

# **Influence of Industrial Effluents on Fungal Populations in the Agricultural Soils of Anantapuramu District in Andhra Pradesh**

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**ABSTRACT:** An evidence suggests that micro organisms are far more sensitive to industrial effluents than 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. The present study was based on variations of fungal 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 fungal populations of polluted and non polluted soil samples were enumerated by serial dilution technique. After incubation period, colonies formed on the surface of the Czapek's dox agar medium were enumerated. The experimental results indicated that most of the physico-chemical properties were significantly higher in polluted soil samples than in non polluted soils. Additionally the fungal populations were increased with increasing the incubation period up to 20 days compared to 0 day, however, population count was decreased after 20 days of incubation. The fungal 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.

**KEY WORDS:** Soil microorganisms, Industrial effluents, fungal populations, Polluted and Non-polluted soils.

## **I. INTRODUCTION**

Soil is the key component of natural ecosystem (Adedokun and Ataga, 2007; Adenipekun, 2008 and Onuh et al., 2008), 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 (Brady et al., 2012 and Day et al., 1994). 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 (Butler and Mason, 1997 and Ellis, 2000). 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.

Fungi are fundamental for soil ecosystem (Warcup, 1951). Fungi are the important components of the soil microbiota (Ainsworth et al., 1995). Micro fungi play a focal role in nutrient cycling by regulating soil biological activity (Arunachalam et al., 1997). The quantities of organic and inorganic materials present in the soil have a direct effect on fungal population of the soil. In addition to the chemicals and wide range of pollutants shows adverse effect

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on mycoflora which are much useful to maintain soil fertility and eco-balance in the soil atmosphere. The members and kinds of microorganisms present in the soil are dependent on many environmental factors such as the amount and type of nutrients, moisture, degree of aeration, pH and temperature etc.

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 fungal 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 (Narasimha et al., 1999) indicates that they cause marked changes in physico-chemical biological and enzymatic properties.

Environmental pollution is the monitoring tools to assess the soil quality (Dick, 1994). 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 (Mishra and Sunandasahoo, 1989 and Singh et al., 2005), Tannary and chromate industry (Nandakumar, 1990), petro chemical industries (Andrade, 2002) and cotton ginning mills (Narasimha et al., 1999) influence the physico-chemical properties of soil. Application of sodium based black liquor from fiber pulping for paper making increased soil pH, electrical conductivity (Xiao et al., 2005).

## II. 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 Oxytocin. Oxytocin is an antihelminthic (flukcide) well reported in British pharmacopoeia (veterinary). Oxytocin is a pale cream color, odorless powder.

### Soil used in the present study

Soil samples were collected from the surrounding areas (1/4<sup>th</sup> 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 (Alexander, 1961). 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 by (Johnson and Ulrich, 1960). 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

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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 (Jackson, 1971). The total nitrogen content in soil samples was determined by the micro- Kjelhdal method reported by Jackson (1971). The inorganic ammonium nitrogen content in soil samples after extraction of 1 M KCl by the Nesslerization method, (Jackson, 1971) and the contents of nitrite nitrogen were determined by the method reported by (Barnes and Folkard, 1951), and the contents of nitrate nitrogen by Brucine method (Ranney and Bartlett, 1972) after extraction with distilled water were determined.

The physico-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 <sup>-1</sup> 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 <sup>a</sup>	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 (%) <sup>b</sup>	2.76	0.82	0.77	0.41
8	Total nitrogen (%) <sup>c</sup>	0.079	0.050	0.65	0.37
9	NH <sub>4</sub> <sup>+</sup> -N (µg g <sup>-1</sup> soil) <sup>d</sup>	7.10	6.22	1.60	2.21
10	NO <sub>2</sub> <sup>-</sup> -N(µg g <sup>-1</sup> soil) <sup>e</sup>	0.64	0.41	0.76	0.65
11	NO <sub>3</sub> <sup>-</sup> -N(µg g <sup>-1</sup> soil) <sup>f</sup>	0.99	0.80	0.93	0.84
12	Sulphur (%)	1.62	2.13	1.23	1.83

<sup>a</sup>1:1.25 (soil:water) ; <sup>b</sup>Walkey-Black method (Jackson, 1971) ; <sup>c</sup>Micro-Kjeldhal method (Jackson, 1971) ; <sup>d</sup>Nesslerization method (Jackson, 1971) ; <sup>e</sup>Diazotization method (Barnes and Folkard, 1951) ; <sup>f</sup>Brucine method (Ranney and Bartlett, 1972)

### Enumeration of fungal 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 °C). Triplicate soil samples of each polluted and non polluted soil samples were withdrawn at periodic intervals such as (0, 10<sup>th</sup>, 20<sup>th</sup>, 30<sup>th</sup>, and 40<sup>th</sup> days) for the enumeration of fungal population.

#### Nutrient medium used:

A wide range of media are used for enumeration and isolation of different groups of fungi that influence the vegetative growth and colony morphology. The medium used was Czapek's dox agar medium (sodium nitrate 2.0gms,

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dipotassium hydrogen phosphate 1.0gm, magnesium sulphate 0.5gm, potassium chloride 0.5gm, ferrous sulphate 0.01gm, sucrose 30gms, agar 15.0gms, distilled water 1000ml). The pH of the medium was maintained 7.3.

## The soil dilution plate method:

Soil dilutions (Waksman, 1922) were made by suspending 1gm of soil of each sample in 10 ml of sterile distilled water. Dilutions from  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$  and  $10^{-8}$  were used for enumeration of fungi in order to avoid over crowding of fungal colonies. 1ml of the suspension of each concentration was added to sterile petri dishes, in triplicates of each dilution, containing sterile Czapek's dox agar medium. 1% streptomycin solution was added to the medium for preventing bacterial growth, before pouring into petri plates. The plates were then incubated at  $28 \pm 2$  °C for 4-7 days. Fungal colonies were enumerated because they form surface colonies that were dispersed, particularly at higher dilutions. After successful growth of micro organisms the fungal colonies of both polluted and non polluted soil samples of Saptagir Camphor and Siflon Drug industries were enumerated by colony counter (Narasimha et al., 1999).

## 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 (Megharaj et al., 1999, Gooty Jaffer Mohiddin et al., 2011 and Farhadsultana and Rangaswamy, 2017).

## III. RESULTS AND DISCUSSION

Acid soils have higher number of fungi compared to alkaline soils. The number of fungi typically found in 1gm of top soil is approximately 106 Cfu. However the concentration of spores and their diversity in soil are not completely known and is dependent on the amount of humidity, temperature and composition of nutrient elements and bio environmental factors (Ayerst, 1969). Soil microbes acts as essential determinants of plant community variety and productivity (Wardle et al., 2004). The environmental factors such as soil pH, moisture, temperature, organic carbon and nitrogen play an important role in the distribution of micro flora (Gaddeyya et al., 2012).

Fungal diversity of any soil depends on a large number of factors of soil such as pH, organic content and moisture (Alexander, 1977 and Rangaswami et al., 1998). Longterm industrial sewage application with increasing concentration of pollutants produced a significant decrease in size of fungal population of soil. But fungi never disappeared completely in soils amended with highest rates of pollutants, suggesting certain adaptations of fungi to such environmental stress. The reasons underlying stress related changes in the diversity of fungal population, particularly those due to the presence of industrial pollution are not completely understood. It is well known that heavy metals cannot be chemically degraded. The number and diversity of fungi in soil can be extremely large, but often a small portion (generally <10%) of them form colonies on used medium plates. Many fungi from natural environment do not grow on synthetic media. We tried to compare the abundance of fungi in soil samples collected from two industrial polluted and non polluted soils.

Polluted and non polluted soil samples of both the industries were analyzed for their physico chemical properties and their results were represented in table 1. The majority of physico 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., (Reddi Pradeep and Narasimha, 2012), (Nagaraju et al, 2007), Poonkothai and Parvatham (Nizammuddin et al., 2008), and Xiao et al (Speir and Ross, 1975), 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 (Nagaraju et al., 2007) and (Johnson and Ulrich, 1960) made similar reports on discharge of effluents from cotton ginning and distillery industries, respectively. Discharge of industrial effluents may have profound influence on physical, chemical and biological properties of soil related to soil fertility (Nagaraju et al., 2009). Similarly, discharge of effluents from various industries like sugar industry (Lowry et al., 1951), dairy factory (Nelson, 1944) and petro chemical industry (Andrade, 2012) influenced the physico 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 (Bollag et al., 2002).

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**Table 2: Population of fungi in polluted and non polluted soils of Saptagir Camphor industry**

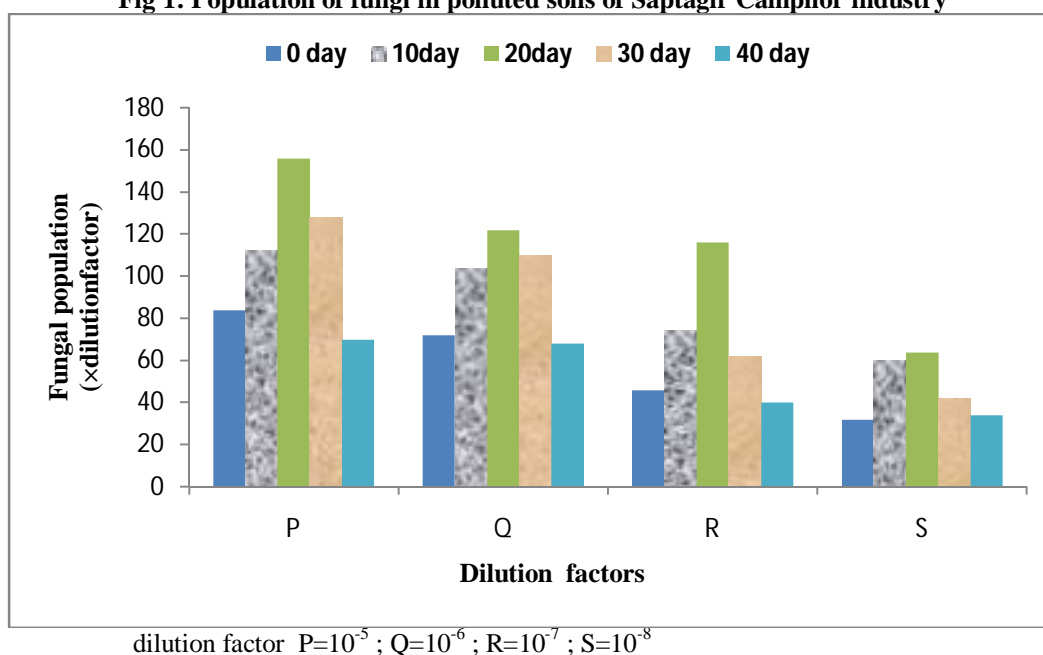
Incubation days	Saptagir Camphor LTD (Garladinne, Anantapur)							
	Polluted Soil sample				Non Polluted Soil sample			
	$\times 10^{-5}$ g <sup>-1</sup>	$\times 10^{-6}$ g <sup>-1</sup>	$\times 10^{-7}$ g <sup>-1</sup>	$\times 10^{-8}$ g <sup>-1</sup>	$\times 10^{-5}$ g <sup>-1</sup>	$\times 10^{-6}$ g <sup>-1</sup>	$\times 10^{-7}$ g <sup>-1</sup>	$\times 10^{-8}$ g <sup>-1</sup>
<b>0 day</b>	84 <sup>a</sup>	76 <sup>a</sup>	46 <sup>a</sup>	32 <sup>a</sup>	78 <sup>a</sup>	52 <sup>a</sup>	36 <sup>a</sup>	24 <sup>a</sup>
<b>10th day</b>	112 <sup>b</sup>	104 <sup>b</sup>	74 <sup>b</sup>	50 <sup>b</sup>	92 <sup>b</sup>	68 <sup>b</sup>	54 <sup>b</sup>	48 <sup>b</sup>
<b>20th day</b>	156 <sup>c</sup>	122 <sup>c</sup>	116 <sup>c</sup>	64 <sup>c</sup>	124 <sup>c</sup>	102 <sup>c</sup>	100 <sup>c</sup>	90 <sup>c</sup>
<b>30th day</b>	128 <sup>d</sup>	110 <sup>d</sup>	62 <sup>d</sup>	42 <sup>d</sup>	106 <sup>d</sup>	82 <sup>d</sup>	72 <sup>d</sup>	46 <sup>d</sup>
<b>40th day</b>	70 <sup>a</sup>	68 <sup>a</sup>	40 <sup>a</sup>	34 <sup>a</sup>	66 <sup>a</sup>	48 <sup>a</sup>	40 <sup>a</sup>	22 <sup>a</sup>

Means, in each column, followed by the same letter are not significantly different ( $P \leq 0.005$ ), from each other according to Duncan's Multiple Range (DMR) test. (Megharaj et al., 1999 and Gooty Jaffer Mohiddin et al., 2011 and Farhadsultana and Rangaswamy, 2017).

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

Population of fungi (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 fungi than in non polluted soils of the same industries. The fungal populations of Saptagir Camphor and Siflon Drug industries were increased with increasing the incubation period up to 20 days compared to 0 day, however, population count was decreased after 20 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.

**Fig 1: Population of fungi in polluted soils of Saptagir Camphor industry**



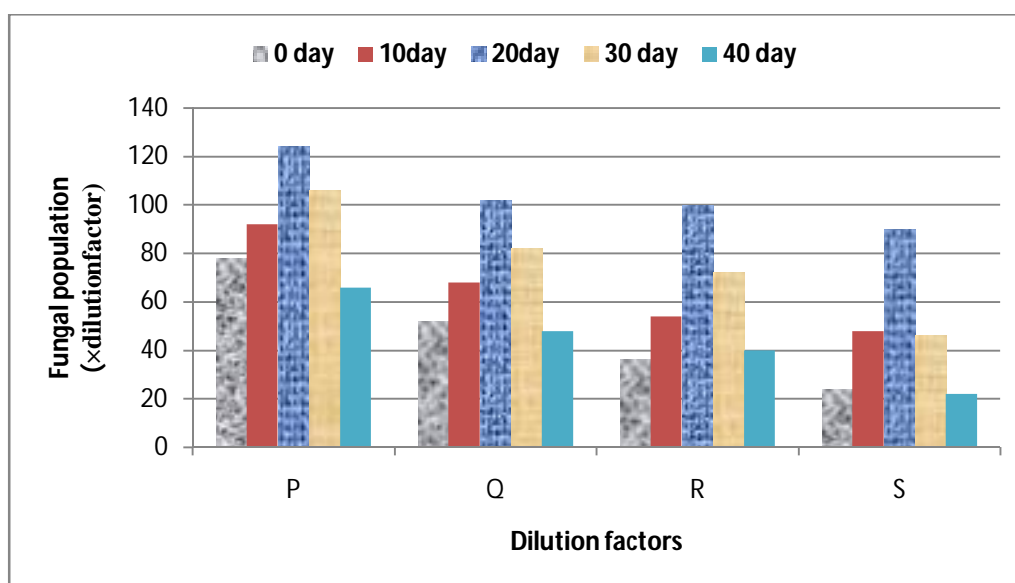
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**Fig 2: Population of fungi in non polluted soils of Saptagir Camphor industry**



dilution factor P=  $10^{-5}$ ; Q= $10^{-6}$ ; R= $10^{-7}$ ; S= $10^{-8}$

**Table 4: Population of fungi 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	80 <sup>a</sup>	74 <sup>a</sup>	42 <sup>a</sup>	28 <sup>a</sup>	64 <sup>a</sup>	50 <sup>a</sup>	28 <sup>a</sup>	14 <sup>a</sup>
10th day	104 <sup>b</sup>	92 <sup>b</sup>	86 <sup>b</sup>	46 <sup>b</sup>	92 <sup>b</sup>	68 <sup>b</sup>	52 <sup>b</sup>	36 <sup>b</sup>
20th day	126 <sup>c</sup>	116 <sup>c</sup>	106 <sup>c</sup>	72 <sup>c</sup>	118 <sup>c</sup>	92 <sup>c</sup>	74 <sup>c</sup>	52 <sup>c</sup>
30th day	100 <sup>d</sup>	94 <sup>b</sup>	62 <sup>b</sup>	42 <sup>d</sup>	72 <sup>d</sup>	62 <sup>d</sup>	48 <sup>d</sup>	20 <sup>d</sup>
40th day	76 <sup>a</sup>	68 <sup>a</sup>	40 <sup>a</sup>	24 <sup>a</sup>	54 <sup>a</sup>	42 <sup>a</sup>	38 <sup>a</sup>	12 <sup>a</sup>

Means, in each column, followed by the same letter are not significantly different ( $P \leq 0.005$ ), from each other according to Duncan's Multiple Range (DMR) test (Megharaj et al., 1999 and Gooty Jaffer Mohiddin et al., 2011 and Farhadsultana and Rangaswamy, 2017).

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



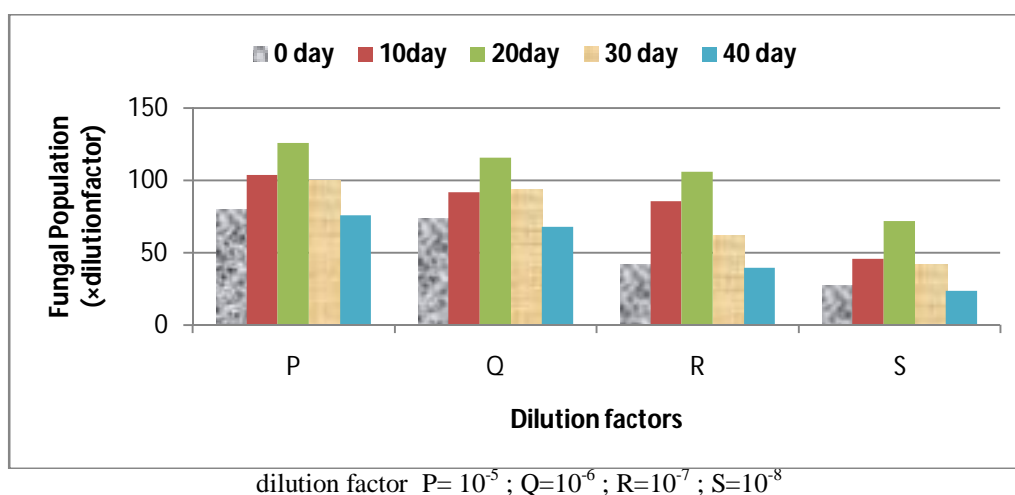
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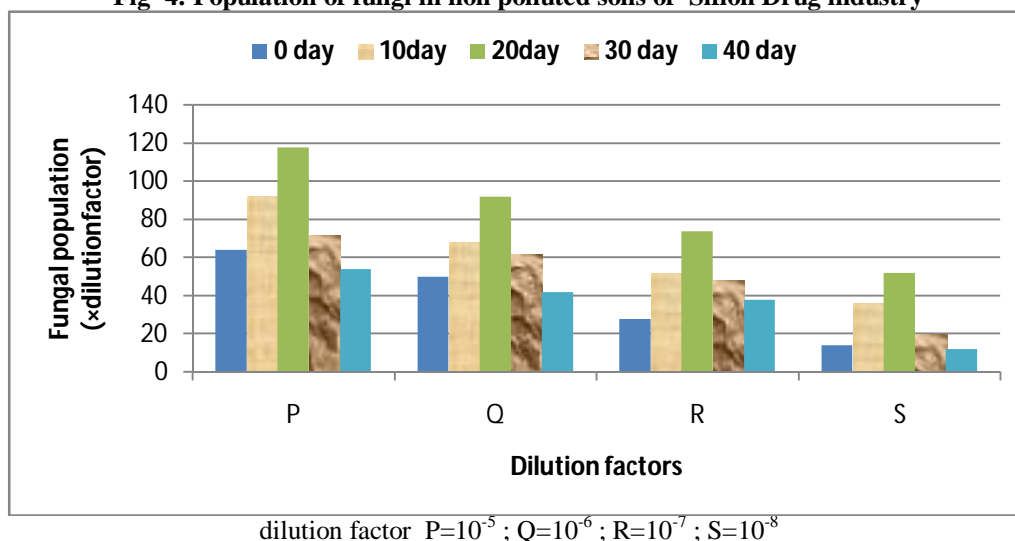
**Fig 3: Population of fungi in polluted soils of Siflon Drug industry**



The effect of long term industrial contamination on soil microorganisms was assessed with a view of determining whether the analysis of these organisms could be used for ecological assessment of contaminated sites. Toxic effects of heavy metals on soil microorganisms have been extensively studied in the past (Baath, 1989; Giller et al., 1998; Williamson et al., 1998), and almost every group of organism has been studied in terms of resistance. Fungi and bacteria constitute the main components of the soil microbial biomass.

It has often been stated that fungal populations are more tolerant to heavy metals than bacterial populations (Hiroki, 1992; Doelman et al., 1994; Brokes, 1995; Rajapaksha et al., 2004 and Stefanowicz et al., 2008). Similar conclusions were made when phospho lipid fatty acid (PLFA) analysis was used to differentiate between the fungal and bacterial components of the soil microbial biomass (Frostegard et al., 1993, 1996; Pennanen et al., 1996 and Kelly et al., 1999), and after the investigation of soils treated with a waste sludge (Pennanen et al., 1996 and Baath, 1998). Because of the plate technique for determining populations of soil fungi, the results can be taken only as indication of what is likely to occur in the soil. Industrial pollution decrease lignin and cellulose degrading microorganisms.

**Fig 4: Population of fungi in non polluted soils of Siflon Drug industry**



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## IV. 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 fungi of polluted soils over non polluted soils. As the dilution rate increases the population of fungi decreases in both polluted and non polluted soils of two industries. The fungal population increases as incubation days increases upto 20 days over 0 day. Thereby population decreases after 20 days of both polluted and non polluted soils of two industries. This observation clearly indicates that the fungal 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.

The higher fungal concentrations were observed in the polluted soils than in non polluted soils. However differences were not statistically significant. The higher fungal counts were due to high organic matter content and higher water content in the polluted soils than in non polluted soils. Average fungal counts were distinctly lower in non polluted soils than in polluted soils. However variability of some results were rather low and the differences were not statistically significant. Fungal population is dominant in polluted soils. Fungal population is dependent in the highest degree on the soil characteristics and especially in the total concentration of nutrients and organic matter content.

There is no significant difference in pH of polluted soils and non polluted soils. Hence pH does not have any effect on the fungal population in both polluted soils and non polluted soils of both the industries. Changes of relative fungi were observed in polluted soils and characterized by different chemical compositions water content, humus content in soil. Their abundance and distribution can explain possible effect of industrial pollution on microorganisms inhibiting the soil, their resistance and activity.

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