Microbial diversity in firework chemical exposed soil and water samples collected in Virudhunagar district, Tamil Nadu, India

P. Dhasarathan · P. Theriappan · C. Ashokraja

Received: 13 October 2008 / Accepted: 9 January 2009

Abstract Microbial diversity of soil and water samples collected from pyrochemicals exposed areas of Virudhunagar district (Tamil Nadu, India) was studied. Soil and water samples from cultivable area, waste land and city area of the same region were also studied for a comparative account. There is a remarkable reduction in total heterotrophic bacterial population (THB) in pyrochemicals exposed soil and water samples (42×10^4 CFU/g and 5.6×10^4 CFU/ml respectively), compared to the THB of cultivable area soil and water samples (98×10^7 CFU/g and 38.6×10^7 CFU/ml). The generic composition the THB of the pyrochemicals exposed samples too exhibited considerable change compared to other samples. *Pseudomonas* sp. was the predominant one (41.6%) followed by *Achromobacter* sp. (25%) in pyrochemical exposed soil and *Pseudomonas* sp. was the predominant one (25%) in pyrochemical exposed water samples followed by *Bacillus* sp. (25%) and *Micrococcus* sp. (16.6%). It was observed that *Corynebacterium* sp. and *Micrococcus* sp. were absent completely in pyrochemical exposed soil and *Achromobacter* sp. was missing in the pyrochemical exposed water samples, which were present in the other samples. The outcome of this study clearly demonstrates that pollutants such as chemicals used in pyrotechniques affect the microbial biodiversity and suitable measures have to be taken to control the pollution level and to save biodiversity.

Keywords Pyrochemicals · Microbial diversity · Bacterial genera · Total heterotrophic bacterial population

Introduction

Bacteria are the most dominant group of microorganisms in soil; water and probably equal one half of the microbial biomass in soil. They are present in all types of soil but their population decreases as the depth of soil increases. Under anaerobic condition, bacteria dominate the scene and carry microbiological activities in soil since fungi and actinomycetes do not grow in the absence of oxygen [1].

In the soil the microbes are in dynamic equilibrium. The microbes require some basic nutrients such as nitrogen, phosphorous and potassium for normal growth. The fertility level of soil is influenced by the availability of these elements [2, 3]. The temperature changes can also influence microorganisms both quantitatively and qualitatively [4]. The organic matter influences the nature and properties of soil and affects the activity of microorganism in the soil and the soil pH also plays an important role in microbial activity [5].

Water bodies receive microorganisms from air, soil, sewage, organic wastes, dead plants and animals etc. It is obvious that at times almost any organism may be found in water, those finding unfavorable conditions die and the others finding favorable condition grow and multiply to increase their population.

Pyrochemicals are the chemicals which were used in the fire works industry; zinc oxide and ammonium oxalate are also the chemicals which are used in pyrotechnics. Though 80% of pyrochemical produced annually in the world is used in developed countries, less than half of all pyrochemicals induced deaths occur in these countries [6–8]. In the present

P. Dhasarathan (✉) · P. Theriappan · C. Ashokraja
Sri Kaliswari College,
Sivakasi - 626 130, India

E-mail: dhasa_rathan@yahoo.co.in

study attempts were made to study the microbial population and diversity in samples collected from pyrochemicals contaminated areas, cultivable area, city area and waste land area.

Materials and methods

Sample collection

Both soil and water samples from different vicinity such as fireworks; cultivable farms, wasteland and city area were collected in sterile polythene and conical flasks separately. The samples were immediately taken to the laboratory and subjected to analysis.

Enumeration of bacterial population

One gm of soil sample was weighed and suspended in 100 ml of sterile water. The above sample was serially diluted with sterile water upto 10^{-9} dilutions by transferring 1 ml of sample from the 100 ml sample to the test tubes containing 9 ml of sterile water. Similarly, water samples drawn were also serially diluted.

Plating and enumeration of viable bacterial population

The viable number of the bacterial counts from soil and water samples was enumerated using pour plate method. One ml aliquots from the appropriate dilutions were pipetted out into sterile petri dishes. 20 ml of sterile molten nutrient agar was aseptically poured into the petri plates and the petri plates were rotated in clockwise and anti-clockwise direction and allowed to solidify. For each dilution, three replicates were maintained. The inoculated plates were incubated at 37°C for 48–72 hours.

Bacterial colonies were counted using bacteriological colony counter after incubation period. The petri dishes, which contained 30–300 bacterial colonies, were selected for enumeration of bacterial colonies and the bacterial populations were expressed as number of colony forming units per gram or ml (CFU/gm or ml) of the sample analysed.

Generic composition of the bacterial strains

Well-isolated bacterial colonies with varying morphological growth characteristics were selected and restreaked in air-dried nutrient agar plates to check the purity of bacterial strains. The pure culture were then restreaked in nutrient agar slants and stored at 4°C for further studies. The bacterial strains isolated were identified up to generic level following the scheme of Simudu and Aiso [9] and Gilmour et al. [10]. The Bergey's manual of determinative bacteri-

ology (1984–1989) was also referred in the identification process.

Result and discussion

Total heterotrophic bacterial population

The total heterotrophic bacterial population in soil samples of fireworks area, cultivable land, wasteland and city area in Sivakasi are illustrated in Table 1. Of all the four regions the total viable count was found to be high in cultivable soils (98×10^7) followed by wasteland (93.2×10^6) city area (78.4×10^4) and fireworks (42×10^4). A similar trend was also observed in case of water samples. The total viable count found to be higher in cultivable area than wasteland, city and fireworks. The total viable count was found to be high in sample from cultivable area (38.6×10^7), followed by wasteland (18.08×10^6) city area (17.76×10^6) and fireworks area (55.6×10^4) as illustrated in Table 1.

Bacterial growth gradually increased in the order of fireworks, city area, wasteland and agriculture land. This extreme variation could be due to the unfavorable condition for the growth of microorganism in fireworks and city area. The increase in the number of bacterial colonies from fireworks to cultivable lands could be due to many reasons.

Basically, Sivakasi is a very dry area with scanty rainfall. More over it is highly industrial as well. So the toxic and chemical effluence liberated might have reduced the microbial population considerably to a very low level in fireworks and city area. The main factor that increase the microbial population are a) amount and type of nutrients available, b) availability of moisture and c) temperature and pH [11]. These factors do not seem to be so well in Sivakasi area. As mentioned above the industrialized effluents and toxic substances must have reduced the amount and type of nutrients needed. Since there is a better availability of the favorable factors, sample drawn from cultivable and wasteland give a reasonably high population of microbial species.

Generic composition

Well isolated bacterial colonies with varying morphological growth characterization were selected for identification. A

Table 1 Bacterial density of the soil and water sample at different sites in and around Sivakasi

S. No	Sample	Bacterial density (cfu/gm)			
		Fireworks	Cultivable	Wasteland	City area
1	Soil	42×10^4	98×10^7	93.2×10^6	78.4×10^4
2	Water	55.6×10^4	38.6×10^7	18.08×10^6	17.76×10^6

total of 12 such colonies were selected from the samples and identified. The generic composition of different samples were analysed and recorded in Table 2.

Generic composition in soil samples

The bacterial genera of the fireworks soil sample were comprised of *Flavobacterium* sp. (41.6%), *Achromobacter* sp. (25%), *Bacillus* sp. (16.6%), *Corynebacterium* sp. (16.6%) and *Pseudomonas* sp. (16.6%). The bacterial genera of the cultivable soil sample comprised *Corynebacterium* sp. (66.6%), *Pseudomonas* sp. (25%), *Bacillus* sp. (16.6%), *Flavobacterium* sp. (16.6%), *Achromobacter* sp. (16.6%) and *Micrococcus* sp. (8.33%). The bacterial genera of the wasteland soil sample comprised *Bacillus* sp. (50%) and *Corynebacterium* sp. (33.3%). The bacterial genera of the city area soil sample comprised *Bacillus* sp. (66.6%), *Corynebacterium* sp. (33.3%), *Micrococcus* sp. (8.33%) and *Pseudomonas* sp. (8.33%).

Generic composition in water sample

The bacterial genera of the fireworks sample comprised *Pseudomonas* sp. (38.4%), *Bacillus* sp. (25%), *Corynebacterium* sp. (8.33%), *Micrococcus* sp. (16.6%) and *Flavobacterium* sp. (16.6%). The bacterial genera of the cultivable area water sample comprised *Corynebacterium* sp. (58.6%), *Pseudomonas* sp. (25%), *Achromobacter* sp. (16.6%), *Flavobacterium* sp. (16.6%) and *Bacillus* sp. (8.33%). The bacterial genera of the wasteland water sample comprised *Corynebacterium* sp. (45%), *Bacillus* sp. (32%), *Achromobacter* sp. (8.33%) and *Micrococcus* sp. (16.6%).

The bacterial genera of the city area water sample comprised *Bacillus* sp. (72%), *Pseudomonas* sp. (16.6%), *Achromobacter* sp. (8.33%), *Flavobacterium* sp. (8.33%) and *Corynebacterium* sp. (3.33%). Soil microorganisms

play an important role in soil process that determines plant productivity. For successful functioning of introducing microbial bioinoculants and their influence on health, exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behavior in soil habitats [12].

With the extensive use of pesticides and other chemicals such as pyrochemicals in cultivable area and industrialization of urban soils, the pollution of the environment with man made complex organic compounds has become a major environmental concern. Many of these novel compounds called xenobiotics (Xenos-Gr Foreign), are not easily degraded by the indigenous microbiota. The polluting industries that have created this mess are fireworks, textile mills, paper and pulp industries and electroplating units.

Conclusion

On the basis of the information available to date a common message emerges that biodiversity is in decline at all levels and geographical scales, but targeted response options whether through protected soils, or resource management and pollution prevention programme can reverse this trend for specific habitats. In the present investigation it is found that the total heterotrophic bacterial density, generic composition and physiological grouping in the fireworks soil had considerably decreased when compared to that of cultivable soil. In terms of extreme influence of pollutants, it is absolutely necessary to monitor the concentration in terrestrial environment. Hence, this study strongly recommends the necessity for monitoring the concentration of pollutants in terrestrial system and to apply appropriate control measures to ensure the safety of terrestrial biodiversity and the ecosystem at large.

Acknowledgement The authors are grateful Shri A. P. Selvarajan, Secretary, Sri Kaliswari College, Sivakasi for providing facility to complete this work.

Table 2 Generic composition of bacterial strains isolated from soil and water sample at different soils in and around Sivakasi

S. No.	Sample	Percentage of bacterial generic composition (%)					
		<i>Bacillus</i> sp.	<i>Corynebacterium</i> sp.	<i>Pseudomonas</i> sp.	<i>Flavobacterium</i> sp.	<i>Achromobacter</i> sp.	<i>Micrococcus</i> sp.
1.	Soil						
	Fireworks	16.6	-	41.6	16.6	25.0	-
2.	Cultivable	16.6	66.6	25.0	16.6	16.6	8.33
3.	Wasteland	50.0	33.3	-	-	-	-
4.	City	66.0	33.3	8.33	-	-	8.33
5.	Water						
	Fireworks	25.0	8.33	38.4	16.6	-	16.6
6.	Cultivable	8.33	58.6	25.0	16.6	16.6	-
7.	Wasteland	32.0	45.0	-	-	16.6	16.6
8.	City	72.0	33.3	16.6	8.33	8.33	-

References

1. Subba Rao NS (2002) Soil Microbiology (Fourth edition) IBH Publishing, New Delhi
2. Subba Rao NS (1995) Soil Microbiology Oxford and IBH Publishing, New Delhi
3. Bhatnagar A and Bhatnagar M (2005) Microbial diversity in desert ecosystem. *Curr Sci* 89(1):91–100
4. Dhasarathan P, Uma Gowsalya G and Rajkumar K (2006) Seasonal variations in microbial population in sivakasi soil with reference to the influence of temperature. *Poll Res* 25(1): 41–45
5. Baker KF and Cook BJ (1974) Biological control of plant pathogens WH Free Man, San Francisco, pp. 433–436
6. Zehr JP, Carpenter EJ and Villareal TA (2000) New perspectives on nitrogen fixing microorganisms in tropical and sub-tropical oceans. *Trends Microbiol* 8(2):68–73
7. Donahue BA, Yin S, Taylor JS, Reines D, Hanawalt PC, Mitchell DL, Vaughan JE and Nairn RS (1989) Inhibition of transient gene expression in Chinese hamster ovary cells by cyclobutane dimers and (6–4) photoproducts in transfected ultraviolet-irradiated plasmid DNA. *Plasmid* 21:21–30
8. Lehmann AR (1995) Nucleotide excision repair and the link with transcription. *Trends Biochem Sci* 20:402–405
9. Simidu V and Aiso K (1962) Occurrence and distribution of heterotrophic bacteria in sea water from the Kangawa Bay. *Bull Jap Sci fish* 28:1137
10. Gilmour A, McCallum F and Allan MC (1976) A study of the bacterial types occurring in the intestine of farmed plaice. *Aquaculture* 161–172
11. Prabakaran G (2004) Introduction to Soil Agricultural Microbiology (First edition) Himalayan Publishing House, New Delhi
12. Hill GT (2000) Methods for assessing the composition and diversity of soil microbial communities. *Appl Soil Microbiol* 4:19–23