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# Biodegradation of Alachlor and Endosulfan Using Environmental Bacterial Strains

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**Abstract:** Soil samples as well as water samples from Ochlocknee River at Florida, were collected and analyzed to check their contamination with pesticides. Two different enrichments from both types of samples were tested on batch experiment to biodegrade alachlor and endosulfan *in vitro*. The biodegradation capabilities of both enrichments were followed up by HPLC. The bacterial consortium of water enrichment could be remove 80% of alachlor and 94% of endosulfan (of initial concentration 100 $mgL^{-1}$) while the bacterial consortium of soil enrichment couldn't remove any of endosulfan but 34% of alachlor. The 16S rRNA sequence analysis identified the eleven bacterial isolates shared in the biodegradation process as; *Ancylobacter* sp. S15, *Agrobacterium* sp. BD-32, *Burkholderia* sp. 2385, *Agrobacterium* sp. CZBSA1, *Psuedomonas* sp. W15Feb9B, *Agrobacterium* sp. CZBSA1, *Agrobacterium* sp. 2367, Beta Proteobacterium PII_ GH1.2.B8, *Agrobacterium* sp. CZBSA1, *Micobacterium olievorans* (strain DSR8) and *Agrobacterium* sp. NB-2A. The best biodegrading bacteria for alachlor and endosulfan were *Burkholderia*, *Psuedomonas* and Beta Proteobacterium.

**Key words:** Bacteria · Biodegradation · Enrichment · Water · Soil

## INTRODUCTION

Alachlor (2-chloro-2`,6`-diethylphenyl-N-(methoxymethyl)acetanilide) is an extremely toxic herbicide that widely used for the control of broad-leafed weeds and grasses in corn, soybean and many other crops in many countries all over the world. Alachlor had the highest concentration detected (165-254 ppb) in a study conducted by Selim and Popendorf [1] on different Egyptian governorates (Cairo, Alexandria, Damietta and Manzala lake). Damietta branch of River Nile and Manzala Lake were detected as the most polluted locations with Alachlor and other pesticides in Egypt [1]. It is a selective systemic herbicide, absorbed by germinating shoots and roots, interfering with the plant's ability to produce protein and elongate roots [2]. It is classified as a B-2 carcinogen [3] and a suspected endocrine disrupter [4]; it also causes liver toxicity and eye lesions [5].

Due to its highly water solubility (242 mg/L), it can be detected in surface water, groundwater and also in the finished water in many countries [6, 7]. Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzo(e) di-oxathiepin-3-oxide) is an organochlorine insecticide, used throughout the world to increase agricultural production [8]. The extensive agriculture production in south Florida leads to the contamination of C-111 canal and associated sites in Florida Bay, where the major pesticide of concern was endosulfan, which was detected at 100% of the sites tested [9]. The decomposition of hazardous substances in soil and water environments through the microbial processes has become an increasingly important research field nowadays. Alachlor and its derivatives, as well as, endosulfan had extensively studied to be biodegraded by soil microorganisms [2, 10-15]. The present research is concerning; the contamination of some soil and water sites with alachlor and endosulfan, the isolation and identification of highly efficient bacterial strains capable of degrading alachlor and endosulfan into less toxic compounds, selecting the best isolates performing the biodegradation process and suggestion of applying of such research on Egypt case.

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## MATERIALS AND METHODS

**Media and Chemicals:** Analytical grade alachlor and endosulfan (as endosulfan sulfate) were purchased from Sigma-Aldrich, USA. All media used through the study, solvents for HPLC and the other constituents of the mineral salt base medium were also of the same origin. Stock solutions of alachlor and endosulfan were prepared in acetone [8] and have been filter sterilized and added to the enrichments to get final concentration in each flask 100 $mgL^{-1}$. The composition of the different media used in the experiments were as follows; mineral salt base medium (per liter of de-ionized water) described by Rousseaux *et al*. [11]: 1.6 g $K_2HPO_4$; 0.4 g $KH_2PO_4$; 0.2 g $MgSO_4.7H_2O$; 0.1g NaCl; 0.02 g $CaCl_2$; 10 mL of sodium citrate stock solution; 1 mL of a salt stock solution; 1 mL of a vitamin stock solution; 1 mL of $FeSO_4.6H_2O$ stock solution. The sodium citrate stock solution was prepared by dissolving 100 g Na-citrate in 1 L de-ionized water. The salt stock solution contained 2 g $L^{-1}$ boric acid; 1.8 $gL^{-1}$ $MnSO_4.H_2O$; 0.2 $gL^{-1}$ $ZnSO_4$; 0.1 $gL^{-1}$ $CuSO_4$; 0.25 g $L^{-1}$ $Na_2MoO_4$. The vitamin stock solution contained 100 mg $L^{-1}$ thiamine-HCl and 40 $mgL^{-1}$ biotin. The $FeSO_4.6H_2O$ stock solution contained 5 $gL^{-1}$ of $FeSO_4.6H_2O$. The vitamin and $FeSO_4.6H_2O$ stock solutions were filter sterilized and kept at 4°C and supplemented to the main MSM after autoclaving. Nutrient agar medium (peptic digest of animal tissue 5g, beef extract 3g and agar 15 g in 1L distilled water). Luria-Bertani medium (LB medium); peptone 10 g, yeast extract 5 g and NaCl 5g in 1L of distilled water.

**Environmental Sampling Sites**

**Soil Samples:** Soil samples were collected from a farm in Tallahassee, Florida, in which a lot of corn crops were planted and irrigated with recycled sewage water of the city after the third stage of treatment. A mixture of all soil samples was prepared and checked for alachlor or endosulfan contamination, using HPLC instrument.

**Water Samples:** Water samples were collected from Ochlocknee River, Tallahassee Florida. This river is well known as a highly contaminated water resource in the city of Tallahassee (selected as an example of polluted rivers receiving agricultural wastewater). A mixture of all water samples was prepared and checked for alachlor or endosulfan contamination, using HPLC instrument.

**Enrichment and Isolation:** Ten grams of soil (mixture of all soil samples) and 10 mL of water (mixture of all river water samples) were used to inoculate 250 mL Erlenmeyer flasks containing 90 mL of mineral salt base medium (MSM) (separately), some of them are supplemented with alachlor and the others are supplemented with endosulfan. The pesticide concentration in each enrichment adjusted at 100 $mgL^{-1}$. Triplicates for both types of enrichment were prepared. All enrichments were incubated aerobically at 30°C with shaking at 150 rpm, in dark for 28 days (first enrichment). The second enrichments were applied by inoculating new 250 mL Erlenmeyer flasks containing 90 mL MSM with 10 mL solution of first enrichments and incubated at 30°C with shaking at 150 rpm, in dark for another 28 days. The fall down of the concentration of alachlor and endosulfan in both enrichments was followed up by HPLC instrument. After the second enrichment, 0.1 mL from each flask was applied on plates of mineral salt agar medium, allowed to be dried and then alachlor (or endosulfan) stock solution was sprayed on the surface of each plate to get pesticide concentration on each plate 100 $mgL^{-1}$. All the plates were incubated at 30°C for 48h. The colonies that showed the best growth were isolated. All isolates subjected for further purification steps to get pure culture from each.

**Degradation of Alachlor and Endosulfan Using Ochlocknee River Water as Enrichment:** The process of alachlor and endosulsfan biodegradation was performed using 100 mL of Ochlocknee river water as enrichment medium and to them, the alachlor (or endosulfan) was added to get the final concentration of pesticides in each flask 100 $mgL^{-1}$. The process was carried out under the same conditions mentioned above.

**Identification of the Isolated Alachlor or Endosulfan-Degrading Bacteria:** The different isolated bacterial strains from each enrichment, which were able to degrade alachlor or endosulfan were characterized by 16S rRNA sequence analysis. After extraction and clean up of genomic DNA for each isolate, they were used as templates to amplify the 16S rRNA gene by polymerase chain reaction (PCR). The universal primers 27F (5`-AGA GTT TGA TCC TGG CTC AG-3`) and 1492R (5`-CGG YTA CCT TGT TAC GAC TT-3`) were used to amplify the 16S rRNA. The PCR reaction was run with the following thermal profile in Bio-Rad thermal cycler; initial denaturation for 5 min at 94°C, denaturation

for 1 min at 94°C, annealing for 1 min at 55°C and extension for 2 min at 72°C and the final extension for 10 min at 72°C. These cycles were repeated for 30 times.

**Comparison Between the Biodegrading Ability of the Isolated Bacterial Strains:** Each selected bacterial strain was grown up in two different 15 mL conical centrifuge tubes containing 10 mL nutrient broth one of them supplemented with alachlor and the other with endosulfan, incubated at 30°C for 24h. The bacterial cells were harvested from each tube, washed many times and re-suspended in distilled water. The optical densities (OD) were measured for each isolate using spectrophotometer at wave length 600 (Spectramax M5, USA). Two flasks containing MSM (one supplemented with alachlor and the other with endosulfan, with initial concentration of 100 $mgL^{-1}$) were inoculated with the same bacterial isolate ($OD_{600}$ 0.5 for all). All the flasks were incubated in shaking incubators 150 rpm, at 30°C for 28 days, the concentration of alachlor and endosulfan was followed up weekly by HPLC instrument.

**Biodegradation Assays:** Experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of biodegradation solution (MSM 90 mL, soil sample 10g (or Ochlocknee river water 10 mL) with pesticide (alachlor or endosulfan) initial concentration 100 $mgL^{-1}$). The flasks were incubated in shaking incubator at 150 rpm and 30°C in dark. Each experiment was carried out in triplicate and necessary control samples were included. At regular time interval, 2 mL of growth medium was processed for detecting the presence of the parent compounds with HPLC instrument.

**Analytical Methods:** Ten grams of soil samples were taken in refluxed flasks with addition of sodium sulphide with 100 mL n-Hexane. They were refluxed for 1 hr and then the filtrates were taken in separating funnels and extracted with 50 mL and 25 mL acetonitrile. The acetonitrile layers were mixed with 500 mL DM water with 2.5 mL saturated sodium sulphide and again shaken in a separating funnels with n-Hexane and evaporated on water bath. The residues were dissolved separately in 1 mL acetonitrile and were used for the pesticide analysis in soil. One litter of each water sample was mixed in separating funnel with the same volume of acetonitrile, shaken for 1h. The organic layer was drained out and concentrated to 1 mL [16], which then used to check the presence of alachlor and endosulfan by HPLC, as mention above. One milliliter of first and second enrichments (containing alachlor) were centrifuged (15000 g 10 $min^{-1}$), filtered (0.22µm Millipore filter). Another 1 mL of first and second enrichments (containing endosulfan) was mixed with equal volume of ethyl acetate, shaked for 1h. The pooled organic phase was concentrated in a rotary evaporator and the residue was dissolved in 0.5 mL ethyl acetate, the extracts were filtered through 0.22µm Millipore filter. Then 20µl aliquots were analyzed by HPLC (WATER, USA) instrument equipped with a C18 column (25 cmX4.6 mm diameter) and a UV-visible absorbance detector set at 220 for alachlor and at 214 for endosulfan. The mobile phase used was Acetonitrile/water (50:50 v/v for alachlor) and (70:30 v/v for endosulfan) at a flow rate of 1 mL $min^{-1}$ at ambient temperature [17]. The extent of degradation was expressed relative to the concentration (peak area) of control samples.

## RESULTS

The analysis of the original soil samples had indicated that there are some organic compounds but no contamination with alachlor or endosulfan, while the analysis of river water samples indicated the presence of alachlor (in range of 5µg $mL^{-1}$) and endosulfan (in range of 3µg $mL^{-1}$) with a lot of other organic compounds.

**Biodegradation of Alachlor:** The biodegradation of alachlor using soil enrichment through first and second enrichments did not make a considerable difference, where the residual concentration of alachlor after the first enrichment was 70µg $mL^{-1}$ and become 66µg $mL^{-1}$ after the end of the second enrichment. So, the maximum removing capacity of soil enrichment for alachlor was 34%. Using of river water enrichment was more effective than soil, where the residual concentration of alachlor at the end of first enrichment was 75µg $mL^{-1}$ and become 55µg $mL^{-1}$ at the end of the second one, with the acceleration of biodegradation rate. So the maximum removing capacity of river water enrichment for alachlor was 45% (Fig. 1).

**Biodegradation of Endosulfan:** As shown in Fig. 2 the effect of soil enrichments on endosulfan biodegradation was almost nil. While water enrichments have been showing high removing capacity for endosulfan where the remaining concentration of endosulfan at the end of first enrichment was 7µg $mL^{-1}$ and become 6µg $mL^{-1}$ at the end of the second. In both enrichments the concentration of endosulfan was fall down between days 7 to 14

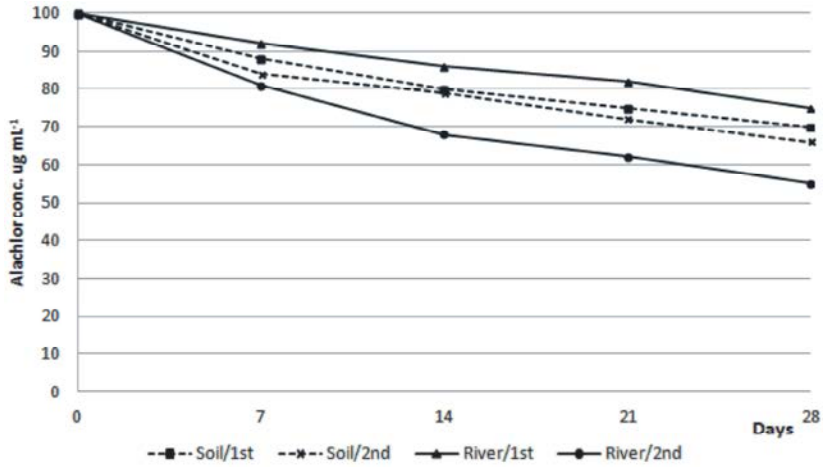


Fig. 1: Biodegradation of Alachlor; 1st and 2nd enrichments of soil and river water samples.

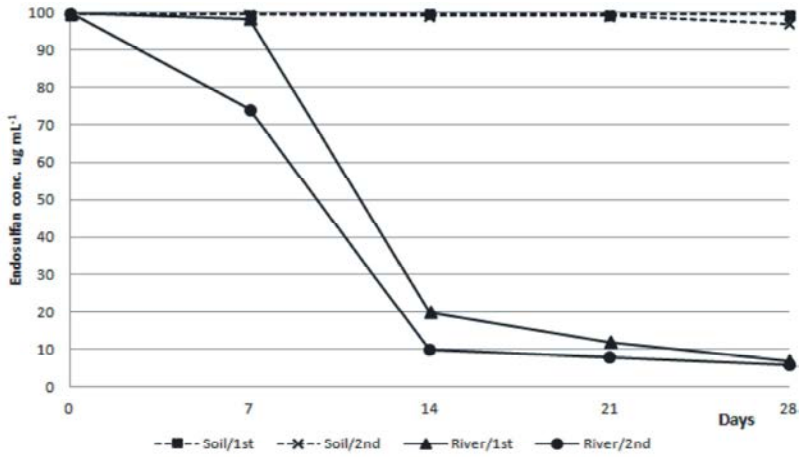


Fig. 2: Biodegradation of Endosulfan; 1st and 2nd enrichments of soil and river water samples.

(in first enrichment endosulfan remaining concentration become 20 while it become 10 in the second), the biodegradation rate was quietly accelerated during the second enrichment. The maximum removing capacity of endosulfan using river water enrichments was 94%.

**Degradation of Alachlor and Endosulfan Using Ochlocknee River Water as Enrichment:** The degradation of alachlor using Ochlocknee river water was weak through the first enrichment (only 11%) while at the end of the second enrichment it becomes 80%. In comparison with the results obtained by using MSM with 10% river water where the maximum removing capacity of alachlor was 45%, these results are considered better. On the other hand, endosulfan degradation was 20% in first enrichment and become 48% in second enrichment. Comparing such results with those obtained by MSM with 10% river water (where it was 94%), concluded that the effect of microbial consortium in biodegradation was magnified by MSM constituents (Fig. 3).

**Identification of the Isolated Alachlor or Endosulfan-Degrading Bacteria:** In this study, eleven bacterial strains were isolated (two from soil enrichments and nine others from river water enrichments). They all identified using 16S rRNA sequence analysis and Mega 6 soft ware program. Six of them were belonged to genus *Agrobacterium*, while the others were different, as indicated in Table 1.

**Comparison Between the Biodegrading Ability of the Isolated Bacterial Strains:** All the identified bacterial isolates were tested for biodegradation of both pesticides separately, at the same time. All the strains belonged to

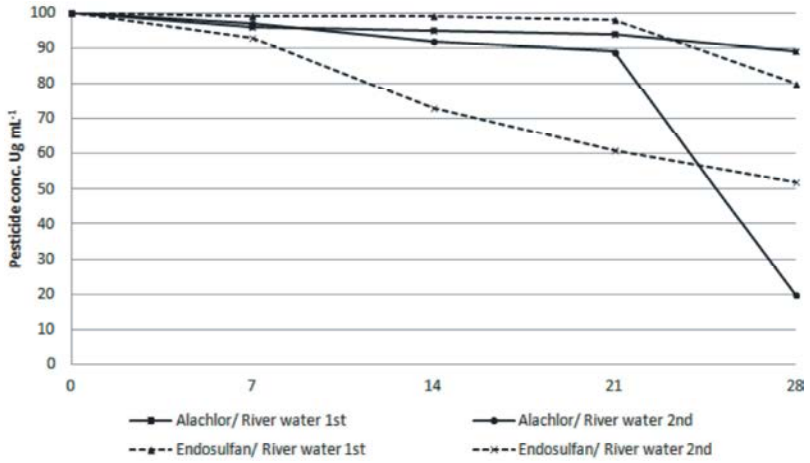


Fig. 3: Biodegradation of alachlor and endosulfan using Ochlocknee river water

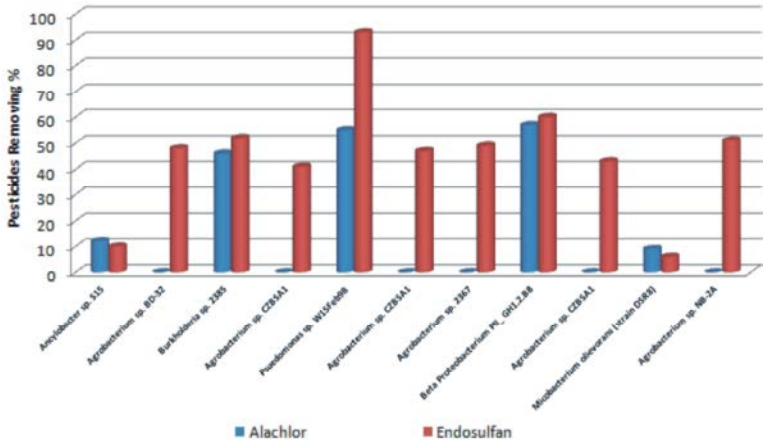


Fig. 4: Biodegrading ability of the identified bacterial strains for both alachlor and endosulfan.

Table 1: Identification of isolated alachlor or endosulfan-degrading bacteria

| Code | Isolate name | GenBank Accession No. | Source |
|---|---|---|---|
| 1 | *Ancylobacter* sp. S15 | KC243678.1 | Soil |
| 2A | *Agrobacterium* sp. BD-32 | GU085230.1 | River |
| 2B | *Burkholderia* sp. 2385 | JX174262.1 | River |
| 3A | *Agrobacterium* sp. CZBSA1 | KJ184900.1 | River |
| 3B | *Psuedomonas* sp. W15Feb9B | EU680989.1 | River |
| 4 | *Agrobacterium* sp. CZBSA1 | KJ184900.1 | Soil |
| 5 | *Agrobacterium* sp. 2367 | JX174244.1 | River |
| 6 | *Beta Proteobacterium* PII_ GH1.2.B8 | AY162061.1 | River |
| 7A | *Agrobacterium* sp. CZBSA1 | KJ184900.1 | River |
| 7B | *Micobacterium olievorans* (strain DSR8) | JQ342859.1 | River |
| 8 | *Agrobacterium* sp. NB-2A | EU155127.2 | River |

*Agrobacterium* sp. showed biodegrading ability only on endosulfan in range of 40 to 50% removing capacity (Fig. 4). The best isolates expressed their removing capacities' for both alachlor and endosulfan were *Psuedomonas* sp. W15Feb9B with removing percentage 55 and 93%, respectively and *Burkholderia* sp. 2385 with removing percentage 46 and 52%, respectively. Both strains were isolated from river water.

## DISCUSSION

Alachlor is a poorly biodegradable organic compound [18]. In soil treated with 10 mg acetochlor $kg^{-1}$ soil, 66% of the parent herbicide remained without degradation one month after treatment [19]. To date, no mixed cultures able to extensively degrade alachlor or its isomers have been definitely described in the literature

[13]. In the present research the bacterial consortium of Ochlocknee river water was found to be able to remove 80% of 100 $mgL^{-1}$ of alachlor through 28 days in the second enrichment. From this consortium, *Agrobacterium* sp. CZBSA1, *Micobacterium olievorans* (strain DSR8), *Burkholderia* sp. 2385, *Psuedomonas* sp. W15Feb9B and Beta Proteobacterium PII_ GH1.2.B8 were isolated and identified then tested for single strain-alachlor degradation. Their alachlor removing capacities were 0%, 9%, 46%, 55% and 57%, respectively. It is clear that *Agromobacterium* and *M. olievorans* are weakly (or completely not) included in alachlor degradation while *Burkholderia, Psuedomonas* and Beta Proteobacterium are mostly do. In contrary to these achievements, Xu *et al.* [20] isolated and identified *Psuedomonas oleovorans* which was capable of degrading acetochlor, but unfortunately, it couldn't transform acetochlor efficiently. Also, there was a hypothesis that a much wider genetic potential for biodegradation might carried within microbial communities than within a single organism [21, 22]. The soil enrichment showed less biodegrading ability for alachlor, where 34% removed only from the initial concentration 100 $mgL^{-1}$ after the second enrichment. The identified bacterial isolates from the soil consortium were *Ancylobacter* sp. S15 and *Agrobacterium* sp. CZBSA1both of them did not show high ability as a single biodegrader.

On the other hand, endosulfan degradation was also achieved through this study, the microbial consortia of the tested soil samples didn't showed any abilities for endosulfan biodegradation, while the river water microbial consortia were capable of removing 80 to 90% of endosulfan initial concentration 100 $mgL^{-1}$ through 14 days. The ability of the different strains of *Agrobacterium* for endosulfan biodegradation was ranged between 40-50%, while those of *Burkholderia, Psuedomonas* and Beta Proteobacterium were 52%, 93% and 60%, respectively. Indeed, the literature contains a lot of works concerning the degradation of endosulfan using soil bacteria [2, 14, 23], while a few or none had concerning endosulfan degradation using river water [8]. Previous publications have reported that endosulfan could be degraded by many bacterial strains, but with different efficiency; *Achromobacter xylosoxidans* 24.8 $mgL^{-1}$ [24], *Pseudomonas aeruginosa* 43% of initial concentration 80 $mgL^{-1}$ [25] and *Alcaligenes faecalis* JBW4 87.5% of initial concentration 200 $mgL^{-1}$ [8]. It is worth to mention that, *Pseudomonas* sp. W15Feb9B, *Beta Proteobacterium* PII_ GH1.2.B8 and *Burkholderia* sp. 2385 were the best isolates shown their capabilities to degrade both alachlor and endosulfan (55% and 93%, respectively) for the former, (46% and 52%, respectively) for the second and (57% and 60%, respectively) for the third. From the literature, it have been noted that Pseudomonads are extensively included in biodegradation of pesticides and other complex organic matters [14, 25, 26]. Also Beta proteobacteria (which may be *Burkholderia*) and *Burkholderia* are reported as highly versatile bacteria in their degradation capacities [27].

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## CONCLUSION

Several soil and water samples were collected from Tallahassee, Fl, USA, they all checked for pesticide contamination. The river water samples were found to be contaminated with alachlor and endosulfan. The bacterial consortium of water enrichment could be remove 80% of alachlor and 94% of endosulfan (of initial concentration 100 $mgL^{-1}$) while the bacterial consortium of soil enrichment couldn't remove any of endosulfan but 34% of alachlor. The 16S rRNA sequence analysis identified the eleven bacterial isolates shared in the biodegradation process as; *Ancylobacter* sp. S15, *Agrobacterium* sp. BD-32, *Burkholderia* sp. 2385, *Agrobacterium* sp. CZBSA1, *Psuedomonas* sp. W15Feb9B, *Agrobacterium* sp. CZBSA1, *Agrobacterium* sp. 2367, Beta Proteobacterium PII_ GH1.2.B8, *Agrobacterium* sp. CZBSA1, *Micobacterium olievorans* (strain DSR8) and *Agrobacterium* sp. NB-2A. The best biodegrading bacteria for alachlor and endosulfan were *Burkholderia, Psuedomonas* and Beta Proteobacterium.

**Recommendations:** The author wants to recommend the use of the same Bio-technology to apply in Egyptian scientific platform, for enhancement of wastewater reuse and control of river Nile water contamination by agricultural wastes.

## REFERENCES


1. Salim, M.I. and W.J. Popendorf, 2010. Pesticide contamination of surface water in Egypt and potential impact. Catrina J., 4(1): 1-9.
2. Sette, L.D., L.A. da Costa, A.J. Marsaioli and G.P. Manfio, 2004. Biodegradation of alachlor by soil streptomyces. J. Environ. Biotechnol., 64: 712-717.
3. U.S. Environmental Protection Agency (EPA), 1990. Drinking Water Regulations and Health Advisories. Chelsea, MI: Lewis Publishers.
4. U.S. EPA, 1997. Special report on environmental endocrine disruption: an effects assessment and analysis. Washington, DC: USEPA report no. EPA/630/R-96/012.
5. U.S. EPA, 1988. Alachlor. Rev. Environ. Contam. Toxicol., 104: 9-20.
6. Michelle, L., E.J. Haldik, A. Bouwer and L. Roberts, 2008. Neutral degradation of chloroacetamide herbicide: Occurrence in drinking water and removal during conventional water treatment. Water Res., 42: 4905-4914.
7. Vryzas, Z., E.N. Papadakis and P. Mourkidou, 2012. Leaching of Br-, metachlor, alachlor, atrazine, deethylatrazine and deisopropylatrazine in clayey vadoze zone: a field scale experiment in North-East Greece. Water Res., 46(6): 1979-1989.
8. Kong, L., S. Zhu, L. Zhu, H. Xie, K. Su, T. Yan, J. Wang, J.I. Wang, F. Wang and F. Sun, 2013. Biodegradation of organochlorine pesticide endosulfan by bacterial strain *Alcaligenes faecalis* JBW4. J. of Environ. Sci., 25(11): 2257-2264.
9. Scott, G.I., M.H. Fulton, E.F. Wirth, G.T. Chandler, P.B. Key, J.W. Daugomah, D. Bearden, K.W. Chung, E.D. Strozier, M. DeLorenzo, S. Sivertsen, A. Dias, M. Sanders, J.M. Macauley, L.R. Goodman, M.W. LaCroix, G.W. Thayer and J. Kucklick, 2002. Toxicological studies in tropical ecosystems: an ecotoxicological risk assessment of pesticide runoff in South Florida estuarine ecosystem. J. Agric. Food Chem., 50(15): 4400-4408.
10. Tiedje, J.M. and M.L. Hagedron, 1975. Degradation of alachlor by a soil fungus, *Chaetomium globsum*. J. Agric. Food Chem., 23: 77-81.
11. Rousseaux, S., H. Alain and G. Soulas, 2001. Isolation and characterization of new Gram-negative and Gram-positive atrazine degrading bacteria from different French soils. FEMS Microbiol. Ecol. J., 36: 211-222.
12. Ismail, B.S. and N. Shamsuddin, 2005. Effect of alachlor and metolachlor on microbial populations in the soil. Mal. J. Microbiol., 1: 36-41.
13. Xu, J., M. Yang, J.Y. Dai, H. Cao, C. Pan, X.H. Qiu and M.Q. Xu, 2008. Degradation of acetochlor by four microbial communities. Biores. Technol. J., 99: 7797-7802.
14. Sunitha, S., V. KrishnaMurthy and R. Mahmood, 2012. Degradation of endosulfan by mixed bacterial cultures enriched from endosulfan contaminated soils of southern India. Inter. J. Biosci. Biochem. and BioInform., 2(1): 31-34.
15. Dehghani, M., S. Nasseri and Z. Zamanian, 2013. Biodegradation of alachlor in liquid and soil cultures under variable carbon and nitrogen sources by bacterial consortium isolated from corn field soil. Iran. J. Environ. Heal. Sci. and Engin., 10(21): 1-9.
16. American Public Health Association "APHA", 2005. Standard Methods for the Examination of Water and Wastewater (22$^{th}$ Ed.). Washington, D.C.
17. Arshad, M., S. Hussain, M. Saleem and A. Khalid, 2007. Biodegradation of $\alpha$-and $\beta$-endosulfan by soil bacteria. J. of Biodegradation, (18): 731-740.
18. Qu, F.P., Y.Y. Yang and X.D. Feng, 1999. Aerobic biodegradation of pesticides containing nitrogen and counter measure for treatment of pesticide wastewater. Environ. Sci. J., 20(4): 12-15.
19. Istvan, J., 2000. Microbial and photolytic degradation of herbicide acetochlor. Int. J. Environ. Anal. Chem., 78(1): 1-8.
20. Xu, J., X.H. Qiu, J.Y. Dai, H. Cao, M. Yang, J. Zhang and M.Q. Xu, 2006. Isolation and characterization of a *Psuedomonas oleovorans* degradation the chloroacetamide herbicide acetochlor. J. Biodegradation, 17(3): 219-225.
21. Liu, S.Y., Z. Zheng, R. Zhang and J.M. Bollag, 1989. Sorption and metabolism of metolachlor by a bacterial community. Appl. Environ. Microbiol., 55(3): 733-740.
22. Sun, H.L., T.J. Sheets and F.T. Corbin, 1990. Transformation of alachlor by microbial communities. Weed Sci. J., 38: 416-420.
23. Shivaramaiah, H.M. and I.R. Kennedy, 2006. Biodegradation of endosulfan by a soil bacterium. J. Environ. Sci. and Health B, (41): 895-905.
24. Li, W., B.B. Xue, Y. Li, X. Peng, J.S. Zhang and Y.C. Yan, 2009. Biodegradation and detoxification of endosulfan in aqueous medium and soil by Achromobacter xylosoxidans strain CS5. J. Hazard. Mat., 167(1-3): 209-216.

25. Hussain, S., M. Arshad, B. Shaharoona, M. Saaleem and A. Khalid, 2009. Concentration dependent growth/non-growth linked kinetics of endosulfan biodegradation by *Pseudomonas aeruginosa*. World J. Microbiol. and Biotechnol., 25(5): 853-858.
26. Bajaj, A., A. Pathak, M.R. Mudiam, S. Mayilraj and N. Manickam, 2010. Isolation and characterization of *Pseudomonas sp.* strain IITRO01 capable of degrading α-endosulfan and endosulfan sulfate. J. Appl. Microbiol., 109: 2135-2143.
27. Velαzquez-Fernαndez, J.B., A.B. Martínez-Rizo, M. Ramírez-Sandoval and D. Domínguez-Ojeda, 2012. Biodegradation and Bioremediation of Organic Pesticides. In Pesticides-Recent Trends in Pesticide Residue Assay. (3rd Ed.). R.P. Soundararjan. INTECH Open Science, pp: 253-272.