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Gas Chromatographic and UV-VIS spectrometric analysis of Bisphenol-A degradation in garden soil collected from Coimbatore district, Tamil Nadu, India

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Abstract

Degradation of Bisphenol A (BPA) was studied under laboratory conditions in garden soil. The degradation of 100ppm BPA was carried out in sterile and non-sterile soil samples. It was found that BPA residue was does not progressively degrade with time. More than 65% of BPA was not degraded within 30 days treatment. The degradation was high in non-sterile sample than in sterile sample. Degradation pattern indicated that BPA was reduced by 5% in sterile soil sample, whereas 26.35% was observed in non sterile soil sample at the end of the experiment. The degradation in non sterile soil may be due to the effect of micro organisms and in sterile soil it may due to photochemical reactions.

Keywords: Degradation, BPA, garden soil, sterile, non sterile.

Introduction

Soil pollution is caused by the presence of man-made chemicals or other alterations in the natural soil environment. This type of contamination typically arises from the rupture of underground storage links, application of pesticides, and percolation of contaminated surface water to subsurface strata, oil and fuel dumping, leaching of wastes from landfills or direct discharge of industrial wastes to the soil[1]. In soil, pollutants and pesticides are subjected to different physiological, biochemical and microbiological processes[2]. Industrial activities produce waste; relatively few industries without pollution control and waste treatment facilities are major source of this pollution[3].

Bisphenol A is one of the endocrine disruptor substances which are released due to industrial activities. An endocrine disrupting chemical (EDC) is a synthetic chemical that when absorbed into the body either mimics or blocks hormones and disrupts the body's normal functions[4]. 2, 2-Bis (4-hydroxyphenyl) propane (Bisphenol A [BPA]) is one of the EDC generally used as a starting material for polymers including polycarbonates, epoxy resins, phenol resins, polyesters, and polyacrylates[5]. This compound is commonly suspected to act as an endocrine disrupter[6]. It has become one of the highest yielding chemicals in the world due to its wide applications and growing demand.

High levels of BPA were identified in leachates from a waste landfill[7,8]. It has been reported that the levels of BPA in the leachates of a hazardous waste landfill range from 1.3 to 17,200 ng/ml (average 269 ng/ml)[9,10]. Due to wide range of negative effects created by BPA, it is necessary to remove BPA from industrial effluents before discharging them into the environment[11]. Perusal of literature revealed that information available on the degradation of BPA in garden soil is limited. Therefore, the present study under taken investigates the degradation of BPA in garden soil under laboratory conditions.

Materials and Methods

Chemical and reagents: Technical grade Bisphenol A was obtained from Sigma- Aldrich, India. Anhydrous sodium sulphate for drying solvent extract prior to GC analysis was of AR grade, purchased from Rankem, India. Silica (60-120 mesh) for cleaning of samples was purchased from Sigma. Solvent ethyl acetate was purchased from Nice chemicals Pvt Ltd, India.

Soil sample collection: Soil sample was obtained from the garden region, Karpagam University, Coimbatore, India. Soil samples were taken by using a core sampler from the top 10 cm of field plots, air dried and passed through a sieve with 2 mm mesh.

Degradation of BPA in the soil: 100g of soil sample was transferred to 1000ml Erlenmeyer flask in triplicate. The flasks were cotton plugged and properly covered. These flasks were sterilized in autoclave at 121°C for 1 hour and other three flasks were kept as control. All flasks were incubated at room temperature. 100ppm concentration of BPA was spiked in each flask, mixed uniformly and incubated at room temperature for a period of 30 days.

Sample extraction: The samples were drawn at 0, 10, 20, and 30days of treatment and analysed for BPA residues. 100g of soil sample was extracted with equal volume of ethyl acetate thrice and kept in rotary shaker at 170 rpm overnight. The extracts were filtered through Whatman No.1 filter paper. The filtrate

was pooled together and concentrated to 1 ml. The concentrated sample was suspended in ethyl acetate and further used for clean up.

Clean up procedure: Glass column of 30cm x 20mm chromatographic column fitted with draw-off valve was used for clean up. Glass wool was placed at the bottom of the column. Na_2So_4 and 10g activated silica were mixed with 2.5 ml of HCl and kept in room temperature for 20 min. Then the mixture was used to fill the column and 2g of anhydrous sodium sulphate was added over the column to remove the moisture content present in the sample. Just prior to use, the column containing absorbent was washed with ethyl acetate. After preparation of the column the sample extract was transferred to the column along the sides of the column without disturbing it. The sample was eluted with 100ml of ethyl acetate. The eluted samples were pooled and concentrated and it was reduced to 1 ml. The sample was used for Gas Chromatographic analysis[2, 12].

Gas Chromatographic analysis: The concentrated extracts were analyzed using gas chromatography[13]. For gas chromatographic analysis Shimadzu-2014AF model with flame ionization detector was used and operating parameters were as follows: Carrier Gas- Ultra Pure Nitrogen, Flow rate of gas- Nitrogen -40ml /min, Flame Source -Hydrogen and Zero air (60ml/min). Injection temperature -275°C Column temperature – 240°C, Detector temperature – 310°C, Sample injection volume- 1µl.

Spectrometric analysis: 100µl of samples were made up to 3 ml by using ethyl acetate and scanned in the range of 200nm to 800 by using UV-Vis spectrophotometer instrument (Model – Shimadzu UV2450). The peak area was measured and degradation percentage was calculated as follows:

$$\text{Percentage of degradation} - \frac{\text{Initial peak area - Final peak area}}{\text{Initial peak area}} \text{ X } 100$$

Results and Discussion

Soil is the main reservoir for the microbes and pesticides are well known to be degraded by microorganisms present in the soil[14]. Microbial transformation has long been beneficial in many ways, actively involved in the degradation of many natural and man-made toxicants and xenobiotics[15]. Microbial metabolism of pesticides has been reviewed extensively[16, 17].Degradation of BPA in river water and seawater has been performed in previous studies[5]. In this work, degradation of BPA was examined in garden soil under laboratory conditions.

BPA standard analyzed in GC showed peak at the retention time of 15.22min. The peaks observed in the same retention time in all other samples indicated the presence of BPA in sterile and non-sterile soil (figures 1 and 2). In all the samples, peak was observed in the retention time of 15 min. It showed the presence of BPA in all samples, but the peak area reduced from 1st day to 30th day. The concentrations of peaks were high in sterile soil than non sterile soil. Result suggested that the BPA has been degraded more in 30th day in non sterile soil, at the same time it not has been completely degraded.

The peak area of the samples were observed using UV-Vis spectrophotometer and recorded. The standard BPA was subjected to analysis and it showed peak in the region of 245nm to 301nm. The same region was fixed for analysis of further samples. The peak areas of all sterile and non sterile samples were measured (figures 3-5). The percentage of remaining BPA in soil was calculated and reported in table 1. On 10th day about 14.72% of BPA was degraded in non-sterile sample where as in sterile soil 0.7% degradation was noticed. After 20 days the degradation increased to 18.60 % in non-sterile sample and 1.5 % of degradation was recorded in sterile sample. On 30th day of incubation the degradation of BPA further increased to 26.35% in non-sterile condition where as in sterile condition the degradation was found to be 5.4%. The results indicated that in sterile soil the degradation of BPA reduced from 100% to 94.6% at the end of the experiment where as in non-sterile soil the degradation of BPA reduced from 100% to 73.65%.

The degradation rate of BPA was higher in the non-sterile soil sample than the sterile sample. Lesser degradation in sterile soil than non- sterile soil is due to the absence of microbial activity. Degradation of BPA in sterile soil sample could be due to photo degradation. The higher degradation rate of BPA in non- sterile soil than sterile soil is attributed to the availability of favourable conditions for biodegradation by native microbes[18]. Microorganism and their enzymes are increasingly being used to reduce pollutants in the environment. These results do not agree with previous studies that BPA can rapidly degrade in river water[19-24]. But this phenomenon was not found in river water and BPA was unchanged for 70 day under aerobic conditions[25]. Like the same, in this study BPA was not been completely degraded by 30 days in soil.

Similar work has been carried out using different pesticide under different conditions. The degradation of soil bound endosulfan was slower than in culture medium and the bacterial colonies were capable of transforming endosulfan into endosulfan sulphate by oxidation[26, 27]. Ample evidence exists in the published literature that many organic chemicals form bound residues in soils by forming stable covalent bonds with organic substances, by polymerizing in soil to form soil organic matter or by cation exchange[28, 29].

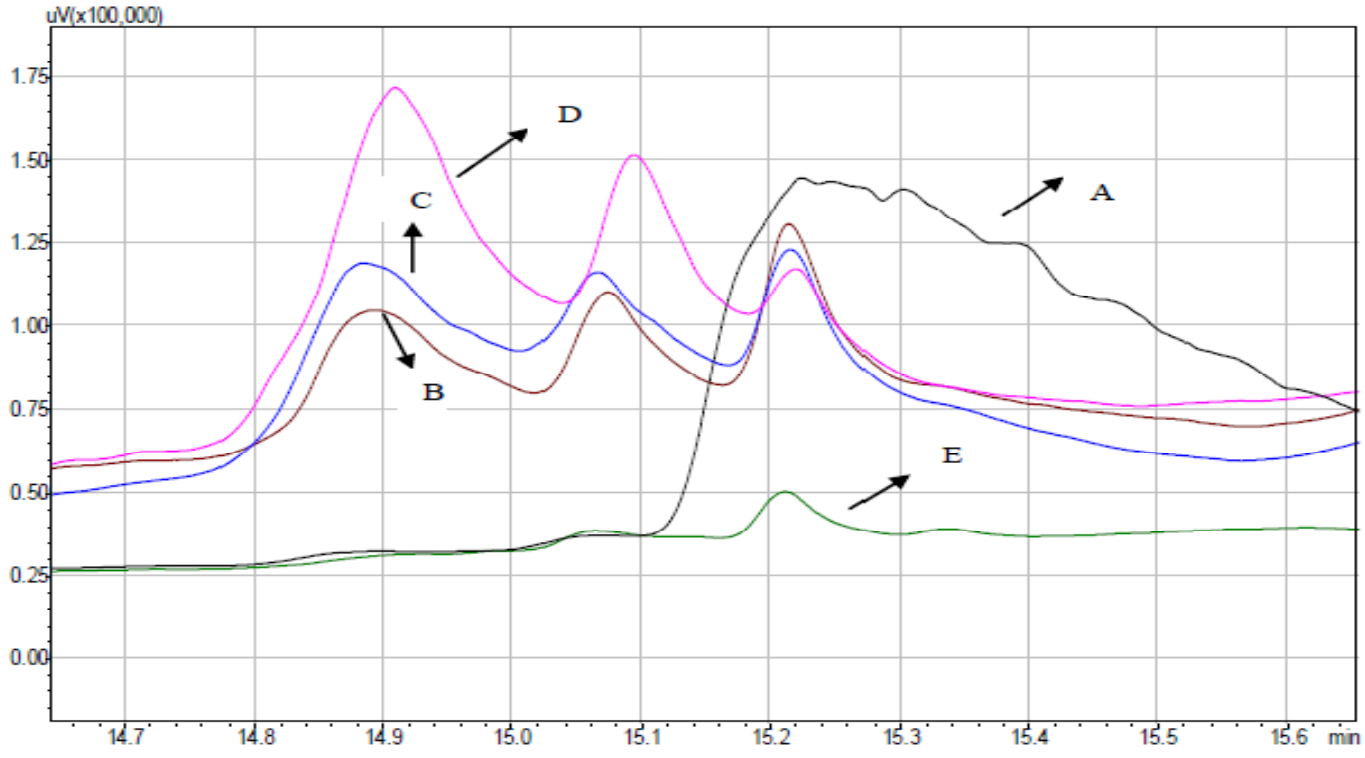
Higher degradation of BPA in non- sterile soil as compared to sterile soil confirms that the soil micro flora play an important role in the degradation of BPA in the environment. The removal of pollutants from the environment is a naturally occurring process where microorganisms utilize the pollutants for their growth. Biological methods for the removal of phenol are possible because some organisms have the capacity to degrade BPA. Many scientists have isolated microorganisms from nature

and obtained good degradation yields[30-32]. The biodegradation pathway of BPA has been studied with a specific strain of gram-negative bacteria and identified four primary metabolites[33, 34]. These metabolites were also rapidly degraded to CO_2 and water or were incorporated into bacterial biomass. The principle of biochemical reaction associated with the microbial metabolism of pesticides includes alkylation, dealkylation, dehalogenations, dehydro-halogenations, oxidation, reduction, hydroxyl ring cleavage and ether cleavage[2].

Rapid dissipation was observed in all soil types, indicating that the metabolic capability of soils to degrade BPA would appear to be widespread in nature. Similar half-lives in a range of 2.5–4.0 days were determined for surface waters in riverway[21]. On the basis of our data and reports, we hypothesize that higher percentage of BPA in soil were not degraded in a sufficient manner in garden soil used in this study under laboratory conditions.

Table 1
Degradation of BPA in sterile and non-sterile soil samples

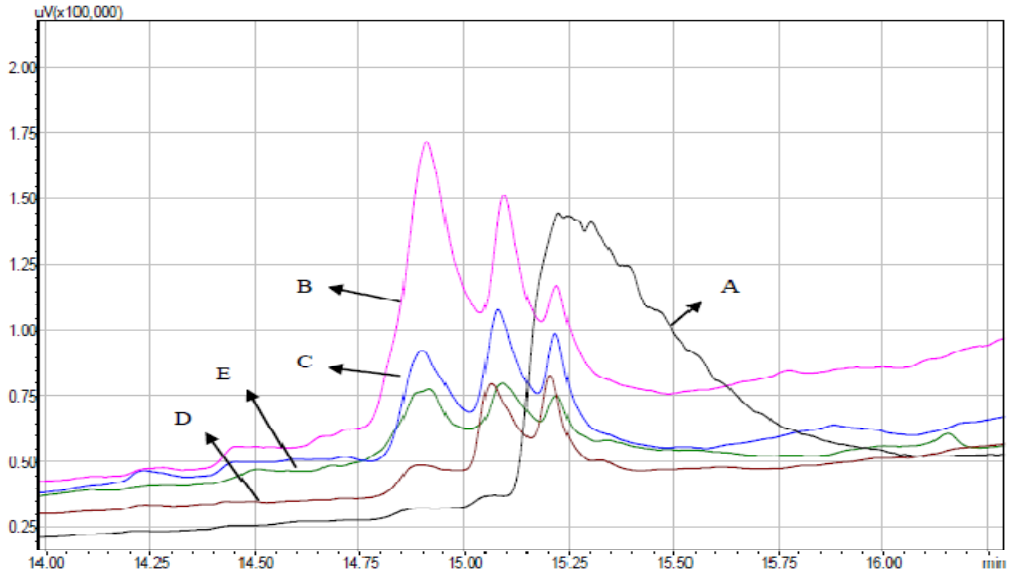
Number of days	Sterile Soil			Non Sterile Soil		
	Peak area	BPA degradation (%)	Remaining BPA (%)	Peak area	BPA degradation (%)	Remaining BPA (%)
0	129	0	100	129	0	100
10	128	0.7	99.3	110	14.72	85.28
20	127	1.5	98.5	105	18.60	81.4
30	122	5.4	94.6	95	26.35	73.65



Retention time (min)

A- Standard, B - Control, C– 10th Day, D- 20th Day, 30- 30th Day

Figure 1
GC Analysis of BPA in non sterile garden soil

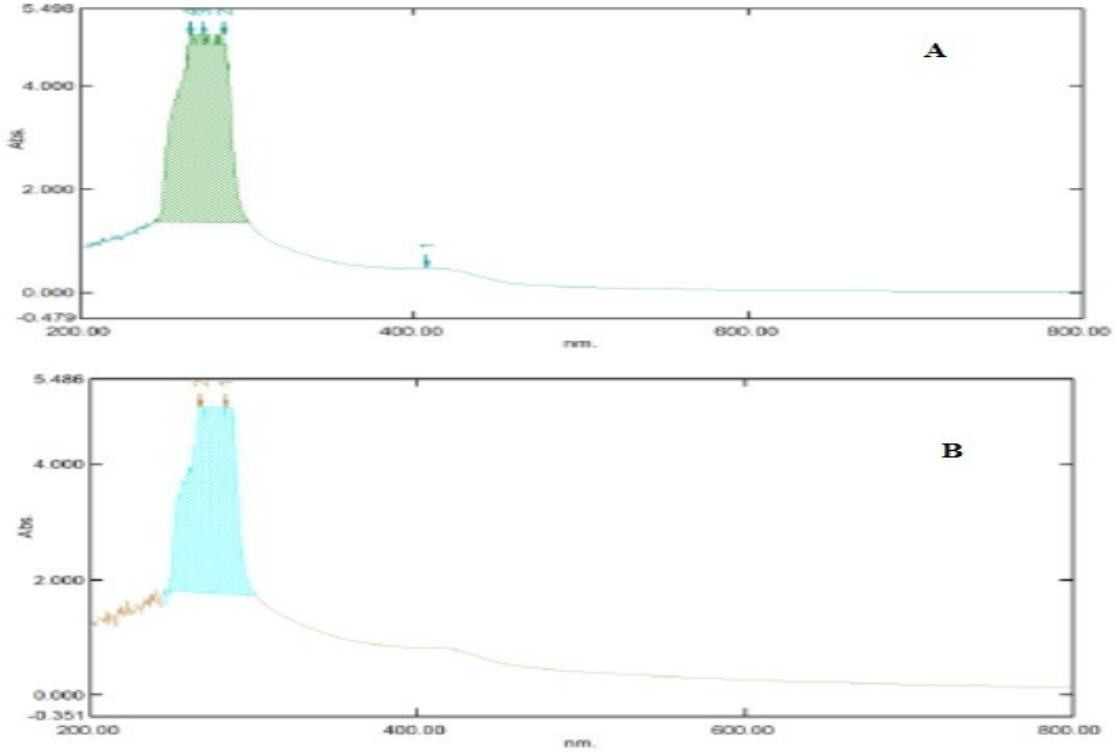


Retention time (min)

A- Standard, B - Control, C– 10th Day, D- 20th Day, 30- 30th Day

Figure 2

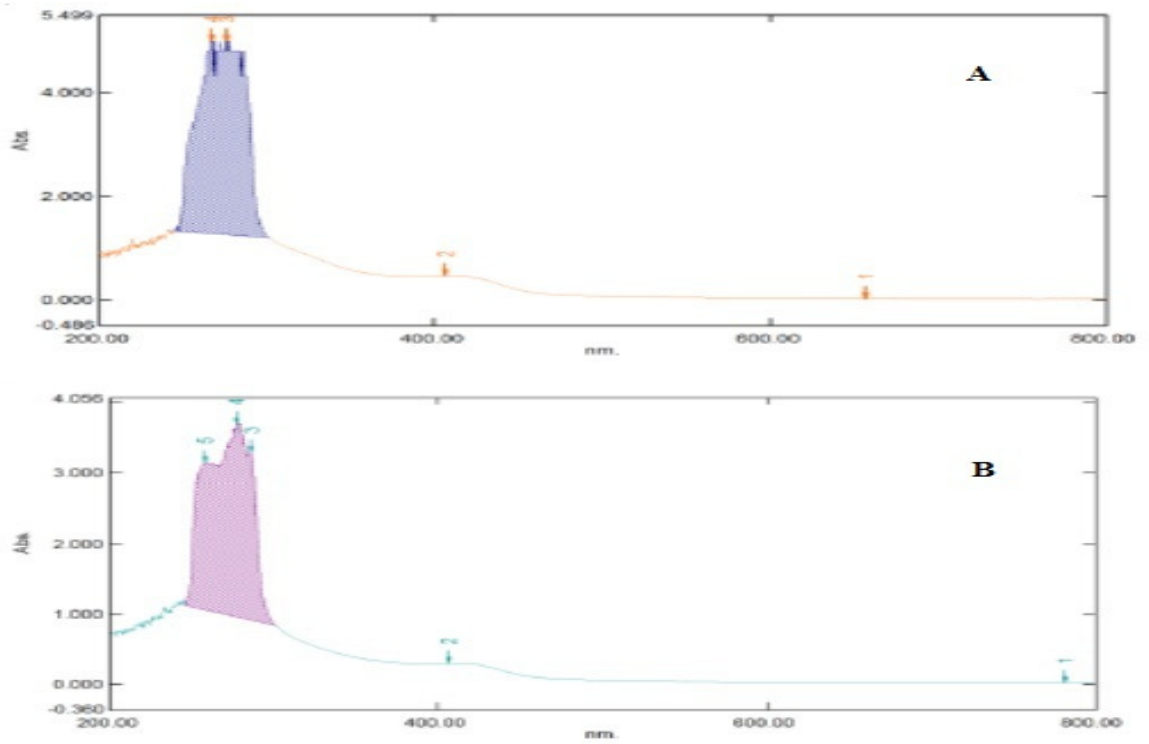
GC Analysis of BPA in sterile garden soil



(A- Sterile soil, B- Non sterile soil)

Figure 3

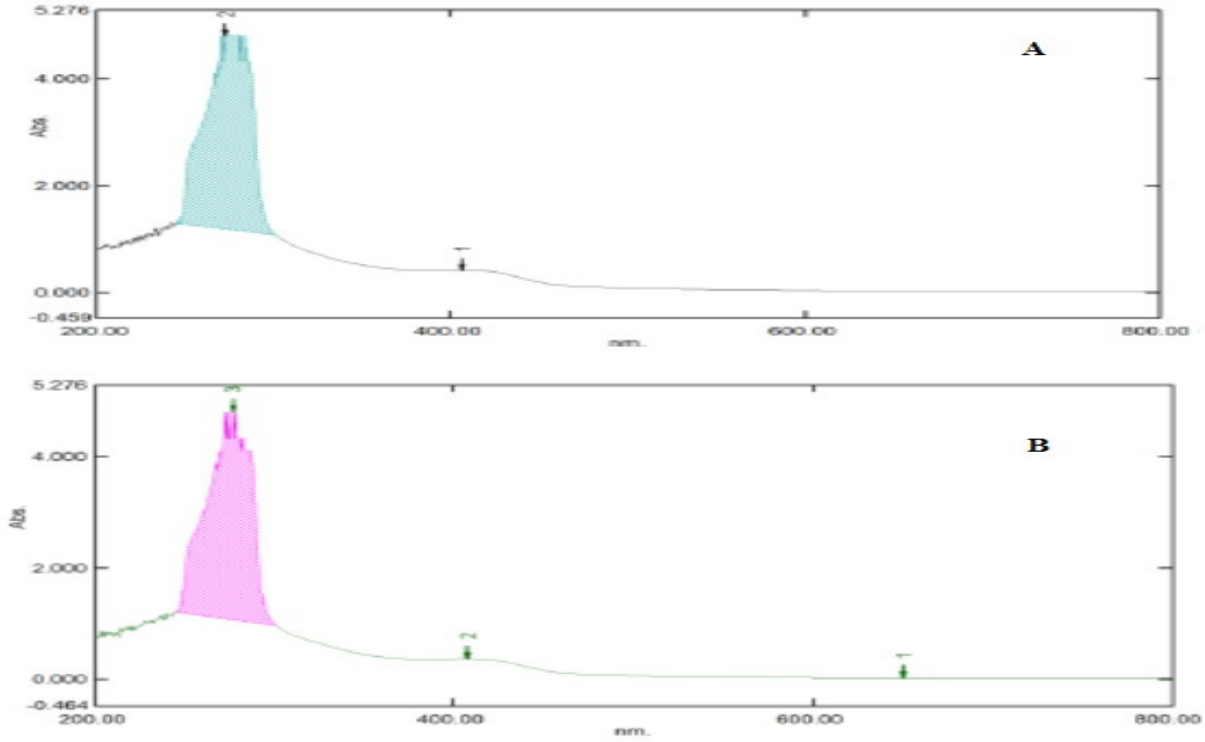
Peak area of BPA available in 10th day garden soil



(A-Sterile soil, B- Non sterile soil)

Figure 4

Peak area of BPA available in 20th day garden soil



(A-Sterile soil, B- Non sterile soil)

Figure 5

Peak area of BPA available in 30th day garden soil

Conclusion

Anthropogenic compounds number in thousands and are used in everyday life in every industry and cause pollution in the environment. In this study, degradation of BPA (100ppm) in garden soil was tested by using the sterile and non sterile soil. Column chromatography was used for the analytical method and ethyl acetate used as a solvent to extract BPA residue in soil. GC analysis showed the presence of BPA in 30th day of both sterile and non sterile soil. Remaining percentage of BPA in soil was measured by the peak area analysis using UV –Vis spectrometer. In sterile soil the degradation was from 100% to 94.6% during the incubation period of 30 days where as in non-sterile the degradation of BPA was from 100% to 73.65%. Further analytical studies are essential to understand the clear degradation mechanism of BPA in garden soil.

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References

1. Brussaard L., Behan-Pelletier V. M., Bignell D. E et al., Biodiversity and ecosystem functioning in soil, *AMBIO*, **26**, 563-570 **(1997)**

2. Shivaramaiah H. M and Arulganesh, Degradation of quinalphos in soil, *Green Farming*, **2 (2)**, 94-96 **(2008)**

3. World Health Organisation, Rapid assessment of sources of air, water and land pollution, WHO offset Publication, Geneva, No.**62,** 113 (**1982**)

4. Chen J., Huang X and Lee D., Bisphenol A removal by membrane bioreactor, *Process Biochemistry*, **43**, 451-456 **(2008)**

5. Kang J.H and Kondo F., Bisphenol A degradation in river water is different from that in seawater, *Chemosphere*, **60**, 1288–1292 **(2005)**

6. Alexander H. C., Dill D. C., Smith L. W., Guiney P. D. and Dorn P., Bisphenol A: acute aquatic toxicity, *Environ.Toxicol. Chem.*, **7 (1)**, 19–26 **(1988)**

7. Yamada K., Urase T., Matsuo T. and Suzuki N., Constituents of organic pollutions in leachates from different types of landfill sites and their fate in the treatment processes, *Journal of Japan Society on Water Environment*, **22 (1)**, 40–45 **(1999)**

8. Behnisch P. A., Fujii K., Shiozaki K., Kawakami I., and Sakai S.I., Estrogenic and dioxin-like potency in each step of a controlled landfill leachate treatment plant in Japan, *Chemosphere*, **43**, 977–984 **(2001)**

9. Filho I.N., Muhlen C.V, Schossler P.V and Caramao E.B., Identification of some plasticizers compounds landfill leachate, . *Chemosphere*, **50**, 657–663 **(2003)**

10. Yamamoto T., Yasuhara A., Shiraishi H and Nakasugi O., Bisphenol A in hazardous waste landfill leachates, *Chemosphere*, **42 (4)**, 415– 418 **(2001)**

11. Rubin B. S and Soto A. M., Bisphenol A: perinatal exposure and body weight, *Mol Cell Endocrinol*., **304 (1-2)**, 55–62 **(2009)**

12. Brunete C.S.,Miguel E and Tadeo J.L., Determination of tetrabromobisphenol-A, tetrachlorobisphenol-A and bisphenol-A in soil by ultrasonic assisted extraction and gas chromatography–mass spectrometry, *Journal of Chromatography A*, **1216 (29)**, 5497–5503 **(2009)**

13. Masuda M., Yamasaki Y., Ueno S. and Inoue A., Isolation of bisphenol A-tolerant/ degrading *Pseudomonas monteilii* strain N-502, *Extremophiles*, **11 (2)**, 355-362 **(2007)**

14. Munnecke D. M., Johnson H. W., Tal B. Barik, Microbial metabolism and enzymology of selected pesticides. In biodegradation and detoxification of environmental pollutants, Edited Chakrabarty A. M., CRC, Press Boca Raton, FL **(1982)**

15. Alexander M., Biodegradation of Chemicals of Environmental Concern, *Science*, **211 (4478)**, 132-138 **(1981)**

16. Katayama A and Matsumura F, Degradation of organochlorine pesticides particularly endosulfan by *Trichoderma harzianum*, *Environmental Toxicology and Chemistry*, **12 (6)**, 1059-1065 **(1993)**

17. Kullman S. W. and Matsumura F., Identification of a Novel Cytochrome P-450 Gene from the White Rot Fungus *Phanerochaete chrysosporium*, *Appl Environ Microbiol*., **63 (7)**, 2741-2746 **(1997)**

18. Shalini Singh, Dureja P. and Kumar S., Biodegradation of endosulfan and endosulfan sulphate in Indian soils, *J Environ Sci Health B*, **35 (3)**, 337-346 **(2000)**

19. Dorn P. B., Chou C. and Gentempo J. J., Degradation of Bisphenol A in natural waters, *Chemosphere*, **16 (7)**, 1501-1507 **(1987)**

20. Ike M., Jin C. S. and Fujita M., Biodegradation of bisphenol A in the aquatic environment, *Water Science and Technology*, **42 (7-8)**, 31-38 **(2000)**

21. Klecka G. M., Gonsior S. J., West R. J., Goodwin P. A. and Markham D. A., Biodegradation of bisphenol A in aquatic environments: river die-away, *Environ Toxicol Chem*., **20 (12)**, 2725-2735 **(2001)**

22. Kang J. H., Ri N. and Kondo F., *Streptomyces* sp. strain isolated from river has high bisphenol A degradability, *Lett Appl Microbiol*., **39 (2)**, 178-180 **(2004)**

23. Kang J.H and Kondo F., Effects of bacterial counts on the biodegradation of bisphenol A in river water, *Chemosphere*, **49 (2)**, 493-498 **(2002)**

24. Kang J. H. and Kondo F., Bisphenol A degradation by bacteria isolated from river water, *Archives of Environmental Contaminations and Toxicology*, **43 (3)**, 265-269 **(2002)**

25. Yin G. G., Kookana R. S. and Ru Y. J., Occurrence and fate of hormone steroids in the environment, *Environ Int*., **28 (6)**, 545-551 **(2002)**

26. Awasthi N., Manikam N. and Kumar A., Biodegradation of endosulfan by a bacterial coculture, *Bulletin of Environmental Contamination and Toxicology*, **59 (6)**, 928-934 **(1997)**

27. Shivramaiah H. M., Organochlorine Pesticides in Agroecosystem: Monitoring Residues with Suitable Strategies for Their Management and Remediation, Department of Agricultural chemistry and soil science, University of Sydney Australia **(1999)**

28. Dragun J., The Soil Chemistry of Hazardous Materials, Hazardous Materials Control Research Institute, Silver Spring, MD, 319–320 **(1988)**

29. Burauel P., Wais A., Fuhr F., Soil-Bound Residues, The Lysimeter Concept, ACS Symposium Series, Edited Fuhr F., Hance R. J., Plimmer J. R., Nelson J. O., **699 (13)**, 177–188 **(1997)**

30. Bettmann H. and Rehm H. J., Degradation of phenol by polymer entrapped microorganisms, *Applied Microbiology and Biotechnology*, **20 (5)**, 285-290 **(1984)**

31. Lallai A and Mura G., pH variation during phenol biodegradation in mixed cultures of microorganisms, *Water Research*, **23 (11)**, 1335-1338 **(1989)**

32. Sokal R.R. and Rohlf F.J., Biometry, second ed. WH Freeman and Co., New York **(1981)**

33. Lobos J. H., Leib T. K. and Su T., Biodegradation of bisphenol A and other bisphenols by a gram-negative aerobic bacterium, *Appl Environ Microbiol*., **58 (6)**, 1823-1831 **(1992)**

34. Spivack J., Leib T. K. and Lobus J. H., Novel pathway for bacterial metabolism of bisphenol A. Rearrangements and stilbene cleavage in bisphenol A metabolism, *J Biol Chem*., **269 (10)**, 7323- 7329 **(1994)**