

Original Research Article

Persistence of Sencor herbicide in Streptomyces-inoculated soil and its effect on some microbial soil

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ABSTRACT

Keywords

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Bioremediation

This study mainly focused on the persistence of Sencor as a herbicide in soil inoculated with some streptomycetes (*S. albosporus subsp abilomycaticus*, *S. herbaricolor* and *S. aureomonopodiales*). Half life period of the herbicide was reached after 15 days in *S. aureomonopodiales* and mixture treatments, while was reached in the other treatments after 30 days. Data showed that the tested *Streptomyces* strains varied greatly in their abilities to degrade Sencor as indexed by the number of compounds produced from Sencor degradation at interval days. The total microbial flora in Sencor amended soil was considerably lower than in uninoculated control during all the experimental periods. This indicates the toxicity of Sencor herbicide to the soil microbial flora and low incidence of indigenous Sencor-degraders in soil. Densities of the nitrogen-fixing bacterium were considerably higher in most *Streptomyces* treatments than noted for uninoculated control or Sencor-amended control on the 15th and 30th day. Actinomycetes densities progressively increased to a peak on the 30th day, then decline thereafter. It is recommended to apply *S. herbaricolor* for the bioremediation of the Sencor herbicide in contaminated soils.

Introduction

Atrazine is a member of the s-triazines herbicide family and is used extensively throughout the world as photosynthesis inhibitor of broad-leaf weeds in crops such as maize, sorghum and sugarcane. Due to its widespread use over the last thirty years, residues of atrazine and its

derivatives have been frequently detected in soils, surface water and groundwater (Silva *et al.* 2009).

Several investigators pointed to that biodegradation of pesticides as well as herbicides is proposed to reduce the

persistence of these chemicals in soil (Castillo *et al.*, 2006, Mohamed *et al.* 2012 and Zaki *et al.* 2012). These chemicals might affect on the soil microbial flora activities (Dubova and Zarina 2001, Guo *et al.* 2009 and Fang and Si 2011). There is an increasing attention in this area of research and numerous reports are found in the literature reported by Harada (2007). The high degradation activity observed by Castillo *et al.* (2006) via the streptomycete strains showed a good potential for bioremediation of soils contaminated with diuron.

On the contrary, researches concerning actinomycetes are somewhat limited. Grossbard (1976) reported that simazine (8 kg/ha) stimulated the total propagules of actinomycetes in soil, while ammonium thiocyanate (462 ppm), bentazon (10 ppm) methurin (3 kg/ha) and metoxuron (500 ppm) caused a negative effect on actinomycetes in soil. Prokopenko (1986) studied the effect of 1-3 kg/ha pendimethalin or 0.5-1 kg/ha metribuzin on the soil microbial flora including actinomycetes. He found that increasing rates of pendimethalin decreased the abundance of all microorganisms, while Sencor stimulated the bacterial and actinomycetes growth but not that of spore-forming bacteria.

Allievi *et al.* (1996) reported that changes in microbial numbers and activities in a pesticide-free agricultural soil from Italy in response to bentazon(e) applications at 10 and 100 ppm were studied after 4 and 30 weeks of incubation in laboratory conditions. Singh *et al.* (1996) revealed that populations of bacteria, actinomycetes, fungi and *Azotobacter* were studied in the soil of a wheat field at harvest following application of post-emergence diclofop-methyl at 0.75, 1.50

and 3.00 kg/ha, that is, the recommended, double and four times the recommended dose, respectively. Shelton *et al.* (1996) conducted an experiment to assess the ability of *Streptomyces* (strain PS1/5) to metabolize atrazine contaminated soil. Inoculation of soil amended with 20 micro g/g of atrazine and 5% chitin as carbon source resulted in ca. 78% removal of atrazine within 28 days. These data suggest that *Streptomyces* species may be potential candidates for soil inoculation to bioremediate herbicide contaminated soils. Kristek *et al.* (2004) studied the influence of fertilization and herbicides on soil microbial flora and elements of sugar beet yield.

An increase in the number of bacteria, actinomycetes was observed as well as aerobic asymbiotic nitrogen fixing bacteria-*Azotobacter chroococcum*. Hu *et al.* (2005) evaluated the effects of atrazine and alachlor on the soil microbial flora of a maize field. Bacteria and fungi were isolated in malt extract and soil extract agar by the soil washing technique. Herbicide applications to the soil decreased fungal populations in the 1st year of the study, without altering bacterial populations. Ulea *et al.* (2010) determined the influence of chlorsulfuron on the total number of microorganisms, on the relationship between the main groups (bacteria and fungi), and on the micromycetes spectrum determined in each variant of our experiment.

The aim of this work was determination of the ability of some *Streptomyces* isolates to degrade the Sencor herbicide in the soil and its half life during 60 days was also determined. The effect of the herbicide on the total microbial counts, *Azotobacter* and actinomycetes was also studied.

Materials and Methods

Streptomycete isolates

Three *Streptomyces* isolates (*S.albosporus* subsp *abilomycaticus*, *S.herbaricolor* and *S. aureomonopodiales*) which were able to utilize Sencor herbicide as a sole carbon and/or nitrogen source were tested for their abilities to degrade this herbicide in soil, *in vitro*.

Mycelium inoculums

Based on the method of Waksman and Lechevalier (1961) standard spore inoculums of the three *Streptomyces* isolates was prepared. Each ml of this suspension contains about 10^5 spores. To prepare mycelium inoculums, three conical flasks (250 ml) containing 50 ml of starch nitrate broth for each *Streptomyces* isolate were used and inoculated with 2.5 ml standard spore inoculum, and then incubated on a rotary shaker (160 rpm min⁻¹) at 28°C±2 for 6 days. Cultures growing in the four flasks were mixed, then 150 ml of the mixture were filtered and the wet mycelium was added to 2 kg soil.

Persistence of Sencor in soil treated with the most active Sencor-degrading *Streptomyces* isolates

Glass jars, each contains 200 g clay loamy soil was amended with Sencor at a rate equivalent to ten folds of the recommended dose (0.15 g/200 g soil). Soil moisture was adjusted to 60% of water holding capacity. Mycelium inoculum of each isolate was inoculated into 8 jars. Two controls were used, *i.e.*, soil alone and soil amended with Sencor only. Soil samples (2 jars for each treatment) were taken as well as controls at intervals 0, 15, 30 and 45 days for

microbiological analyses and gas liquid chromatography.

Extraction of herbicide and its degradation products from soil

Extraction of Sencor and its products were carried out as recommended by Thornton and Stanley (1977).

Gas liquid chromatography (GLC) analysis.

The gas liquid chromatography was carried out as recommended by Thornton and Stanley (1977). Effect of herbicide on the total microbial counts, *Azotobacter* and actinomycetes. To reach such goal, microbiological analysis include the determinations of total microbial counts on nutrient agar medium (Jacobs and Gerstein 1960), *Azotobacter* counts on the modified Ashby's medium (Abdel Malek and Ishac 1988) and actinomycetes counts on starch nitrate agar medium (Waksman and Lechevalier 1961) was done.

Results and Discussion

Pesticides are usually applied simultaneously or one after another for crop protection, and this type of pesticide application often leads to a combined contamination of these compound residues in the soil environment (Chu *et al.* 2008).

Isolation of pesticide-degrading actinomycetes from a contaminated site: bacterial growth, removal and dechlorination of organochlorine pesticides were reported by Fuentes *et al.* (2010), Fuentes *et al.* (2011) and Saez *et al.* (2012).

Persistence of Sencor herbicide in soil amended with some *Streptomyces* species

It is well known that the adsorption of herbicides to soil components reduces

their phytotoxicity and biodegradations, so higher rates of application are often required to produce adequate weed control in soils. Publications have demonstrated that atrazine has toxic effects in algae, aquatic plants, aquatic insects, fishes and mammals. Due to the toxicity and persistence of atrazine in the environment, the search of microbial strains capable of degrading it is fundamental to the development of bioremediation processes, as corrective tools to solve the current problems of the irrational use of agrochemicals (Sene *et al.* 2010).

Engelhardt *et al.* (1982) stated that, degradation of the triazinone herbicide metatriton by an *Arthrobacter* sp. has been demonstrated with formation of benzoyl formic acid acetylhydrazone and benzoyl formic acid as major metabolites. Giardina *et al.* (1982) described the degradation of the pre- and postemergence herbicide atrazine by a *Nocardia* sp. N-Dealkylation of the atrazine molecule yielded 4-amino-2-chloro-1,3,5-triazine as the major route of metabolism by the actinomycete. Qu and Wu (2002) showed that three *Pseudomonas* spp. strains (PD1, PD2 and PD3), which degraded herbicide waste water including atrazine, propazine and prometryn. These strains reached the highest rate of degradation in 2-6 days and the CODCr of the waste water dropped to 79.54% after 12 days. The present experiment was conducted to study the persistence of Sencor in soil amended with the herbicide (0.75g/kg) and separately inoculated with active Sencor-degrading *Streptomyces* species, *i.e.*, *S. albosporus* subsp *abilomycaticus*, *S. herbaricolor*, *S. aureomonopodiales* and mixture of them. Uninoculated soil amended with Sencor served as a control.

Generally speaking, persistence of Sencor herbicide decreased in soil as time

proceeded in all treatments including the control but the disappearance of the herbicide was faster in *Streptomyces* inoculated treatments (Table 1). After 15 days, fast degradation rates occurred in *Streptomyces* treatments being more obvious in the mixture and *S. aureomonopodiales* inoculated treatments. Half life of Sencor was reached in these latter two treatments where only 48.76 and 51.23% of initial Sencor was present in mixture and *S. aureomonopodiales* treatments respectively. The present results are in good harmony with the observed higher proliferation of total microbial flora, actinomycetes and *Azotobacter* in the same two treatments at the same period. It seems likely, therefore, that higher activity in the biodegradation of the herbicide would produce metabolites favoring the growing microbial flora. As time proceeded up to 30 days, higher degradation rates of herbicide continued and half life was reached in *S. albosporus* subsp *abilomycaticus* and *S. herbaricolor* treatments where persisted Sencor represented only 42.64 and 32.87 % of initial amount respectively. However, *S. aureomonopodiales* was still leading in this respect where only 28.27 % of the herbicide persisted. The activity of *Streptomyces* mixture in Sencor degradation was overcome by *S. aureomonopodiales* and *S. herbaricolor*. The present data might be taken as a clue for the antagonism which might exist between *Streptomyces* species in the mixture treatment. At the end of the experiment (45 days), about 25% only of the herbicide persisted in soil inoculated with *Streptomyces*. As far the uninoculated control, 54.75 % of initial Sencor was still present in soil indicating low incidence of Sencor-degraders in soil. The present results are in line with those of

microbiological determinations. These values suggest considerably high capabilities of the *Streptomyces* species used in Sencor degradation especially for *S. aureomonopodiales*.

The results of this study could be confirmed by that found by Castillo *et al.* (2006) who reported that the diuron degrading activity of 17 streptomycete strains, obtained from agricultural and non-agricultural soils, was determined in the laboratory. All strains were identified as *Streptomyces* sp. by phenotypic characteristics and PCR-based assays. The strains were cultivated in liquid medium with diuron (4 mg L⁻¹) at 25 °C for 15 days. Biodegradation activity was determined by high-performance liquid chromatography. The results indicated that all strains were able to degrade diuron, but to different amounts. Twelve strains degraded the herbicide by up to 50% and four of them by up to 70%.

Compounds produced from biodegradation of Sencor herbicide

Periodic determinations of products produced from Sencor degradation in soil by each of tested *Streptomyces* species are given Figures 1, 2 and 3. Results of gas liquid chromatographic analysis revealed a degree of similarity as well as differences in the nature of compounds produced by tested strains at different intervals of determinations. Two compounds having retention times of 1.54 (compound I) and 3.916 (compound III) were always found in all treatments at the three intervals of determinations. Moreover, one compound having retention time 5.678 (compound VII) was detected in all treatments after 30 days except for *S. albosporeus* subsp *abilomyceticus*. In the other direction, some products were unique for each tested

strain: a. Compound V (RT 5.322) for *S. albosporeus* subsp *abilomyceticus* after 15 days, b. Compound IV (RT 5.070) for *S. herbaricolor* after 30 days, and c. Compound VII (RT 5.964) for *S. aureomonopodiales* after 45 days. Variations in products of Sencor degradation between *Streptomyces* strains might explain their different capabilities in the removal of 50% of Sencor (half-life).

It should be kept in mind that the possibility still exists that some compounds might be produced and completely degraded by soil microbial flora and/or *Streptomyces* inoculated in the soil in between the intervals of determination. However, the present results could be taken as a good proof for the variation between *Streptomyces* strains used as indexed by differences in detectable compounds. The results of the present work demonstrate the superiority of *S. aureomonopodiales* strain in fast Sencor-degrader in soil being active in the removal of about 50 and 72% of the herbicide after 15 and 30 days respectively. Similar results was reached by Shelton *et al.* (1996) where *Streptomyces* PS1/15 strain metabolized 78% of atrazine in soil within 28 days.

Finally, it is suggested to inoculate soil with *S. aureomonopodiales* or a mixture of *S. albosporeus* subsp *abilomyceticus*, *S. herbaricolor* and *S. aureomonopodiales* for biodegradation of Sencor from contaminated soil with herbicide. Future studies are necessary to elucidate metabolic pathways of Sencor biodegradation by *Streptomyces* and utilization of bar gene for the induction of transgenic plants.

Data in Table (2) show that the tested *Streptomyces* strains varied greatly in their

abilities to degrade Sencor as indexed by the number of compounds produced from Sencor degradation at interval days. After 15 days from incubation the strains as well as the mixture do not reveal any differences as two compounds (I and III) were found in all treatment. Results showed that the degradation of sencor herbicide was highly observed after 30 days from *Streptomyces* inoculation. *Streptomyces herbaricolor* was found to be the most effective strain. Yang *et al.* (2010) observed a high atrazine mineralizing efficiency when amixed culture of *Klebsiella* sp. A1 and *Comamonas* sp. A2 was used. However, when these authors used pure cultures, they obtained no or poor growth and no or less atrazine degrading ability. Also, Ana *et al.* (2013) reported that a molinate mineralizing culture (mixed culture DC) was used as inoculum in the bioaugmentation assays. Significantly higher removal of molinate was observed in bioaugmentation than in natural attenuation microcosms (63 and 39 %, respectively) after 42 days of incubation at 22 °C.

Effect of Sencor on total and some microbial flora

Lew *et al.* (2013) analyzed the effect of pesticide contamination of the littoral zone on the population of bacteria and fungi were using the example of a eutrophic water reservoir exposed for [30 years to the influence of expired crop protection chemicals, mainly DDT. For three consecutive years, quantity analyses of bacteria and fungi were conducted and the composition of the microorganism population analyzed against seasonal dynamics. The data showed the effectiveness of aquatic microorganism-community analyses as a tool for

indicating changes in the water environment caused by pesticide contamination. Wu *et al.* (2009) studied the effects of butralin on 3 major groups of soil microorganism population. Results showed that the inhibition effect of butralin to aerobic microorganisms in soil was actinomyces > bacteria > fungi, but all aerobic microorganisms could recover to normal level as control after 28 days of butralin treatment.

In this study, the total microbial flora in Sencor amended soil was considerably lower than in uninoculated control during all the experimental periods (Table 3). This indicates the toxicity of Sencor herbicide to the soil microbial flora and low incidence of indigenous Sencor-degraders in soil. As for *Streptomyces* treatments, densities of total microbial were always higher than in the two respective controls. Counts were at their highest levels in *S. aureomonopodiales* and mixture treatments especially after 30 days of inoculation. Such increase in counts might result from artificial inoculation with *Streptomyces* and/or proliferation of microbial flora due to the utilization of metabolites produced from Sencor degradation by *Streptomyces*.

Effect on actinomycetes

Actinomycetes densities progressively increased to a peak on the 30th day, then decline thereafter (Table 4). Counts were considerably higher in *Streptomyces* inoculated treatments than noted for the two corresponding controls during all experimental periods. This was expected due to artificial inoculation with *Streptomyces* strains. However, highest counts were observed in *S. aureomonopodiales* and mixture treatments

Table.1 Persistence rate of Sencor (0.75 g/kg) in soil treated with some *Streptomyces* species

Treatments	Persistence of Sencor (%) at different intervals (days)			
	0	15	30	45
Control	99.98	72.07	65.38	54.75
<i>S. albosporus</i> subsp <i>abilomyceticus</i>	99.81	59.00	42.64	24.93
<i>S. herbaricolor</i>	99.02	62.99	32.87	28.60
<i>S. aureomonopodiales</i>	99.70	51.23	28.27	24.15
Mixture	99.88	48.76	38.09	21.60

Table.2 Number of compounds produced from Sencor degradation in soil amended with three *Streptomyces* strains.

Compounds produced from Sencor degradation	Sencor			Sencor + <i>S.albosporus</i> subsp <i>abilomyceticus</i>			Sencor+ <i>S.herbaricolor</i>			Sencor+ <i>S.aureomonopodiales</i>			Sencor+Mixture of <i>Streptomyces</i> strains		
	15*	30	45	15	30	45	15	30	45	15	30	45	15	30	45
I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
II	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
III	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IV	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
V	-	-	-	+	-	-	-	+	-	-	-	+	-	+	-
VI	-	-	-	-	-	-	-	+	-	-	+	+	-	+	-
Total	2	2	2	3	2	2	2	5	2	2	3	4	2	5	1

*: Days post incubation.

Figure.1 A: Gas liquid chromatographic analyses of two controls (commercial Sencor (Upper) and soil amended with Sencor (Lower)). **B:** Gas liquid chromatographic analysis of soil amended with Sencor at different intervals.

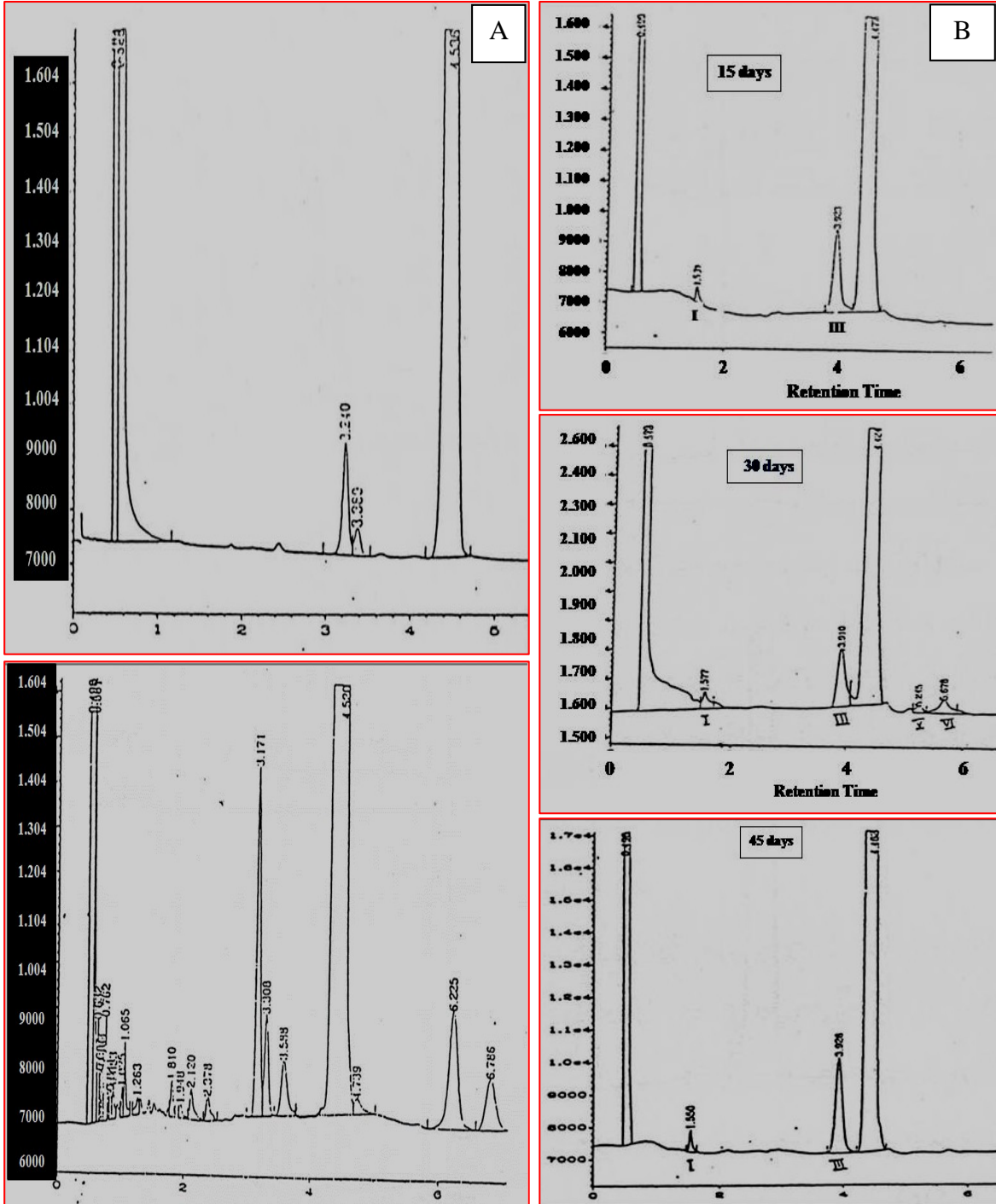


Figure.2 A Gas liquid chromatographic analysis of biodegradation of Sencor by *S. albosporus* subsp *abilomycaticus* at different intervals. B: Gas liquid chromatographic analysis of biodegradation of Sencor by *S. herbaricolor* at different intervals.

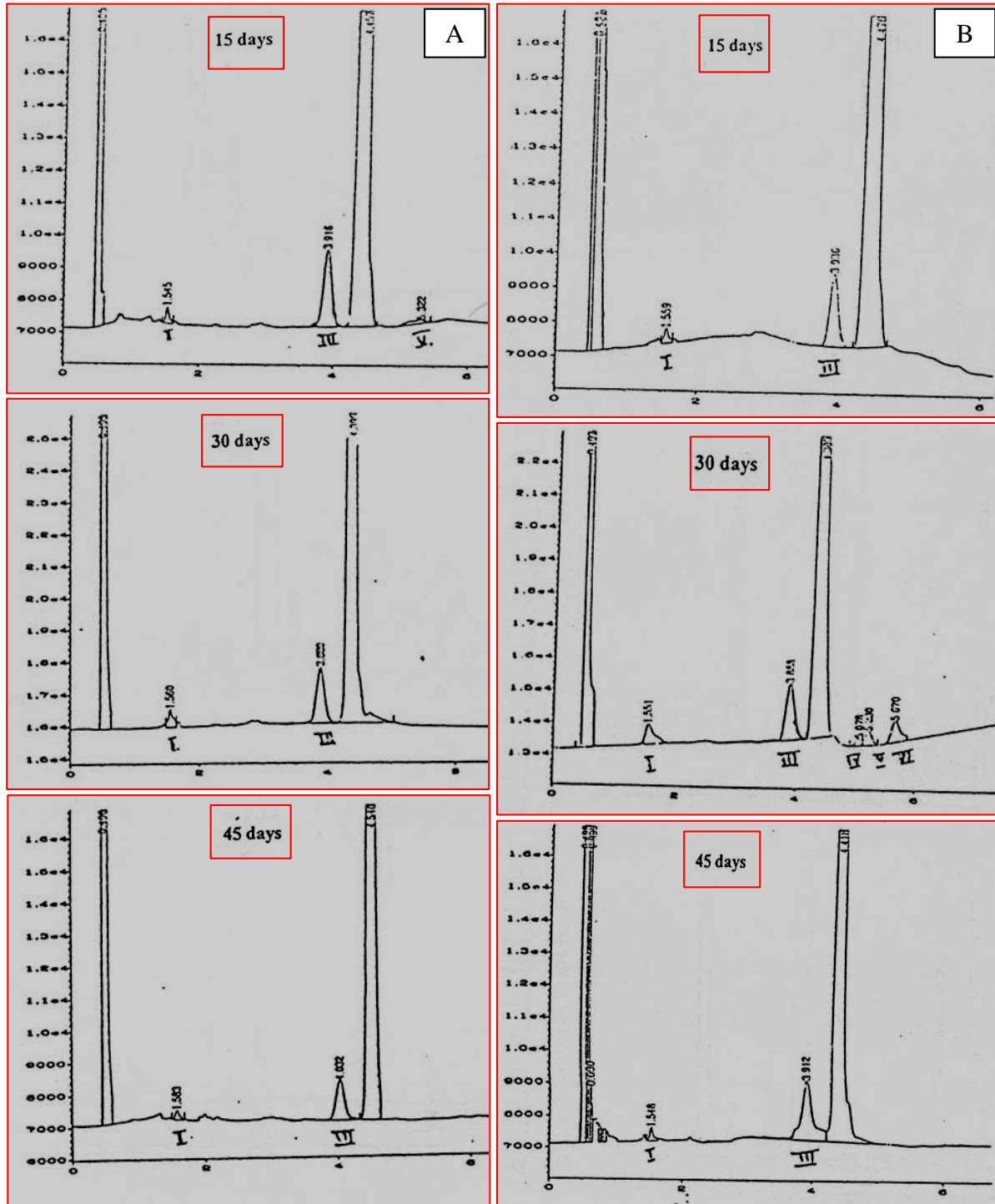
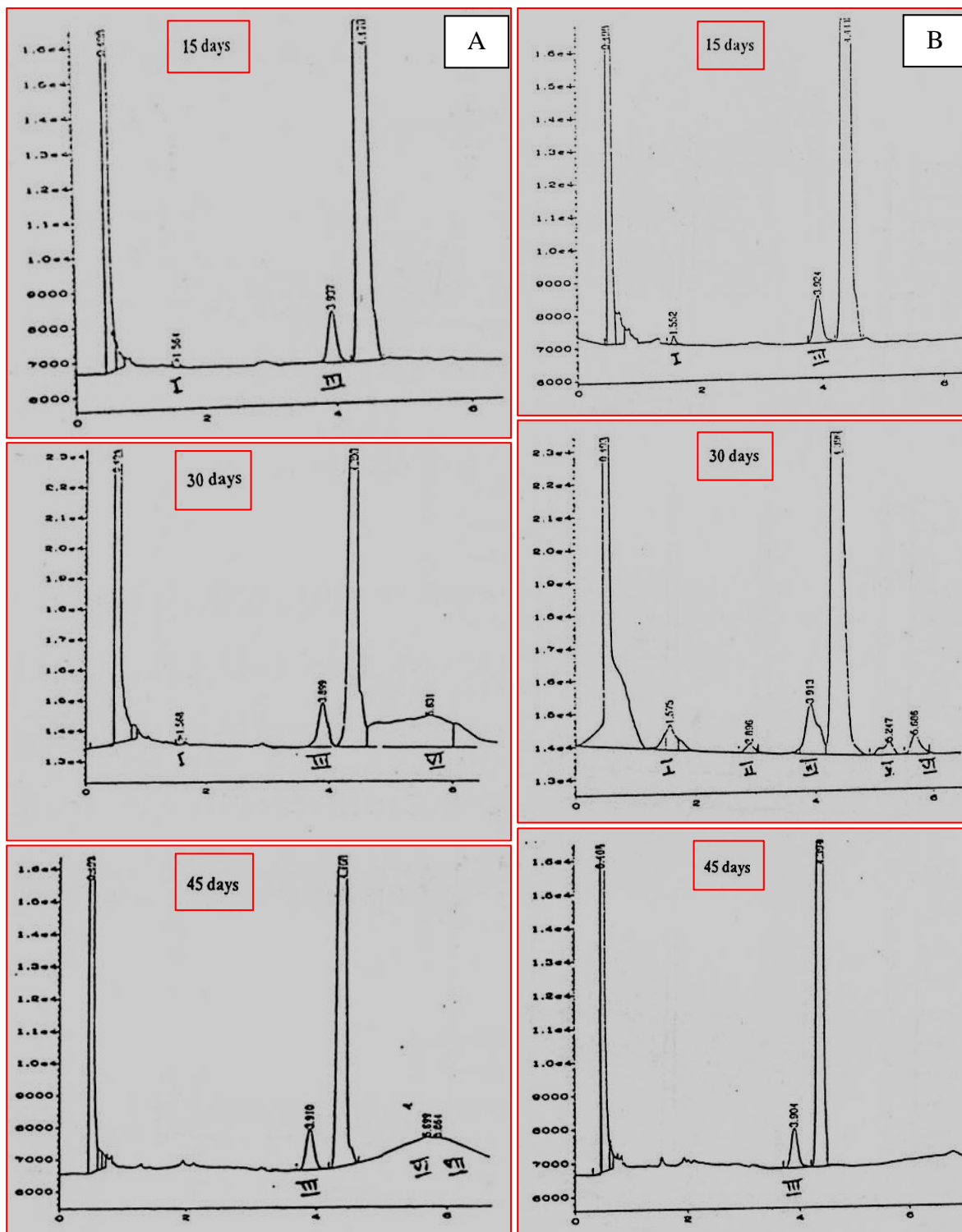


Figure.3 A: Gas liquid chromatographic analysis of biodegradation of Sencor by *S. aureomonopodiales* at different intervals. B: Gas liquid chromatographic analysis of biodegradation of Sencor by mixture of *Streptomyces* species at different intervals



Effect on Azotobacter

Densities of this nitrogen-fixing group were considerably higher in most *Streptomyces* treatments than noted for uninoculated control or Sencor-amended control on the 15th and 30th day (Table 5). Highest counts were recorded for *S. aureomonopodiales* and mixture of treatments. It seems likely that high activity of *Streptomyces* species in the biodegradation of Sencor during this period might result in metabolites favoring high proliferation of this group. Thereafter, counts ceased being more or less around their average counts in the two respective controls. This might reflect depletion of Sencor-degradation products probably due to high degradation rates of the herbicide at earlier periods.

Fang and Si (2011) indicated that the nanoscale Fe₃O₄ treatment had a positive effect on bacteria and actinomycetes; however, the fungi number was decreased by the same treatment. Dubova and Zarina (2001) studied the effect of herbicide pollution on soil microbial flora activity and find out the potential of Toxkit microbiotests as alternative cost-effective methods for the detection and control of herbicides and their decomposition components in agricultural soils. Effects on soil microorganisms strongly depend on the pesticide dosage, but the influence may be varied by its availability in soil. This study recommends applying *S. herbaricolor* for the bioremediation of the atrazine herbicide(s) in contaminated soils.

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