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Effect of bisphenol A on soil bacterial population

M. Kamaraj *, Rajeshwari Sivaraj, M. Manjudevi and L Jansi
Department of Biotechnology, School of Life sciences, Karpagam University, Eachanari, Coimbatore-(Tamilnadu) - India

Abstract

This study examined the effect of Bisphenol A (BPA) on bacterial count in agriculture soil. In addition, the study was also concerned with the inhibition range of BPA on selected bacterial cultures. Synthetic BPA was mixed as 10g/1kg of agriculture soil. Physico-chemical analysis has been performed for BPA inoculated soil and uninoculated soil after 30th day. Available N, P, K values and electrical conductivity in soil was found to decrease in BPA inoculated soil than control soil. Bacterial population was observed on 30th day in the control soil 250 CFU/ml at 10^{-4} dilution and inoculated soil it as 100 CFU/ml. BPA inhibition range against four selected bacterial cultures were tested and zone of inhibition radius measured as nearly 20mm in all these culture for 30mg/ml of BPA.

Key-Words: Bisphenol A, Soil, Bacteria, Inhibition, Analysis

Introduction

Industrial activities cause some pollution and produce waste; relatively few industries without pollution control and waste treatment facilities are responsible for the bulk of the pollution[1]. Bisphenol A is one of the endocrine disruptor substances which are released due to industrial activities. Endocrine disruptors attract the interests of an increasing number of scientists because of their possible negative effects on human health. Some endocrine disruptors have been reported to be environmental pollutants that can give rise to abnormal sexual development and abnormal feminizing on animals Endocrine disruptors attract the interests of an increasing number of scientists because of their possible negative effects on human health. Some endocrine disruptors have been reported to be environmental pollutants that can give rise to abnormal sexual development and abnormal feminizing on animals[2].

BPA is mainly used for the production of polycarbonate plastic, epoxy resins and non-polymer additives[3, 4, 5]. It has become one of the highest yielding chemicals in the world[6] due to its wide applications and growing demand.High levels of BPA were identified in leachates from a waste landfill[7, 8] and reported that the levels of BPA in the leachates of a hazardous waste landfill ranged from 1.3 to 17,200 ng/ml (average 269 ng/ml)[10].

*** Corresponding Author**
E.mail: kamaraj.bt@gmail.com
Mob. +91-97895 05023

BPA in four landfill leachates ranged from 15 to 5400 ng/ml, but ranged from 0.5 to 5.1 ng/ml in effluents after treatment[7].

Soil pollution is caused by the presence of man-made chemicals or other alteration 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[11].

BPA released to ground or surface water can be absorbed to soil or sediments. In fact the levels of BPA in sediments are higher than those in surface waters[12, 13, 14]. However, BPA contamination in soil can be positively correlated with human densities because of an increase in BPA pollution by human wastes such as domestic and/or industrial wastes[15].

Despite several studies concerned with determination of BPA in soil, but there are few data on effects of BPA in soil. The purpose of the present study is to identify the effects of BPA on bacterial population in soil and their inhibition behaviour against selected bacterial cultures.

Material and Methods

Chemicals

BPA (GC grade >99%) was purchased from Sigma-Aldrich (India). Nutrient agar (NA) (HiMedia Laboratories Pvt Ltd, India) medium contained per liter of distilled water: peptic digest of animal tissue (5.0 g), beef extract (1.5g), yeast extract (1.5 g), NaCl (5.0 g), and agar (15.0 g) was prepared according to the

recommendation of the manufacturers and used for bacterial growth. Water was distilled and then purified by a Milli-Q water purification system (Nihon Millipore, Yonezawa, Japan). Ampicilline was purchased from HiMedia Laboratories Pvt Ltd, India.

Sample collection and preparation

Soil used in the study was collected from the plough layer (0–10 cm) of an experimental plot located in the region of Coimbatore district, Tamilnadu, India. Soil samples were air dried, sieved (2mm) and stored in separate containers at room temperature. 10 g of BPA was mixed with 1kg soil and another 1kg of soil was maintained as control. After 30th day, basic physico-chemical characteristics of soil were tested and soil bacterial colonies were recorded.

Bacteriological examination

The pour plate method was used for the bacteriological examination of the soil samples in accordance with the Standard methods (American Public Health Association, 1998). The samples were diluted with distilled water by serial dilutions and added to each of the two replicate petri dishes. The prepared agar was poured to each of the petri dishes, mixed, and incubated at 35^{0}C for 48 h. The petri dishes having 30–300 colonies were counted and the results are reported as colony forming units (CFU) per milliliter.

Bacterial inhibition

Bacterial inhibition of BPA was determined by agar-well diffusion method. BPA standards were prepared in different concentration like 100µg, 1mg, 10mg, 15mg and 30 mg per ml of double sterile distilled water. Four strains of bacteria namely *Streptococcus* sp, *Staphylococcus* sp, *Salmonella* sp and *Bacillus* sp were used for the experiment. All these microbes were inoculated in nutrient broth and were incubated at 37^{0}C for 24 h the earlier day. These fresh cultures were swabbed on nutrient agar medium and each sample was filled into the duplicate wells of agar plate directly and incubated for 24 h. At the end of the incubation period, inhibition zone formed on the medium were measured in millimetre (mm). The experiments were performed in duplicate and inhibition zone was also compared with Ampicillin as a reference standards.

Statistical analysis

The statistical analysis of the data was carried out for completely randomized design[16] as well as for Dunkun's multiple range test[17].

Results and Discussion

Due to the industrialization and intensities of chemical usage, BPA can be found in wastewater from factories and it is not completely removed during wastewater treatment. This wastewater containing BPA can be a source of contamination of the soil environment[3, 18.]There is increasing concern that pollutants affect the microbial community present in soil, and that these non-target effects may degrade the performance of important soil functions. These critical soil functions include organic matter degradation, nitrogen cycle and methane oxidation[10].

In this present study, at the end of 30th day physico-chemical properties of soil were tested and the results are tabulated (Table 1).Furthermore, it has been reported that soil properties such as soil texture, micro aggregates, pH, presence of key cations and organic matter can affect bacterial community directly or indirectly[19] .However, soil pH has been reported not only determine H^+ ion concentration but also to influence the concentration of these cations[20]. According to Ashman and Puri (2002), a soil with pH range of 6.5-7.5 will have high concentrations of Ca^{2+}, Mg^{2+} and K^+ compared to an acidic soil (4.0-5.5), which they reported as being characteristic of agricultural soils .The emerging interest of soil microbial ecology and diversity is due to the functional roles they play in biogeochemical functioning[19,21].

In the present study, the pour plate method was attempted to investigate the bacterial community in the BPA inoculated and control soil. The importance of microbes in agro ecosystems has led to their use in land management strategies and as indicators of disturbances such as changes in agronomic practices [22, 23] reduced or no tillage approaches[24, 25]. However, the inability to culture most environmental samples is a fundamental problem to understanding their ecological significance[26, 27]. In this study four different dilutions like 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} were used and the colonies ranged from the number 30 – 300 were measured. Results showed the significant decrease in the bacterial community from the 1st day to 30th day (Fig.1). In initial day the colony count is around 390 in 10^{-4} dilutions. After 30th day nearly 250 colonies were counted in control soil and the count was reduced to nearly 30 colonies in BPA inoculated soil.

This study highlighted the relationship between bacterial numbers obtained using full strength NA in BPA inoculated and control soil. This was done to determine the effect of BPA in soil microbial diversity studies. This NA media was used in an effort to increase the chances of isolating more culturable bacterial species [28].NA was universal media that differ with other media in compositions. The constituents present in the media are complex compounds that supply organisms with nutrients required for growth. The amount of constituents present in media types can either be sufficient or minimal for the growth of some

organisms[28] .The higher number of colonies recorded on control soil was an indication that the use of BPA may have important effects on the growth of bacteria. Furthermore, the low colony count and poor growth obtained on BPA inoculated soil was probably because of the full strength concentration and toxicity of BPA. This means that the presence of BPA stimulate or inhibit the numbers of bacterial community in soil that are estimated by the plate counts (CFU). However, from the statistical result there was a significant effect of this treatment at the 30th day which was indicated in the overall decrease of bacterial numbers at that period (Fig. 1). More direct evaluation between control and inoculated soils achieved by significant results.

Many soil bacteria have been referred to as culturable or "unculturable" organisms. There have been arguments that certain groups of organisms are viable but not culturable on media and can only be identified molecularly[28]. Improving media composition would increase the chances of obtaining these "unculturable" organisms[30].

The BPA inhibition efficiency for selected bacterial cultures was tested and the zone of inhibiton radius was measured in a millimetre. The different concentration of BPA has been tested against these cultures (Fig 2).In 100µg/ml of BPA the minimum radius level measured as 1mm against Staphylococcus sp and Bacillus sp and 3mm for Streptococcus sp and Salmonella sp. In 30mg/ml of BPA maximum radius level of nearly 20mm was measured against Streptococcus sp, Salmonella sp, Staphylococcus sp and Bacillus sp. The inhibition level was significantly increased with the higher concentrations of BPA in all organisms used in this study. The results are compared with the positive control Ampicilline (1mg/ml) showed nearly 20mm radius of inhibition zone in all the four bacterial species. The bacterial cultures used in the study are pathogenic organisms which are isolated from various sources. The inhibition zone indicated that the BPA kills or inhibits the growth of bacteria. This inhibition activity depends on the concentration, while the higher concentration and the larger the surface area, the better the antibacterial activity obtained[31].Generally, the results reported from different studies are difficult to compare because of the use of different test methods, bacterial strains and sources of antimicrobial samples used. Special attention has been paid to bacterial groups such as the nitrifying bacteria that are believed to play key roles in agriculture, especially since the diversity of bacteria involved is very small.

Our results indicate that long term treatment of soils with BPA may have affected the size of the soil bacterial population but it is uncertain whether such a small difference would have any effect on soil fertility. The pH, soil moisture doesn't alter but the electrical conductivity, available N, P, K values altered significantly. The bacterial population, based on pour plate colony count method was different in the BPA-treated and control soils. The inhibition range of BPA against selected bacterial cultures were tested and reported. However, further work is needed to clarify whether this is due to the effects of BPA, rather than the heterogeneity of soil itself.

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Table 1: Analysis of Agriculture soil

Soil characteristics	Control	30th day soil incubated with 10g BPA
Texture	Clay Loam	Sandy Clay Loam
Lime	Non-Calcareous	Non-Calcareous
pH	8.44±0.2	8.39±0.2
Electrical conductivity (dSm^{-1})	0.14±0.02	0.02±0.01
Soil moisture (%)	105.75±1.2	105.75±1.2
Available N ($kg\ ha^{-1}$)	218±3	514±4
Available P ($kg\ ha^{-1}$)	30.0±2	20.0±1
Available K ($kg\ ha^{-1}$)	394±4	454±4

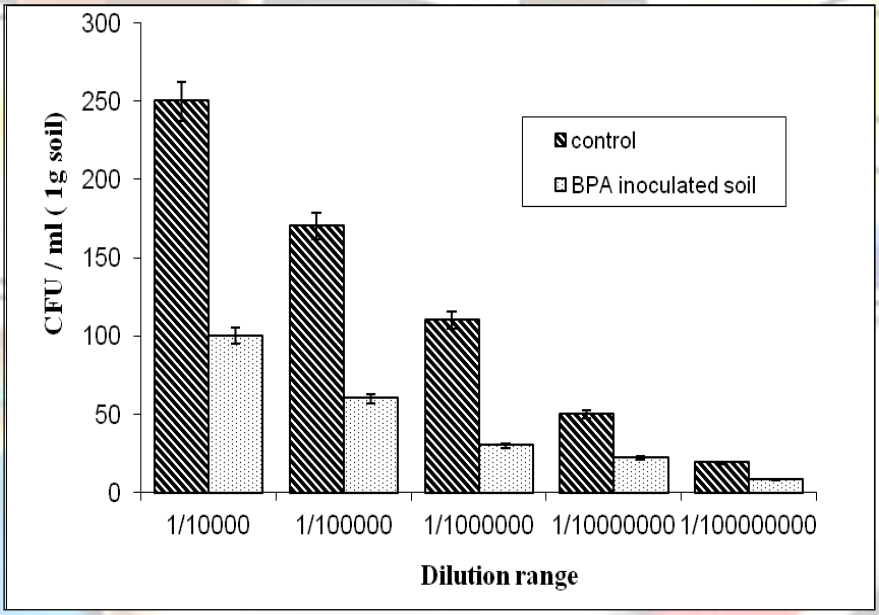

Fig. 1: CFU of Bacteria in 1g of 30th day soil

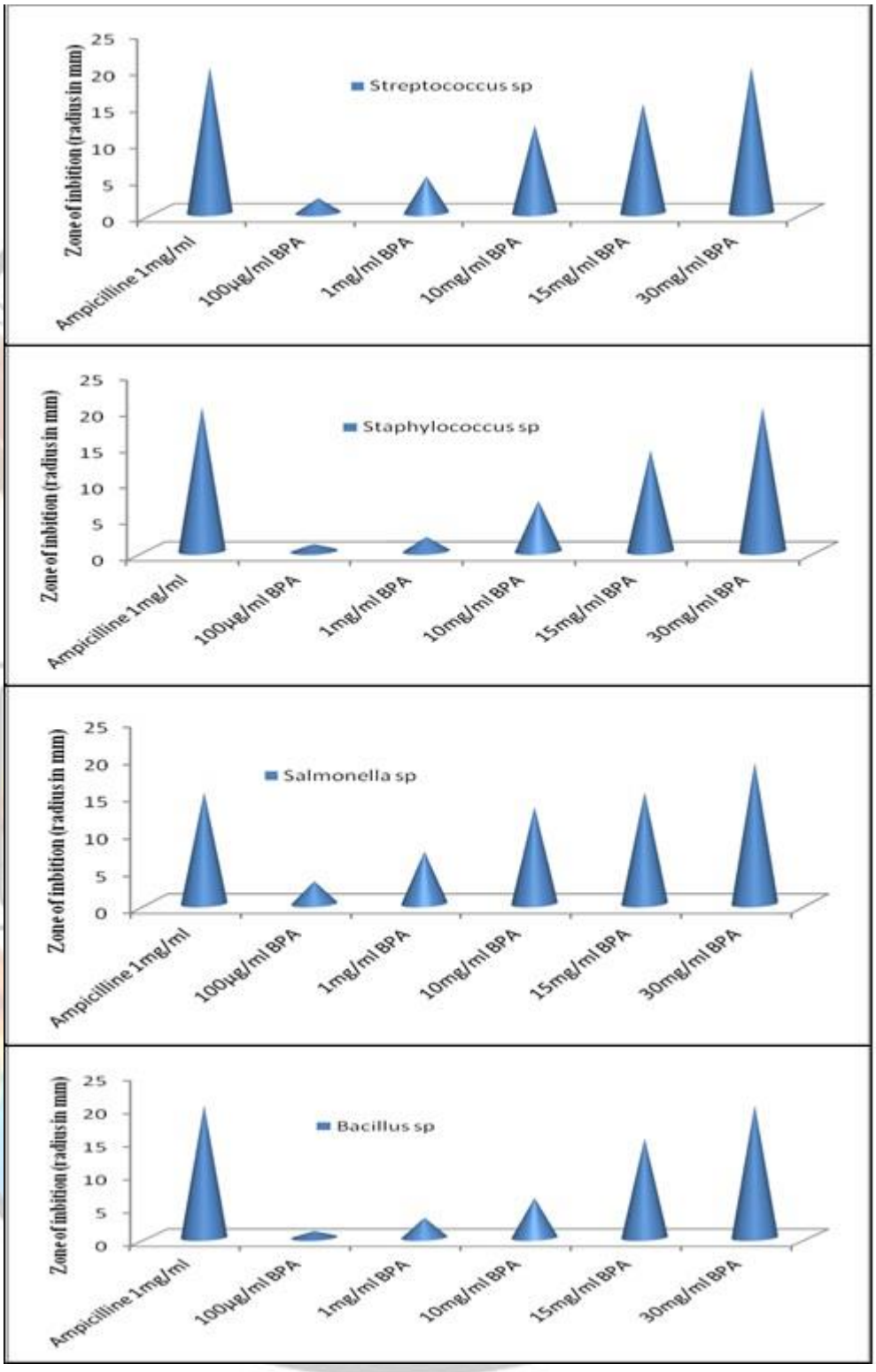

Fig. 2: The inhibition efficiency of BPA for selected bacterial species