], Azhari Omer Abdelbagi[2], -. Elsiddig Ahmed Elmustafa Elsheikhc[3], khogali Mahmoud Khogali Saliheen[4]

1,4 Faculty of Agricultural Technology and Fish Sciences, Al-Neelain University, Khartoum, Sudan
2,3 Faculty of Agriculture, University of Khartoum, Khartoum, Sudan.
*__Corresponding author__:

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Abstracts

Study of microbial degradation using high concentration (500 mg/l) of endosulfan in carbon free by four types of soil microorganisms (inorganic nitrogen bacteria and actinomycetes, bacteria and actinomycetes which live in poor medium, organic nitrogen bacteria and fungi) isolated from highly polluted soil (Gezira Scheme Pesticides Stores) by selective medium (Starch agar, Nitrate agar, Meat Peptone Agar, Chabecks medium and Carbon Free Medium). The results indicated the rate of reduction of half lives ranged between 64.4 - 72.9% for α- endosulfan and 55.5 - 71.3% for β-endosulfan and results indicated that high concentration of β-endosulfan caused reduction in the microbial capability. The higher reduction occurred in the Organic nitrogen bacteria activity while the lowest reduction was noting in the activity of in Fungi. Mixing of various groups of microorganism together did 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 of the Pesticide.

Keywords: Endosulfan, Biodegradation, Sudan.

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 (Miles and Moy, 1979). 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, has been implicated in mammalian gonadal toxicity, genotoxicity and neurotoxicity (Sutherland et al., 2000). These health and environmental concerns

have led to an interest in detoxification of endosulfan in the environment.

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 endosufan degrading activity. Microorganisms have increasingly been investigated as a source of xenobiotic-degrading enzymes.

Several studies have reported the isolation of bacteria co-culture (Awasthi, 1997) and mixed culture capable of degrading endosulfan(Sutherland et al., 2000). The degradation of β-endosulfan has been reported by Aspergillus Niger , Tirchoderma harzianum and Mucor thermohylospora MTCC 1384 (Katayama and Matsumura, 1993; Shetty et al., 2000) have been examined for endosulfan degradation.

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. Microorganisms selected for their ability to release the sulfite group from endosulfan(Sutherland et al., 2000) and to use this insecticide as a source of sulfur for bacterial growth Bacterial co-culture . Isolated using endosufan as a sole carbon source (Awasthi, 1997).

The purpose of this experiment to study the microbial degradation at elevated concentration (500 mg/l) of endosulfan in carbon free medium.

Materials 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 and 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 medium were prepared in four conical flasks (1500 ml) following the method of these include:

(a) Starch agar (SAA)

This medium was used for isolation of 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 isolation of 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 isolation of 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 medium (CHA)

This medium was used for isolation of fungi. The medium 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 flask containing this medium has autoclaved for 20 minutes at 121°C, then allowed to cool at room temperature and kept in the refrigerator as stock medium at 5° C.

(E) Carbon Free Medium (CFM):

CFM 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. This medium has autoclaved for 20 minutes, at 121°C then allowed to cool at room temperature and kept in refrigerator at 5 ºC for further use.

Isolation of the microbial inoculums

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

Four quantities of the medium (Starch agar (SAA), Nitrate agar (NA), Meat peptone agar (MPA), Chabecks medium (CHA), each 200 ml 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 biodegradation experiment.

Microbial degradation of 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 medium was taken from the stock flasks into each test tube. About one ml of inoculums was added to each test tube as following.

1) Three test tubes each containing 10 ml medium treated with endosulfan (500mg/l medium) and inoculated with one ml fungi.
2) Three test tubes each containing 10 ml medium treated with endosulfan (500mg/l medium) and inoculated with one ml organic nitrogen bacteria.
3) Three test tubes each containing 10 ml medium treated with endosulfan (500mg/l media) and inoculated with one ml inorganic nitrogen bacteria and actinomycetes.
4) Three test tubes each containing 10 ml medium treated with endosulfan (500mg/l medium) and inoculated with one ml Bacteria and actinomycetes which live in poor media.
5) Three test tubes each containing 10 ml medium treated with endosulfan (500mg/l medium) and inoculated with one ml mixed microorganisms (i.e. including all of the previous microorganisms).
6) Three test tubes each containing 10 ml media treated with endosulfan (500mg/l medium) and inoculated with one ml Distilled water as control.

All test tubes were incubated at 30 °C for 30 days and contents of endosulfan (α and β) and endosulfan sulphate were analyzed every 10 days using GLC (gas liquid chromatograph) apparatus.

Results

Tables 1 and 2 showed 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 ranged 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 of this chemical. The higher reduction occurred in the organic nitrogen bacteria activity, while the lowest reduction was notied in the activity in Fungi. Mixing the various groups of microorganism together did 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. The higher level of sulphate generated was 0.2 m Ml/l.

Table 1. Half live (days) and percentage reduction in half live of α-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.

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

Microorganisms	R2	Slope	τ½(days)	Reduction in τ½ %
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 lives.

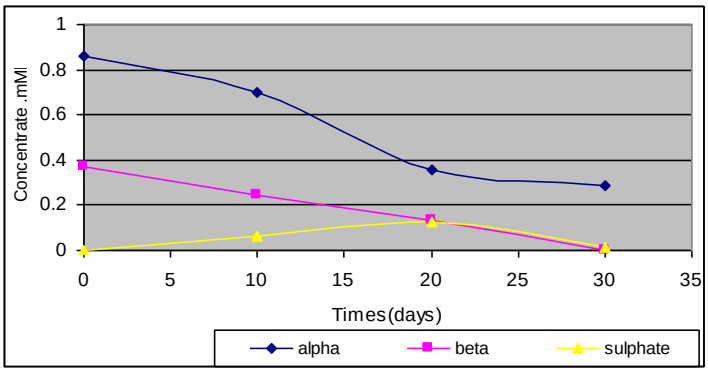


Fig. 1.Degradation of α & β-endosulfan and sulphate generation by soil fungi exposed to endosulfan (500 mg/l) in carbon-free medium.

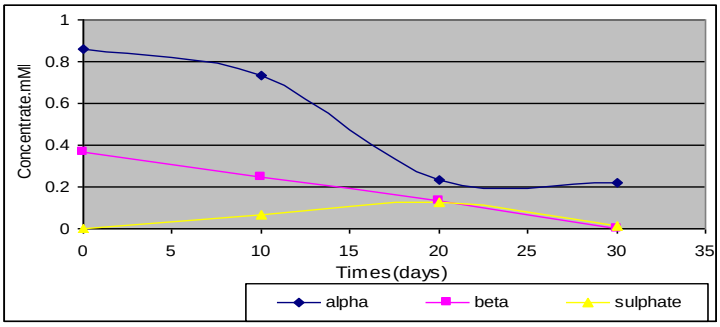


Fig. 2.Degradation of α & β-endosulfan and sulphate generation by soil inorganic nitrogen `actinomycetes and bacteria exposed to endosulfan (500 mg/l) in carbon-free medium.

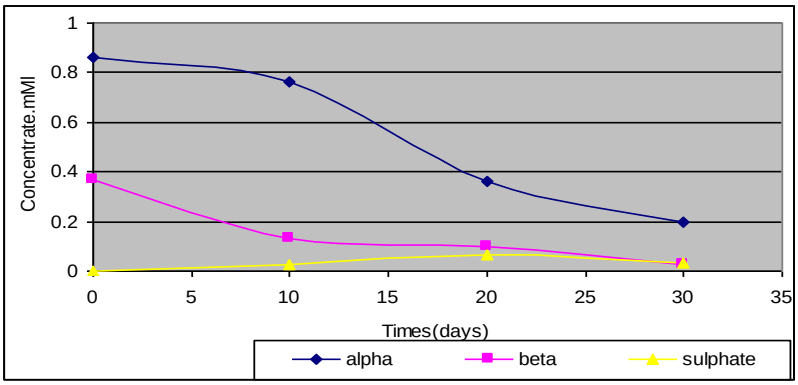
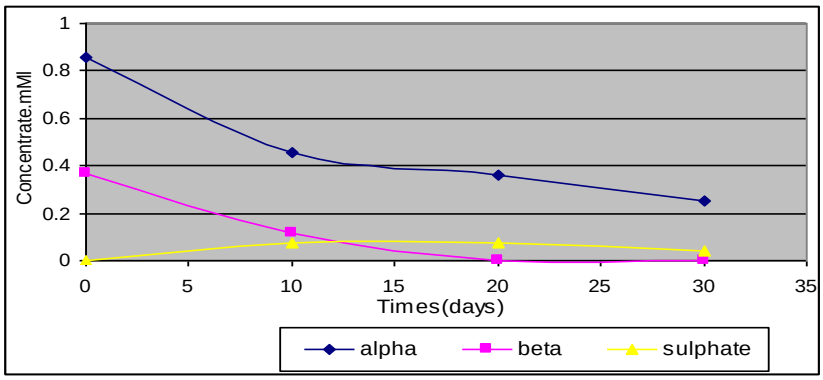


Fig. 3.Degradation of α & β-endosulfan and Sulphate generation by soil bacteria and Actinomycetes which lives in poor medium. Exposed to endosulfan (500 mg/l) in carbon-free medium.



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

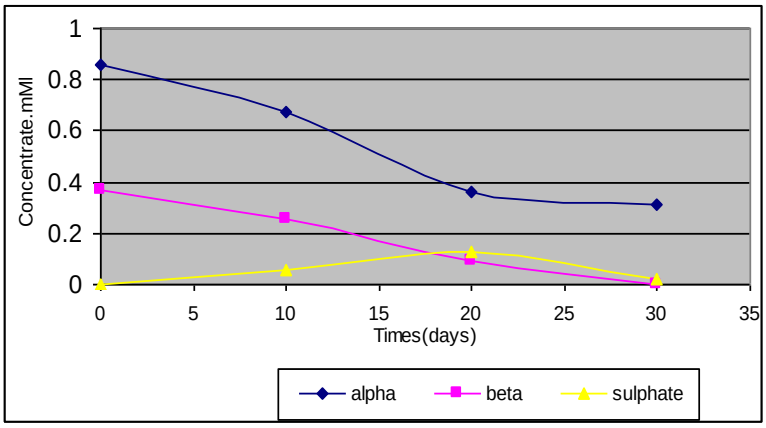


Fig. 5.Degradation of α & β-endosulfan and sulphate generation by soil Mixture microorganism exposed to endosulfan (500 mg/l) in carbon- free medium.

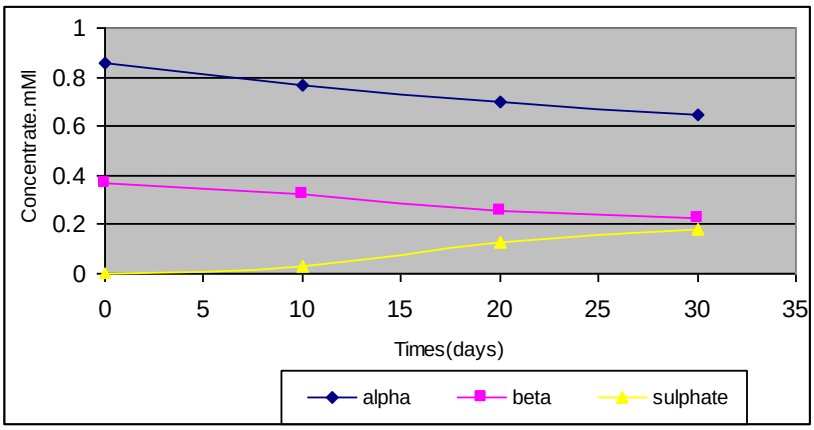


Fig. 6. Degradation of α & β-endosulfan and sulphate generation in sterilized treated carbon-free medium.

Discussion

The storage of pesticides in Sudan has created many problems. The total amounts of the stored pesticides in Sudan was estimated at 666 tones, 77.5% in liquid state and 22.5% as solids with about 6459 cubic meters of contaminated soil scattered over 43 major and minor sites in the country (Butrous, 1999).

Microbial degradation at elevated concentration of endosulfan in medium free from carbon sources was studied. The rate of reduction in half lives ranged 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 *et al*., 2010a; Elsaid *et al*., 2010b). Reduction in half lives was greater in α-endosulfan compared to β- endosulfan this results is in conformity with the results of Tariq *et al*., (2000) who mentioned that bacteria degraded α-endosulfan more than β isomer. Previous work by Ali (2005) reported the presence of organic nitrogen bacteria, inorganic nitrogen bacteria and actinomycetes and bacteria and actinomycetes which lives in poor medium in three types of Sudanese soil (Gorashi pesticide store, cotton field near Hasahissa and residential soil from Hasahissa town). Generally the result indicated that microorganisms isolated from highly contaminated soils (stores and cotton soils) showed superior capability in degrading the two isomers of endosulfan this results is in conformity with the report of Awasthi, (1997) who mentioned that microorganism isolated from contaminated areas are relatively more adapted and consequently have a greater potential for cleaning highly polluted soil. Similar conclusions were reported by other author (Tariq, *et al*. 2000, Ali 2005, and Shivaramaiah and Kennedy 2006). Previous work by Ali (2005) reported the presence of organic nitrogen bacteria, inorganic nitrogen bacteria and actinomycetes and bacteria and actinomycetes which lives in poor media in three types of Sudanese soil (Gorashi pesticide store, cotton field near Hasahissa and residential soil from Hasahissa town).

Although there is no significance in half lives but looking the cures in figures 1-5. In conclusion 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 and they became highly capable of degrading the pesticides, evident by the delayed sharp drop in degradation rate.

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الهدم الميكروبي لمبيد إندوسلفان عالي التركيز في وسط خالي من الكربون

أسامة الجيلاني السيد[1]، أزهري عمر عبد الباقي[2]، الصديق أحمد المصطفي الشيخ[3] وخوجلي محمود خوجلي صالحين[4]

1-4: كلية التقانة الزراعية وعلوم الاسماك ، جامعة النيلين ، الخرطوم ، السودان.

2-3: كلية الزراعة ، جامعة الخرطوم ، الخرطوم ، السودان

المستخلص

تمت دراسة معدل هدم مبيد الاندوسلفان مستخدماً تركيز عالي من المبيد (500 ملجرام/لتر) في وسط خالي من الكربون الحر بواسطة اربعة انواع من الاحياء الدقيقة في التربة(بكتريا النايتروجين اللاعضوية، بكتريا الاكتينومايسيتس، الاكتينومايسيتس الي تعيش في وسط فقير، بكتيريا النايتروجين العضوية و الفطريات) التي تم عزلها من اماكن عالية التلوث بالمبيدات (مخازن المبيدات مشروع الجزيرة) في أوساط غذائية مختارة (Starch agar, Nitrate agar, Meat Peptone Agar, Chabecks medium and Carbon Free Medium). اوضحت النتائج ان معدل مدى هدم المبيد في نقص العمر بين 72.9% – 64.4في الفا اندوسلفان و 71.3% – 55.5 لبيتا اندوسلفان و اوضحت النتائج ان التركيز العالي لبيتا اندوسلفان اثرت علي القدرة الميكروبية. حدث الهدم الاكبر في نشاط بكتريا النايتروجين العضوية، بينما الهدم الاقل لوحظ في نشاط الفطريات. خلط المجموعات الاربعة المتنوعة لم يظهر اي زيادة في كفاءة الاربعة مجموعات علي معدل هدم المبيد ومن ناحية اخرى لم تظهر النتائج أي فروقات معنوية في نقص عمر المبيد عند التركيز العالي (500 ملم/لتر) والتركيز المنخفض (100 ملم/لتر).