RESEARCH ARTICLE

EVALUATION OF MYCOBIOTA OF SOIL FUNGI ISOLATED FROM GARDEN SOIL AND FROM SOIL CONTAMINATED WITH PAPER MILL EFFLUENTS

Amit Kumar[1]*, Sanjay Kumar[1], Ashu Tyagi[2], Raj Singh[3], Permod Kumar[4] and M.U. Charaya[5]

Department of Botany, M.S. College Saharanpur
Department of Biotechnology, M.M.U. Mullana, Haryana
Email: amitsaini.saini421@gmail.com

Received-19.07.2021, Revised-13.08.2021, Accepted-24.08.2021

Abstract: The present communication deals with a comparison of the Mycobiota of garden soil with the soil contaminated by effluents of paper industry. A lesser number of fungal isolates were obtained from soils under the impact of pulp and paper mill effluents as compared to that from normal garden soil. The Shannon's diversity index of polluted soil was also lowest than that of garden soils. *Aspergillus terreus*, *Aspergillus flavus* and *Aspergillus niger* dominated the Mycobiota of polluted soils. These species can be utilized for *in situ* bioremediation of pulp and paper mill effluents. Alternatively, their biomass may be tried for developing –biosorption- based treatment plant for the effluents. Such under the impact of these effluents had higher pH than of garden soils.

Keywords: Garden Soil, Polluted Soil, Paper and Pulp mill Effluents, Shannon's Diversity Index, Soil Fungi

INTRODUCTION

Unprecedented urbanization as well as indiscriminate industrialization especially in the preceding century has resulted in negative impact on our environment which has become highly discernible in the past few decades. Pulp and paper industries are amongst the major industries contributing to this pollution. The consumption of the paper products has increased fourfold during last fifty years. The global market for paper and pulp stood at USD 518.82 billion in 2019, and is expected to reach at USD 679.72 billion by 2027 (Anon, 2020). In India also, paper industry occupies an important position in the economy by contributing 500 billion to GDP. Paper and pulp production in India is expected to reach 39.18 million tonnes by 2030 (Anon, 2018). Paper industry utilize enormous amount of water as different steps of processing. Generally, these generate huge amount of effluents which are highly coloured and contain lignins, xylans, resin acids, phenols, furans and halogens (Chaudhary and Paliwal, 2018). The application of waste water to land has been advocated as one of the options for its disposal because soil is considered to possess substantial potential for accommodating and degrading the pollutants (Martin, 1998; Comberato, 2006). Adoption of such practices must be preceded by a thorough evaluation of the impact of these effluents on physicochemical and biological properties of the soils. A limited number of studies have been carried out in this direction including that by Kannan and Oblisami (1990), Singh (2007), Dhevagi and Oblisami (2008), Reddy *et al*, (2013), Singh *et al*, (2013), Kumar *et al*, (2016). However, most of the studies deal with physicochemical characters and at the most bacterial and fungal populations. A little attention has been given to the analysis of Mycobiota by Rameshvara Reddy *et al*, (2018). Considering the role of fungi in maintenance of soil composition and fertility, the impact of the effluent on soil Mycobiota needs to be investigated.

MATERIALS AND METHODS

Three sets (A_1, A_2 and A_3) were marked in home garden – the distance between any two sets being 10 meters. Similarly, three sets (B_1, B_2 and B_3) were marked in the land exposed to effluent from Paper Mill (Saharanpur, U.P.). About 100 gm of soil was collected aseptically in sterile polythene bags with the help of sterilized trowel. Care was taken to remove upper layer of litter before taking the samples. Samples from the sets A_1, A_2 and A_3 were mixed to obtain a composite sample 'A' from garden soil. Similarly, a composite sample 'B' was obtained for soil polluted with paper mill effluents. The temperature of soil at each set was measured *in situ* using soil thermometer. Both the composite samples were analyzed for mycobiota, following dilution plate method (Waksman, 1927). 20 gm of soil sample from a given composite sample were transferred to 200 ml of distilled water followed by stirring for half an hour to facilitate the detachment of fungal propagules from the soil. 10 ml of this suspension were transferred to an Erlenmeyer flask containing 90 ml of sterilized distilled water to obtain a suspension of 1:100 dilution. From this suspension, further dilutions (1:1000 and 1:10000) were obtained. From the suspension of each dilution (1:100, 1:1000 and 1:10000), one ml dilution was transferred to each of a set of three petri dished followed by the addition of 20 ml of sterilized and cooled Potato Dextrose Agar medium (Raper and Thom, 1949) with 30 ppm of Rose bengal and 30 ppm of streptomycin. The petri plates containing the medium and inocula were incubated at 25± 1° C for 6-8 days. Fungal species growing in the petri dishes

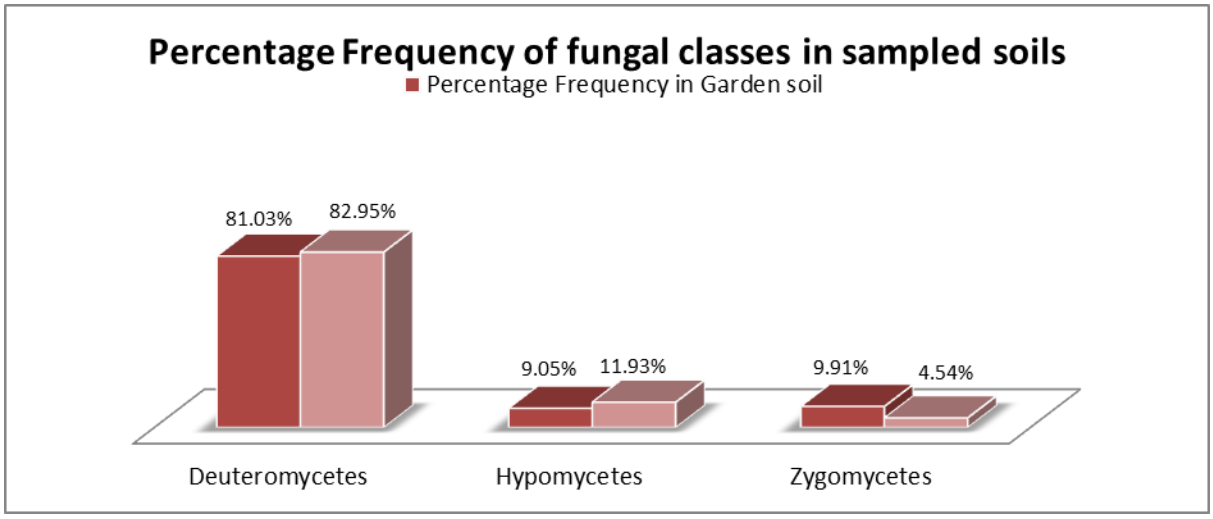
*Corresponding Author

were identified with the standard keys (Gilman, 1957; Nagmani *et al.*, 2006). Total numbers of colonies of each fungal species were also counted. The pH of the composite samples was determined using standard electric pH meter.

RESULTS AND DISCUSSION

In all, 3a species of fungi were obtained from the soil samples under study table only two or three *i.e. Mucor heimalis* and *Rhizopus oryzae* belong to Zygomycetes. This is in agreement with earlier observations (Galloway, 1935; Singh and Charaya, 1975; Charaya, 2006) that there is paucity of Mucorales in India. The remaining 29 species were anamorphic fungi (Deuteromycetes). A number of earlier workers have also observed the dominance of Duteromycetes (Hudorn, 1968; Dubey *et al.*, 1980; Kumar and Charaya, 2012). Amongst the anamorphic fungi, the Hyphomycetes comprised the major fraction. The aspergilli were represented by 13 species while the penicillia were represented by 6 species only. Many workers in the past including Waksman (1927), Sing and Charaya (1975) have suggested aspergilla are more abundant in the warmer regions of the world. The results of the present study are in agreement with the above observations.

A total of 232 CFU were obtained from garden soil while the polluted soil yielded only 176 isolates. Thus an adverse effect of paper mill effluent on fungal population was observed. This is in contrast with the earlier observation of Reddy *et al.*, (2013) who recorded higher fungal population in soils affected by industrial effluents. Earlier, Dhevagi *et al.*, (2008) had reported that irrigation with treated paper mill effluent was not harmful to soil microbial population. As far as the number of species is concerned, 26 species were isolated from garden soil as well as from polluted soils. The Shannon diversity of polluted soil was also lower (H=2.45) as compared to that of garden soil (H=2.62).



Graph 1: The Percentage Contribution of Different Fungal Classes in Garden Soil and Contaminated Soil.

Table 1. Mycobiota isolated from Garden soil and from the soil contaminated with the effluent of Star Paper Mil Saharanpur (TI= Total Isolates; PI= Percentage Isolates)

Fungal Species	Garden Soil		Soil contaminated with Star Paper Mil Effluent	
	TI	PI	TI	PI
Alternaria alternata	-	-	08	4.54
Alternaria brassicicola	-	-	06	3.40
Alternaria sp.	-	-	04	2.27
Aspergillus candidus	02	.86	-	-
Aspergillus fischeri	01	.43	-	-
Aspergillus flavipes			02	1.13
Aspergillus flavus	32	13.79	33	18.75
Aspergillus fumigatus	02	.86	02	1.13
Aspergillus humicola	05	2.15	03	1.70
Aspergillus nidulans			01	.56
Aspergillus niger	39	16.81	26	14.77
Aspergillus ochraceous	19	8.18	-	-
Aspergillus terrus	05	2.15	44	25
Aspergillus sydowi	01	.43	-	-
Aspergillus tamari	-	-	01	.56

Aspergillus versicolor	-	-	04	2.27
Cladosporium herbarum	01	.43	-	-
Curvularia clavata	01	.43	-	-
Curvularia lunata	01	.43	01	.56
Fusarium chlamydosporum	03	1.29	-	-
Fusarium equisetii	01	.43	-	-
Fusarium moniliforme	06	2.58	01	.56
Fusarium oxysporum	08	3.44	01	.56
Fusarium sp.	-	-	01	.56
Geotrichum candidum	27	11.63	04	2.27
Mucor heimalis	23	9.91	05	2.84
Penicillium chrysogenum	19	8.18	10	5.68
Penicillium digitatum	02	.86	-	-
Penicillium italicum	-	-	03	1.70
Penicillium lignorum	05	2.15	-	-
Penicillium glabrum	11	4.74	04	2.27
Penicillium sp.	10	4.31	-	-
Phoema glomerata	-	-	03	1.70
Rhizopus oryzae	-	-	03	1.70
Trichoderma atrinoviride	-	-	01	.56
Trichoderma lignorum	02	.86	-	-
Trichoderma ressei	03	1.29	04	2.27
Trichoderma sp,	03	1.29	-	-
Verticillium sp.			01	.56
No. of Species	**26**	**-**	**26**	-
Total Isolates	**232**	**-**	**176**	-
Shannon Diversity Index(H)	**2.623**		**2.451**	

The fungal composition of polluted soil differed markedly from garden soils. The mycobiota of garden soil was dominated by *Aspergillus niger* followed by *Aspergillus flavus*, *Geotrichum candidum*, *Mucor heimalis*, *Aspergillus ochraceous* and *Penicillium chrysogenum*. The mycobiota of polluted soil were dominated by *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillium chrysogenum*. The percentage of by *Aspergillus terreus* and *Aspergillus flavus* were much higher in polluted soils. Rameshwara Reddy *et al*, (2018) also found *Aspergillus niger* and *Aspergillus flavus* to be amongst its a dominated fungi in pulp and paper industrial effluents. Madan *et al*., (2018) have suggested that the application of native fungal isolates might be utilized for the treatment of effluents emanating paper and pulp industries. *A. niger* has been reported to be capable of biodegrading pulp and paper mill effluents (Emtiazi *et al*., 2001; Saritha *et al*., 2010; Kamali and Khodaprast, 2015). Lokeshwari *et al*., (2013) found *A. flavus* to most effective in biodegradation of lignin of pulp industry. These three fungal species -singly or in combinations- may be tried as bioinoculants for *in situ* bioremediation of paper mill effluents. Alternatively, the biomass of these fungal species may be tried for biosorptive removal of pollutants from the effluents. *Aspergillus niger* and *Emericella nidulans* have already been recommended for the purpose (Grainger *et al*., 2011; Singhal *et al*., 2016).

As far as the effect of effluents on soil pH is concerned, studies by different worked have yielded variable results. Reddy *et al*., (2013) and Harshini *et al*., (2014) did not observe any observable effect. Kannan and Oblisami (1990), Singh *et al*, (2013) have recorded an increase in the pH of soils under the impact of paper mill waste water. In the present study also, pH of polluted soil was much greater (8.35) than that of garden soil (7.22).

REFERENCES

Almeida, I.C.C., Fernandes, B.A., Júlio Neves, J.C.L., Ruiz, H.A., de Lima, T.L.B. and Hoogmoed, W. (2017). Soil Quality after Six Years of Paper Mill Industrial Wastewater Application. *Revista Brasileira de Ciência do Solo*, **(4):** article number: e0160017.

Anonymous (2018). Pulp and paper Normalization Document and Monitoring and Verification Guidelines. Bureau of Energy Efficiency, Government of India.

Anonymous (2020). Paper and Pulp Market To Reach USD 679.72 Billion By 2027 | Reports and Data. *Globe Newswire*, (March 16, 2020), New York.

Camberato, J.J., Gagnon, B., Angers, D.A., Chantigny, M.H. and Pan, W. L. (2006). Pulp and paper mill by-products as soil amendments and plant nutrient sources. *Canadian Journal f Soil Science*, **(86)**: 641-653.

Chaudhry, S. and Paliwal, R. (2018). Techniques for Remediation of Paper and Pulp Mill Effluents: Processes and Constraints. In *Handbook of Environmental Materials Management* (Ed. Hussain C.) Springer, Cham. https://doi.org/10.1007/978-3-319-58538-3_134-1.

Dhevagi, P. and Oblismani, G. (2008). Impact of paper mill effluent on groundnut soil microflora and soil enzymes. *Asian Journal of Microbiology, Biotechnology & Environmental Sciences Paper*, **(10)**: 371-377.

Emtiazi, G., Naghavi, N.S. and Bordbar, A.K. (2001). Biodegradation of lignocellulosic waste by *Aspergillus terreus*. *Biodegradation*, **(12)**: 259-263.

Gilman, J.C. (1957) *A Manual of Soil Fungi* (Revised 2nd Edn), Oxford & IBH Publishing Co., New Delhi, 392 pp

Grainger, S., Fu, G.Y. and Hall, E.R. (2011). Biosorption of Colour-Imparting Substances in Biologically Treated Pulp Mill Effluent Using *Aspergillus niger* Fungal Biomass. *Water Air and Soil Pollution*, **(217)**: 233-244.

Harshini, T., Anusha, B., Saida, N.L. and Venkateswar Reddy, K. (2014). Influence of Paper And Pulp Industry Effluents On Physico-Chemical & Biological Properties Of Soil. *International Journal of Bioassays*, **(3)**: 3408-3412.

Kamali, M. and Khodaparast, Z. (2015). Review on recent developments on pulp and paper mill wastewater treatment. *Ecotoxicology and Environmental Safety*, **(114)**: 326-342.

Kannan, K. and Oblisami, G. (1998). Influence of irrigation with pulp and paper mill effluent on soil chemical and microbiological properties. *Biology and Fertility of Soil*, **(10)**: 147-201.

Kumar, V., Chopra A.K., Kumar, S., Singh, S. and Thakur, R.K. (2015). Effects of pulp and paper mill effluent disposal on soil characteristics in the vicinity of Uttaranchal Pulp and Paper Mill, Haridwar (Uttarakhand), India. *International Journal of Agricultural Science Research*, **(4)**: 117-125.

Lokeshwari, N., Sriniketh,G., Joshi, S.G., Inamdard, S., Srikanth, B., Bashirahmed and Sushma, L. (2013). Isolation of fungi for delignification of Pulp and Paper mill effluent. *International Journal of Current Engineering and Technology*, special issue, 124-128.

Madan, S., Sachan, P. and Singh, U. (2018). A review on bioremediation of pulp and paper mill effluent – An alternative to conventional remedial technologies. *Journal of Applied and Natural Science*, **(10)**: 367 – 374.

Martin, P. (1998). River pollution in India: An Overview. *Employment News*, **(82)**: 1-2.

Nagamani, A., Kumar, I.K. and Manoharachary, C. (2006). Hand Book of Soil Fungi, I.K. International Publishing House Pvt Ltd, New Delhi, India.

Rameswara Reddy, N., Srinivas, G. and Rajakumar, R. (2018). Isolation and identification of microorganism from pulp and paper mill effluent contaminated soil. International journal of basic and applied research, **(8)**: 91-97.

Reddy, K.V., Vijayalakshmi, T., Giri, A. and Bhavani, A.G. (2013). Effect of Paper Industry Effluents on Physico-chemical and Biological Properties of Soil**.** *The International Journal of Innovative Research in Science Engineering and Technology*, **(2)**: 3811-3815.

Singh, S.K. (2007). Effect of irrigation with paper mill effluent on the nutrient status of soil. *International Journal of Soil Science*, **(2)**: 74-77.

Singh, P.K., Ladwani, K. and Deshbhratar, P.B. and Ramteke, D.S. (2012). Impact of paper mill wastewater on soil properties and crop yield through lysimeter studies. *Environmental technology*, **(34)**: 599-606.

Singhal, A., Jha, P.K. and Thakur, I.S. (2016). Biosorption of pulp and paper mill effluent by *Emericella nidulans*: isotherms, kinetics and mechanism. *Desalination and water treatment*, **(57)**: 22413-22428.

Vara Saritha, V., Maruthit, Y.A. and Mukkanti, K. (2010). Potential fungi for bioremediation of industrial effluents. *Bio Resources*, **(5)**: 8-22.

Waksman, S.A. (1917). Is there any fungal flora of the soil? *Soil Sci*. **(2)**: 103–155.