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Influence of industrial effluents on bacterial populations in the agricultural soils of Anantapuramu district in Andhra Pradesh

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Abstract

Introduction: An increasing body of evidence suggests that micro organisms are far more sensitive to industrial effluents than soil animals or plants growing on the same soil. Setting up of new industries resulted in the disposal of industrial effluents causing air, water and soil pollution. Microbes play a crucial role in decontaminating polluted sites.

Materials & Methods: The present study was based on variations of bacterial populations in polluted and non polluted soils of Saptagir Camphor and Siflon Drug industries. In this direction, soil samples were collected from Saptagir Camphor industry near Garladinne, Anantapuramu, Andhra Pradesh and Siflon Drug industry near Rachanapalli, Anantapuramu, Andhra Pradesh. The bacterial populations of polluted and non polluted soil samples were enumerated by serial dilution technique. After incubation period, colonies formed on the surface of the nutrient agar medium were enumerated.

Results: The experimental results indicated that most of the physico-chemical properties, bacterial populations were significantly higher in polluted soil samples than in non polluted soils. Additionally the bacterial populations were increased with increasing the incubation period up to 30 days compared to 0 day, however, population count was decreased after 30 days of incubation.

Conclusion: The bacterial population increases s they utilizes the nutrients in the soil, as incubation period increases the depletion of nutrients occurs and hence the population decreases on prolonged incubation.

Keywords: Soil microorganisms, Industrial effluents, Bacterial populations, Polluted and Non–polluted soils

Introduction

Soil is the key component of natural ecosystem [1, 2, 25], and as such environmental sustainability depends largely on a sustainable soil ecosystems. Soil is one of the most vital natural resources and soil productivity is considered as important factor for success of agricultural production rather than soil fertility [6, 9]. It is a vibrant habitat for huge diversity of life forms. It is also habitat of micro organisms. Microbial diversity constitutes the most extraordinary reservoir of life in the biosphere. Over millennia microbes have adapted to extremely diverse environment, and developed an extensive range of new metabolic pathways or library of catabolic enzymes [8,12]. The expedited advent of urbanization and industrialization for economic growth has adversely affected the biological diversity, which is one of the major concerns of developing countries. Soil produces food for teeming millions and supplies raw materials for a large number of industries on which world economy is sustained. In fact on the other hand, progress of civilization and rapid industrialization brought within the danger of soil pollution.

Industrial effluents are nothing but the materials generally discarded from industrial operations or derived from manufacturing processes. The wastes of such activities are finally discharged into the soil and natural water courses resulted the undesired influence on environment particularly on soil micro flora and soil enzymes. In recent years microbes have been drawing tremendous attention due to their ability to degrade waste materials and their by improving soil quality. Taking the above facts into consideration, a research was undertaken in Saptagir Camphor and Siflon Drug industrial effluents to explain the influence of camphor and drug industrial effluents on the bacterial populations.

Agro industries processing and generate large volume of liquid /solid effluents and release them in to the environment. A perusal of the literature on the discharge of effluents on to the soil [22] indicates that they cause marked changes in physico-chemical biological and enzymatic properties.

Environmental pollution is the monitoring tools to assess the soil quality [10]. Discharge of industrial effluents in to the soil has an impact on soil microbial properties and soil enzymatic activities. Discharge of effluents from various industries like paper mills [18, 29], Tannary and chromate industry [21], petro chemical industries [4] and cotton ginning mills [22] influence the physico-chemical properties of soil. Application of sodium based black liquor from fiber pulping for paper making increased soil pH, electrical conductivity [31].

Discharge of effluents from Camphor and Drug industries to surrounding environment including agriculture, without neutralization has become general practice. Camphor and Drug industrial effluents contain considerable amount of organic and inorganic pollutants. The impact of these pollutants on microbial activities in terrestrial ecosystem is scanty. There is also considerable interest in the study of soil enzymes because such effect reflects the potential capacity of soil to perform certain biological transformations of soil fertility. Soil contamination or soil pollution as a part of land degradation is caused by the presence of xenobionis (human-made) chemicals or other alternations in the natural soil environment. It is typically caused by industrial activity, agricultural chemicals or improper disposal of waste. The most common chemicals involved are petroleum, hydro carbons, poly nuclear aromatic hydrocarbons such as (naphthalene and benzo (a) pyrene), solvents and pesticides, lead and other heavy metals. Contamination is correlated by degree of industrialization and intensity of chemical usage. Industrial activity has been the biggest contributor to the soil pollution in the last century, especially since the amount of mining and manufacturing has increased. Industrial effluents would have adverse impact on agriculture and would cause environmental degradation. In this study the influence of industrial effluents on bacterial populations in both polluted and non polluted soil samples of Saptagir Camphor industry and Siflon Drug industry was investigated.

Materials and methods

Study area

The study area is selected from the soil around Saptagir Camphor factory near Garladinne, Anantapuramu, Andhra Pradesh, India and Siflon Drug industry near Rachanapalli, Anantapuramu, Andhra Pradesh, India.

Saptagir Camphor Limited

Manufacture and supply of terpene chemical and associated products. Camphor is used in traditional pooja rituals as well as pharmaceuticals. Synthetic camphor is white crystalline solid with pharma grade 95% purity.

Siflon Drugs

Manufacture and supply of veterinary drugs such as Oxyclozanide. Oxyclozanide is an anthelminitic (flukcide) well reported in British pharmacopoeia (veterinary). Oxyclozanide is a pale cream color, odorless powder.

Soil used in the present study

Soil samples were collected from the surrounding areas (1/4th km) of Siflon drug industry and Saptagir camphor factory Anantapuramu, Andhra Pradesh, India. Soil samples without effluent discharges served as control were collected from adjacent site (1Km) away from the same camphor and drug industries. Both soil samples were collected using trowel at a depth of 0-12 cms and mixed thoroughly to prepare a homogenous composite sample. Soil samples were air dried at room temperature and they were cleaned to remove plant debris and other waste materials and passed through 2 mm sieve and stored at 4˚C prior to analysis.

Physico-chemical properties

Physico chemical properties include soil texture, pH, water holding capacity, electrical conductivity, organic matter, total nitrogen etc. The soil particles like sand, silt and clay contents were analyzed with the use of different sieves by the method of Alexander [3]. The water holding capacity (WHC) of soil samples was determined by adding distilled water up to the saturation point and then 60 water-holding capacity of soil samples was calculated [15]. Soil pH was measured by mixing soil and water in the ratio of 1:1.25 using Systronics digital pH meter with calomel gas electrode assembly. Electric conductivity of soil samples was determined by the addition of distilled water to 1 gram of soil sample was measured by Conductivity Bridge. Organic carbon contents in soil samples was estimated by Walkley and Black method and the organic matter of soil was calculated by multiplying the values with 1.72 [18]. The total nitrogen content in soil samples was determined by the micro- Kjelhdal method [14]. The inorganic ammonium nitrogen content in soil samples after extraction of 1 M KCl by the Nesslerization method [14] and the contents of nitrite nitrogen were determined by the method reported [7], and the contents of nitrate nitrogen by Brucine method [27] after extraction with distilled water were determined.

The physic-chemical properties of both polluted and non polluted soil samples of Saptagir Camphor and Siflon Drug industries were listed in table 1.

Table 1: Saptagir Camphor soil physico chemical properties.

S. No	Physico Chemical Properties	Saptagir Camphor polluted soil	Saptagir Camphor non-polluted soil	Siflon drug polluted soil	Siflon drug non-polluted soil
1	Color	red	Red	black	Black
2	Water holding capacity (ml g^{-1} soil)	42.39	37.97	39.53	39.95
3	Texture				
	Sand (%)	54.3	52.2	57.5	56.5
	Silt (%)	32.4	34.5	35.4	33.4
	Clay (%)	13.3	13.3	7.1	10.1
4	pH[a]	8.58	8.5	8.21	8.53
5	Electrical conductivity (m.mhos)	223	202	0.12	0.08
6	Organic carbon (%)	1.61	0.48	0.45	0.24
7	Organic matter (%)[b]	2.76	0.82	0.77	0.41
8	Total nitrogen (%)[c]	0.079	0.050	0.65	0.37
9	NH_4^+-N (µg g^{-1} soil)[d]	7.10	6.22	1.60	2.21
10	NO_2^--N(µg g^{-1} soil)[e]	0.64	0.41	0.76	0.65
11	NO_3^--N(µg g^{-1} soil)[f]	0.99	0.80	0.93	0.84
12	Sulphur (%)	1.62	2.13	1.23	1.83

[a]1:1.25 (soil:water) ; [b]Walkey-Black method (Jackson, 1971) ; [c]Micro-Kjeldhal method (Jackson, 1971) ; [d]Nesslerization method (Jackson, 1971) ; [e]Diazotization method (Barnes and Folkard, 1951) ; [f]Bricine method (Ranney and Bartler, 1972)

Enumeration of bacterial population in polluted and non polluted soils

Incubation

10 gms of soil samples contaminated with /without effluents of camphor and drug industries were transferred to test tubes. Soil samples were maintained at 60% water holding capacity at room temperature in the laboratory (28 ± 4 ºC). Triplicate soil samples of each polluted and non polluted soil samples were withdrawn at periodic intervals such as (0, 10th, 20th, 30th, and 40th days) for the enumeration of bacterial population.

Serial dilution method

10 gms of incubated soil samples is mixed with 100 ml of distilled water and then the serial dilution blanks were prepared in test tubes and marked sequentially starting from 10^{-1} to 10^{-8} dilutions . 1 ml of soil sample solution was dissolved in 9ml of water i.e. 10^{-1} dilution, 1 ml from this was then transferred to 9 ml of the 10^{-2} labeled test tube i.e. 10^{-2} dilution, using a sterile pipette; and this was repeated for each succeeding step till 10^{-8}. Nutrient agar medium petri plates were prepared and used for the enumeration of bacteria, from 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} dilution tubes, 0.5 ml of dilution liquid was then spread on nutrient agar medium petri plates in triplicates using the standard spread plate technique.

The nutrient agar plates were then incubated at 37˚ C for 24 hrs. After successful growth of micro organisms the bacterial colonies of both polluted and non polluted soil samples of Saptagir Camphor and Siflon Drug industries were enumerated by colony counter [22].

Statistical Analysis

The microbial populations in polluted and non polluted soils of Saptagir Camphor and Siflon Drug industries are contrasted using Duncan's multiple range (DMR) test [13, 17].

Results and discussion

Polluted and non polluted soil samples of both the industries were analyzed for their physic chemical properties and their results were represented in table 1. The majority of physic chemical properties of polluted and non polluted soils of both the industries were approximately similar. There were no noticeable changes in the pH of the polluted soil over non polluted soils of both industries. Soil texture of Saptagir Camphor industry in terms of percentage of sand, silt, and clay are 54.3, 32.4 and 13.3 in polluted soil and 52.2, 34.5 and 13.3 in non polluted soil, respectively. Soil texture of Siflon Drug industry in terms of percentage of sand, silt, and clay are 57.5, 35.4 and 7.1 in polluted soil and 56.5, 33.4 and 10.1 in non polluted soils, respectively. There were no noticeable changes in electrical conductivity in polluted and non polluted soils of both the industries. The results were in conformity with the studies of Sparling et al., [19, 24, 26, 28, 30, 31], had increased electrical conductivity in soils contaminated by the effluents of dairy, cotton ginning, automobile, and black liquor for straw pulping industries, respectively. But content of organic carbon is higher in polluted soils than in non polluted soils of both the industries. Sulphur content is higher in non polluted soils than in polluted soils of both the industries. Higher organic matter of polluted soil may be due to discharge of waste water in organic nature. Increased organic matter enhances soil enzymatic activity [15, 19] made similar reports on discharge of effluents from cotton ginning and distillery industries, respectively. Discharge of industrial effluents may have profound influence on physic chemical and biological properties of soil related to soil fertility [20]. Similarly, discharge of effluents from various industries like sugar industry [16], dairy factory [23] and petro chemical industry [4] influenced the physic chemical properties of soil. This is due to organic waste that may contribute to maintain or increase the organic matter and nutrient content in soil [5].

Population of bacteria (cfu/ml) has been decreasing with increasing dilution factor. The polluted soils of the two industries (Saptagir Camphor and Siflon Drug) shows higher population of bacteria than in non polluted soils of the same industries. The bacterial populations of Saptagir Camphor and Siflon Drug industries were increased with increasing the incubation period up to 30 days compared to over 0 day, however, population count was decreased after 30 days of incubation.

The direct discharge of effluents in to the soil has many complications over the microbial populations both qualitatively and quantitatively. The microbial diversity was expressed in colony forming units per ml (cfu/ml) at the sampling sites and shown in table 2 and 3.

Table 2: Population of bacteria in polluted and non polluted soils of Saptagir Camphor industry.

Incubation days	Saptagir Camphor LTD (Garladinne, Anantapur)							
	Polluted Soil sample				Non Polluted Soil sample			
	$\times 10^{-5}$ g^{-1}	$\times 10^{-6}$ g^{-1}	$\times 10^{-7}$ g^{-1}	$\times 10^{-8}$ g^{-1}	$\times 10^{-5}$ g^{-1}	$\times 10^{-6}$ g^{-1}	$\times 10^{-7}$ g^{-1}	$\times 10^{-8}$ g^{-1}
0 day	336[a]	272[a]	184[a]	148[a]	226[a]	164[a]	132[a]	64[a]
10th day	408[b]	386[b]	324[b]	226[b]	292[b]	228[b]	222[b]	186[b]
20th day	498[c]	410[c]	392[c]	384[c]	432[c]	326[c]	314[c]	274[c]
30th day	366[d]	334[d]	308[d]	256[d]	392[d]	266[d]	212[b]	146[d]
40th day	324[a]	266[a]	192[a]	136[a]	284[b]	230[b]	148[a]	72[a]

Means, in each column, followed by the same letter are not significantly different (P≤ 0.005), from each other according to Duncan's Multiple Range (DMR) test [11].

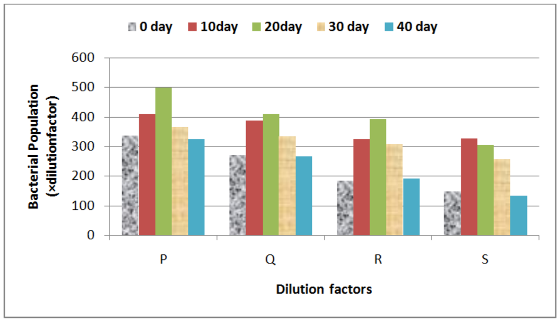
$$\text{Number of colonies per gram soil} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Volume of the plate}}$$

Table 3: Population of bacteria in polluted and non polluted soils of Siflon Drug industry.

Incubation days	Siflon Drugs and Pharmaceuticals PVT.LTD (Rachanapalli, Anantapur)							
	Polluted Soil sample				Non Polluted Soil sample			
	$\times 10^{-5}$ g^{-1}	$\times 10^{-6}$ g^{-1}	$\times 10^{-7}$ g^{-1}	$\times 10^{-8}$ g^{-1}	$\times 10^{-5}$ g^{-1}	$\times 10^{-6}$ g^{-1}	$\times 10^{-7}$ g^{-1}	$\times 10^{-8}$ g^{-1}
0 day	230[a]	196[a]	136[a]	68[a]	212[a]	188[a]	152[a]	56[a]
10th day	406[b]	292[b]	226[b]	134[b]	346[b]	262[b]	218[b]	92[b]
20th day	526[c]	432[c]	258[c]	266[c]	428[c]	384[c]	306[c]	192[c]
30th day	444[d]	302[b]	210[b]	188[d]	328[b]	352[c]	192[b]	126[d]
40th day	382[e]	234[d]	166[d]	92[e]	210[a]	174[a]	128[a]	64[a]

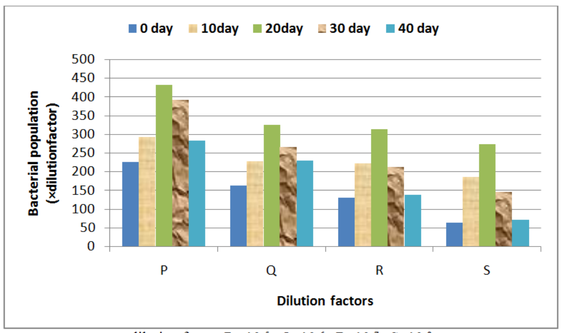
Means, in each column, followed by the same letter are not significantly different (P ≤ 0.005), from each other according to Duncan's Multiple Range (DMR) test [11].

$$\text{Number of colonies per gram soil} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Volume of the plate}}$$



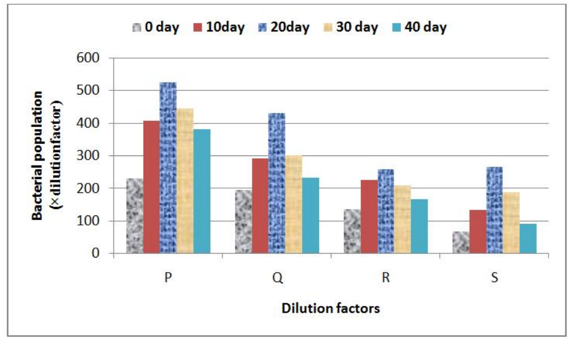
dilution factor P=10⁻⁵ ; Q=10⁻⁶ ; R=10⁻⁷ ; S=10⁻⁸

Figure 1: Population of bacteria in polluted soils of Saptagir Camphor industry.



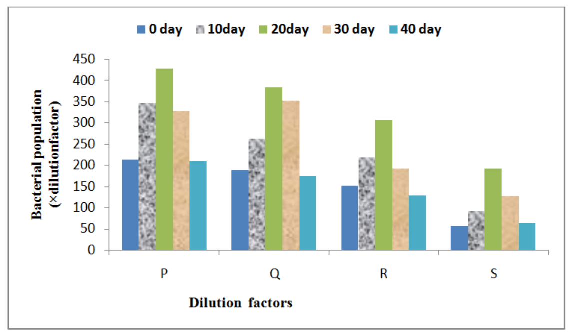
dilution factor P= 10⁻⁵ ; Q=10⁻⁶ ; R=10⁻⁷ ; S=10⁻⁸

Figure 2: Population of bacteria in non polluted soils of Saptagir Camphor industry.



dilution factor P= 10⁻⁵ ; Q=10⁻⁶ ; R=10⁻⁷ ; S=10⁻⁸

Figure 3: Population of bacteria in polluted soils of Siflon Drug industry.



dilution factor P=10⁻⁵ ; Q=10⁻⁶ ; R=10⁻⁷ ; S=10⁻⁸

Figure 4: Population of bacteria in non polluted soils of Siflon Drug industry.

Conclusion

The present study clearly indicates that the disposal of effluents from Saptagir Camphor industry and Siflon Drug industry alters the physical, chemical, and biological properties and also population of bacteria of polluted soils over non polluted soils. As the dilution rate increases the population of bacteria decreases in both polluted and non polluted soils of two industries. The bacterial population increases as incubation days increases upto 30 days over 0 day. Thereby population decreases after 30 days of both polluted and non polluted soils of two industries. This observation clearly indicates that the bacterial population increases as they utilizes the nutrients in the soil, as incubation period increases the depletion of nutrients occurs and hence the population decreases on prolonged incubation.

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