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# BIODEGRADATION KINETICS OF BROMOXYNIL AS A POLLUTION CONTROL TECHNOLOGY

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## ABSTRACT

Nonpoint source (NPS) pollution from agriculture is the leading source of impairment to Survey Rivers and lakes. Pesticides are one of the major NPS pollutants that result from agricultural activities. Among those pesticides, is Bromoxynil (BRMX) which is a widely used herbicide. The present study was carried out to determine the capability of selected biological control agents to degrade BRMX at different incubation periods. Microbial degradation of BRMX at the rate of 100 ppm in pure liquid culture media of either seven isolates of bacteria; *Azotobacter chroococcum*, *Azospirillium barasilense, Klebsilense pneumoneae*, *Pseudomonas cepacia*, *Pseudomonas fluoresences, Bacillus subtilis*, and *Bacillus polymyxa* or two isolates of fungi; *Trichoderma viride* and *Trichoderma harzianum* has been determined at different time intervals using high performance liquid chromatography (HPLC) with UV detection, and solid phase extraction (SPE) technique. A biphasic model was assumed in order to carry out the statistical study of the loss of BRMX from the media enriched with either bacteria or fungi. The results showed that, the percentages of residual amount of BRMX from media enriched with bacteria ranged from 29.51 -71.94, 18.89-43.88, 9.82-35.07, 3.47-31.90 and 1.80-19.24% after 3, 7, 14, 21 and 28 days of incubation, respectively. On the other hand, the residual amount of BRMX from media enriched with fungi ranged from 45.61-60.26, 21.25-30.56, 6.48-20.63, 1.25-10.49 and 0.63-1.56% after 3, 7, 14, 21 and 28 days of incubation, respectively. The data indicate that there was a faster rate of BRMX loss in the first phase than the second one. This is clearly reflected in the $t_{1/2}$ values. Therefore, it is recommended to use these microorganisms as biological control agents for the integrated pest management (IPM) programs to reduce agricultural non point source (NPS) pollution to prevent pesticides from reaching ground and surface water.

## 1. INTRODUCTION

The most recent *National Water Quality Inventory* reports that agricultural nonpoint source (NPS) pollution is the leading source of water quality impacts to survey rivers and lakes, the third largest source of impairments to survey estuaries, and also a major contributor to ground water contamination and wetlands degradation.( EPA, 2004).

NPS pollution occurs when water run over land or through the ground, picks up pollutants and deposits them in surface waters and introduce them into ground water.

The major NPS pollutants that result from agricultural activities are sediment, nutrients, pathogens, pesticides, and salts. To reduce NPS contamination from pesticides, people can apply Integrated Pest Management (IPM) techniques based on the specific soils, climate, pest history, and crop for a particular field. IPM helps limit pesticide use and manages necessary applications to minimize pesticide movement from the field.

Several studies were conducted to examine the degradation of pesticides in soil (Bartha *et al*., 1967; Johnson and Camper, 1991, Yarden *et al*., 1990, Salama *et al*., 1999). Bioremediation is a pollution control technology that uses biological systems to

catalyze the degradation or transformation of various toxic chemicals. Bioremediation is limited in the number of toxic materials it can handle, but where applicable, it is cost-effective (Atlas and Unterman, 1999). Many microorganisms play a double role in the cultivated soils, as plant pathogens and organic materials degrading agents (Garrett, 1970). The fungi are unique among microorganisms in that they secrete a variety of extracellular enzymes (White and Aust, 1994). The ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them in bioremediation (Alexander, 1994). The fungi use an extracellular, free radical, nonspecific mode of degradation, which allow them to degrade both soluble and insoluble contaminants (White and Aust, 1994).

Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) is a widely used herbicide. Biodegradation experiments showed a rapid disappearance of bromoxynil (Smith, 1971) and a high mineralization of bromoxyniloctanoate (Collins, 1973) in soil. The main degradation and transformation pathways of directly applied bromoxynil in soil were mineralization and formation of bound residues (Rosenbrock *et al*., 2004).

In a biofilm reactor *Agrobacterium radiobacter* was able to degrade bromoxynil within a short time (Müller and Gabriel, 1999). In soil and in cultures of *Pseudomonas putida* 13XF, various metabolites resulted from stepwise hydrolysis of the cyano group (formation of the corresponding benzamide and benzoic acid), from partial debromination, and from hydroxyl methylation (Golovleva et al., 1988).

Unfortunately, no data are available on *in vitro* biodegradation of BRMX by pure cultures of biological control agents of plant pathogens or plant growth promoting rhizobacteria, which constitute one of the main techniques involved in the IPM.

The present study was carried out to determine the capability of selected biological control agents to degrade BRMX at different incubation periods. BRMX disappearance was measured using HPLC and SPE technique.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and Standard

Technical Bromoxynil (BRMX), [3,5-dibromo-4-hydroxybenzonitrile], was provided by Environmental Protection Agency (EPA, USA) with purity of 98%. Certified HPLC-grade of methylene chloride, methanol and acetonitrile (ACN) and granular AR anhydrous sodium sulfate were purchased from BDH Company, while the Baker SPE-12G Column Processor designed vacuum manifold capable of processing up to 12 solid phase extraction columns and solid phase extraction column (BAKERBOND spe$^{TM}$, octadecyl (C18), 500 mg per column) was purchased from J.T.Baker, Mallibnckrodt Baker, Inc., Phillipsburg, NJ, USA. Ultra-pure deionized water of 15 MΩ.cm resistivity was obtained from a water purification system (PURELAB Option-R, ELGA, UK).

### 2.2. Selected microorganisms and their sources

#### *2.2.1. Selected microorganisms*

*Bacillus subtilis* (Ehrenberg) Cohn culture BI, *Pseudomonas fluoresences*, *Pseudomonas cepacia* (*Burkholderia cepacia* ex Burkholder), *Bacillus polymyxa* and three growth promoting nitrogen fixing rhizobacteria, namely *Azospirillium barasilense*, *Azotobacter chroococcum*, and *Klebsilense pneumoneae* as well as two isolates of fungi; *Trichoderma viride* and *T. harzianum*

#### *2.2.2. Sources of microorganisms*

*Bacillus subtilis* (Ehrenberg) Cohn culture BI (B.S.I.) and *Pseudomonas fluoresences* (P.F.) were originally isolated from soil of the Experimental Farm of Sabahia Research

Station, Alexandria, Egypt. *Pseudomonas cepacia* (*Burkholderia cepacia* ex Burkholder) isolate was obtained from the Plant Pathology Dept., North Carolina State University, USA, type culture collection (ATCC55344). *Bacillus polymyxa* was kindly provided by Dr. M.A. El-Meleigy, Plant Production and Protection Dept., Faculty of Agriculture & Vetrinary Medicine, Al-Qassim University, KSA, this isolate was originally isolated from wheat roots of Al-Qassim fields, Central Saudi Arabia. Three growth promoting nitrogen fixing rhizobacteria, namely *Azospirillium barasilense*, *Azotobacter chroococcum*, and *Klebsilense pneumoneae* were kindly provided by Dr M.G. Hassonna, Plant Pathology Dept., Faculty of Agriculture, Alexandria University, Alex., Egypt. *Trichoderma viride* and *T. harzianum* were obtained from Plant Pathology & Weed Science Dept., Colorado State University, Fort Collins, Colorado, USA.

#### *2.2.3. Media used*

Fungi cultures, 7 days old, were maintained on potato dextrose agar (PDA) medium at 27±1°C to serve as a source of inoculum. The fungi were grown in 250 ml flasks containing 100 ml of Czapek-Dox medium. On the other hand, bacteria isolates were grown on nutrient agar (NA) medium at 27±1°C for 48 hrs.

### 2.3. Design of biodegradation experiments

Bacteria growth was suspended in sterilized distillated water and adjusted to its optical density at $A_{650}$ nm to 0.01 ($10^7$ cell/ml). Bacteria were grown in 250 ml flasks containing 100 ml of nutrient broth medium under aseptic conditions. The pH of either PDA or NA media was adjusted to 7.0 using phosphate buffer. Inoculation was accomplished by adding either 1ml of bacteria suspension or one disc (5 mm, diameter) of the fungus under aseptic conditions. BRMX was added at the rate of 100 ppm. The flasks were kept at 27±1°C during a period of 28 days for both bacteria and fungi without shaking. Aliquots (20 ml each) of media were withdrawn by micropipette 3, 7, 14, 21 and 28 days after incubation and subjected to chromatographic analysis. Control flasks were prepared by adding BRMX to media without microorganisms. Treatments were done in triplicate for each time.

### 2.4. Determination of Bromoxynil Residues

#### *2.4.1. Extraction procedure*

Three 20-ml aliquots of media from each time interval were used. Aliquots were filtered under vacuum through Whatman No. 2 filter paper and then subjected to solid phase extraction (SPE). Each cartridge was conditioned with two times of 5 ml of methanol and then with two times of 5 ml of ultra-pure deionized water of 15 MΩ.cm resistivity and slowly aspirated. Aliquots were loaded and vacuumed at a rate of 5 ml/min. Subsequently, the elution took place with two times of 5 ml of methylene chloride into glass vials (12 ml), followed by evaporation to dryness under vacuum. The dry residues of BRMX were redissolved in 1 ml of methanol and after agitation by vortex were analyzed by HPLC.

#### *2.4.2. Preparation of standard solutions*

The HPLC system was standardized on the same day as the samples were analyzed by injecting 20µl of standard seven solutions of freshly prepared BRMX in methanol with concentrations ranging from 0.0 to 10 ppm from a stock solution of 1000 ppm. Areas under the peak (uV.sec) versus concentrations (µg) were plotted and fit by simple linear regression to obtain an equation for the standard curve. The amount of BRMX in each sample was thus calculated based on the slope of the standard curve.

#### *2.4.3. High Performance Liquid Chromatography (HPLC) Analysis*

Extracts were chromatographed on a Perkin Elmer HPLC system model 200 equipped with a degasser, quaternary LC pump model 2000Q/410, 20 µl loop, Spheri-5 RP-18 column (15 cm x 4.6 mm i.d., 5 µm, Perkin Elmer), oven column, a LC200 UV detector. The Turbochrom Workstation Software package was used for instrument control, data acquisition, and data analysis. The column temperature was kept at 25 °C. BRMX was eluted using an isocratic gradient of 78:22 v/v ACN: H2O. BRMX was detected at wavelength (λ) of 220 nm with flow rate of 1.1 ml/min according to Al-Rehiayani and Osman, 2005, the retention time for BRMX was 3.2 min.

#### *2.4.4. Limits of Detection and Quantification*

The limits of detection (LOD) and limits of quantification (LOQ) with this procedure were defined as the concentration (expressed as ppm) that gave signals of 3 and 10 times the noise, respectively, within its retention time ($t_R$) window (Falqui-Cao *et al*, 2001).

### 2.5. Statistical Analysis

The data were calculated as mean ± S.D and analyzed using analysis of variance technique (ANOVA). Probability of 0.05 or less was considered significant. All statistical analyses were done with Costat Program (Version 2, Cohort software, 1986) on a personal computer.

## 3. RESULTS AND DISCUSSION

### 3.1. Residual BRMX from media inoculated with bacteria

Data in Table 1 illustrate that the residual amount of BRMX declined with increasing the incubation period in the media amended with bacteria. Results showed that the remaining amount of BRMX were 71.94, 43.88, 35.07, 31.90 and 19.24 ppm, at 3, 7, 14, 21 and 28 days of incubation , after administration of 100 ppm of BRMX (initial concentration) to media inoculated with *Azotobacter chroococcum*, respectively (Table 1). Although from day 7 on, the decrease was slow, there were significant differences between the levels of BRMX. Also, data in Table 1 illustrate that less than 20% of the initial concentration was detected 28 days after BRMX application.

In case of *Azospirillium barasilense,* the remaining amounts of BRMX were 57.54, 31.11, 21.25, 7.57 and 3.06 ppm after 3, 7, 14, 21 and 28 days of incubation, respectively. At 28 days of incubation, about 3% of the initial concentration was detected. The capability of *Klebsilense pneumoneae* to degrade BRMX is illustrated in Table 1. The remaining amounts of BRMX were 47.90, 20.35, 9.82, 6.74 and 1.80 after 3, 7, 14, 21 and 28 days of incubation, respectively. Less than 2% of the initial concentration was detected 28 days after BRMX application.

*Pseudomonas cepacia* degraded the initial concentration of BRMX to levels of 63.10, 34.25, 28.16, 16.22 and 7.11 ppm after 3, 7, 14, 21 and 28 days of incubation, respectively (Table 1). From day 7 on, the degradation was low. Also, data revealed that about 7% of the initial concentration was detected after 28 days. The obtained results showed that *Pseudomonas fluoresences* degraded BRMX to levels of 29.51, 18.89, 10.07, 3.47 and 2.85 ppm after 3, 7, 14, 21 and 28 days of incubation, respectively (Table 1). About 3% of the initial concentration was detected after 28 days. Data in Table 1 illustrate that the remaining amount of BRMX were 56.23, 31.62, 22.15, 16.18 and 4.85 ppm after 3, 7, 14, 21 and 28 days of incubation, respectively, when inoculated with *Bacillus subtilis*. At day 28 about 4.85% of the initial concentration was detected.

Data in Table 1 also illustrate that there were significant differences between the

remaining amounts of BRMX during all the tested times, where the levels of BRMX were 53.70, 24.79, 23.89, 16.39 and 7.51 ppm after 3, 7, 14, 21 and 28 days of incubation, respectively, when the media was inoculated with *Bacillus polymyxa* (B.P.). Also, data revealed that about 7.5% of the initial concentration was detected after 28 days.

### 3.2. Recovered BRMX from media inoculated with fungi

Data in Tables 1 illustrate that the recovered amount of BRMX declined with increasing the incubation period in the media amended with fungi. Results showed that, 3, 7, 14, 21 and 28 days after administration of 100 ppm of BRMX (initial concentration) to media inoculated with *Trichoderma viride*, the remaining amounts of BRMX differed significantly, where the detected levels were 60.26, 30.56, 6.48, 1.25 and 0.63 ppm, respectively (Table 1). From day 7 on, the degradation was very fast. The percentages of initial concentrations were less than 1% of the initial concentration 28 days after incubation.

In case of *T. harzianum,* the remaining amounts of BRMX were 45.61, 21.25, 20.63, 10.49 and 1.56 ppm after 3, 7, 14, 21 and 28 days of incubation, respectively (Table 1). During the periods of 7-14 on, the degradation was very steep, where the percentages of initial concentrations ranged from 21.25-20.63% of the initial concentration. Less than 2% of the initial concentration was detected after 28 days.

### 3.3. Kinetic Studies

A biphasic model was assumed in order to carry out the statistical study of the loss of BRMX from the media enriched with either bacteria or fungi according to the equation (1).

$$R= A_0e^{-\alpha t} + B_0e^{-\beta t} \quad (1)$$

In case of *Trichoderma viride* a monophasic model was assumed in order to carry out the statistical study of the loss of BRMX according to the equation (2).

$$R= A_0e^{-\alpha t} \quad (2)$$

Where R is the recovered amount of BRMX at t days, $A_0$ and $B_0$ are the concentrations of BRMX at t=0 and $\alpha$ and $\beta$ are the disappearance rate constants for the first and second phase model, respectively. The half-life ($t_{1/2}$) of the exponential decay was calculated according to the equation (3).

$$t_{1/2}= (2.303 \log 2)/ \text{rate constant} \quad (3)$$

The data indicate that there was a faster rate of BRMX disappearance in the first phase than the second one (Table 2). This is clearly reflected in the half- life values of BRMX in the first phase ($t_{1/2\ \alpha}$), where estimated to be 13.12, 7.64, 10.70, 8.80, 6.95, 9.27 and 11.40 days in media amended with *Azotobacter chroococcum*, *Klebsilense pneumoneae*, *Pseudomonas cepacia*, *Bacillus subtilis*, *Pseudomonas fluoresences*, *Bacillus polymyxa* and *Azospirillium barasilense*, while they were 9.52 and 8.69 days in culture amended with *Trichoderma viride* and *Trichoderma harzianum*, respectively. However, the half-life values ($t_{1/2\ \beta}$) for BRMX in the second phase model were 49.66, 27.81, 26.74, 24.83, 19.87, 49.66 and 30.23 days in culture amended with *Azotobacter chroococcum*, *Klebsilense pneumoneae*, *Pseudomonas cepacia*, *Bacillus subtilis*, *Pseudomonas fluoresences*, *Bacillus polymyxa* and *Azospirillium barasilense*,, respectively, while it was 38.63 days in media amended with *Trichoderma harzianum*.

Bromoxynil was a focus of several biodegradation studies. Vokounova et al. 1992; Gabriel et al. 1996 found *Pseudomonas putida* 13XF to be able to convert bromoxynil to 3,5-dibromo-4-hydroxybenzamide and 3,5-dibromo-4-hydroxybenzoic acid in the presence of a carbon and energy source, indicating that this strain possesses nitrile hydratase activity (formation of the benzamide) as well as amidase activity (formation of the benzoic acid). *Agrobacterium radiobacter* 8/4 however

lacks the amidase activity, since 3,5-dibromo-4-hydroxybenzamide was found as the only metabolite resulting from bromoxynil degradation (Gabriel *et al*., 1996). *Klebsiella pneumoniae* sub sp. *ozaenae* uses bromoxynil as nitrogen source by direct formation of 3,5 dibromo-4-hydroxybenzoic acid, with the enzyme nitrilase (McBride *et al*., 1986). Smith and Cullimore (1974) observed a bromoxynil degradation to 3,5-dibromo-4-hydroxybenzamide and 3,5-dibromo-4 hydroxybenzoic acid and a further non identified metabolite by a strain of *Flexibacterium*. In contrast, a *Flavobacterium* strain degrades bromoxynil by cleavage of the nitrile group and formation of cyanide and 2,6-dibromohydrochinone (Topp *et al*., 1992). The responsible enzyme is a hydroxylase capable to transform pentachlorophenol. In model aquifers two main pathways of bromoxynil metabolism were found: hydrolysis of the nitrile group and replacement of bromine by chlorine when chloride was present in the matrix (Graß *et al*., 2000). Abiotic degradation of bromoxynil by photolysis is also reported (Millet *et al*., 1998; Texier *et al*., 1999).

The half-life of Bromoxynil in soil is generally several days, and its biodegradation is the primary mechanism of dissipation (Smith, 1971). Bromoxynil may be degraded by a *Flexibacterium* sp. and an isolate of *Klebsiella pneumoniae* sub sp. *ozanenae* (Cullimore and Kohout, 1974; Smith and Cullimore, 1974; McBride *et al*., 1986) and the metabolism was initiated by hydrolysis of the cyano group to the carboxylate (Smith and Cullimore, 1974; McBride *et al*., 1986). Moreover, bromoxynil was degraded more slowly by the *Flavobacterium* sp. than pentachlorophenol due to the accumulation of deleterious concentrations of cyanide (Topp et al., 1992). An increasingly popular approach for the remediation of halogenated environmental pollutants is biological reductive dehalogenation (Cupples et al., 2005). *Desulfitobacterium chloroespirans* uses bromoxynil as an electron acceptor for growth and it could promote debromination in soil subjected to anaerobic conditions and transient aerobic conditions (Cupples *et al*., 2005).

## 4. CONCLUSION

Bromoxynil disappears rapidly from pure media cultures of either bacteria or fungi supporting microbial growth. During the 28-day incubation period, approximately 81-98 and >98% of BRMX was degraded by bacteria and fungi, respectively, as measured by BRMX disappearance. Our results showed that biodegradation of bromoxynil was rapid and its persistence was less in media amended with *Pseudomonas fluoresences*, *Klebsilense pneumoneae*, *Bacillus subtilis and Trichoderma harzianum.* Therefore, it is recommended to use these microorganisms as biological control agents for the integrated pest management programs to reduce agricultural non point source (NPS) pollution to prevent pesticides from reaching ground and surface water**.**

**Table (1): Bromoxynil residues (ppm) after microbial degradation at different times of incubation.**

| Incubation Time (day) | Bacteria | | | | | | | Fungi | |
|---|---|---|---|---|---|---|---|---|---|
| | *A. chroococcum* | *A. barasilense* | *K. pneumoneae* | *P. cepacia* | *P. fluoresences* | *B. subtilis* | *B. polymyxa* | *T. viride* | *T. harzianum* |
| 0 | 100±0.0$^{f}$ | 100±0.0$^{e}$ | 100±0.0$^{e}$ | 100±0.0$^{d}$ | 100±0.0$^{e}$ | 100±0.0$^{e}$ | 100±0.0$^{f}$ | 100±0.0$^{f}$ | 100±0.0$^{e}$ |
| 3 | 71.94±0.33$^{e}$ | 57.54±0.91$^{d}$ | 47.90±0.12$^{d}$ | 63.10±0.12$^{c}$ | 29.51±0.52$^{d}$ | 56.23±0.25$^{d}$ | 53.70±0.35$^{e}$ | 60.26±0.35$^{e}$ | 45.61±1.22$^{d}$ |
| 7 | 43.88±0.50$^{d}$ | 31.11±0.51$^{c}$ | 20.35±0.15$^{c}$ | 4.25±0.26$^{b}$ | 18.89±0.34$^{c}$ | 31.62±0.46$^{c}$ | 24.79±0.74$^{d}$ | 30.56±0.40$^{d}$ | 21.25±0.66b$^{c}$ |
| 14 | 35.07±0.49$^{c}$ | 21.25±0.54$^{b}$ | 9.82±0.25$^{b}$ | 28.16±0.35$^{a}$ | 10.07±0.22$^{c}$ | 22.15±0.49$^{b}$ | 23.89±0.52$^{c}$ | 6.48±0.24$^{c}$ | 20.63±0.12$^{b}$ |
| 21 | 31.90±0.30$^{b}$ | 7.57±0.50$^{a}$ | 6.74±0.13$^{b}$ | 16.22±0.27$^{a}$ | 3.47±0.42$^{b}$ | 16.18±0.22$^{a}$ | 16.39±0.14$^{b}$ | 1.25±0.20$^{b}$ | 10.49±0.71$^{b}$ |
| 28 | 19.24±0.13$^{a}$ | 3.06±0.49$^{a}$ | 1.80±0.23$^{a}$ | 7.11±0.22$^{a}$ | 2.85±0.33$^{a}$ | 4.85±0.29$^{a}$ | 7.51±0.33$^{a}$ | 0.63±0.16$^{a}$ | 1.56±0.58$^{a}$ |

Data are expressed as ppm and calculated as means ± S.D. Means in a column with different coefficients (a-f) are significantly different ($p \leq 0.05$).

**Table (2): Statistical parameters of BRMX dissipation.**

| Statistical parameters | Bacteria | | | | | | | Fungi | |
|---|---|---|---|---|---|---|---|---|---|
| | *A. chroococcum* | *K. pneumoneae* | *P. cepacia* | *B. subtilis* | *P. fluoresences* | *B. polymyxa* | *A.barasilense* | *T.viride* | *T.harzianum* |
| $A_0$ (ppm) | 95.50 | 93.32 | 97.72 | 85.11 | 56.23 | 81.28 | 89.13 | 70.79 | 79.43 |
| $B_0$ (ppm) | 53.70 | 21.63 | 51.29 | 50.12 | 20.89 | 29.85 | 44.67 | - | 31.62 |
| $\alpha$ ($days^{-1}$) | 0.053 | 0.091 | 0.065 | 0.079 | 0.100 | 0.075 | 0.061 | 0.073 | 0.080 |
| $\beta$ ($days^{-1}$) | 0.014 | 0.025 | 0.026 | 0.028 | 0.035 | 0.014 | 0.023 | - | 0.018 |
| $t_{1/2\alpha}$ (days) | 13.12 | 7.64 | 10.70 | 8.80 | 6.95 | 9.27 | 11.40 | 9.52 | 8.69 |
| $t_{1/2\beta}$ (days) | 49.66 | 27.81 | 26.74 | 24.83 | 19.87 | 49.66 | 30.23 | - | 38.63 |
| Regression coefficient | 0.889 | 0.814 | 0.888 | 0.865 | 0.746 | 0.822 | 0.880 | 0.871 | 0.852 |

$A_0$ and $B_0$ are the concentrations of BRMX at t=0 and $\alpha$ and $\beta$ are the disappearance rate constants for the first and second phase model, respectively

## REFERENCES


Alexander, M.: 1994, Biodegradation and Bioremediation. Academic Press. San Diego, CA, USA.pp.368-379

Al-Rehiayani, S. and Osman, K.A.: 2005, Fate of preharvest-sprayed dicofol in date fruits: Residue analysis by HPLC. *Agric. Marine Sci, Sultan Qaboos Univ.,* **10**: 21-26.

Atlas, R.M. and Unterman, R.: 1999, Bioremediation In: Industrial Microbiology & Biotechnology, 2nd Ed. ASM Press, Washington, pp .666-681.

Bartha, R, Landzilota, R.P. and Parmer, D.: 1967, Stability and Effect of Pesticides in Soil. *Appl. Microbiol*., **15**: 67-75.

Collins, R.F.: 1973, Perfusion studies with bromoxynil octanoate in soil. *Pestic. Sci.* **4**: 181–192.

Costat Program: 1986, Version 2, Cohort Software.

Cullimore, D.R. and Kohout, M.: 1974, Isolation of a bacterial degrader of the herbicide bromoxynil from a Saskatchewan soil. *Can. J. Microbiol*. **20**:1449-1452.

Cupples, A.M., Sanford, R.A., and Sims, G.K.: 2005, Dehalogenated of the herbicide bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) and ioxynil (3,5-diiodo-4-hydroxybenzonitrile) by *Desulfitobacterium chlororespirans*. *Appl. Environ. Microbiol.,* **71(7)**: 3741-3746.

EPA: 2004, Managing Nonpoint Source Pollution from Agriculture**.** EPA Pointer No. **6**: 841-F-96-004F, 2004

Falqui-Cao, C., Wang, Z., Urruty, L., Pommier, J.-J., and Montury, M.: 2001, Focused microwave assistance for extracting pesticide residues from strawberries into water before their determination by SPME/HPLC/DAD. *J. Agric. Food Chem.* **49**: 5092-5097.

Gabriel, J., J. Vekovaa and Vosaahlo J.: 1996, High-performance liquid chromatographic study of the aromatic nitrile metabolism in soil bacteria. *J.Chromatogr*. **B681**: 191–195.

Garrett, S.D.: 1970, Pathogenic root-infection. Cambridge University Press, pp. 294- 312

Golovleva, L.A., Pertsova, R.N., Kunc, F., and Vokounova, M.: 1988, Decomposition of the herbicide bromoxynil in soil and in bacterial cultures. *Folia Microbiol*. **33**: 491–499.

Graß, B., Mayer, H., Nolte, J., Preuß, G., and Zullei Seibert, N.: 2000, Studies on the metabolism of hydroxybenzonitrile herbicides: I. mass spectrometric identification, *Pest.Manag. Sci.* **56**: 49–59.

Johnson, W.H. and Camper, N.D.: 1991, Microbial degradative activity in pesticides soil. *J. Environ. Sci. Health,* **B26**, 1-4.

McBride, K.E., Kenny, J.W. and Stalker, D.M.: 1986, Metabolism of the herbicide bromoxynil by *Klebsiella pneumoniae* subsp. *ozaenae*. *Appl. Environ Microbiol*. **52**: 325-330.

Millet, M., Palm, W.U., and Zetzsch, C.: 1998, Abiotic degradation of halobenzonitriles: investigation of the photolysis in solution. *Ecotoxicol. Environ. Saf.* **41**: 44–50.

Müller, D. and Gabriel, J.: 1999, Bacterial degradation of the herbicide bromoxynil by *Agrobacterium radiobacter* in biofilm. *Folia Microbiol*. **44**: 377–379.

Rosenbrock, P., Munch, J.C., Scheunert, I. and Deoorfler, U.: 2004, Biodegradation of the herbicide bromoxynil and its plant cell wall bound residues in an agricultural soil. *Pesticide Biochem. and Physiol.* **78**: 49–57.

Salama, A.K., Al-Mihanna, A.A. and Abdalla, M.Y.: 1999, Microbial degradation of pirimiphos-methyl and carbrayl by pure culture of two soil fungi. *J. King Saud Univ., Agric. Sci.,* **11(1)**: 25-32.

Smith, A.E.: 1971, Degradation of

bromoxynil in Regina heavy clay. *Weed Res.***11**: 276–282.

Smith, A.E.and Cullimore, D.R.: 1974, The *in vitro* degradation of the herbicide bromoxynil. *Can. J. Microbiol*. **20**: 773-776.

Texier, I., Giannotti, C., Malato, S., Richter, C. and Delaire, J.: 1999, Solar photodegradation of pesticides in water by sodium decatungstate, *Catal. Today* **54**: 297–307.

Topp, E., Xun, L. and Orser, C.S.: 1992, Biodegradation of the herbicide bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) by purified pentachlorophenol hydroxylase and whole cells of *Flavobacterium* sp. strain ATCC 39723 is accompanied by cynogenesis. *Appl. Environ. Microbiol.* **58 (2)**: 502-506.

Vokounova, M., Vacek, O.and Kunc, F.: 1992, Effect of glucose and ribose on microbial degradation of the herbicide bromoxynil continuously added to soil. *Folia Microbiol.,* **37**: 128–132.

White, R.B. and Aust, S.D.: 1994, Biodegradation of environmental contaminants using rot fungi. *AAPg Bulletin,* **78 (8)**: 1335- 1341

Yarden, O., Salomon, R., Katan, J. and Ahronson, N.: 1990, Involvement of fungi and bacteria in enhanced and nonenhanced biodegradation of carbandazim and other benzimidazole compounds in soil. *Can. J. Microbiol.,* **36**: 15-23.