ISSN: 0976-3376

Available Online at http://www.journalajst.com

Asian Journal of Science and Technology
Vol. 07, Issue, 05, pp.2903-2911, May, 2016

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE

DEGRADATION OF ATRAZINE BY *PSEUDOMONAS SPP* AND *BACILLUS* SPP FROM *SACCHARUM OFFICINARUM* (SUGAR CANE) FIELDS OF SOUTHERN INDIA AND ITS POTENTIAL APPLICATION IN BIOREMEDIATION

[1,*]Lavanya Balakrishnan and [2]Athilakshmi, N.

[1]Department of Microbiology, Hindustan College of Arts and Science, Chennai, India
[2]Department of Microbiology, Valliammal College for Women, Chennai, India

ARTICLE INFO

Article History:

Received 12th February, 2016
Received in revised form
14th March, 2016
Accepted 19th April, 2016
Published online 30th May, 2016

Key words:

Herbicide,
Atrazine,
Pseudomonas spp,
Bacillus spp,
Saccharum officinarum,
Bioremediation.

ABSTRACT

Herbicide Atrazine is widely used in *Saccharum Officinarum* (sugar fields) in South India. Atrazine has been found as a residue in soil and water bodies of sugarcane fields for up to 2 years. Prolonged exposure to atrazine has been found to cause many harmful effects including cancer. This study evaluates the capacity of bacteria to degrade Atrazine in soil from South Indian Sugar cane fields and hence study its application in bioremediation. Soil was tested for Atrazine residue 2 weeks after spraying. Soil bacteria were enumerated by serial dilution on Trypticase soy agar and the resulting colonies were grown in nutrient agar with varying concentrations of Atrazine to detect Atrazine tolerant bacteria. The Atrazine tolerant bacteria were identified by Biochemical tests. Finally, an assay was performed to study degradation of Atrazine by the bacteria in a Minimal salt medium supplemented with Glucose. It was found the bacteria were capable of degrading Atrazine by using it as its Nitrogen source for growth. *Pseudomonas* spp and *Bacillus* spp were found to be resistant to Atrazine and able to degrade Atrazine optimally. Results suggested that an emulsion of the bacteria can possibly be used for bioremediation of Atrazine soil fields and water bodies.

Copyright © 2016 *Lavanya Balakrishnan and Athilakshmi.* This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Bioremediation is the utilization of microorganisms to remove pollutants like Pesticides, Alkyl Benzene Sulfonates (ABS), Chlorinated hydrocarbons or Oil pollutants from the environment. The natural capacity of diverse microorganisms to degrade numerous organic compounds ranging from petroleum hydrocarbons to chlorinated solvents, and to transform various inorganic substances, including metals; forms the basis for bioremediation (Dileep, 2008). Most bioremediation projects have relied on naturally occurring microorganisms at the contaminate site. Bioremediation can hence be used to reclaim soils and waters polluted by substances hazardous to human health and the environment. Bioremediation leads to the actual reduction and detoxification of wastes. Ideally, wastes can be completely degraded or mineralized to their simple inorganic constituents which are carbon dioxide and water in the case of pure organic compounds. Bioremediation has been recognized as one of the more cost-effective and environmentally desirable clean up alternatives. Bacteria, fungi, algae, lichens and higher plants variedly respond to environmental pollutants and act as bio-remedials to help purify the atmosphere, either Though accumulation or assimilation of the pollutants into their systems. (Ravindra *et al*., 2014). Pesticide, a common pollutant under extensive studies because of its adverse side-effects; is any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest (insects, animals, weeds, fungi or microorganisms like bacteria and viruses). The term pesticide also applies to herbicides, fungicides and various other substances used to control pests. Most pesticides create some risk of harm to humans, animals, or the environment as they are designed to kill living organisms. In recent years, pesticide compounds are being increasingly used with the result that many of these chemicals, reach the soil or water bodies and persist for long periods causing harm to us. Bioremediation plays a vital role in the cleaning up of the soils contaminated with pesticides (Kavitha and Geetha, 2014). The residue pesticides in soil or on food were found to have adverse effects on the humans. One such herbicide is Atrazine, which is widely used in India on *Saccharum officinarum* (sugar cane) fields to control weeds. In the USA it is under controversy but still used for Maize and Sugarcane, whilst it was banned in Europe from 2003. Growers consider atrazine essential in their weed control program. But its residue can be detected in soil and ground water and hence plays havoc in human health (Jennifer and

Corresponding author: Lavanya Balakrishnan
Department of Microbiology, Hindustan College of Arts and Science, Chennai, India.

Aaron, 2006). *Saccharum officinarum*, (Sugar cane) a perennial plant of the family Graminaeae, is cultivated in Asia as a commercial sugar source. The sugarcane plant requires the use of herbicides to prevent growth of weeds, for its proper and effective growth. Loss of sugarcane crops due to weeds accounts for upto 25% yearly (Sugarcane status report, 2013). Herbicide Atrazine that has been used in this study is part of Triazine group which are six-member rings containing three Nitrogen and Azine (a nitrogen containing ring), make up heterocyclic Nitrogen. Atrazine is a selective broadleaf herbicide that is commonly applied to crops world-wide. Atrazine has staying power. It persists in soil and water for over a year, and traces have been found in water supplies and fruits, vegetables, meat and dairy products. When water near agricultural fields was analyzed it was found that the recurrent pesticide detected were Carabamates, Trazines such as Atrazine, Phosphoric esters. Atrazine is active in the soil for about 5 to 7 months. Soil microorganisms can break down atrazine. Sunlight may also break down atrazine to a small degree. Atrazine dissolves in water, can move easily in soil and hence can leach into ground water. Atrazine is slightly toxic to fish and birds. (Alfred *et al*., 2003). Environmental Protection Agency (EPA) has found atrazine to potentially cause the following health effects when people are exposed at levels above the maximum contaminant level (MCL) for short periods of time: congestion of heart, lungs, kidneys, low blood pressure, muscle spasms, weight loss, damage to adrenal glands. Effects from a lifetime exposure are weight loss, cardiovascular damage, retinal and some muscle degeneration, cancer. Atrazine can cause various kinds of cancer, including cancer of the breast, ovaries, uterus, testicles as well as leukemia and lymphoma. Atrazine, like DDT, is an endocrine disrupting chemical. It interrupts regular hormone function, causing birth defects, reproductive tumors, and weight loss in mothers and embryos (Frank, 2007).

Pseudomonas is a genus of bacteria that thrive in environments where few other micro-organisms can survive. They are used in the bioremediation efforts due to their ability to use a wide range of organic molecules as carbon sources. Several species of *Pseudomonas* found in the soil can produce non-fluorescing and fluorescing pigments. *Pseudomonas* is commonly found in soil and water. It cannot tolerate conditions where pH is less than 4.5. Best growth conditions have a single carbon source, low iron content, pH 7.2, available oxygen and incubation temperature between 25-30°c. *Pseudomonas* spp have been extensively studied in the bioremediation of pesticides (Grit *et al.,* 2004, Scott Stelting *et al*., 2010, Nikolina *et al*., 2012, and Mansooreh *et al*., 2013). *Bacillus* spp are ubiquitous in nature (i.e.; it is found abundantly in soil, water and airborne dust). They are strictly aerobic or facultatively anaerobic. They are Chemoorganotrophic (i.e.; requiring an organic source of carbon and metabolic energy.) (Jinhua *et al*., 2014). Both organisms are ideal for bio-remediation studies (Shahitha, 2012). The degradation of pesticides left over in soil using microorganisms came to form the basis of this project work. The aim of this study was to degrade herbicide atrazine, using soil bacteria from sugarcane fields in South India. A total of 5 soil samples were collected from various sugarcane fields from Mandya (Karnataka), Kanchipuram and Bhuvanagiri (Tamil Nadu). The main objectives of the study was to see if atrazine was present in the soil sample using Thin Layer Chromatography (TLC), Isolate bacteria from the soil samples by spread plate technique, Isolate bacteria that could tolerate atrazine by the use of various concentrations of atrazine in Nutrient agar, degrade atrazine using the atrazine tolerant bacteria by growth with various concentrations of atrazine in the presence or absence of glucose, as individual bacterial species or in combination in a Minimal salt medium and measuring their optical density and finally confirm the degradation of atrazine by urease test.

MATERIALS AND METHODS

Materials

Soil samples were collected from various sugarcane fields in Kanchipuram, Bhuvanagiri (Tamil Nadu) and Mandya (Karnataka). The soil was taken from fields two weeks after harvesting (Fig 1).

Fig. 1. Sugarcane fields sprayed with Atrazine in Mandya (Karnataka)

Fig. 2. Atrazine under Trade name 'Atrataf'

The top soil was collected aseptically in plastic containers. For study purposes, pesticide ATRAZINE was obtained from Kanchipuram under the tradename 'ATRATAF' from TATA Rallis, India (Fig 2). Silica Gel coated slides for Thin layer

chromatography (TLC) were prepared as per standard procedures. Ninhydrin reagent was obtained to develop the TLC spots (Egon, 1969). For optimal bacterial growth, Nutrient agar, Trypticase soy agar and Minimal salt medium was prepared using standard procedures using HIMEDIA. Grams's stain, Biochemicals like Catalase, Oxidase, Indole, Methyl Red, Voges Proskauer, Citrate, Christensens' urea medium, Nitrate and Triple sugar iron agar and Sugars like Glucose, Maltose and Lactose were all prepared using standard procedures using HIMEDIA, for the identification and confirmation of the soil bacteria.

Detection of Atrazine in soil

Each Soil sample from Mandya, Kanchipuram and Bhuvanagiri were macerated in solvents chloroform, diethyl ether and ethyl alcohol for 3 days and then filtered. The Atrazine to be used as the standard for TLC, was also dissolved in the same solvents. The filtrate and Atrazine solution were spotted onto TLC plates and allowed to run in TLC chambers previously saturated with the same solvent mixture. After development of the chromatogram the plates were dried and sprayed with Ninhydrin reagent to reveal the spots.

Enumeration of soil bacteria from Trypticase Soy Agar

1gm of the soil sample was serially diluted in distilled water for up to 5 dilutions (10^{-1} to 10^{-5}). 0.1ml from 10^{-3},10^{-4} and 10^{-5} was spread plated onto Trypticase Soy agar. Triplicates were made and incubated at room temperature for 24 hours.

Isolation of Atrazine tolerant bacteria

Five sets of 100ml Nutrient agar (100ml x 5) were prepared and sterilized. After it was cooled atrazine was added in various concentrations to each conical flask (0.05gm, 0.04gm, 0.03gm, 0.02gm, 0.01gm) (Table 1) and it was shaken well to mix and then dispersed into sterile petri-plates. The colonies from spread plate technique were streaked onto the Nutrient agar plates containing the various concentrations of atrazine. Plates were incubated at room temperature for 24 hours.

Table 1. Varying concentrations of Atrazine

Colony Type	*Atrazine (in gms)*				
	Plate 1	***Plate 2***	***Plate 3***	***Plate 4***	***Plate 5***
1	0.05	0.040	0.03	0.02	0.01
2	0.05	0.040	0.03	0.02	0.01
3	0.05	0.040	0.03	0.02	0.01

Biochemical tests for Identification of Atrazine tolerant bacteria

Based on the Colony morphology and Gram's staining, various standard biochemical tests (IMVIC) were run on the various colonies after plating them on to Nutrient Agar.

Biodegradation Assay of Atrazine in Minimal salt medium

An Assay was performed on a 96-well micro-titer plate. The control was Minimal salt medium (Focht, 1994). Atrazine in varying concentrations (0.01, 0.02, 0.03 and 0.04 gm/ml) was added, Glucose (0.01 gm/ml) was added to some wells to determine if the microbes could degrade the atrazine but required additional nutrients for survival. Bacteria 1 (*Pseudomonas* spp), Bacteria 2 (*Bacillus* spp) and a combination of Bacteria 1 and 2 were used (Table 2 and Fig 3). The test was performed in triplicate. The plate was incubated at room temperature for 72 hours and then the optical density was measured at 620nm.

Table 2. Biodegradation Assay of Atrazine in Minimal salt medium

	1	2	3	4	5	6	7	8	9	10	11	12	
A	M	M	A	A	AG	AG	MP	MP	MB	MB	MC	MC	A
B	0.01 AP	0.02 AP	0.03 AP	0.04 AP	0.01 AGP	0.02 AGP	0.03 AGP	0.04 AGP	0.01 AC	0.02 AC	0.03 AC	0.04 AC	B
C	0.01 AP	0.02 AP	0.03 AP	0.04 AP	0.01 AGP	0.02 AGP	0.03 AGP	0.04 AGP	0.01 AC	0.02 AC	0.03 AC	0.04 AC	C
D	0.01 AP	0.02 AP	0.03 AP	0.04 AP	0.01 AGP	0.02 AGP	0.03 AGP	0.04 AGP	0.01 AC	0.02 AC	0.03 AC	0.04 AC	D
E													E
F	0.01 AB	0.02 AB	0.03 AB	0.04 AB	0.01 AGB	0.02 AGB	0.03 AGB	0.04 AGB	0.01 AGC	0.02 AGC	0.03 AGC	0.04 AGC	F
G	0.01 AB	0.02 AB	0.03 AB	0.04 AB	0.01 AGB	0.02 AGB	0.03 AGB	0.04 AGB	0.01 AGC	0.02 AGC	0.03 AGC	0.04 AGC	G
H	0.01 AB	0.02 AB	0.03 AB	0.04 AB	0.01 AGB	0.02 AGB	0.03 AGB	0.04 AGB	0.01 AGC	0.02 AGC	0.03 AGC	0.04 AGC	H
	1	2	3	4	5	6	7	8	9	10	11	12	

** M = Minimal Salt Medium, A = Atrazine (0.01 gm/ml), G = Glucose (0.01gm/ml), P = Psuedomonas spp, B = Bacillus spp, C = Combination of Psuedomonas & Bacills spP, ** A = Concentrations of Atrazine 0.01, 0.02, 0.03, 0.04 gm / per ml of Minimum salt medium*

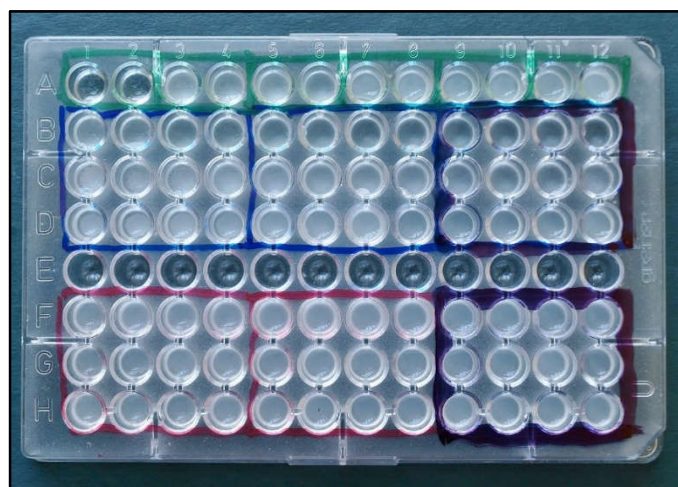


Fig. 3. Assay map

Detection of urease

A slant was prepared using Christensen's Urea medium. The slant was inoculated with a drop of inoculum from the biodegradation assay and incubated at room temperature for 18-24 hours or longer.

RESULTS AND DISCUSSION

Detection of Atrazine in soil

Thin layer chromatography (TLC) produced a band by the test sample (i.e. soil sample) on the Silica plates. The band was purple in color after spraying with Ninhydrin. The band was found to look relatively similar and at the same level as that of

the standard sample (i.e. atrazine). TLC showed the presence of bands similar to the standard run along with it (Fig 4). It hence proved the presence of atrazine residue in all the 3 soil samples (From Mandya, Kanchipuram and Bhuvanagiri) even after 2 weeks of harvesting.

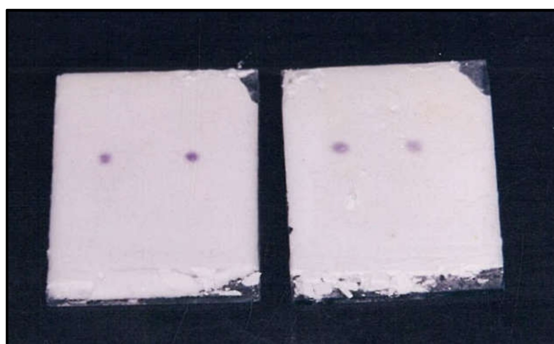


Fig. 4a. Sample 1 (Soil from Mandya – Karnataka)

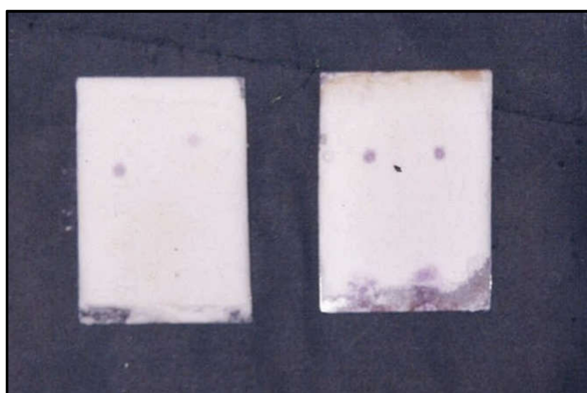


Fig. 4b. Sample 2 (Soil from Kanchipuram - Tamilnadu)

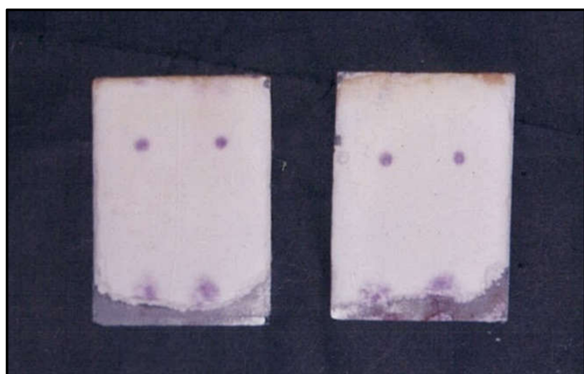


Fig. 4c. Sample 3 (Soil from Bhuvanagiri – Tamilnadu)

Enumeration of soil bacteria from Trypticase Soy Agar

The bacteria in the soil sample was enumerated by serially diluting the soil sample and plating it onto Trypticase soy agar, which can be used to enumerate soil organisms as it has KH_2PO_4 a pH buffer that provides an ideal phosphorous and potassium source to soil bacteria. White colored colonies, bright greenish-yellow colored colonies and cream colored colonies were seen (Table 3). Only three types of colonies were seen on these plates, this low number maybe due to the presence of atrazine in the soil (maybe due to the fact not all bacteria can tolerate atrazine) (Fig 5).

Table 3. Colony Morphology on Trypticase Soy Agar

Colony 1	Colony 2	Colony 3
White colored, round, large, convex colonies with regular edges	Bright greenish-yellow colored, large, low-convex colonies, with irregular edges	Large, white colored, low-convex, rough looking colonies, with irregular edges

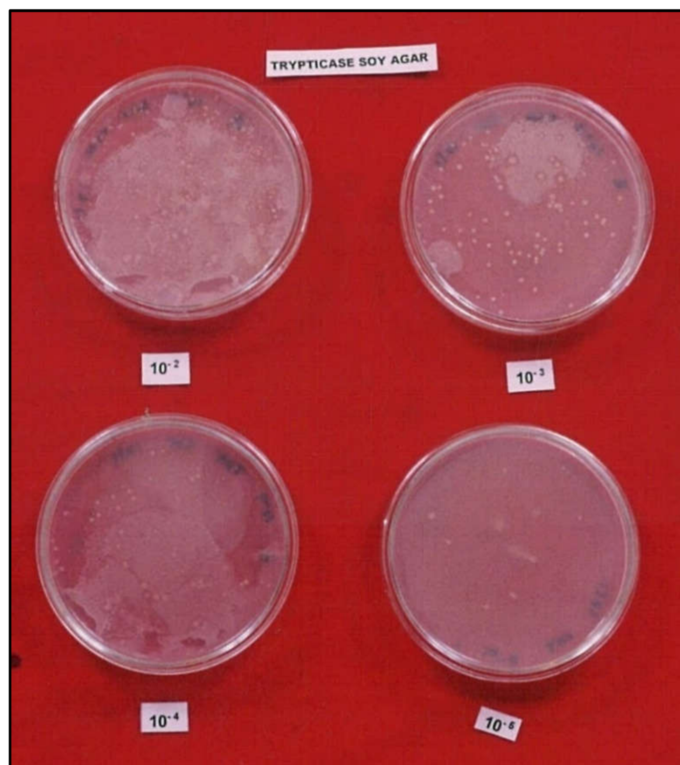


Fig. 5. Enumeration of soil bacteria from Trypticase Soy Agar by serial dilution

Isolation of Atrazine Tolerant Bacteria

The three types of colonies were grown in nutrient agar that contained various amounts of atrazine to identify the bacteria that could tolerate atrazine. Growth occurred in two sets of the plates (Colony Type 2 and 3) whilst the Colony Type 1 did not grow on the plates. It was hence decided that only two of the three bacteria could tolerate atrazine (Table 4).

Table 4. Growth of bacteria in the presence of varying concentrations of Atrazine

Colony	Atrazine (in gms)				
Type	0.05	0.04	0.03	0.02	0.01
1	N	N	N	N	N
2	N	N	Y	Y	Y
3	N	N	N	Y	Y

No growth was observed by Colony 1 in any of the plates containing atrazine, indicating the bacteria were not tolerant to atrazine. Growth was observed on plates with the concentrations 0.01 and 0.02gms by Colony 2 (Fig 6a). Indicating this bacteria was atrazine tolerant. Growth was observed on plates with the concentrations 0.01, 0.02 and 0.03gms by Colony 3 (Fig 6b). Indicating this bacteria was also atrazine tolerant. It was also noted that higher the Atrazine content, lower the growth of the bacteria.

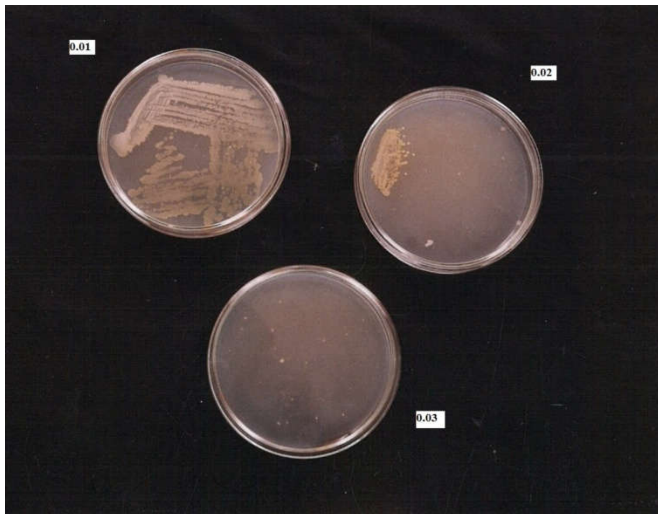


Fig. 6a. Atrazine tolerant bacteria – Colony 2

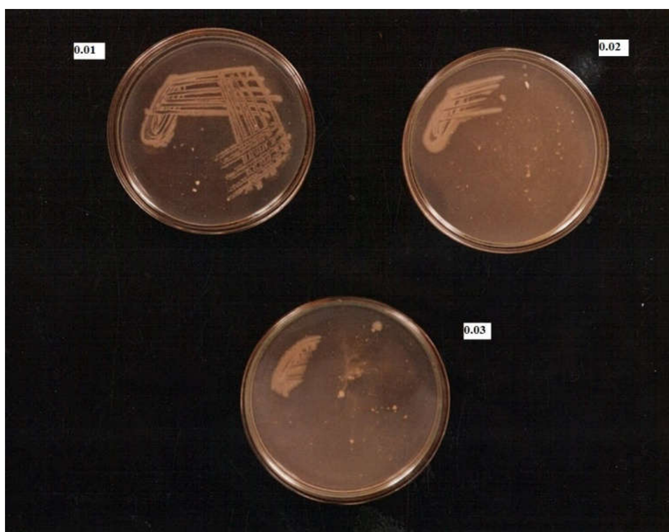


Fig. 6b. Atrazine tolerant bacteria – Colony 3

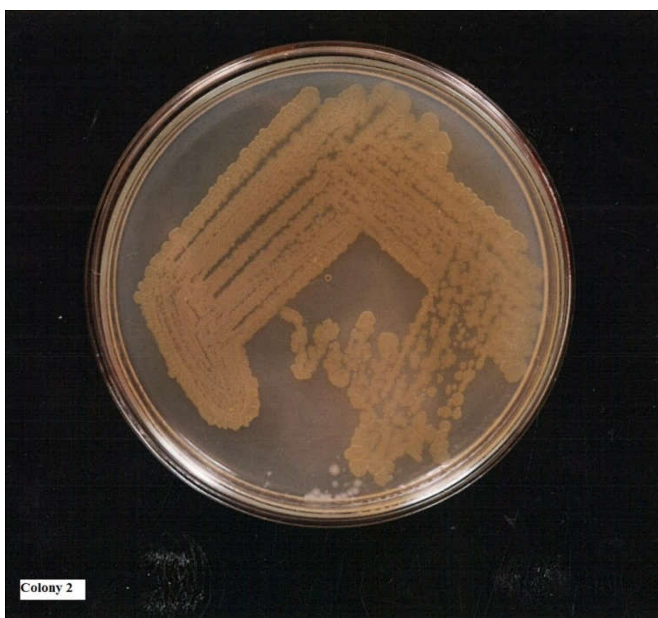


Fig. 7a. Colony 2 on Nutrient Agar

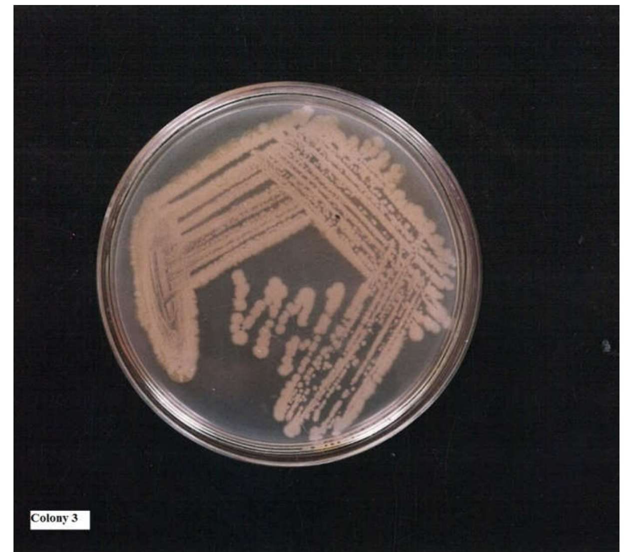


Fig. 7b. Colony 3 on Nutrient Agar

Biochemical tests for the identification of Atrazine tolerant bacteria

To assist in identification of the bacteria, each was subjected to standard identification procedures such as staining, biochemical and sugar tests. As there was no growth observed in the plate of Colony Type 1, we concluded that the organism is Atrazine intolerant and hence ceased to study it further. In order to identify Colony type 2 and 3, they were plated onto Nutrient Agar (Fig 7) and subjected to Grams staining. The Colony Type 2 showed Gram negative bacilli whilst the Colony Type 3 showed Gram positive bacilli. Further biochemical tests were performed on them (Table 5).

Table 5. Biochemical test results of Colony 2 and 3

	Colony 2	Colony 3
Gram's staining	Pink colored, long, large, Gram negative bacilli	Purple colored, long, large, Gram positive bacilli
Catalase	+	-
Oxidase	+	NA
Indole	+	NA
MR	-	NA
VP	-	NA
Citrate	Utilized	NA
Nitrate	Reduced	Reduced
TSI	Alkaline slant, Alkaline butt	NA
Gelatin	NA	Hydrolyzed
Glucose	Acid, no gas	Acid, no gas
Maltose	NA	Acid, no gas
Lactose	NA	Acid, no gas

Colony type 2's biochemical tests concluded that Catalase was produced and was indicated by the presence of effervescence, Oxidase was produced and was indicated by the formation of a colored indophenol (a purple color), A red ring was formed indicating that tryptophan was converted to indole, MR test was found to give a negative result, VP test was found to give a negative result, Citrate was utilized indicated by a color change to blue, Nitrate was reduced indicated by the red color, Triple Sugar Iron showed alkaline butt / alkaline slant with gas or H_2S formation, indicating no fermentation of any carbohydrates. Based on the above tests we concluded Colony

type 2 bacteria was *Pseudomonas* spp (Fig 8). Colony Type 3's biochemical tests concluded that Catalase was produced and was indicated by the presence of effervescence, Nitrate was reduced indicated by the red color, Gelatin was found to be hydrolyzed. Glucose and Maltose sugars caused acid production but no gas, Lactose caused acid production but no gas. Based on the above tests we concluded that Colony type 3 bacteria was *Bacillus* spp (Fig 9).

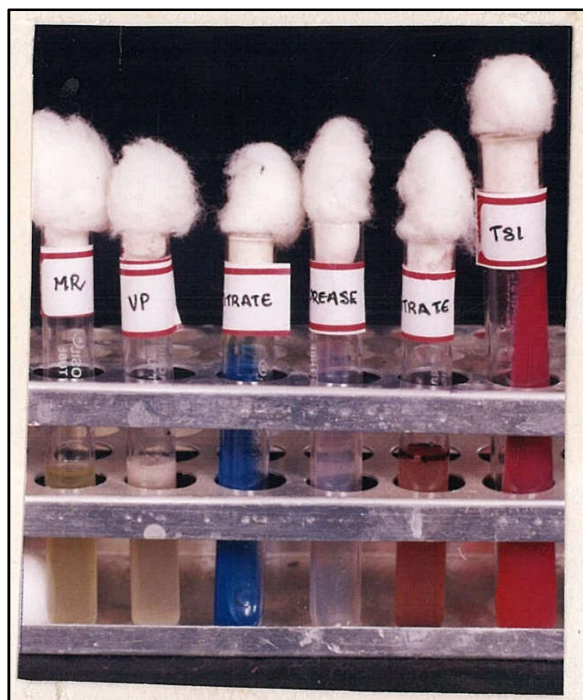


Fig. 8. Biochemical test results for colony 2 (*Pseudomonas* spp)

source for its growth. Its optical density was measured at 620nm after 72hrs of incubation at room temperature.

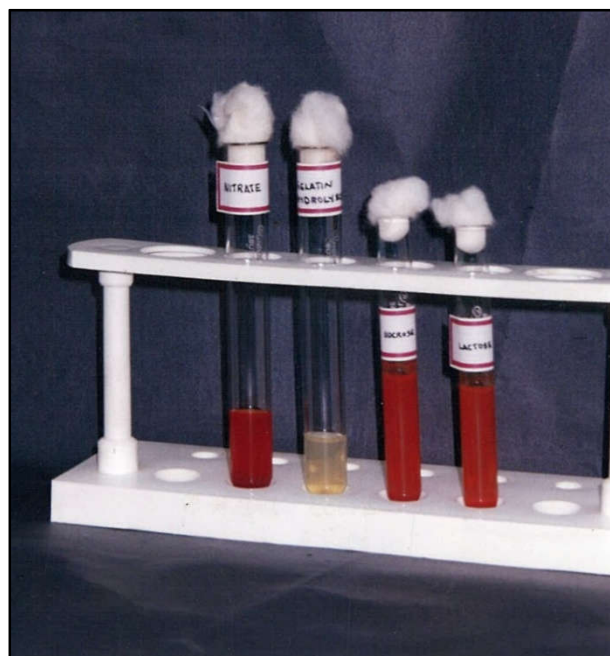


Fig. 9. Biochemical test for colony 3 (*Bacillus* spp)

It was found that as the concentrations of Atrazine were increased more bacterial growth was detected, indicating that Atrazine was being broken down to utilize its Nitrogen and was hence being degraded by the bacteria. Presence of glucose neither decreased nor increased the effectivity of degradation. *Pseudomonas* spp showed a slightly increased growth rate over *Bacillus* spp.

Table 6. Biodegradation Assay of Atrazine in Minimal salt medium: Measurement of Optical Density at 620nm

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.010	0.009	0.021	0.023	0.028	0.027	0.069	0.078	0.019	0.027	0.085	0.091	A
B	0.156	0.173	0.182	0.198	0.159	0.178	0.180	0.195	0.168	0.178	0.195	0.211	B
C	0.161	0.177	0.185	0.197	0.163	0.171	0.188	0.198	0.169	0.177	0.191	0.218	C
D	0.159	0.181	0.188	0.193	0.161	0.173	0.185	0.197	0.167	0.179	0.193	0.219	D
E													E
F	0.139	0.166	0.171	0.182	0.140	0.169	0.177	0.188	0.167	0.177	0.191	0.210	F
G	0.138	0.161	0.173	0.183	0.141	0.168	0.178	0.182	0.168	0.178	0.195	0.205	G
H	0.141	0.168	0.170	0.188	0.144	0.171	0.176	0.189	0.167	0.184	0.192	0.201	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Biodegradation Assay of Atrazine in Minimal salt medium

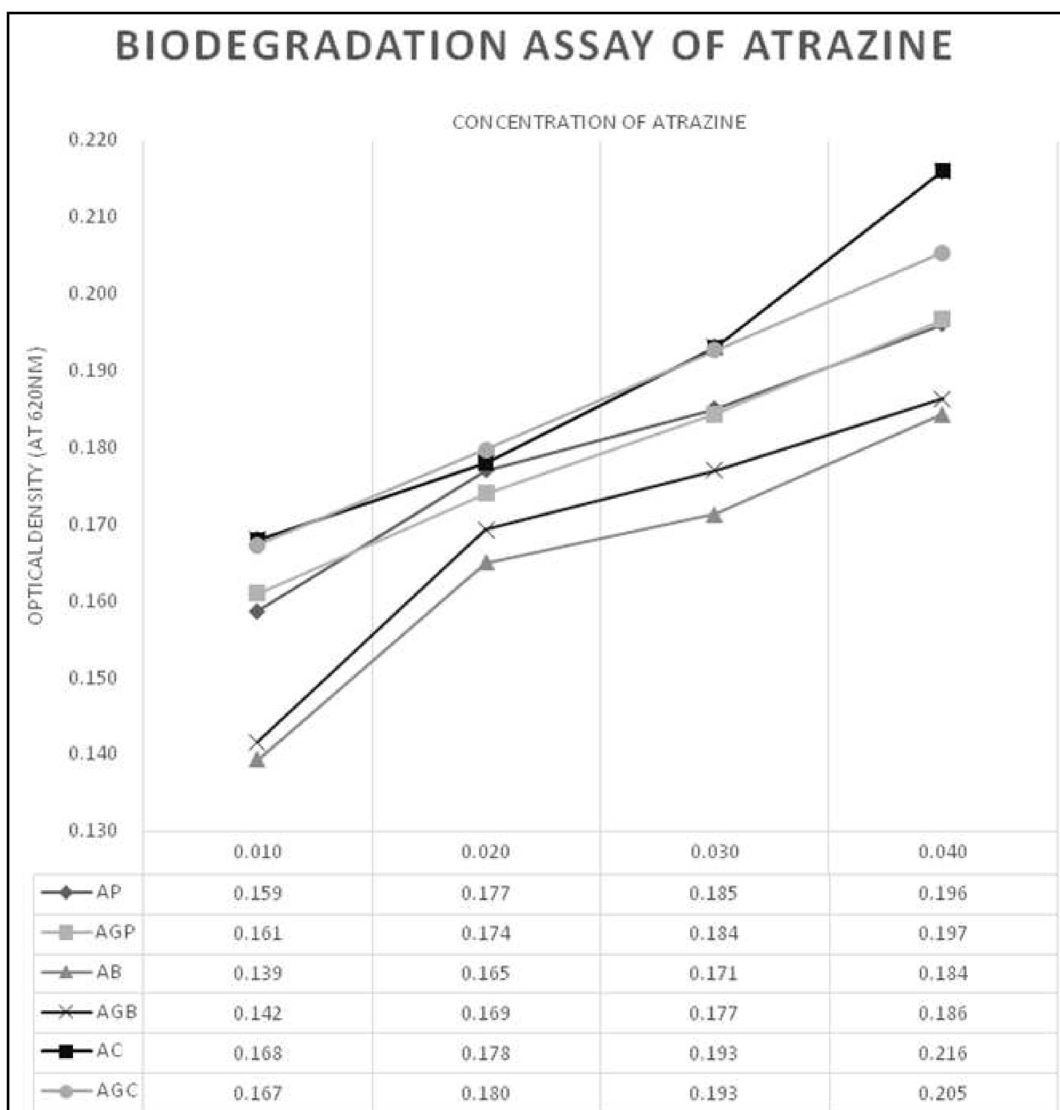
Pseudomonas spp and *Bacillus* spp were incubated separately, and also in combinationin various amounts of atrazine and in the presence or absence of glucose in a Minimal salt medium to detect its capacity to degrade atrazine (Fig 10). This was to prove its ability to grow in the medium. The medium was not provided with many nutrients and glucose was added to only a few tubes to see if it enhanced the degradation of atrazine. The bacteria successfully degraded atrazine to utilize some of its chemicals as nutrient for itself to enhance its growth. The bacteria were forced to utilize Atrazine as a sole Nitrogen

The combination of both the bacteria showed a slightly increased growth rate over the *Pseudomonas* spp growth rate (Table 6) (Graphs 1).

Detection of urease

In the Atrazine breakdown pathway, the bacteria break down the atrazine by various steps into cyanuric acid and this is further broken down into biuret and this into urea. This urea is converted to carbon dioxide by urease (Robinson, 2013). In order to verify whether atrazine was truly degraded various techniques maybe employed such as HPLC or an Immuno-

Fig. 10. Biodegradation Assay



	0.010	0.020	0.030	0.040
AP	0.159	0.177	0.185	0.196
AGP	0.161	0.174	0.184	0.197
AB	0.139	0.165	0.171	0.184
AGB	0.142	0.169	0.177	0.186
AC	0.168	0.178	0.193	0.216
AGC	0.167	0.180	0.193	0.205

Graph 1. Biodegradation Assay of Atrazine

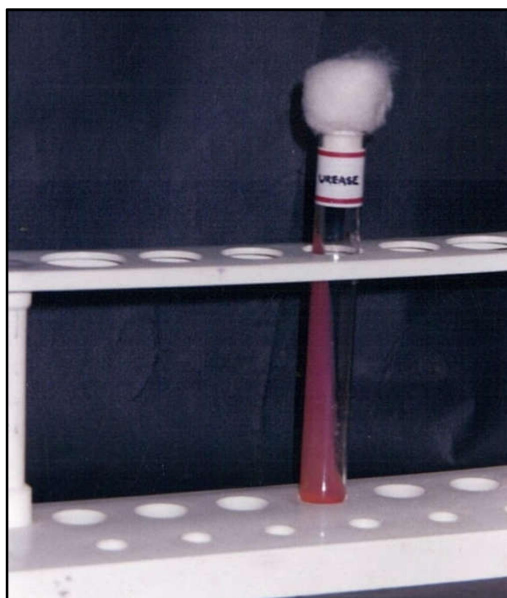


Fig 11. Urease test positive for Urea

assay but here a relatively straight forward urease detection test was performed to confirm the degradation of atrazine. The end product of the degradation namely urea was used for the confirmation (Mark, 1995). The degradation of atrazine gives urea which splits into ammonia and carbon dioxide and this was detected by urease test which came out as positive hence proving that the atrazine was degraded. Urease test media contained urea and the pH indicator phenol red. This indicator turned to a darkened pink color when the pH was greater than 8.4. When urea was hydrolyzed ammonia was produced raising the pH of the medium above 8.4 which caused a dark pink reaction (Fig. 11). Atrazine was hence degraded to form urea and this was split into ammonia and carbon dioxide and further into ammonium carbonate which caused an increase in the pH and hence a change in the indicator in the medium.

Conclusion

Atrazine's residue affects humans in several ways and is said to also trigger breast cancer. Atrazine is a cancer causing toxic herbicide, and has been banned in several countries but is still used in Developing countries like India, as it is highly effective and cheap. Atrazine is appearing in soil and in both surface water and ground water (Frank, 2007). So it is worthwhile to remove its traces by bioremediation. The plan to degrade the pesticide 'ATRAZINE' found remaining in the soil after harvesting was found to be possible by bioremediation. Bioremediation was found to be a quick, safe and economically better way to degrade the left over atrazine. It was found out that the remaining 'Atrazine' could be more effectively degraded by the combination of bacteria *Pseudomonas* spp and *Bacillus* spp than using the individual bacteria. Our data suggests that an emulsion of the organisms studied can be devised and applied to the soil or water bodies to degrade the herbicide.

REFERENCES

Alfred Dorsey, Susan, S. Little, David B Knaebel, Daniel Plewak, Heather Printup and Jennifer Rhoades 2003. Toxicological Profile for Atrazine, US Department of Health and Human Services.

Dileep, K. Singh 2008. Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments, *Indian Journal of Microbiology*, Vol.48, pp.35-40.

Egon Stahl, 1969. Thin Layer Chromatography - A Laboratory Handbook, Springer.

El Sebai, T., Devers-Lamrani, M., Changey, F., Rouard, N. and Martin-Laurent, F. 2011. Evidence of atrazine mineralization in a soil from the Nile Delta: isolation of Arthrobacter sp TES6, an atrazine-degrading strain. *International Biodeterioration & Biodegradation,* Vol. 65, pp. 1249–1255.

Evy Alice Abigail, Jaseetha Abdul Salam and Nilanjana Das 2013. Atrazine degradation in liquid culture and soil by a novel yeast *Pichia kudriavzevii* strain Atz-EN-01 and its potential application for bioremediation, *Journal of Applied Pharmaceutical Science*, Vol. 3, No. 06, pp. 35-43.

Focht, D.D 1994. Microbiological procedures for bio degradation research, pp: 407-426.

Frank Ackerman, 2007. Economics of Atrazine, International Journal of Occupational and Environmental Health. Vol. 13, No. 4, pp. 437-445.

Grit Neumann, Riho Teras, Liis Monson, Maia Kivisaar, Frieder Schauer, and Hermann J. Heipieper 2004. Simultaneous Degradation of Atrazine and Phenol by Pseudomonas sp. Strain ADP: Effects of Toxicity and Adaptation, *Applied and Environmental Microbiology,* Vol. 70, No. 4, pp 1907-1912.

Jennifer Bethsass and Aaron Colangelo, 2006. European Union Bans Atrazine, While the United States Negotiates Continued Use, *International Journal of Occupational and Environmental Health*,Vol. 12. No. 3, pp. 260-267.

Jinhua Wang, Lusheng Zhu, Qi Wang, Jun Wang, and Hui Xie 2014. Isolation and Characterization of Atrazine, Mineralizing *Bacillus subtilis* Strain HB-6, PLOS ONE, Vol.9, No. 9.

Kavita Rani and Geeta Dhania 2014. Bioremediation and biodegradation of Pesticide from Contaminated Soil and Water - A Noval Approach. *International Journal of Current Microbiology and Applied Sciences*, Vol. 3, No. 10, pp. 23-33.

Mansooreh Dehghani, Simin Nasseri, and Hassan Hashemi 2013. Study of the Bioremediation of Atrazine under Variable Carbon and Nitrogen Sources by Mixed Bacterial Consortium Isolated from Corn Field Soil in Fars Province of Iran, *Journal of Environmental and Public Health*, Vol. 2013.

Mark Radosevich, Samuel, J. Traina, Yue Li Hao and Ollu, H. Tuovinen, 1995. Degradation and mineralization of Atrazine by a soil bacteria isolate. *Applied and Environmental Microbiology,* Vol. 61, No. 1, pp. 297-302.

Nikolina Udiković-Kolić, Colin Scott and Fabrice Martin-Laurent 2012. Evolution of atrazine degrading capabilities in the environment. Vol. 96, No. 5, pp.1175-1189.

Ravindra Singh, Pushpendra Singh and Rajesh Sharma 2014. Microorganism as a tool of bioremediation technology for cleaning environment: A review, Proceedings of the *International Academy of Ecology and Environmental Sciencces*, Vol.4, No. 1, pp. 1-6.

Robinson David Jebakumar Solomon, Amit Kumar, and Velayudhan Satheeja Santhi 2013. Atrazine biodegradation efficiency, metabolite detection, and trzD

gene expression by enrichment bacterial cultures from agricultural soil. Journal of Zhejiang University SCIENCE B,Vol. 14, No. 12, pp. 1162-1172.

Samina Wasi, Shams Tabrez and Masood Ahmad 2013. Use of *Pseudomonas* spp. for the bioremediation of environmental pollutants: *A Review*, Vol. 185, No. 4.

Scott Stelting, Richard G. Burns B, Anwar Sunna C, Gabriel Visnovsky A and Craig Bunt D 2010. Immobilization of Pseudomonas sp. strain ADP: a stable inoculant for the bioremediation of atrazine. 19th World Congress of Soil Science, Soil Solutions for a Changing world, Australia.

Shahitha, S. 2012. Studies on Atrazine Mineralization by a consortium of Bacteria isolated from sugarcane field soil. Journal of Applied Pharmaceutical Science, Vol. 2, No. 6, pp 223-226.

Sneha Sagarkar, Aura Nousiainen, Shraddha Shaligram, Katarina Bjorklor, Kristina Lindstrom, Kirsten S Jorgensen and Atya Kapley 2014. Soil Mesocosm studies on Atrazine Bioremediation. *Journal of Environmental Management*. Vol.139, pp. 208-216.

Sugarcane status report 2013. Directorate of Sugarcane Development, Government of India.

****