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MICROBIAL DIVERSITY IN RUBBER INDUSTRY EFFLUENT

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ABSTRACT
The present work was carried out to monitor the impact of rubber effluent on the microbial diversity viz., bacteria, fungi and cyanobacteria. Results of one year ecological study revealed that altogether 10 species of bacteria, 15 species of fungi and 42 species of cyanobacteria were isolated from the effluent stream. Among the bacteria, Pseudomonas with two species and others with single species each were recorded. Aspergillus was dominant among fungi with 7 species followed by Penicillium with two. Cyanobacteria, one of the dominant group of algae, inhabiting all kinds of water (effluents), recorded 42 species. Oscillatoria with 14 species was the dominant genus followed by Lyngbya (8), Phormedium (4), Chroococcus and Microcystis with two species each. Higher amounts of phosphates and nitrates, with sufficient amount of oxidizable organic matter, limited dissolved oxygen content and slightly alkaline pH were probably the factors favoring the growth of microbes especially cyanobacteria. The utilization of dominant species of cyanobacteria to monitor pollution in rubber effluent has also been discussed.

KEYWORDS: *Rubber effluent, biodiversity, microbes and pollution*

INTRODUCTION
The rate of contamination of our natural water bodies increases with increased human activities in the industrial and domestic spheres. The wastes of such activities are finally discharged into the natural water courses resulted the undesired eutrophication. In recent years microbes have been drawing tremendous attention due to their ability to degrade waste materials and thereby improving water quality, [1]. Taking the above facts in to consideration, a survey was under taken in rubber industry effluent to explore the nature of microbial flora such as bacteria, fungi and cyanobacteria in order to exploit them as a tool in treating rubber industry effluent

MATERIALS AND METHODS
Rubber effluent was collected from Nijavalli latex, situated at Cochin, Kerala, India. A sampling programmed consisting of series of monthly water quality and microbial survey was conducted for a one year period from November 2010 to October 2011. Population of bacteria and fungi were isolated from the effluent samples by serial dilution technique. Bacteria were identified based on colony characteristics, Gram staining methods and by various biochemical studies as given [2]. Fungi were identified by using standard manuals, [3 and 4]. For cyanobacterial survey, 10 places were selected along the effluent stream. Samples were collected from the places along with effluents in polythene bags. Standard microbiological methods were followed for the isolation and identification of cyanobacteria [5, 6 and 7]. Effluents samples were collected in duplicate from each station in pre-sterilized bottles.

Physico-chemical characteristics of effluent were analyzed according to the standard methods [8]. Temperature and pH of the effluent were measured at the station itself.

RESULTS AND DISCUSSION:
Physico-chemical characteristics of effluent:
The physico-chemical analysis of the effluent revealed its slightly alkaline nature and also the presence of high quantity of both organic as well as inorganic nutrients in all the seasons examined (Table 1). Values of Do were very low indicating highly obnoxious conditions. Though BOD and COD levels in the present study were high as per IS standards, their levels were not so high as compared to other types of effluent such as dye, paper, pharmaceuticals and sugar [8]. Dairy [1],

distillery [10] and Coir pith effluent [11]. Most of the parameters tested were slightly higher in summer than in rainy and winter seasons. Similarly [1 and 12] reported that results with paper mill and dairy effluent respectively at different seasons. They recorded objectionable amounts of BOD and COD, oil and grease, total dissolved solids, and algal nutrients such as ammonical nitrogen, nitrate nitrogen, silicates, phosphates and calcium. Such a trend was observed in the rubber effluent also. Pollution load of three effluents such as automobile, coir pith and starch effluents were analyzed [13]. Among these, highly objectionable amounts of various pollutants including BOD and COD were recorded in coir pith followed by Automobile and starch effluents. While studying the seasonal variations in physico-chemical parameters of chemical factory and paper mill effluent [14] found a direct relationship between temperature and organic matter. High organic matter was observed during summer and low during winter. This is in keeping with the observations that the course of decomposition of organic matter depends on temperature [15, 16, 17 and 18]. In the present study also, a higher amount of organic matter was observed during summer followed winter and rainy seasons (**Table 1**).

TABLE 1
CHARACTERISTICS OF RUBBER EFFLUENT FOR THE PERIOD FROM
(Mean ± SD values of each)
NO**VEMBER 2010 TO OCTOBER 2011**

S. No.	Parameters	Summer (March – June)	Rainy (July – Nov)	Winter (Dec – Feb.)
1.	Temperature	27.32 ± 3.1	25.64 ± 0.72	27.30 ± 0.49
2.	pH	7.64 ± 2.1	7.28 ± 0.8	7.40 ± 0.23
3.	Total Suspended solids	10.28.50 ±119.24	1137.33±126.16	1042.50 ±219.33
4.	Total dissolved solids	470 ± 130.24	450 ±126.25	410.24 ± 152.91
5.	Free Carbon-di-oxide	90 ± 1.2	7.0 ± 0.1	65 ± 1.7
6.	Carbonate	Nil	Nil	Nil
7.	Biarbonate	280 ± 1.3	260 ± 1.2	240 ± 1.5
8.	BOD	363 ± 1.2	326 ± 0.8	314 ± 2.8
9.	COD	826 ± 1.7	797 ± 1.6	804 ± 2.2
10.	Dissovled Oxygen	1.09 ± 0.2	1.16 ± 0.8	1.06 ± 0.29
11.	Nitrate	168 ± 1.9	149 ± 1.6	152 ± 1.5
12.	Nitrite	72 ± 2.1	59 ± 1.8	60 ± 1.8
13.	Ammonia	296 ± 1.8	230 ± 1.9	286 ± 0.2
14.	Total Phosphate	80 ± 0.1	65 ± 0.4	77 ± 0.1
15.	In organic Phosphate	32 ± 0.8	26 ± 0.5	38 ± 0.01
16.	Organic Phosphate	42 ± 0.8	38 ± 0.8	39 ± 1.7
17.	Calcium	138 ± 0.5	117 + 0.7	123 ± 1.2
18.	Magnesium	98 ± 0.8	78 ± 1.2	81 ± 0.8
19.	Chloride	1642 ± 2.8	1386 ± 3.1	1448 ± 0.8

Except pH and temperature, all values are expressed in mg/l^{-1}

Microbial diversity:
Bacterial diversity has not been studied in detail in waste water. However, a few reports are available on the bacterial flora of certain waste water. Isolation of bacteria belonging to different groups [19] mainly the *Cytophaga, Flavobacterium* – Bacterioides group, γ and β subclass of the class proteobacteria and the green non sulphur bacteria, from a heavily polluted site in a coastal stream. Similarly [1] isolated eight different bacterial genera from dairy wastes in order to

carry out biodegradation of dairy effluent. In the present study also nine different genera were isolated from rubber effluent (**Table 2**). Most of the isolated genera are potential pathogens. Some of these bacteria have previously been reported to be present in wastewaters [20] and oil polluted sites [21 and 22]. However, [23] isolated only two bacterial genera such as *Derxia* and *Beijerinckia* from dye effluent drenched soils. Due to the environmental stress caused by the high level of pollutants, which allowed only a restricted number of species that tolerated such conditions.

TABLE 2
BACTERIAL FLORA FROM RUBBER EFFLUENT
NOVEMBER 2010 TO OCTOBER 2011

S. No.	Name of the Bacteria	Summer (March – June)	Rainy (July – Nov)	Winter (Dec – Feb.)	Percentage occurrence
1.	*Escherichia Coil*	++	+++	+	50
2.	*Enterobacter acrogens*	+++	++	+++	66
3.	*Klebsiella pneumoniae*	++++	++	+++	75
4.	*Lactobacillus sp.*	++	-	++	33
5.	*Pseudomonas putida*	++	++++	+	58
6.	*P.fluorescens*	-	++	-	17
7.	*Proteus vulgaries*	-	-	+	8
8.	*Salmonella sp.*	++++	+++++	+++	100
9.	*Baillus cereus*	+++	++	+	50
10.	*Xanthomonas fragariae*	-	+	+	17

+++++ : Observed in all the five months
++++ : Observed in four months
+++ : Observed in three months
++ : Observed in only Two months
+ : Observed in only one month
- : Not observed

Fungi occurred over different seasons of the year but some of them showed restricted distribution (**Table 3**). They found in maximum numbers and diversity during summer with 15 species followed by winter with 11 and rainy seasons with 9 species. Among the fungal species isolated, *Curvularia* and *Neurospora* species were not reported from winter and rainy seasons. Of the fungal genera, *Aspergillus* was dominant with seven species. Reported that 23 species fungi from dye effluent [24] drenched soil and found *Aspergillus, Curvalaria, Penicillium* and *Trichoderma* were the dominant genera. Similarly [1 and 25] also observed fifteen and eleven pathogenic species of fungi from the effluents of Gelatin factory and dairy effluents respectively with *Aspergillus* as the dominant genus with six species, since it occurred in most of the months studied, thereby lending support to the present observation. Higher concentrations of chlorides, total dissolved solids and BOD were the reasons for their frequent occurrence [26]. Most of the aquatic fungi preferred low temperature between 15 to 30^{0}C has been reported [27]. Whereas higher temperature retarded the growth. In the present study also, the temperature of the rubber effluent ranges between 25^{0}C and 28 ^{0}C thereby favoring the growth of fungi.

TABLE 3
FUNGAL FLORA FROM RUBBER EFFLUENT
NOVEMBER 2010 TO OCTOBER 2011

S. No.	Name of the fungi	Summer (March June)	Rainy (July – Nov)	Winter (Dec – Feb.)	Percentage occurrence
	MONILIACEAE				
1.	*Aspergillus flavus* Link	+++	++++	+++	83
2.	*A. fumigatous* Fresenius	+++	+++	+	50
3.	*A.luchensis* Inui	++	+++	+++	66
4.	*A.nidulans* (Eidam) winter	++++	+++++	+++	100
5.	*A. niger* van Tieghem	++++	+++++	+++	100
6.	*A.Sulphureus* (Fresenius) Thom and Church	++	-	-	33
7.	*A.ustus* (Bainier) Thom and Church	++	+++	++	58
8.	*Penicillium citrinum* Thom	+++	+++	++	66
9.	*P.janthinellum* Biourge	++	-	+++	50
10.	*Trichoderma Koringi* Oudemans	+	-	++	17
11.	*Fusarium oxysporum* schlechlendahl	+++	-	++	42
12.	*Verticillium glaucum* Bonorden	++	+	+	33
	DEMATIACEAE				
13.	*Curvularia lunata* (walker) Boedijn	+	-	-	08
14.	*Helminthosporium oryzae* Breda de Haran	+++	++++	-	58
	FIMETARIACEAE				
15.	***Neurospora crassa* shear and Dodge**	**+**	**-**	**-**	08

+++++ : Observed in all the five months
++++ : Observed in four months
+++ : Observed in three months
++ : Observed in only Two months
+ : Observed in only one month
- : Not observed

Fresh water fungi generally grow at pH 7.0 to 8.5 and the occurrence of species such as *saprolegnia parasitica, Aspergillus, Fusaruim* and *Curvularia* were recorded [25, 27 and 28]. Similarly the pH of the effluent in the present study ranges from 7.2 and 7.6 and fungi such as *Aspesgillus, Fusarium, Curvalaria, Verticillium, Helminthosporium* and *Neurospora* were recorded (**Table**).

Among the species of fungi, *A. nidulans* and *A.niger* was the dominance, which occurred in all the months in all seasons. In stagnant water bodies, the temperature may rise during summer, as a result, the species could be reduced [27]. Contrary to this, in present investigation, the temperature of the effluent did not vary much (**Table 1**) as it was collected from the running stream and hence the observed variation.

Several important publications deal with the ecological distribution of cyanophyceae [9, 13, 28, 29, 30, and 31,]. Many of them emphasize the importance of light, temperature, pH, Carbondioxide, organic matter, alkalinity, nitrates and phosphates as factors important in determining the distribution of blue green algae. The above observations have been made in natural fresh water, lentic and lotic systems. In the manmade system such as the present one, the above factors remain more or less uniform throughout the year. In the present investigations, it was observed that cyanophycean members dominated the effluent stream during all the seasons. During summer 37 species were observed, while their number during rainy and winter seasons was 35 and 34 species respectively, (Table 4). Of the genera, *Chroococcus minor, Microcysis robusta, Oscillatoria chlorina* and *Lyngbya majuscula* observed both in summer and rainy seasons were not recorded in winter. Similarly *O.curviceps, O.formosa* and *Lyngbya confervoides* were not found in rainy seasons. On the other hand *O.claricentrosa, Phormidium corium, P.incrustatum* and *P.jadinianum* was not recorded in summer.

Rich blooms of some cyanobacteria such as *Microcystis acruginosa*, *Synechococcus elongatus*, *Oscillatoria early* and *O.salina*, *O.willei* were observed in all seasons in through and the year. This abundance is attributed to favorable conditions of oxidizable organic matter, less dissolved oxygen and high calcium content (**Table 1**) an observation which supports by [32, 33 and 34]. Observations of [31 and 35] suggest that cyanophyceae grow luxuriously with great variety and abundance in ponds rich in calcium. High orthophosphate levels favored the development of cyanobacterial bloom, [36]. The positive correlation between phosphate and cyanobacteria was observed [37]. The Luxuriant growth of cyanobacteria of low concentration of oxygen and in the presence of high concentrations of nitrogen phosphate has also been reported by [38 and 39] .Similar observations were also made in the present study with reference to various nutrients (**Table 1**). Polluted water rich in nitrogen due to formed in non heterocystous [40 and 1]. Present investigation also showed dominance of non heterocystous forms and not single species of hetrocystous cyanobacteria (**Table 4**).

TABLE 4

CYANOBACTERIAL FLORA FROM RUBBER EFFLUENT

S. No.	Name of the Bacteria	Summer (March – June)	Rainy (July – Nov)	Winter (Dec – Feb.)	Percentage of occurrence
I	**CHROOCOCLACEAE**				
1.	*Chroococcus macrococcus* (kiitz) Rabenh	+++	+++	++	**66**
2.	*C.minor (Kutzing)* Nageli	++	+++++	-	**58**
3.	*Aphanothece pallida* (Kiitz) Rabenh	+	+++	++	**50**
4.	*Microcrstis acruginosa* (Kiitz)	+++	++++	+++	**83**
5.	*M.flos-aquae* (wittr) (Kirchner)	+++	++++	++	**75**
6.	*M.robusta* (clark) Nygarrd	+++	++	-	**42**
7.	*Synechococcus elongatus* Nag	++++	++++	+++	**91**
8.	*Synechocystis Pevalekii* Ercegovi	++	++++	+++	**75**
II	**OSCILLATORIACEAE**				
9.	*Oscillatoria acuminata* Gomont	+++	+++	+	**58**
10.	*O.acuta Bruhl et. Biswas*	++	+++	++	**58**

11.	*O.animalis* Ag.ex Gomont	+++	++	+	**50**
12.	*O.brevis* (Kiitz) Gomont	+++	+++	++	**66**
13.	*O.chlorina* Kiitz ex Gomont	++	+++	-	**42**
14.	*O.claricentrosa* Gardner	-	+++	+	**33**
15.	*O.curviceps* Ag.ex Gomont	+++	-	++	**42**
16.	*O.earlei* Gardner	+++	++++	++	**75**
17.	*O.late-virens* (crouan) Gomont	+++	++++	+++	**83**
18.	*O.princeps* Vaucher ex. Gomont	+++	-	++	**42**
19.	*O.pseudogeminata* G. schmid	+++	+++++	++	**83**
20.	*O.okeni* Ag. ex. Gomont	++	-	-	**16**
21.	*O.formosa*	+++	-	+++	**50**
22.	*O.salina* Biswas	++++	+++++	+++	**100**
23.	*O.subbrevis* schmidle	+++	++++	+	**66**
24.	*O.terebriformis* Ag.ex Gomont	++	++	+	**42**
25.	*O.willei* Gardnet em. Drouct	++++	++++	+++	**91**
26.	*Phormidium anomala* Rao. C.B.	+++	+++	+	**58**
27.	*P.Corium* (Ag.) Goment	-	+++	-	**25**
28.	*P.fragile* (Meneghini) Gomont	++	+	+	**33**
29.	*P.incrustatum* (Ag.) Gomont	-	-	+	**08**
30.	*P.tenue* (Menegh.) Gomont	++	++	+	**42**
31.	*P.uncinatum* (Ag.) Gomont	+++	-	-	**33**
32.	*P.jadinianum* Gomont	-	+	+++	**33**
33.	*P.mucosum* Gardner	++	+++	+	**50**
34.	*Lyngbya borgerti* Lemmermann	+++	+++	++	**66**

35.	*L.ceylanica* Fremy	++	++	++	**50**
36.	*L.connectens* Biswas	+	+	++	**42**
37.	*L.Confervoides* C Ag. ex. Gomont	++	-	+	**25**
38.	*L.connectens digueti* Gomont	+++	++++	++	**75**
39.	*L.infixa* Fremy	++	++	++	**50**
40.	*L.majuscula* Harvey ex. Gomont	++	++	-	**42**
III	**SCYTONEMATACEAE**				
41.	*Plectonema radiosum* (S. Chiedem) Gomont	++++	++	+++	**75**
42.	***P.wollei* Farlow ex.Gomont**	**++**	**++**	**-**	**42**

+++++ : Observed in all the five months
++++ : Observed in four months
+++ : Observed in three months
++ : Observed in only Two months
+ : Observed in only one month
- : Not observed

Genus Oscillatoria has been found to be tolerant to pollution which frequently inhabits the polluted water [13 and 41]. In the present study also *Oscillatoira* was found dominating the rubber effluent represented by 17 species. Emphasize the use of algae as reliable indicators of pollution [11]. In the present study, the percentage value of algae above 65 (**Table 4**) should be considered as indicator species of rubber effluent. Their high representation indicates their capacity to thrive in this type of manmade habitats. Such species can be used as marker species or indicators of particular habitats [41]. Besides *Oscillatoria,* many other cyanobacteira were also found to be tolerant to rubber effluent as evidenced by their occurrence (**Table 4**).

The Cyanobacteria that are isolated from an effluent stream could be grown on large scale in controlled waste stabilization ponds and thus pollution is taken care of to certain extent. Several investigations [10 and 42] have pointed out that the indicator species could be used to monitor pollution in controlled waste stabilization ponds.From the foregoing investigation, *O.salina* was observed in all the months at all seasons (**Table 4**) and hence it could be treated as an indicator species of rubber effluent. On the basis of this fact it is suggested that the indicator species could be used for pollution abatement programmes.

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