International Journal
of Research in
Pharmacy and Science

Research Article

Analysis of metal ions physicochemical parameters and mycocommunities from industrial effluents of Bareilly

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ABSTRACT

The physicochemical parameters, concentration of heavy metals and mycocommunities in industrial effluents of Bareilly has been investigated and presented in this paper. Samples from 15 different industrial sites of Bareilly were assessed for their physiochemical parameters. pH was found to vary between 6.4 to 9.1, temperature between 27.1- 35°C, EC, TDS and salinity of the studied sites ranged between 465.1-1321.3 µs cm^{-1}, 482- 1030 ppm, 1.0-6.8 respectively. The concentration of heavy metals was estimated by Atomic Absorption Spectrophotometer (ECIL 4141). Ca, Mg, Cd, Mo, Ni , Pb and Zn were found in the range of 34.92 to12.1, 6.19 to 0.24, 3.940 to 0.015, 2.721 to 0.124, 3.32 to 0.02, 0.01 to 0.70 and 3.26 to 3.0 µg/ml respectively. Cd, Pb and Ni were present above the permissible limit given by National Environmental Regulation (1999) and W.H.O water quality standard. Isolation of mycocommunities from collected samples were undertaken by direct and dilution plate method on YpSs medium. 14 fungi were isolated from collected samples. Among them *Aspergillus niger, Aspergillus flavus, Rhizopus oryzae, Penicillium chrysogenum* and *Humicola grisea* were more prevalent.

Key words: *Heavy metals; AAS; YpSs medium; Physicochemical Parameters; Fungi.*

INTRODUCTION

Rapid industrial development is directly related with the economic growth of country. Industrial development results in generation of polluted effluents, which are being directly discharged into the surroundings land, Agricultural field, surface water, irrigation channel and finally rivers after partial treatment or without any kind of treatment. These effluents may cause extinction of many organisms (both aquatic and terrestrial) because of presence of many toxic and hazardous substances which are also harmful to human health[1, 2, 3]. Among these contaminants, heavy metals are the most hazardous pollutant because they are non degradable and get accumulate to the toxic level and cause chronic poisoning in plants and aquatic animals[4].
Any toxic metal may be called heavy metals irrespective of their density or atomic mass[5]. Heavy metals are the member of an ill defined subset of elements that exhibit metallic properties. These include the transition metal, some metalloids, lanthanides and actinides. Any metal or metalloid may be considered a contaminant if it occurs in a concentration that causes a detrimental effect on human or environment[6].
High concentration of heavy metals in soil and water may suppress the growth of microorganisms. Hence the frequency and occurrence of microorganisms in industrial areas get affected because of toxic metals. But in recent years microbial metal accumulation received much attention due to the potential use of microbes like bacteria, fungi and yeast for treatment of metal polluted waste and water.
Effluents of chemical industries contain high concentration of hazardous heavy metals such as Cd, Hg, Ni, Cr, Pb, As, Zn. Once heavy metal enters the food chain, they may be accumulated in the human

body through the process of biomagnification through food chain. Metals such as zinc, copper, chromium and manganese are essential nutrients however an increase in their uptake beyond the permissible limit can become toxic[7].

The objective of present study is to determine the physicochemical parameters and presence of heavy metals in industrial effluents and their effect on the growth and occurrence of mycocommunities in the habitat, which might be helpful in their remediation.

MATERIAL AND METHOD

Physicochemical parameters

Forty five samples from 15 industrial sites were collected in different months and their physiochemical parameters were analyzed with the help of conductivity meter (PCS tester 35 WP).The industrial sites used were **S1-** Vinayak oil industry, **S2-** Aromatic and allied chemicals, **S3-** Opal soya food, **S4-** Shivam essential oils, **S5-** Camphor and allied products ltd, **S6-** Rama shyam paper industry, **S7-** Indian surgical, **S8-** kelidable fertilizer, **S9-** Golden soap industry, **S10-** Brindawan bevarages (coca cola), **S11-** Rattan sugar industry, **S12-** Prakash bakery, **S13-** Pashupati plywood, **S14-** N.P Agro industries ltd, **S15-** B.L Agro oils ltd.

The organic matter of effluent was evaluated by Walky and Black method[8]. 0.5 ml water sample was taken by micro pipette in 500 ml conical flask and add 10 ml of potassium dichromate solution and shake with swirling motion. Thereafter, 20 ml conc. H_2SO_4 was added. This suspension was kept for 30 minutes and 200 ml of distilled water was added to it, subsequently, 10 ml of 85% o-H_3PO_4 (ortho phosphoric acid) and 0.2 gm of sodium fluoride was added. Thereafter, 30 drops of diphenyl amine indicator was added. The above contents was titrate against 0.5 N Ferrous ammonium sulphate solution until the colour of mixture changed in dull green to bright green as end point. Similar procedure was followed with a blank preparation as well. The per cent organic matter (OM %) was calculated using following equation-

$$\text{OM (\%)} = 6.791/V\ [1 - T1/T2]$$

Where,
OM = Organic matter
V = water samples (ml)
T1 = Volume of titrate used against sample (ml)
T2 = Volume of titrate used against water blank (ml)

Analysis of industrial effluent for metal concentration:

Concentration of different metal ions present in the sample was analyzed with the help of AAS. The samples prior to analyze were digested by Nitric-perchloric acid digestion method[9]. 25 ml of sample was transferred into 250 ml Erlenmeyer flask and evaporated by heating on hot plate. After the entire quantity of water samples were dried, it was cooled and 0.5 ml of perchloric acid and 5 ml nitric acid (conc.) were added to it and evaporated till the dark brown color fainted, cooled again and 2.5 ml of concentrated nitric acid was again added. Heated again, rest the material changed to light yellow/colorless. At this stage, 5 drops of perchloric acid was added. Evaporated gently on a hot plate until fumes of perchloric acid no longer comes out. After cooling, it was diluted with 50 ml double distilled water and boiled again for 2-3 minutes. Digested samples were analyzed by AAS for different heavy metals.

Isolation of fungal species:

Fungal forms were isolated by direct and dilution plate method on YpSs medium from the collected samples. Media contained (per liter of distilled water) yeast extract, 4.0 gm; $MgSO_4$. $7H_2O$, 0.5 gm; K_2HPO_4, 1.0 gm; Soluble starch, 15.0 gm; Agar - Agar, 20.0 gm and pH was adjusted as 7.0. Direct and diluted (1/10, 1/100 and 1/1000) water samples used for isolation. Triplicate plates were taken for each enrichment of sample. Petriplates were incubated at 45 °C and observed for seven days regularly after every 24 hrs. Appearing fungal colonies were isolated and transferred on to agar slants in pure forms and identified.

RESULT AND DISCUSSION

Physiochemical analysis of industrial effluents

Data on physiochemical analysis of the tested samples are presented in table -1, it is apparent from tabulated data that pH ranged between 6.4 to 9.1, temperature from 27.1 to 35° C. EC and TDS were found between the limits of 465.1 to 1321.3 µs cm^{-1}, and 482 to 1030 respectively. Total amount of organic matter and salinity varied between 0.52-1.82%, and 1.0- 6.8% respectively.

Heavy metal analysis of industrial effluents

Concentrations of different heavy metals detected from industrial effluents are presented in table 2 with their accepted values as given by W.H.O, water quality standard[10] and National Environmental Regulation Act[11].

Table 1: Physicochemical parameter and number of fungi from different industrial sites of Bareilly

Collection sites	pH	Temperature (°C)	Month	Organic matter (%)	Salinity (%)	EC (µs cm^{-1})	TDS (PPM)	Total no. of Fungi
S1	8.0	31.2	August	0.89	4.8	985.1	700	8
S2	7.6	35.0	August	1.07	3.9	812.5	560	10
S3	7.5	33.0	August	1.04	4.2	560.7	868	6
S4	7.3	29.0	September	1.11	5.9	868.8	590	12
S5	8.0	30.1	September	0.53	2.2	804.5	1030	10
S6	9.1	32.1	August	0.73	1.0	465.1	635	8
S7	8.0	28.2	August	0.52	6.8	823.7	812.4	11
S8	7.8	27.1	September	0.95	2.1	465.5	619	7
S9	7.9	35.4	October	0.58	2.6	1122.8	482	15
S10	6.4	31.6	October	0.73	3.5	765.3	629	6
S11	7.5	29.8	October	1.05	2.4	1127.1	785.3	9
S12	7.0	31.3	October	0.94	1.9	1321.3	985	12
S13	7.3	32.0	November	1.17	2.7	658.7	689	10
S14	9.1	28.6	November	1.82	4.3	1254.6	893.5	8
S15	8.0	27.2	December	1.73	1.5	728.9	874	10

Table 2 - Concentration of metals (µg/ml) in different industrial water samples

Collection sites	Ca	Mg	Cd	Mo	Ni	Pb	Zn
S1	25.73	0.342	0.015	0.124	0.678	0.020	1.725
S2	13.36	0.43	0.121	0.520	1.848	0.06	0.85
S3	32.29	0.725	0.142	1.320	2.32	ND	1.032
S4	34.92	2.252	0.245	1.410	0.417	0.025	1.38
S5	12.01	1.560	0.275	0.750	0.305	0.015	0.43
S6	33.93	0.427	ND	0.177	0.462	0.08	1.92
S7	15.28	0.619	2.721	2.721	1.243	0.70	2.312
S8	13.42	4.705	3.041	1.717	0.025	0.16	2.024
S9	25.92	3.810	3.940	2.293	1.6	0.24	3.26
S10	17.92	1.24	0.230	0.483	3.32	ND	1.36
S11	24.30	1.43	ND	0.236	0.157	0.026	3.10
S12	26.10	0.24	2.07	1.631	0.32	2.235	0.521
S13	30.71	6.19	3.32	1.020	1.43	0.21	1.43
S14	28.92	1.19	1.70	0.204	3.19	0.01	1.38
S15	19.29	2.95	0.290	1.449	2.30	0.11	1.01
Min	**12.01**	**0.24**	**0.015**	**0.124**	**0.025**	**0.01**	**0.43**
max	**34.92**	**6.19**	**3.940**	**2.721**	**3.32**	**0.70**	**3.26**
W.H.O Water quality standard (2004)	**75**	**30**	**-**	**.07**	**0.02**	**0.01**	**3.0**
National environmental regulation (1999)	**-**	**30**	**2**	**-**	**-**	**-**	**-**

Fig. 4: CVD drugs in the form of tablet

***Calcium (Ca)*:** Hardness of water is generally attributed by Ca. The concentration of Ca in industrial effluent was found higher 34.92 in S4 while the lowest one is recorded in S5. Ca is essential component for normal growth and development of plants and is the main constituents of bones and teeths in humans and it also has many metabolic functions. Generally there is no adverse impact of calcium in higher concentration but sometime kidney stone has been reported because of excess calcium. It's increasing intake help in preventing osteoporosis and lowering of blood pressure[12].

***Magnesium (Mg)*:** Among the industrial effluents the concentration of Mg in S13 was found to be very high 6.19 µg/ml and the lowest in S12 i.e. 0.24 µg/ml. This value is within the recommended permissible limit (30 mg/l) IS. 10500, (1992-1993)[13] and W.H.O. Dietary dose of Mg does not cause any health risk however pharmaceutical dose of Mg in supplement can impose adverse effect such as hypotension, nausea, vomiting, facial flushing, urine retention, lethargy, muscle weakness, respiratory depression, arrhythmia, cardiac arrest, coma, and death[14].

Table 3 - Isolated fungi from different industrial sites and their per cent frequency

Fungal isolate	Frequency (%)
Zygomycotina	
Rhizomucor species	6
Mucor species	9
Ascomycotina	
Chaetomium thermophilum	12
Emericella nidulans	4
Thermoascus aurantiacus	10
Talaromyces emersonii	4
Deutromycotina	
Aspergillus flavus	12
A. niger	15
A. fumigatus	13
A. terreus	5
Humicola grisea	12
H. insolens	6
Penicillium spinulosum	8
P. chrysogenum	21

***Cadmium (Cd)*:** Industrial waste water was found to contain Cd as high as 3.940 µg/ml from Golden Soap Industry i.e. (S9) which is more than tolerable limit (2 mg/l) as given by National Environmental Regulation[11]. S1 contain the lowest value of Cd which is safe as compared to the threshold limit given by IS10500 (1992- 1993)[13]. No traces of Cd were recorded in S11 Ratan sugar industry. Cadmium is suspected to be carcinogenic; has a long biological half life (< 10 years). High concentration of cadmium causes failure of kidney in humans. It accumulates in kidney for a long time causing impairment of the renal tract of kidney[15, 16].

***Molybdenum (Mo)*:** Among the industrial site the Mo concentration has been found to ranged between 2.721µg/ml to 0.124 µg/ml. Highest concentration of Mo was recorded in S7 (Indian surgical) and the lowest at S1 (Vinayak oil industry). Trace amounts of molybdenum are necessary for human health. Residences are advised to avoid the Mo exposure by not consuming water having Mo above the Wisconsin health advisory level of 90 µg/l.

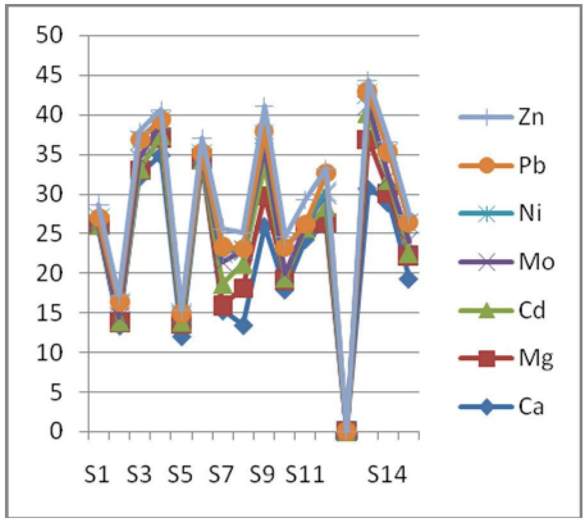

Figure 1 - Metal concentration in fifteen industries

***Nickel (Ni)*:** Concentration of nickel in industrial waste water has found to vary from 0.025 µg/ml to 3.32 µg/ml. The maximum concentration of Ni has been observed in Brindawan beverages (S10) and minimum 0.025 µg/ml in Kelidable fertilizer (S8). The acceptable limit for nickel is 0.02mg/l[10]. Nickel concentration found in studied site was much higher than the acceptable limit. The most common harmful effect of nickel in humans is an allergic skin reaction in those who are sensitive to nickel. Nickel is an allergen as well as a potential immunomodulatory and immunologic agent in humans[17].

***Lead (Pb)*:** lead compounds are reported to be mutagenic and genotoxic causing disturption of enzymatic function and chromosomal aberration[18]. Excess of lead concentration in human may cause liver, kidney, immune system, cardiovascular system and gastrointestinal damage[19, 20]. Lead exposures to early childhood are said to be associated with retarded cognitive development and learning disability[21]. The highest lead concentration 0.70 µg/ml was observed from effluent of industry S7 (Indian surgical) and the lowest 0.01 µg/ml was recorded from S14 (NP Agro Industry). This value is slightly higher as compare to acceptable value for lead given by W.H.O[10].

Zinc (Zn)**:** Zn is one of the important trace elements that have a vital role in several metabolic processes in plant and animals but its higher concentration may be harmful and toxic for health. Among the industrial effluent the Zn concentration was found to be the highest 3.26 µg/ml in S9 (Golden soap industry) and was lowest 0.43 µg/ml in S5 (Camphor and Allied Products ltd). Result showed that Zn concentration was slightly higher than the W.H.O water quality standard, which is 3.0 mg/L.

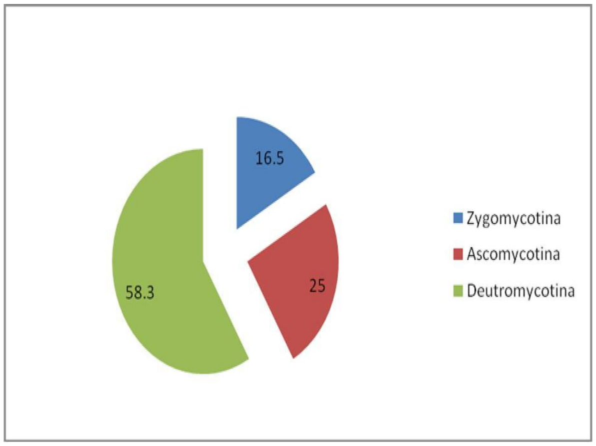

Figure 2. Frequency of isolated fungi

Isolation and identification of mycocommunities

A total of 14 fungi were isolated from the collected samples of industrial effluents, of which two fungal forms belonged to Zygomycotina (16.5 %) four to Ascomycotina (25 %) and eight belonged to Deutromycotina (58.3%). Among Deutromycotina species of *Aspergillus* were most frequent. *Penicillium chrysogenum* was encountered in the highest frequency followed by *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium spinulosum*, and *Aspergillus terreus* was in least frequency. Among Ascomycotina *Chaetomium thermophilum* was most prevalent followed by *Thermoascus aurantiacus* and *Emericella nidulans* and *Talaromyces emersonii*. Two member of Zygomycotina were observed, of which *Mucor* was frequently isolated followed by *Rhizomucor* species.

CONCLUSION

The industries dispose a huge amount of liquid waste through waste water system, which flows down to the sewer, ultimately to the river and other waste system. The observed concentration of metals like calcium, magnesium, zinc and molybdenum were within the acceptable limits given by W.H.O and National Environmental Regulation in industrial waste water. But Ni, Pb and Zn have concentration higher the recommended value as given by W.H.O and National Environmental Regulation[11]. So the ground water suffers at a great risk of being contaminated with these metals discharge in industrial effluents. Therefore it is advised that the effluents should be further treated so that it will reduce the concentration of trace metal which are hazardous for human and animals as well as for society.

A total of 14 fungi were isolated from industrial effluents (table 3). These isolated fungal forms occurred in different frequency. This may be due to different physicochemical parameters of the collected samples. Tabulated data showed that high frequency of fungi were isolated from the sites having high temperature and organic matter. This observation was also confirmed by Maheshwari[21] and Anastasi et al[22]. Least number of fungi was encountered from the sites having low organic matter and high salinity. Which indicate that these are the unfavorable condition for microbial growth. These results indicate that pH, temperature, salinity and organic matter play a key role on growth of microbes[23]. The Highest frequency of species of *Aspergillus* and *Penicillium* was recorded from Industrial sites having higher concentration of toxic metals like cadmium lead and nickel i.e. S8, S9, S10, S12, S13, S14 and S15. Which indicate that these fungal species may have ability to tolerate high concentration of toxic metals. Different industrial sites have different frequency of fungi this may be because of different concentration of metal ions at each sites and their role in metabolism of fungi. Fungi are known to tolerate the heavy metals[24, 25] and can remove the metals by the process of Biosorption. So we will use the above isolated fungi in our proposed work for mycoremediation of heavy metals in order to clean the environment.

ACKNOWLEDGEMENT

We are very thankful to State Government for funding this project under the scheme of centre of excellence.

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