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SEASONAL VARIATION AND ALGAL DIVERSITY IN THE HIGHLY ALKALINE SOLID WASTES FROM A PAPER MILL IN SOUTHERN ASSAM (INDIA)

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ABSTRACT

Algae represent cosmopolitan prokaryotes, occurring in almost every aquatic and terrestrial environment including soil, in fresh water, in salt water and as well as in several surface types. The capability of cyanobacteria to fix molecular nitrogen together with their desiccation tolerance, renders them to be the most successful colonizers in low-fertility, high-pH sites. Current research has addressed to recover, stabilize and utilize degraded soils through the use of various algae. Besides chemical and physical methods, such algae based biological remediation processes are considered eco-friendly and cost effective. In this study, we investigated the soil algae inhabiting lime sludge waste generated by a Paper Mill, in Hailakandi district, Assam in India. Studies conducted on the seasonal distribution of algal communities revealed the occurrence of a total of 18 algal species belonging to 14 genera with predominant presence of Cyanobacteria followed by Chlorophyceae and Bacillariophyceae. Distribution of the algal genera was varied in different seasons, *i.e*. highest in monsoon and post-monsoon period and least in pre-monsoon period. Predominant blue - green alga was *Oscillatoria*, while *Cosmarium* was the most dominant green alga recorded. Different diversity indices were ascertained and correlation study made. Highest diversity was noted during the post-monsoon period.

KEY WORDS: **Algal diversity, Lime sludge wastes, Paper Mill, Southern Assam**

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INTRODUCTION

Very little information is available about algal community structure and diversity in industrial wastes and especially in solid wastes. Algae represent cosmopolitan prokaryotes, occurring in almost every aquatic and terrestrial environment including soil, in fresh water, in salt water and as well as in several surface types. [1] They represent the first community to colonise bare soil and newly exposed substrata of both natural and technogenic origin. [2] As photoautotrophs, they generate organic matter from the inorganic substances and subsequently help establish higher plant communities. [3] Moreover, the inherent capability of cyanobacteria to fix molecular nitrogen together with their desiccation tolerance, enables them to be the most successful colonizers in low-fertility, high-pH sites.[4-5] The microbiotic crust study is currently receiving greater attention for bioremediation. The substrates characterized by the accumulation of alkaline salts in the soil profile generally show restricted plant growth. Research has been directed to recover, stabilize and utilize such degraded soils through the use of various bio-fertilizers like algae.[6] Besides chemical and physical methods, algae based biological processes can be eco-friendly and also economical.[7-9] Though research on algae *vis-a-vis* paper mill effluent has been extensively documented those from solid waste are scant.[10] In Barak valley, the paper mill at Panchgram generates both solid and liquid waste. The solid wastes in the form of lime sludge are disposed outside the mill campus across the main road. Algal growth is found on these lime sludge sub-stratums during rainy seasons though less growth is observed during winter and pre-monsoon period due to scarcity of water. Accordingly, the present study aims to assess the soil algal flora of lime sludge waste of the paper mill and also the seasonal variation. The Shannon diversity index, Simpson dominance index and evenness index were used to estimate the algal diversity. Correlation analyses were performed to ascertain the influence of soil parameters on algal diversity.

MATERIALS AND METHODS

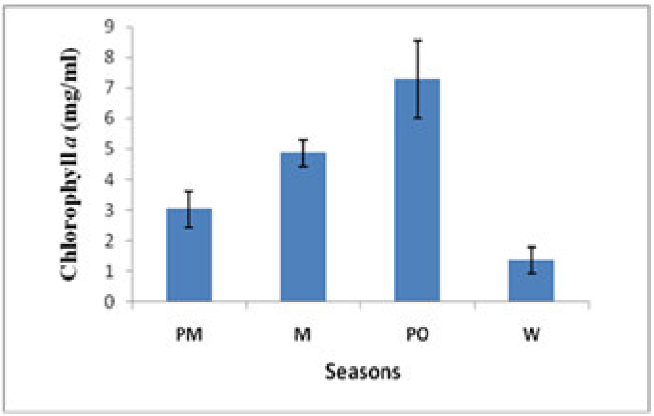
The soil samples were collected from the crusts of lime sludge wastes in different seasons (Premonsoon, Monsoon, Post monsoon and winter) from the dump area near the paper mill. Geographic details of the study sites were recorded using GPS (Germin eterex). The study area is located at the longitude of 92° 36′ E and the latitude 24° 52′ N at an elevation of 68 feet. Soil pH and conductivity were measured by electrometric method. Soil bulk density was estimated by soil core method[11] while the moisture content of the soil was determined by oven drying method.[12] Soil organic carbon was determined by Walkey and Black's rapid titration method.[13] The texture of the soil was analyzed by Bouyoucos soil hydrometer method.[14] The algal samples collected from different sites were observed under microscope and identified using standard keys[15-17] and preserved in 4.5% formalin for further study. The algal samples were finally counted following Lackey's drop method.[18] Chlorophyll *a* was determined using Trichromatic equation.[19] Statistical analyses were made using methods outlined by Snedecor and Cochran (1967).[20] Pearson correlation analyses were made to study the inter-relationships of the various parameters.

RESULTS AND DISCUSSION

The physico-chemical parameters of lime sludge deposit of the study sites are shown in Table 1. The seasonal abundance, density and frequency of algae of study sites of lime sludge are shown in Table 2 – 4 and the seasonal variation of chlorophyll *a* is depicted in Figure 1. A total of 18 algal species belonging to 13 genera under three classes of algae had been enumerated from the study sites. As the pH of lime sludge waste is >11, the species detected are high pH tolerant. Maximum numbers of species were observed in Cyanophyceae (10) followed by Chlorophyceae (4) and Bacillariophyceae (4). Maximum number of species belonged to the genus *Oscillatoria* (3), followed by *Cosmarium* and *Navicula* each of which contained 2 species while the other genera (*Anabaena*, *Aphanothece*, *Chroococcus*, *Cylindrospermum*, *Calothryx, Leptolyngbya, Melosira*, *Nitzschia*, *Nostoc, Oocystis, Pediestrum* and *Scytonema*) were represented by only one genera. The photomicrographs of algae encountered in this study are shown in Plate 1 and the algal colonization in Plate 2. The algal occurrence were more abundant during the monsoon and post monsoon season. *Nostoc* and *Oscillatoria* were found to be present irrespective of all the seasons. In case of Chlorophyceae, except *Pediestrum*, other three species (*Cosmarium*, *Navicula* and *Nitzschia*) were found to colonize in most of the seasons. Among Bacillariophyceae, *Navicula mutica* were found to be present throughout the seasons. In the present study cyanobacteria showed relatively high abundance and density. Both the abundance and density were observed to be highest in *Aphanothece* and *Leptolyngbya* among cyanobacteria and quite less in Chlorophyceae and Bacillariophyceae. However, Bacillariophyceae showed more frequency than Chlorophyceae. Among Chlorophyceae, *Oocystis eremosphaeria* and *Cosmarium pachydermum* were found to have higher abundance and density. Species of *Leptolyngbya* and *Oscillatoria* were found to occur most frequently among Cyanophyceae, while two species of *Cosmarium* showed higher frequency in case of Chlorophyceae. Lukešova (2001)[2] investigated the algal communities in two contrasting chronosequences established on reclaimed spoils in Czech Republic and in Germany. The Sokolov chronosequence was characterized by tertiary cypric clay substrate and high pH, while in Germany, chronosequence by pyritic sand of extremely low pH. Out of 122 species of algae studied, green algae prevailed in both areas, but in Sokolov, cyanobacteria and diatoms were also quite diverse, and in younger sites they were abundant. The seasonal fluctuation of chlorophyll *a* concentration (Fig. 1) has been monitored in the present study showing highest value in post-monsoon period. Presence of algae in the dry period in the study sites might be attributable to the availability of water in some of the low lying water logged areas for prolonged period. In the present study waste soils were found to be highly alkaline. The lime sludge waste dump is characterized by a distinctive substrate quality, like very strong alkaline pH (~11). Several other reports mentioned optimum growth of algae including cyanobacteria to be favoured by neutral to slightly alkaline pH.[21-25] The soil organic matter was found to be quite low in the study site (0.35%). Role of cyanobacteria in reclamation of highly sodic and alkaline soil has been documented.[6,26,21] Laboratory and field level investigation on amelioration of sodic or alkaline soil by cyanobacteria through the accumulation of inorganic ions, organic compounds (sugars, polyols, quaternary amines) and osmoregulators has been reported.[27-28]

Table 1
Physico-chemical parameters of soil of the study area

Parameters	Average ± SD
pH	12.03±0.4
Conductivity (ms)	17.99±2.98
Moisture content (%)	18.22±1.92
Bulk Density (gm cm^{-3})	0.90±0.52
Organic Carbon (%)	0.35±0.06
Textural class	Sandy



PM= Pre monsoon; M= Monsoon; PO= Post monsoon; W= Winter

Figure 1
Seasonal variation of chlorophyll a

Table 2
Seasonal variation of algal abundance in the study area

Sl. no.	**Class**	**Abundance (ind X10²cm^{-2})**			
	Cyanophyceae	**PM**	**M**	**PO**	**W**
1	*Anabaena orientalis* Dixit (after Dixit)	3.50	22.23	82.98	
2	*Aphanothece microscopica*		152.53	22.08	
3	*Croococcus limneticus* Lemm. (after Smith)			18.53	4.00
4	*Cylindrospermum muscicola* Kuetzing		62.66	20.53	10.39
5	*Leptolyngbya sp.*		106.52		28.92
6	*Nostoc sphaericum* Vaucher		25.89	46.02	4.94
7	*Oscillatoria amphibia* Ag. (orig.)	4.41	48.32	22.58	
8	*Oscillatoria subbrevis* Schmidle	7.32	9.39	47.17	9.91
9	*Oscillatoria tenuis* var. *tergestina* (Kuetz.)			33.52	8.60
10	*Scytonema zeilerianum*	2.51	49.38		
	Chlorophyceae				
11	*Cosmarium pachydermum*		7.60	22.46	4.97
12	*Cosmarium subimpressulum* Borge	1.45	2.37	3.49	
13	*Oocystis eremosphaeria* G. M. Smith		34.33	92.16	58.26
14	*Pediastrum integrum*		1.73	4.43	
	Bacillariophyceae				
15	*Melosira juergensii* Agarth		6.77	4.69	
16	*Navicula mutica* Kuetz. *f. goeppertiana* (Bleisch) Grun	0.86	3.43	9.69	1.85
17	*Navicula radiosa* Kutz. v. *tenella* (Breb. Ex. Kutz.) Grun.			4.56	14.27
18	*Nitzchia intermedia* Hantzsch		4.62	3.43	

PM= Pre monsoon; M= Monsoon; PO= Post monsoon; W= Winter

Table 3
Seasonal variation of algal density in the study area

Sl. no.	Class	Density (ind X10²cm⁻²)			
	Cyanophyceae	**PM**	**M**	**PO**	**W**
1	*Anabaena orientalis* Dixit (after Dixit)	2.72	5.56	47.70	
2	*Aphanothece microscopica*		43.38	13.66	
3	*Croococcus limneticus* Lemm. (after Smith)			10.29	2.01
4	*Cylindrospermum muscicola* Kuetzing		31.71	37.54	6.21
5	*Leptolyngbya sp.*		38.40		14.46
6	*Nostoc sphaericum* Vaucher	3.75	11.18	22.52	2.47
7	*Oscillatoria amphibia* Ag. (orig.)	2.20	23.20	9.62	
8	*Oscillatoria subbrevis* Schmidle	4.78	3.86	12.43	4.09
9	*Oscillatoria tenuis* var. *tergestina* (Kuetz.)			22.88	5.94
10	*Scytonema zeilerianum*	1.53	14.05		
	Chlorophyceae				
11	*Cosmarium pachydermum*		4.03	13.54	1.81
12	*Cosmarium subimpressulum* Borge	0.82	0.83	0.93	
13	*Oocystis eremosphaeria* G. M. Smith		14.62	41.36	15.75
14	*Pediastrum integrum*		0.85	1.77	
	Bacillariophyceae				
15	*Melosira juergensii* Agarth		2.27	1.80	
16	*Navicula mutica* Kuetz. *f. goeppertiana* (Bleisch) Grun	0.47	1.79	5.28	0.96
17	*Navicula radiosa* Kutz. v. *tenella* (Breb. Ex. Kutz.) Grun.			2.18	4.55
18	*Nitzchia intermedia* Hantzsch		2.31	2.57	

PM= Pre monsoon; M= Monsoon; PO= Post monsoon; W= Winter

Table 4
Seasonal variation of algal frequency in the study area

Sl. no.	Class	Frequency (ind X10²cm⁻²)			
	Cyanophyceae	**PM**	**M**	**PO**	**W**
1	*Anabaena orientalis* Dixit (after Dixit)	56.25	33.00	40.33	
2	*Aphanothece microscopica*		46.22	50.00	
3	*Croococcus limneticus* Lemm. (after Smith)			50.00	50.00
4	*Cylindrospermum muscicola* Kuetzing		54.12	50.00	60.42
5	*Leptolyngbya sp.*		62.28		50.00
6	*Nostoc sphaericum* Vaucher		56.50	44.62	50.00
7	*Oscillatoria amphibia* Ag. (orig.)	50.00	45.75	58.50	
8	*Oscillatoria subbrevis* Schmidle	63.58	56.25	58.50	50.00
9	*Oscillatoria tenuis* var. *tergestina* (Kuetz.)			64.25	62.50
10	*Scytonema zeilerianum*	53.42	43.75		
	Chlorophyceae				
11	*Cosmarium pachydermum*		66.88	50.06	50.00
12	*Cosmarium subimpressulum* Borge	59.72	51.33	37.50	
13	*Oocystis eremosphaeria* G. M. Smith		49.25	47.33	44.44
14	*Pediastrum integrum*		54.33	34.38	
	Bacillariophyceae				
15	*Melosira juergensii* Agarth		56.25	34.38	
16	*Navicula mutica* Kuetz. *f. goeppertiana* (Bleisch) Grun	53.33	64.67	52.83	48.61
17	*Navicula radiosa* Kutz. v. *tenella* (Breb. Ex. Kutz.) Grun.			45.83	41.67
18	*Nitzchia intermedia* Hantzsch		67.00	75.00	

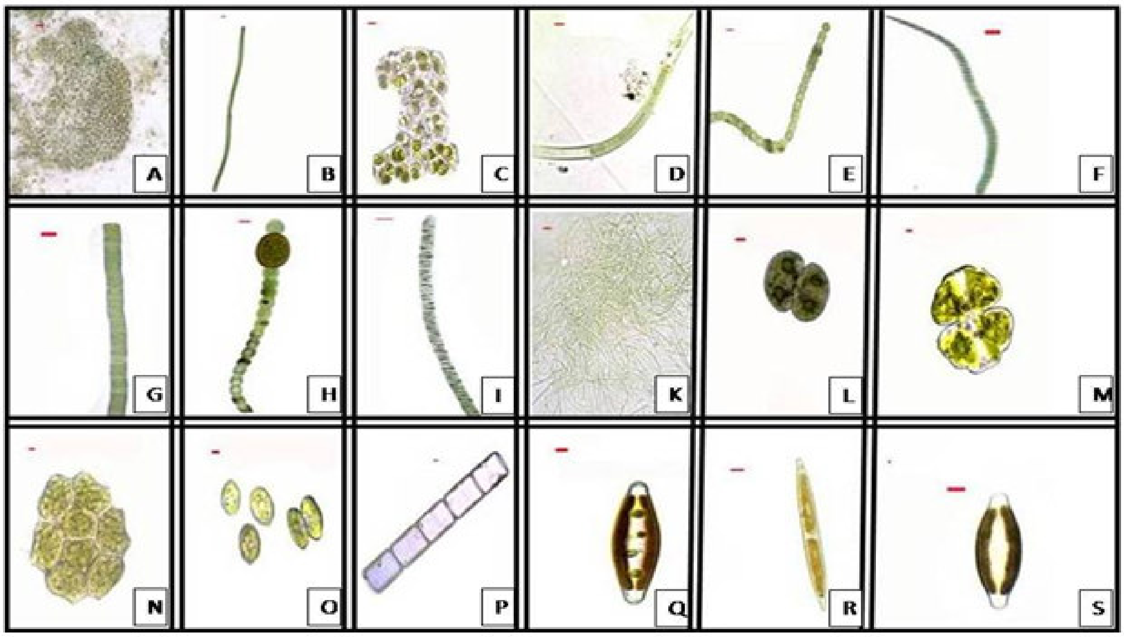


Plate 1
Photomicrographs of the algal species A: *Aphanothece microscopica; B: Oscillatoria tenuis var. tergestina* (Kuetz) C: *Croococcus limneticus* Lemm. (after Smith); D: *Scytonema zeilerianum;* E: *Nostoc sphaericum*Vaucher; F: *Anabaena orientalis* Dixit (after Dixit); G: *Oscillatoria subbrevis* Schmidle; H: *Cylindrospermum muscicola* Kuetzing; I: *Oscillatoria amphibia* Ag. (orig.); J: *Leptolyngbya* sp.; K: *Cosmarium pachydermum*; L: *Cosmarium subimpressulum;* M: *Pediastrum integrum*; N: *Oocystis eremosphaeria* G. M. Smith; O: *Melosira juergensii* Agarth; P: *Navicula radiosa* Kutz. v. *tenella* (Breb. Ex. Kutz.) Grun; Q: *Nitzchia intermedia* Hantzsch; R: *Navicula mutica* Kuetz. *f. goeppertiana* (Bleisch) Grun).

Plate 2
View of the lime sludge deposition area (study site) adjoining the paper mill

Abundance of algal species shows positive relationship with soil pH (r=0.85; p=0.042; R 2=0.701); relationship between abundance and soil conductivity is positive (r=0.395; p=0.181; R2=0.56); Moisture content (MC) shows negative relationship with abundance of soil algae (r=-0.199; p0.515; R2=0.08); Soil Bulk Density (SBD) shows negative relationship with abundance of different algal species (r=-0.099; p=0.09; R2=0.25). Water Holding Capacity (WHC) shows a positive relationship with algal species (r=0.088; p=0.2; R2=0.26); Organic Carbon (OC) concentration (%) shows negative relationship with abundance of algal species (r=-0.860; p=0.03; R2=0.67). The relationship among abundance of different algal species and soil pH and organic carbon is statistically significant at 0.05 levels. Table 5 depicts the diversity indices of algal communities in the various seasons. Highest algal diversity was observed during post monsoon season with maximum Shannon-Wiener diversity index (H=1.38), minimum Simpson's dominance index (D=0.34) and maximum Pielou's evenness index (J=0.70). The algal distribution during winter was observed to be least diverse with minimum Shannon-Wiener diversity index (H=0.66), maximum Simpson's dominance index (D=0.64) with Pielou's evenness index of J=0.43. These results are consistent with the fact that wet conditions following monsoon provides an ideal condition for algae to grow.

Table 5
Seasonal variation of diversity indices

Diversity index	PM	M	PO	W
Shannon- Wiener Diversity Index (H)	0.67 ± 0.28	1.20 ± 0.14	1.38 ± 0.07	0.66 ± 0.11
Simpson's dominance index (D)	0.63 ± 0.18	0.41 ± 0.09	0.34 ± 0.01	0.64 ± 0.07
Pielou's evenness index (J)	0.55 ± 0.24	0.66 ± 0.14	0.70 ± 0.03	0.51 ± 0.05

PM= Pre monsoon; M= Monsoon; PO= Post monsoon; W= Winter

Table 6
Bivariate correlation analysis of physico-chemical and biological parameters of lime sludge using Pearson correlation coefficients

Correlation matrix (Pearson):

Variables	Abundance	Chl *a*	pH	Conductivity	MC	SBD	WHC (%)	OC %
Abundance	**1**							
Chl *a*	-0.436	**1**						
pH	**0.85***	**0.580***	**1**					
Conductivity	0.395	-0.364	-0.303	**1**				
MC	-0.199	0.188	0.301	**-0.935***	**1**			
SBD	-0.099	0.231	0.075	-0.166	0.118	**1**		
WHC (%)	0.088	**-0.623***	**-0.595***	**0.693***	**-0.714**	-0.119	**1**	
OC (%)	-0.**860***	0.056	0.218	**-0.919***	**0.952***	0.070	**-0.557***	**1**

MC : moisture content, SBD : soil bulk density, WHC : water holding capacity, OC : organic carbon
Values in bold* are different from 0 with a significance level alpha=0.05

CONCLUSION

The solid lime sludge waste of the paper mill despite having very high pH does support growth of several blue-green and green algal species. A total of 18 high pH tolerant algal species spread over three families were recorded from the study site. A distinct seasonal variation of algal abundance was observed in the study sites. Post monsoon period favoured richest algal growth while winter and pre-monsoon seasons recorded the least. The solid lime sludge deposits were found to harbour some dominant forms of Cyanophyceae and diatoms which might prove to be relevant for bioremediation in a fast, reliable cost-effective and ecofriendly approach. However, the efficacy of these strains needs to be explored.

CONFLICT OF INTEREST

Conflict of interest declared none.

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