Chapter 5

Results and Discussion

This chapter deals with the results generated out of extensive field study that is starting from the selection of study sites, recording of physico-chemical parameters, isolation-selection-culturing- identification of fungal strain, setting several experimental protocols, steps for bioremediation/ bioaccumulation/antibacterial activities and biomonitoring study.

5.1. Physiography of Subarnarekha River

The Subarnarekha river basin is sited in the middle of $21^{0}33'$ to $23^{0}32'$ North latitude and $85^{0}9'$ to $87^{0}27'$ East longitude covering 0.6% of the geographical area of India. The total annual yield of water about 7940 million m^3 is flowing within the river basins (Giri et al., 2013).

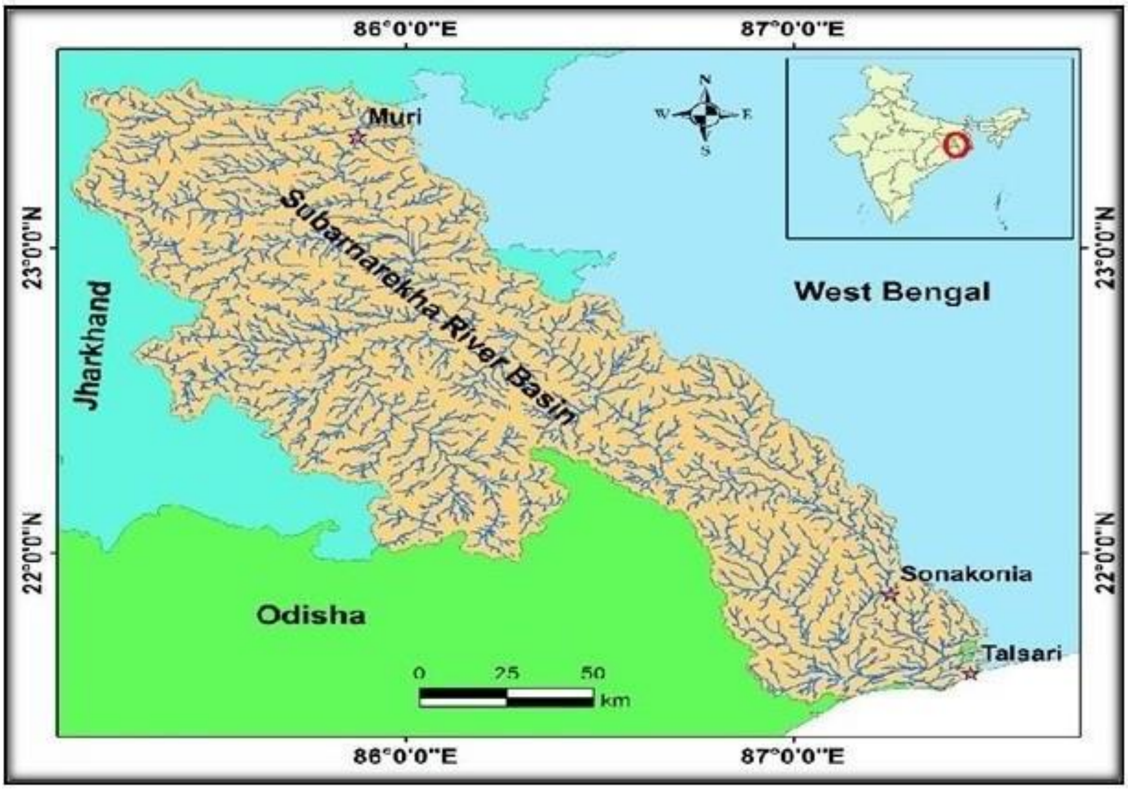


Figure 5.1: Location map of Subarnarekha river basin, India

It is a transboundary river with both freshwater and estuarine influences. It originates from the

Nagri, Ranchi flowing through three states of India such as Jharkhand, Odisha and West Bengal and ends to the Bay of Bengal at Talsari, Odisha (Chakraborty et al., 2013). These three states of south-east part of the India topographically possess the high land of laterite uplands like plateau which occupy a large portion of geomorphic surface of these states (Figure 5.1). The riverine ecosystem are inhabited by a variety of detrivores. It has shown a group of ecological niches where most important fungi and bacterial resources played a prime role. Beside, this river basin along with watersheds and nearby terrestrial landscapes are utilized by a huge number of mineral-based mines and industries in the upstream at Jharkhand state, India, both working and uncontrolled with environmental hazards.

The first study site, at upstream for the present research whose local named as Muri (S-I) of Purulia district are characterized in having so many industries and thereby prove, the river with discharge of industrial effluents especially from the drainage of copper mines. In the middle stretch of the river in Midnapore (West) District West Bengal, India, the second study site, whose local named as Sonakonia (S-II) are free from industrial activities and brackish water influence but are characterized by massive agricultural activities. At extreme downstream of the river the third study site whose local named as Talsari (S-III) of the state of Odisha, India is located at the confluence of the Bay of Bengal with Subarnarekha estuary. It is also under threat of heavy metals discharge mostly out of ecotourism. In S-I, S-II and S-III, the river banks are endowed with a number of nullahs carrying waste water from adjoining human settlements (Figure 5.2). Seasons are pronounced as monsoon (July to October with heavy rainfall and high temperature), post monsoon (November to February with low rainfall and moderate temperature) and premonsoon (March to June with least rainfall and highest temperature).

5.2. Water and Soil quality parameters: Seasonal study on selected study sites

5.2.1. Seasonal fluctuation of water quality parameters from Subarnarekha river

The physicochemical parameters were studied during the whole study period of 24 months from three sampling sites of the Subarnarekha river basin.

Figure 5.2: Representation of selected study sites (SI, S-II and S-III)

5.2.1.1. pH

The pH is the most important factor for monitoring metal precipitation, and sorption. pH being an important water quality parameter also influence the diversity and distribution of organisms by acting on the eco-physiology and also play role in the mobilization along with the accumulation of heavy metals. The seasonal fluctuations of pH of water of three different study sites have been presented in Tables 5.1-5.3. The average pH of water showed a fluctuation in between from 6.2 (post-monsoon 2013-2014) to 7.5 (pre-monsoon 2014) at S-I, from 6.3 (post-monsoon, 2013-14) to 7.6 (monsoon 2013, pre-monsoon 2014) at S-II and from 6.1 (monsoon, 2013) to 7.6 (pre-monsoon 2014) at S-III. Acidic pH helps to heavy metal mobilization. The concentration revealed a higher release rate of metals in the sediment-water system at lower pH than higher pH.

Figure 5.3: Sampling from study site of Subarnarekha river basin

5.2.1.2. Temperature (°C)

Temperature is considered to be one of the most important abiotic factors in the ecological investigation. The temperature being an important ecological parameter determines several chemical reactions in the river water and also regulates the physiological metabolism and distribution of various organisms in the aquatic system. Particularly in fungal ecology as it governs different metabolic enzyme-based activities of living organisms. In the present investigation, a definite seasonal trend of temperature variation has been observed with the highest temperature during pre- monsoon and lowest during post-monsoon. Seasonal variations of temperature of water of three study sites during the study period (July, 2012 to June, 2014) have been presented in Tables 5.1-5.3. Temperature revealed a wide range of variation with a minimum temperature of 14.5^{0}C (post-monsoon 2012-13) and that of a maximum of 34.2^{0}C (pre-monsoon 2013) at S-I; from 18.5^0 C (post-monsoon 2012-13) to 33.7^{0}C (pre-monsoon, 2013) at S-II and from 17.5^{0}C (post-monsoon, 2012-13) to 31.8^{0}C (pre-monsoon, 2014) at S-III respectively. From the correlation coefficient analysis, it has been found that many fungal species of three different study sites showed a positive and negative correlation with water temperature.

5.2.1.3. Alkalinity (mg/l as $CaCO_3$)

Alkalinity of water developed because carbonate and bicarbonate salts are acted as a buffer against acidic pH that promotes the growth and propagation of the aquatic organisms. Seasonal variations of alkalinity of water of three study sites are being presented in Tables 5.1-5.3. The values of alkalinity as recorded during the study period were found highest during the monsoon period and lowest during the post-monsoon period. The alkalinity of water displayed a variation from a minimum of 70.7 mg/l (post-monsoon 2012-13) to a maximum of 98.2 mg/l (monsoon 2013) at S- I; from 63.7 mg/l (post-monsoon 2012-13) to 86.8 mg/l (monsoon, 2012) at S-II and from 65.8 mg/l (pre-monsoon, 2013) to 85.2 mg/l (post-monsoon, 2013-14) at S-III respectively.

5.2.1.4. Ca-Hardness (mg/l as $CaCO_3$)

Seasonal variations of Ca-hardness of water have been presented in Table 5.1-5.3. The values of Ca- hardness of water as recorded during the present study were found to increase during premonsoon period and decrease during the post-monsoon period. Ca-hardness of river water ranged from a minimum of 47.1 mg/l (post-monsoon, 2013-14) to a maximum of 82.1 mg/l (pre-monsoon, 2013) at S-I; from 44.8 mg/l (post-monsoon, 2012-13) to 59.4 mg/l (monsoon, 2012) at S-II and from 48.6 mg/l (post-monsoon, 2013-14) to 77.9 mg/l (premonsoon, 2014) at S-III.

5.2.1.5. Mg-Hardness (mg/l as $CaCO_3$)

Seasonal fluctuations of Mg-hardness of water of three different study sites showed a general trend of increasing during the post-monsoon period and decreasing during the monsoon period which have been presented in Table 5.1-5.3. The values of Mg-hardness of water showed a fluctuation from 2.32 mg/l (monsoon, 2013) to 8.98 mg/l (monsoon, 2012) at S-I; from 2.15 mg/l (monsoon, 2012) to 9.32 mg/l (post-monsoon, 2012-13) at S-II and from 1.04 mg/l (monsoon, 2013) to 6.39 mg/l (post- monsoon, 2013-14) at S-III.

5.2.1.6. Total Hardness (mg/l as $CaCO_3$)

The hardness of water is related to salt concentration. Seasonal variations of total hardness (TH) of water of three different study sites have been shown in Table 5.1-5.3. The recorded total hardness showed a general trend of increasing in the pre-monsoon period and decreasing in the monsoon period. Total hardness fluctuated from a lowest of 63.8 mg/l (post-monsoon 2013-14) to a highest of 105 mg/l (pre-monsoon 2014) at S-I; from 62.7 mg/l (monsoon, 2013) to 93.6 mg/l (post-monsoon, 2013-14) at S-II and from 56.2 mg/l (monsoon, 2013) to 96.5 mg/l (premonsoon, 2014) at S-III.

5.2.1.7. Total Dissolved Solid (mg/l)

Total dissolved solids (TDS) are the combination of completely inorganic and organic materials in water in the form of ionized, micro-granular or molecular suspended form. Due to the increase of dissolved solid TDS should be increased, for that reason growth of aquatic life can be hampered. The values of total dissolved solid ranged from 96 mg/l (post-monsoon, 2012-13) to 173 mg/l (pre- monsoon 2014) at S-I; from 104 mg/l (post-monsoon, 2012-13) to 222 mg/l (pre-monsoon, 2014) at S-II and from 185 mg/l (post-monsoon, 2013-14) to 336 mg/l (pre-monsoon, 2014) at S-III. Those included in Table 5.1-5.3.

5.2.1.8. Total Suspended Solid (mg/l)

Some solid persist in suspended condition due to their more hydrophilic in nature. In general condition, those do not precipitate. Seasonal variations of total suspended solid (TSS) of water have been presented in Table 5.1-5.3. TSS of water displayed a variation from a minimum of 49.2 mg/l (pre-monsoon, 2012-13) to a maximum of 88 mg/l (pre-monsoon, 2014) at S-I; from 68 mg/l (post-monsoon, 2012-13) to 173 mg/l (monsoon, 2012) at S-II and from 152 mg/l (post-monsoon, 2013-14) to 290 mg/l (monsoon, 2012) at S-III.

5.2.1.9. Biological Oxygen Demand (mg/l)

A huge amount of oxygen requires for the respiration of aquatic lives which indicates the biologica l oxygen demand (BOD) of water. Seasonal variations of BOD have been shown in Table 5.1-5.3. According to APHA the acceptable limit for biological oxygen demand is 4 mg/l (APHA 2005). The recorded BOD revealed an increasing trend during premonsoon period and a decreasing trend during the monsoon period. BOD of river water ranged from the lowest value of 0.6 mg/l (monsoon, 2013) to 2.04 mg/l (pre-monsoon, 2014) at S-I; from 1.04 mg/l (post-monsoon, 2012-13) to 1.9 mg/l (pre monsoon, 2013) at S-II and from 1.01 mg/l (pre-monsoon, 2014) to 1.52 mg/l (post-monsoon, 2012- 13) at S-III. High BOD of S-III indicate the amount of aquatic life were more amount.

5.2.1.10. Chemical Oxygen Demand (mg/l)

Chemical oxygen demand (COD) is an important indicator of organic as well as inorganic pollut ion in the river, which estimated throughout the period of this study at all sites. Seasonal variations of COD of water have been represented in Tables 5.1-5.3. The values of COD displayed a variation froma minimum of 20.1 mg/l (monsoon, 2012) to a maximum of 40.6 mg/l (pre-monsoon, 2014) at S-I; from 26.8mg/l (post-monsoon, 2012-13) to 48.5 mg/l (monsoon,

2012) at S-II and from 25.2 mg/l (post-monsoon, 2012-13) to 54.3 mg/l (pre-monsoon, 2013) at S-III.

5.2.1.11. Dissolved Oxygen (mg/l)

Solubility of dissolved oxygen (DO) in water is determined by the temperature and growth of aquatic plants and algae. Seasonal fluctuations of DO of water of three different study sites showed a general trend of increasing during the monsoon period and decreasing during the post-monsoon period which have been presented in Table 5.1-5.3. DO fluctuated from a lowest of 5.2 mg/l (monsoon, 2012) to the highest of 7.2 mg/l (post-monsoon, 2013-14) at S-I; from 4.5 mg/l (post-monsoon, 2012-13) to 7.85 mg/l (monsoon, 2012) at S-II and from 5.5 mg/l (monsoon, 2012) to 6.9 mg/l (pre-monsoon 2013) at S-III.

5.2.1.12. Total kjeldahl nitrogen (mg/l)

Nitrogen mainly participates in protein andnucleic acid anabolism. High amount of nitrogen indicated that the population of aquatic organisms should be high. Seasonal variations of total Kjeldahl nitrogen of water have been represented in Table 5.1-5.3. The values of total Kjeldahl nitrogen displayed a variation from a minimum of 1.28 mg/l (monsoon, 2013) to a maximum of 1.98 mg/l (post-monsoon, 2012-13) at S-I; from 0.78 mg/l (monsoon, 2013) to 1.68 mg/l (monsoon, 2012) at S-II and from 1 mg/l (monsoon, 2012) to 2.65 mg/l (pre-monsoon, 2013) at S-III.

5.2.1.13. Chloride content (mg/l)

Seasonal fluctuation of the chloride content of water of three different study sites showed a general trend of increasing during the premonsoon period and decreasing during the monsoon period which have been presented in Table 5.1-5.3. Available chloride content displayed a variation from a minimum of 17.6 mg/l (monsoon, 2012) to a maximum of 37.6 mg/l (pre-monsoon, 2014) at S-I; from 24.8 mg/l (monsoon, 2013) to 38.2 mg/l (monsoon, 2013) at S-II and from102 mg/l (pre- monsoon, 2013) to 148 mg/l (post-monsoon, 2012-13) at S-III.

5.2.1.14. Total phosphate phosphorous (mg/l)

Phosphate can participate for the synthesis of DNA and RNA, which indicated the multiplication of living organisms. Seasonal fluctuations of total phosphate phosphorus of water of three different study sites showed a general trend of increasing during the post-monsoon period and decreasing during the monsoon period which have been presented in Table 5.1-5.3. Total phosphate phosphorus of river water ranged from a lowest value of 0.05 mg/l (monsoon, 2012; pre-monsoon, 2014; monsoon 2013) to 0.09 mg/l (several season) at S-I; from 0.32 mg/l (pre-monsoon, 2014) to 0.71 mg/l (pre-monsoon, 2014) at S-II and from 0.12 mg/l (post-monsoon, 2012-13) to 0.64 mg/l (pre-monsoon, 2013) at S-III. This reflection have supported by Dutta and Malhotra (1986) who have also established the same outcome at Ramsagar reservoir. Sarwade et al., (2015) found some negligible effect of phosphate on the diversity of living organisms (Pakhira, 2019).

5.2.1.15. Total coliform and faecal coliform

The bacteriological analysis of water samples of Subarnarekha river, collected from the three study sites i.e. S-I; S-II, and S-III have been presented in Table 5.1-5.3. Total coliform bacteria exhibited a minimum of 540 MPN/100ml (monsoon, 2012) to a maximum of >2400 MPN/100ml (several seasons) at S-I; from 920 MPN/100ml (post-monsoon, 2012-13 and 2013-14) to >2400 MPN/100ml (several seasons) at S-II and from 900 MPN/100ml (post-monsoon, 2012-13 and post-monsoon 2013-14) to >2400 MPN/100ml (several seasons) at S-III. The observation of total coliform bacteria showed a general trend of increasing during the monsoon period and decreasing during the post- monsoon period.

Amount of fecal coliform determined the water polluted by fecal matter. Generally in pre-monsoon water level should be low but the concentration of pollutant (fecal matter) was high.

Seasonal variations of fecal coliform bacteria have been shown in Table 5.1-5.3. Faecal coliform bacteria displayed a variation from a minimum of 350 MPN/100ml (post-monsoon, 2012-13 and 2013-14) to a maximum of >2400 MPN/100ml (several seasons) at S-I; from 540 MPN/100ml (several seasons) to 1600 MPN/100ml (several seasons) at S-II and from 540 MPN/100ml (several seasons) to 1600 MPN/100ml (several seasons) at S-III. The observed fecal coliform bacteria exhibited a general trend of increasing during the monsoon period and decreasing during the post-monsoon period.

Table 5.1: Seasonal physico-chemical parameters of water at study site-I (S-I) during the study period July, 12-June, 14

S1		pH	TEM (^{0}C)	ALKA	CaH	MgH	TH	TDS	TSS	BOD	COD	DO	TKN	CHL	TPP	FCOL	TCOL
MON, 12	Range	6.9-7.3	28.4-30.5	91.6-97.2	57.7-72.5	2.64-8.98	68.5-98.5	140-156	59-85	0.7-1.1	20.1-35.8	5.2-6.96	1.34-1.58	17.6-20.30	0.05-0.09	540-1600	920->2400
	Mean	7.07	29.57	94.63	65.32	4.75	83.5	149	71.5	0.88	28.57	6.05	1.44	19.07	0.071	1165	2030
POM, 12-13	Range	6.5-7.1	14.5-20.8	70.7-78.2	48.3-54.7	3.05-6.95	67.2-76.8	96-108	49.2-68.9	1.58-1.96	34.8-38.5	6.24-7.1	1.69-1.98	24.6-30.8	0.07-0.09	350-920	540-1600
	Mean	6.77	17.37	74.52	51.6	4.85	71.5	101.5	58.27	6.67	36.4	6.67	1.81	27.6	0.075	587.5	1165
PREM, 13	Range	6.6-7.4	17.2-34.2	74.8-84.5	74.2-82.1	5.83-6.15	98.1-107	158-172	78-86.8	1.24-1.85	33.8-38.5	5.35-5.98	1.56-1.87	29.8-36.4	0.06-0.08	1600->2400	1600->2400
	Mean	7.07	29	78.45	78.15	5.99	102.7	165	81.65	1.55	35.9	5.52	1.7	33.20	0.066	1800	2200
MON, 13	Range	6.4-6.9	29.1-30.8	93.4-98.2	55.7-70.3	2.32-8.66	65.2-96.4	146-162	55-82	0.6-1.2	21.5-36.7	5.4-6.85	1.28-1.56	19.8-22.2	0.05-0.09	540-920	1600->2400
	Mean	6.6	30.12	95.87	64	5.64	81.3	155.25	70.92	0.91	29.75	0.91	1.41	20.92	0.071	730	2000
POM, 13-14	Range	6.2-6.7	15.2-20.4	74.2-81.9	47.1-52.4	4.07-5.08	63.8-73.2	102-112	51-66.8	1.62-1.88	32.1-36.8	6.18-7.2	1.71-1.94	26.7-31.3	0.07-0.09	350-920	920->2400
	Mean	6.42	17.57	77.77	49.62	4.67	68.8	106.75	59.32	1.75	34.7	6.71	1.83	29.12	0.082	682.5	1630
PREM, 14	Range	6.7-7.5	29.8-33.5	76.8-86.5	72.9-81.2	5.37-5.78	96.4-105	155-173	76-88	1.36-2.04	34.2-40.6	5.68-6.4	1.48-1.85	28.4-37.6	0.05-0.08	1600->2400	1600->2400
	Mean	7.1	31.9	81.1	77.25	5.58	100.15	164.75	81.72	1.40	36.57	1.71	1.70	33.22	0.069	2200	2200

Table 5.2: Seasonal physico-chemical parameters of water at study site-II (S-II) during the study period July, 12-June, 14

S II		pH	TEM (°C)	ALKA	CaH	MgH	TH	TDS	TSS	BOD	COD	DO	TKN	CHL	TPP	FCOL	TCOL
MON, 12	Range	7.1-7.5	28.3-30.2	79.6-86.8	55.1-59.4	2.15-2.46	63.9-68.5	155-201	149-173	1.2-1.8	31.3-48.5	5.21-7.85	0.84-1.68	26.7-36.9	0.44-0.64	920-1600	1600->2400
	Mean	7.32	29.27	83.07	56.62	2.32	66.12	177.25	159.75	1.5	39.45	6.71	1.22	31.55	0.54	1260	2200
POM, 12-13	Range	6.4-7.24	18.5-20.8	63.7-75.1	44.8-53.2	7.54-9.32	76.3-91.4	104-142	68-82	1.04-1.24	26.8-31.2	4.5-5.9	1.32-1.50	28.5-31.9	0.41-0.69	540-1600	920->2400
	Mean	6.89	19.72	69.2	49.8	8.10	83	122	74	1.15	28.7	5.17	1.43	30.07	0.53	900	1460
PREM, 13	Range	6.8-7.6	30.8-33.7	67.9-70.5	51.8-55.2	7.73-8.66	83.6-88.2	188-208	145-165	1.3-1.9	39.8-44.4	6.13-6.65	1.45-1.59	27.5-33.5	0.33-0.68	540-1600	1600->2400
	Mean	7.22	32.12	68.92	53.07	8.04	86.05	197.5	156	1.55	42.3	6.37	1.52	30.5	0.49	1165	2000
MON, 13	Range	6.9-7.6	27.2-30.4	76.2-86.4	53.2-57.4	2.2-2.64	62.7-68.2	148-205	138-165	1.3-1.7	29.5-45.6	5.45-7.23	0.78-1.58	24.8-38.2	0.42-0.68	540-1600	1600->2400
	Mean	7.27	28.87	81.22	55.12	2.36	64.8	178.5	147.75	1.5	36.37	6.45	1.18	31.97	0.53	900	2000
POM, 13-14	Range	6.3-7.2	19.2-20.4	65.9-74.2	46.8-56.8	7.08-8.98	75.8-93.6	112-148	78-92	1.08-1.32	27.2-31.5	4.62-5.85	1.41-1.58	26.5-36.5	0.39-0.66	540-1600	920->2400
	Mean	6.72	19.77	70	51.62	8.06	84.67	130	85	1.20	29.5	5.17	1.5	30.75	0.522	995	1830
PREM, 14	Range	6.7-7.6	30.1-32.9	68.2-72.4	48.1-54.9	8.13-8.61	82.7-90.2	192-222	145-162	1.4-1.85	38.5-45.2	6.21-6.71	1.51-1.62	29.6-37.2	0.32-0.71	540-1600	1600->2400
	Mean	7.1	31.67	70.07	51.87	8.44	86.47	207	153.75	1.60	41.9	6.52	1.56	33.6	0.55	1165	2000

Table 5.3: Seasonal physico-chemical parameters of water at study site-III (S-III) during the study period July, 12-June, 14

S-III		pH	TEM(^{0}C)	ALKA	CaH	MgH	TH	TDS	TSS	BOD	COD	DO	TKN	CHL	TPP	FCOL	TCOL
MON, 12	Range	6.4-7.1	27.5-30.3	76.6-80.4	50.9-52.2	1.78-3.44	58.2-66.3	255-322	242-290	1.18-1.27	43.8-54.2	5.5-6.9	1-1.18	106-136	0.21-0.39	920-1600	1600->2400
	Mean	6.85	28.67	78.75	51.5	2.604	62.17	289.25	270.5	1.22	47.92	6.12	1.065	119.75	0.30	1260	2200
POM, 12-13	Range	7-7.4	17.5-18.5	74-83.6	49.4-58.6	4.81-5.56	72.2-78.5	196-208	168-182	1.15-1.52	25.2-28.8	6.12-6.68	1.23-1.45	135-148	0.12-0.34	540-1600	920->2400
	Mean	7.17	18.22	78.3	54.5	5.19	75.77	201	174.75	1.36	27.47	6.38	1.31	141.25	0.22	900	1630
PREM, 13	Range	6.9-7.5	29.2-31.2	65.8-72.6	71.4-77.6	3.64-3.88	86.7-93.5	321-330	268-285	1.02-1.16	45.2-54.3	6.3-6.9	1.92-2.65	102-132	0.48-0.64	540-1600	1600->2400
	Mean	7.1	30.42	69.27	74.85	3.76	90.27	326	274.7	1.097	49.85	6.57	2.25	119	0.55	1165	2200
MON, 13	Range	6.1-7.2	26.8-30.8	74.8-78.5	52.1-54.8	1.04-2.93	56.2-66.8	254-318	242-282	1.22-1.39	41.6-53.7	5.6-6.5	1.1-1.24	112-138	0.28-0.41	540-1600	1600->2400
	Mean	6.7	28.57	76.57	53.27	1.949	61.22	287.25	266	1.35	47	6.075	1.18	126.5	0.34	1165	2200
POM, 13-14	Range	6.9-7.5	17.9-19.2	75.2-85.2	48.6-57.8	5.32-6.39	74.8-79.6	185-204	152-178	1.12-1.62	29.2-39.4	6.45-6.72	1.32-1.64	129-146	0.13-0.25	540-1600	920->2400
	Mean	7.15	18.47	80.5	53.07	5.78	76.77	93.5	166.75	1.427	34.82	6.58	1.482	137.25	0.195	900	1630
PREM, 14	Range	6.8-7.6	29.4-31.8	68.4-74.8	73.2-77.9	2.78-4.54	84.6-96.5	324-336	262-271	1.01-1.18	46.7-54.2	6.14-6.78	1.87-2.35	110-131	0.44-0.58	540-1600	1600->2400
	Mean	7.2	30.92	71.47	75.33	3.69	90.5	329.25	266.3	1.1	50.7	6.43	2.07	122.75	0.507	995	2000

5.3 Composition of soil sediment

Load of living organisms as well as quality and testing of water depends upon bottom and edge soil of riverian tract. Soil sediment mainly maintain pH and alkalinity of soil, those are important part of diversity of fungus. Soil sediment, the river bed sedimentological structures are important aspects in connection with the different fungal species within their habitat. Textural composition of the study sites were founded to be predominantly sandy and were observed to exhibit a vertical distributional profile due to the discharge from the rocky upper part of the river. Clay content of the sediment tended to show significant changes in different seasons and sites. Both the sand and silt content were varied in fresh water zone (S-I, S-II) to brackish water zone (S-III).

The different study sites are located along the river course of the Subarnarekha from Muri (upper course) to Talsari (lower course) with distinct terrain condition, river bed slope, and morphological structures. Therefore, the concentrations of sand, silt and clay particles within the river bed are varied from upper to lower courses. In this study, the seasonal and site specific percentage distribution are showing within the study area. From the upper to lower courses the percentage distribution of the sand size sediment is gradually decreasing from (S-I) with 97.2% to 91.7 % in S-II and 83.1 in S-III during the monsoon period. Similarly, during post-monsoon periods is varied between 95.9% (S-I) to 91.7 % (S-II) and 83.1 in S-III. Whereas per-monsoon 92.2% (S-I), 90.6 % (S-II) and 87.7% (S-III) respectively. But, the concentrations of silt and clay particles are gradually increasing towards the lower courses of the river that depicted in Table 5.4.

5.4. Identification of both point and nonpoint pollution sources

5.4.1. Non-point source

Table 5.4: Seasonal variations of soil parameters at three sites during the study period (July, 2012-June, 2014)

Site	Season	Soil pH	Soil Temperature(^{0}C)	Sand %	Silt %	Clay %	Salinity	Organic carbon	N	P	K	Total fungal count
Muri	Pre monsoon	6.1	28	92.2	4.5	3.3	21.7	0.03	0.03	0.01	0.01	23
	Monsson	6.8	26.5	97.2	1.3	1.5	10.4	0.05	0.05	0.02	0.01	25
	Post monsoon	7.3	28	95.9	1.4	2.7	15.6	0.06	0.06	0.01	0.02	24
Sonakonia	Pre monsoon	7.2	30.5	90.6	7.6	1.8	25.2	0.05	0.05	0.01	0.04	21
	Monsson	7.1	27	91.7	4.1	4.2	11.2	0.09	0.06	0.03	0.05	25
	Post monsoon	7.6	27	93.2	5.3	1.2	20.1	0.01	0.07	0.02	0.05	25
Talsari	Pre monsoon	7.9	34	87.7	2.1	10.2	30.8	0.08	0.07	0.02	0.05	23
	Monsson	7.6	30.5	83.1	4.6	12.3	15.8	0.12	0.08	0.03	0.08	26
	Post monsoon	8.2	29	85.2	3.1	11.7	25.6	0.10	0.1	0.04	0.07	27

Out of many pollutants having non-point sources such as chemical fertilizers, domestic sewage, petroleum waste persistent pesticides etc. are allowed (Figure 5.4). Several group of pathogenic and non-pathogenic microbes (fungi and bacteria) are important, which play their roles causing several water borne diseases and also serve as an ecological component for river ecosystem integrating themselves into the tropic interaction and nutrient cycling by way of decomposition process.

5.4.2. Point source

The suspended solids and heavy metal concentration in the river water are increased during the monsoon periods due to the loss through land run off, exposed solid waste dumping sites and mining activities and transportation of wastes from different industries (Figure 5.5). In the vast stretches of river basin several environmental problems have been caused for the mining of granites, basalts, quartzite, dolerite, sandstone, limestone, dolomite, gravels, and river sands (Garzanti, 2012).

Figure 5.4: Various non-point sources of pollution a. in S-I discharge of industrial waste, b. in S-II disturbance of the sediment texture and enhancement of turbulence due to sand lifting, c. in S-III discharge of fossil fuel from fishing trawler

Besides that, the domestic and industrial wastewater generated from the urban areas, along the stretch of the river after being discharged into the river pose serious pollution threats to riverine flows in the river (Pakhira, 2019). The non-judicious exploitation of natural resources of Subarnarekha river is consisting of both living (e.g. fishes and microbes) and non-living (e.g. Sand granules, soil) components that have proved to be real threats to the river basin.

Figure 5.5: Various point sources of pollution a. smoke discharge from the industries. b. Stock up of industrial waste water

In the last few decay, a sustainable environment was hampered during the unplanned development of mining and mineral industries upstream of the Subarnarekha river (Paria and Chakraborty, 2019).

5.5. Heavy metals analysis and their seasonal concentration at study sites

5.5.1. Heavy metals analysis of water

Subarnarekha river water is not only contaminated by a coliform group of bacteria but also a high risk of heavy metal contamination during effluent discharge from several mining based industries. Out of several heavy metals Cd, Hg, Pb are recalcitrant and those are more toxic to human that have been taken into consideration different heavy metals are discussed below-

Chromium (Cr)

Available chromium in water displayed the highest value of 1.01 mg/l (monsoon, 2013) that of the lowest value of bdl (monsoon, 2012) at S-I; the minimum value of 0.021 mg/l (monsoon, 2013) and that of a minimum of 0.20 mg/l (post- monsoon, 2013-14) at S-II and highest of 0.05 mg/l (several seasons) and that of the lowest bdl (monsoon, 2012) at S-III respectively. Seasonal variations of chromium in water are being presented in Table 5.5-5.7.

Lead (Pb)

The minimum and maximum values of lead contents in water were estimated to be as 0.25 mg/l (monsoon, 2012) to 0.55 mg/l (premonsoon 2013) at S-I, 0.43 mg/l (monsoon, 2013) to 1.52 mg/l (pre-monsoon, 2013) at S-II and 0.33 mg/l (monsoon, 2012) to 0.41 mg/l (pre-monsoon, 2013) at S-III respectively. Seasonal variations of lead contents in different study sites have been shown in Table 5.5-5.7.

Copper (Cu)

Although copper acts as cofactor of the metabolic enzyme but also a high amount of Cu are the cause of several diseases. Available copper concentration in water exhibited the lowest value of 0.02 mg/l (monsoon, 2012) and highest value of 0.09 mg/l (pre-monsoon, 2013) at S-I, from 0.06 mg/l (several seasons) to 0.09 mg/l (several season) at S-II and also minimum value of mg/l (several seasons) to that of 0.06 mg/l (post-monsoon, 2013-14) at S-III. Seasonal variation of copper in water is being presented in Table 5.5-5.7.

Zinc (Zn)

Seasonal variations of available zinc in water of three different study sites have been presented in Table 5.5-5.7. The values of zinc content ranged from 0.24 mg/l (monsoon, 2012) to 0.35 mg/l (pre-monsoon, 2013) at S-I, from 0.23 mg/l (monsoon, 2012) to 0.60 mg/l (pre-monsoon, 2013) at S-II and from 0.21 mg/l (monsoon, 2012) to 0.31 mg/l (pre-monsoon, 2013) at S-III.

Cadmium (Cd)

Seasonal variations of available cadmium in water of three different study sites have been presented in Table 5.5-5.7. The values cadmium content were fluctuated from bellow detection level (monsoon, 2012) to 0.03 mg/l (several seasons) at S-I; from 0.02 mg/l (several seasons) to 0.03 mg/l (pre-monsoon 2013 and pre-monsoon, 2014) at S-II and from bellow detection level (monsoon, 2013) to 0.03 mg/l (pre-monsoon, 2013- 2014) at S-III.

Mercury (Hg)

Monthly and Seasonal variations of available mercury in the water of three different study sites have been presented in Table 5.5-5.7. The values mercury content were fluctuated from bdl (monsoon, 2012-13) to 0.01 mg/l (several seasons) at S-I; from bdl mg/l (monsoon, 2013) to 0.03 mg/l (pre-monsoon, 2013 and pre-monsoon 2014) at S-II and from bdl (monsoon, 2012 and monsoon 2013) to 0.02 mg/l (pre-monsoon, 2014) at S-III.

5.5.2. Heavy metals analysis of soil

Soils may develop polluted by the build-up of heavy metals and metalloids by releasing from the rapidly growing industrial areas, removal of high metal wastes, mine tailings, and land application of fertilizers, sewage sludge, pesticides, wastewater irrigation, petrochemical spillage and atmospheric deposition.

Lead (Pb)

Available lead in soil was showed the highest value from 1.27 mg/l (monsoon, 2013) to the lowest value of 1.63 mg/l (post-monsoon, 2012-13) at S-I; from 0.51 mg/l (post-monsoon, 2012-13) to 1.82 mg/l (pre-monsoon, 2013) at S-II and from 1.26 mg/l (monsoon, 2012) to 1.63 mg/l (pre-monsoon, 2013) at S-III. Seasonal variations of lead in the soil are being presented in Table 5.5-5.7.

Copper (Cu)

The minimum and maximum values of the copper content in soil varied from 0.09 mg/l (pre-monsoon, 2013) to 0.39 mg/l (post-monsoon, 2012-13) at S-I; from 0.11 mg/l (post-monsoon, 2012-13) to 0.51 mg/l (Pre-monsoon, 2014) at S-II and from 0.05 mg/l (pre-monsoon, 2014) to 0.50 mg/l (pre-monsoon, 2013) at S-III. Seasonal variations of copper content in the soil in different study sites have been shown in Table 5.5-5.7.

Zinc (Zn)

Monthly and Seasonal variations of available zinc in soil of three different study sites have been presented in Table 5.5-5.7. The values of zinc content were fluctuated from 0.41 mg/l (pre-monsoon, 2013) to 0.73 mg/l (pre-monsoon, 2014) at S-I; from 0.31 mg/l (post-monsoon, 2012-13) to 0.60 mg/l (pre-monsoon, 2013) at S-II and from 0.48 mg/l (post- monsoon, 2013-14) to 0.59 mg/l (pre-monsoon, 2013) at S-III.

Chromium (Cr)

Available chromium in soil was showed the lowest value from 0.02 mg/l (several seasons) to the highest value of 0.04 mg/l (pre-monsoon, 2014) at S-I; from 0.05 mg/l (several seasons) to mg/l (pre-monsoon, 2013-14 and pre-monsoon, 2014) at S-II and from 0.04 mg/l (monsoon, 2012) to 0.05 mg/l (several seasons) at S-III. Seasonal variations of chromium in soil are being presented in Table 5.5-5.7.

Cadmium (Cd)

Seasonal variations of available cadmium in soil of three different study sites have been presented in Table 5.5-5.7. The values cadmium content were varied from 0.02 (monsoon, 2012 and post-monsoon 2012-13) to 0.03 mg/l (several seasons) at S-I; from 0.04 mg/l (several seasons) to 0.05 mg/l (several seasons) at S-II and from 0.04 mg/l (several seasons) to 0.05 mg/l (pre-monsoon, 2014) at S-III.

Mercury (Hg)

Mercury is the cause of minamata disease. Monthly and Seasonal variations of available mercury in the soil of three different study sites have been presented in Table 5.5-5.7. The values mercury content were fluctuated from 0.01 (monsoon, 2012) to 0.032 mg/l (post-monsoon 2013-14) at S-I; from 0.01 mg/l (monsoon, 2013) to 0.056 mg/l (pre-monsoon, 2014) at S-II and from 0.01 mg/l (monsoon, 2013) to 0.03 mg/l (pre-monsoon, 2013 and pre-monsoon 2014) at S-III.

Table 5.5: Seasonal variations of different heavy metals in water and soil, at S-I (Muri) duringthe study period (July, 2012-June, 2014)

Heavy metals	MON, 12		POM, 12-13		PREM, 13		MON, 13		POM, 13-14		PREM, 14	
	Water	Soil	Water	Soil	Water	Soil	Water	Soil	Water	Soil	Water	Soil
Cr	bdl	0.02	0.02	0.03	0.01	0.03	1.01	0.02	0.02	0.03	0.02	0.04
Pb	0.25	1.52	0.4	1.63	0.55	0.51	0.33	1.27	0.35	1.32	0.35	1.38
Cu	0.02	0.35	0.07	0.39	0.09	0.09	0.43	0.36	0.04	0.38	0.04	0.38
Zn	0.24	0.69	0.28	0.69	0.35	0.41	0.25	0.68	0.25	0.71	0.31	0.73
Cd	bdl	0.02	0.02	0.02	0.01	0.03	0.02	0.03	0.03	0.03	0.03	0.03
Hg	bdl	0.01	0.01	0.02	0.02	0.03	bdl	0.03	bdl	0.032	0.01	0.023

Table 5.6: Seasonal variations of different heavy metals in water and soil, at S-II during the study period (July, 2012-June, 2014)

Heavy metals	MON, 12		POM, 12-13		PREM,13		MON, 13		POM, 13-14		PREM, 14	
	Water	Soil	Water	Soil	Water	Soil	Water	Soil	Water	Soil	Water	Soil
Cr	0.03	0.05	0.03	0.04	0.04	0.05	0.021	0.05	0.20	0.06	0.03	0.06
Pb	0.45	1.52	0.46	0.51	1.52	1.82	0.43	1.54	0.46	1.64	0.44	1.72
Cu	0.06	0.45	0.09	0.11	0.49	0.49	0.06	0.43	0.06	0.44	0.07	0.51
Zn	0.23	0.55	0.29	0.31	0.6	0.6	0.25	0.53	0.29	0.55	0.3	0.58
Cd	0.02	0.05	0.02	0.05	0.03	0.04	0.02	0.04	0.02	0.04	0.03	0.05
Hg	0.02	0.052	0.02	0.05	0.03	0.06	bdl	0.01	0.02	0.042	0.03	0.056

Table 5.7: Seasonal variations of different heavy metals in water and soil at S-IIIduring the study period (July, 2012-June, 2014)

Heavy metals	**MON 12**		**POM, 12-13**		**PREM, 13**		**MON,13**		**POM, 13-14**		**PREM,14**	
	Water	Soil	Water	Soil	Water	Soil	Water	Soil	Water	Soil	Water	Soil
Cr	bdl	0.04	0.02	0.05	0.02	0.05	0.02	2.05	0.03	0.05	0.03	0.05
Pb	0.33	1.26	0.36	1.35	0.41	1.63	0.37	1.32	0.38	1.43	0.39	1.48
Cu	0.05	0.42	0.05	0.49	0.05	0.5	0.05	00.4	0.06	0.45	0.06	0.05
Zn	0.21	0.52	0.24	0.53	0.31	0.59	0.22	0.5	0.22	0.48	0.23	0.58
Cd	bdl	0.04	0.01	0.04	0.02	0.04	0.02	0.04	0.03	0.04	0.02	0.05
Hg	bdl	0.02	0.01	0.022	0.01	0.03	bdl	0.01	0.01	0.021	0.02	0.023

5.6. Fungal diversity of Subarnarekha river, India

During the whole survey period, a total numbers of 112 fungi were isolated, screened and recorded from the three sampling sites of Subarnarekha river, India). Out of all those species, 28% were found at S-I, 29 % were observed at S-II and 43 % were recorded at S-III (Figure 5.6).

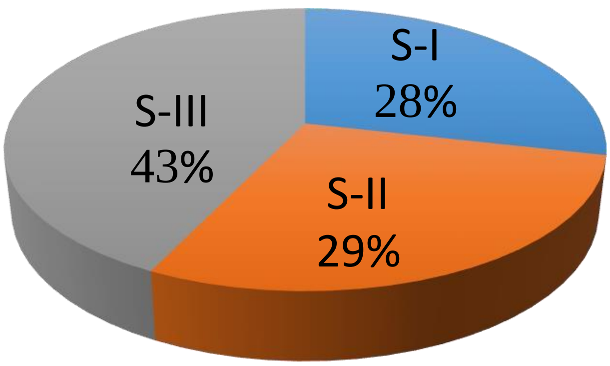


Figure 5.6: Distribution of fungi at three different study site

Most of those recorded fungal species belongs to the *Penicilium sp.* (23), *Aspergillus sp*. (27), *Pythium sp*. (21), *Fusarium sp*. (18), *Rhizopus sp.* (11) and *Tricoderma sp.* (14). In addition radar chart highlighting the distribution of fungus family revealed that the highest fungal diversity was found at S-III followed by S-II and least at S-I (Figure 5.9). Season wise fungal count is mentioned in Table 5.19.

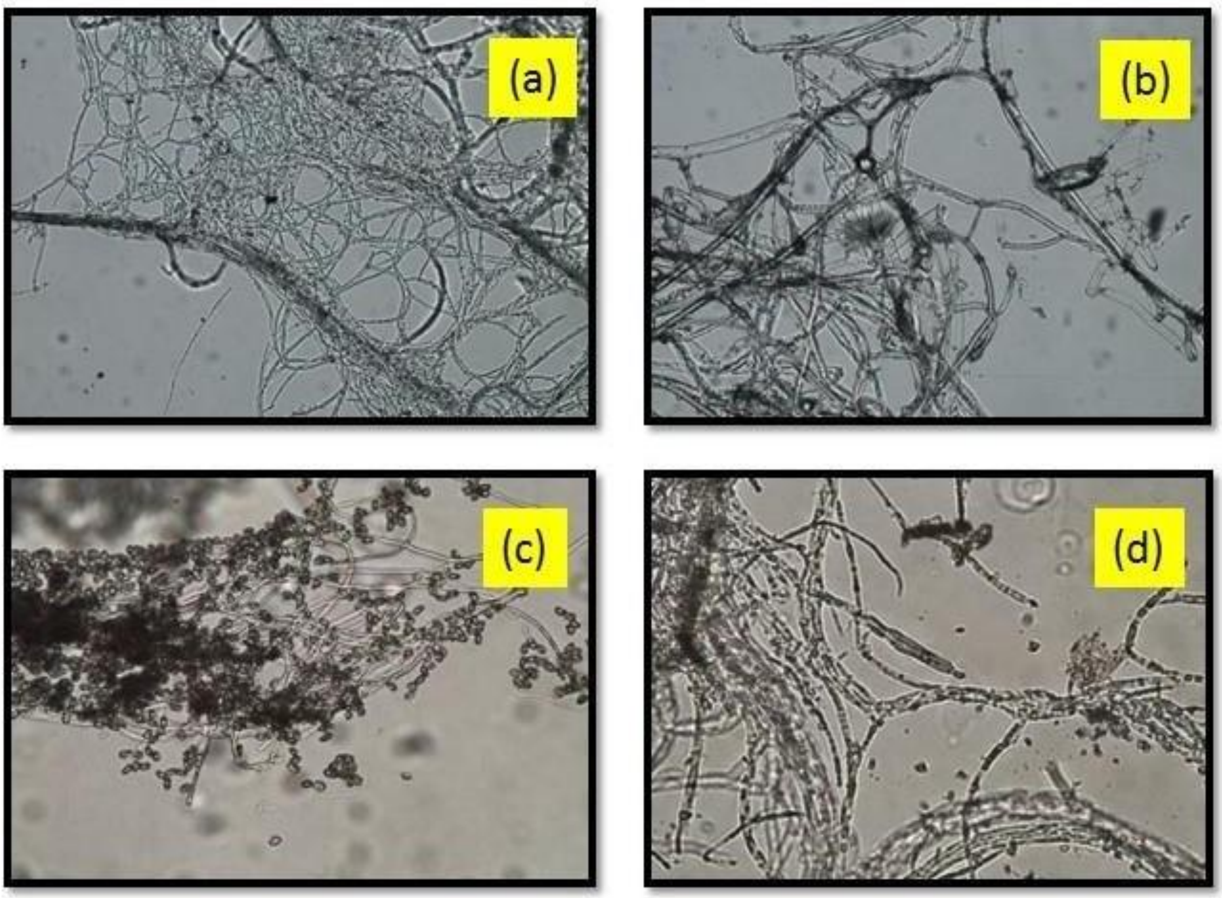


Figure 5.7: Identification of fungal strains (a, b, c and d) under phage contrast microscope

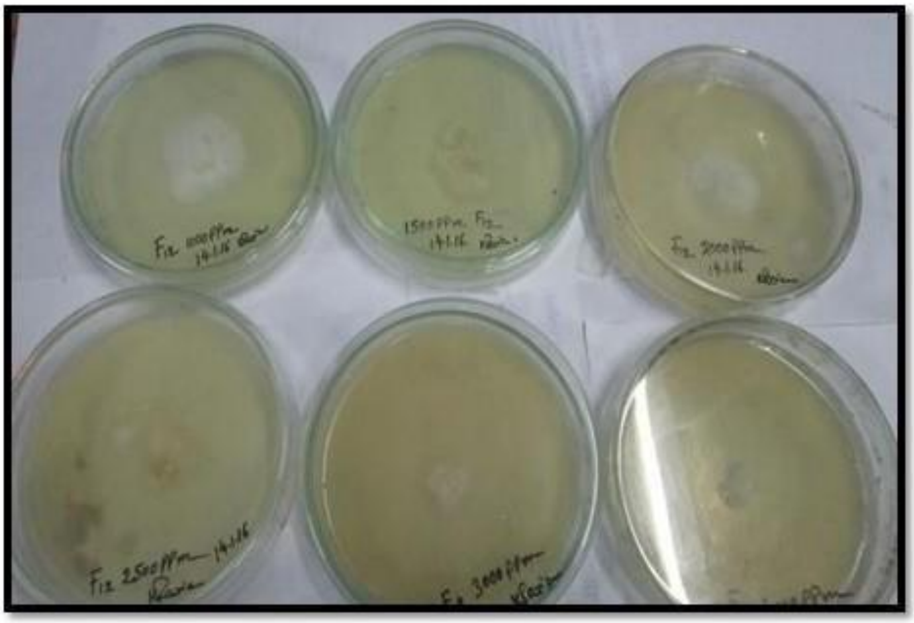


Figure 5.8: Isolation of fungi in potato dextrose agar medium

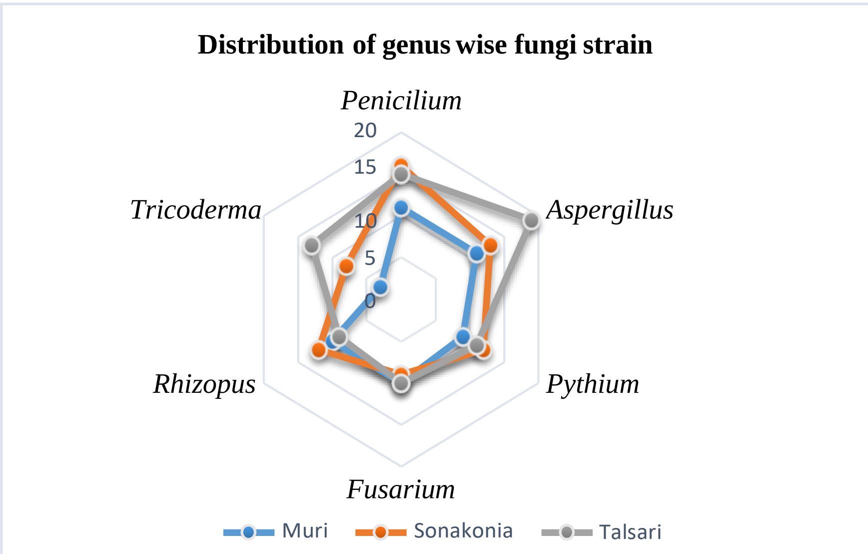


Figure 5.9: Genus wise distribution of fungal strains by radar chart

5.7. Diversity of metal tolerance fungi in different study sites of Subarnarekha river

It has a unique observation from the present study, that several fungal species have the ability to bioaccumulation and removal of heavy metals from the surrounding water.

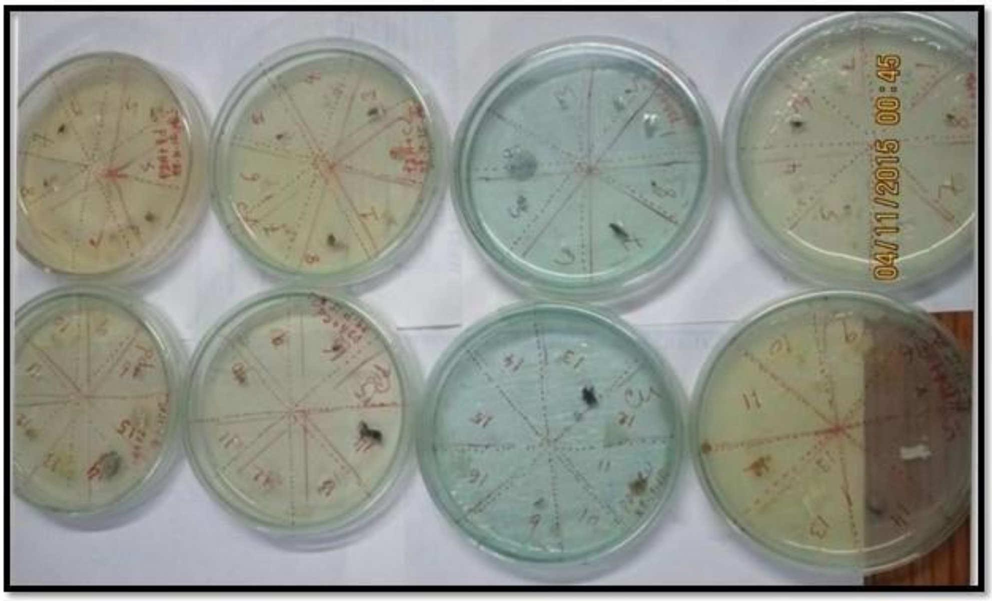


Figure 5.10: Isolation method of metal tolerant fungi

In order to remove the metals from the aqueous culture media, functional groups of fungus cell surface actas legends (Ajitha, et al., 2014). Experimental studies have revealed that such

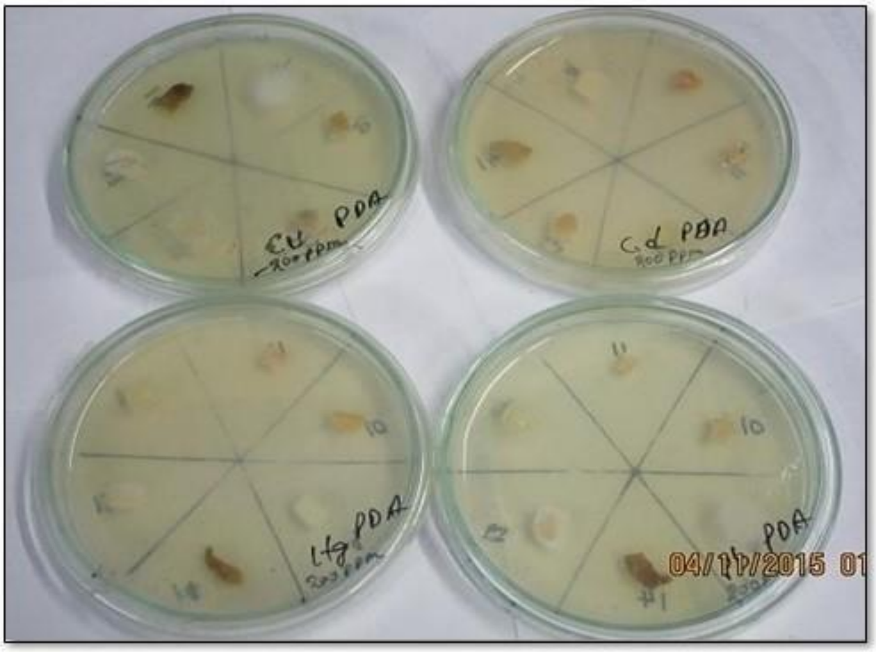


Figure 5.11: Isolation of heavy metal tolerant fungi at different metal concentrations

heavy metals tolerant fungi were not only present in the industrial effluents, but also have in sewage, sludge (Mani D. 2014).Different fungal strains tolerate lower concentration of metals but it also decrease in the presence of higher concentration heavy metal rich sampling sites (Iram et al., 2012). A number of fungi have been isolated from three stretches of the sample sites i.e. (1) S- I at upstream (2) S-II, at the middle part (3) S-III at downstream of the Subarnarekha river(Figure 5.10). Several species of soil-inhabiting fungi (Figure 5.11) viz. *Aspergillus sp.,Pythium sp., Rhizopus sp., Penicillium sp.* and *Fusarium 5sp., Tricoderma sp.* have been isolated from the collected soil samples. From which, *Aspergillus penicillioides* (F12) has exhibited maximum tolerant activities up to 1000 ppm of heavy metals of Cd (II), and Pb (II) but up to 200 ppm of Hg. This strain was identified with the ITS genetic system by amplification and sequencing. It has also been observed that *Aspergillus penicillioides* (F12) secrete huge amounts of exopolysaccharide that supports in immobilization of heavy metals and showed high adhesive activity of heavy metal. Consequently, the result demonstrates that both biomass and exopolysaccharides are responsible for heavy metal bioremediation from river bank. In this respect, various species of *Aspergillus sp*. have been described as effective heavy metals reducers (Kalin, et al., 2005). Then, the present study was also revealed the wide distribution pattern of fungi in the riverian ecosystems those were found in S-I, S-II and S-III sites.

5.8. Selection of metal tolerant fungus *Aspergillus penicillioides* for detailed study

DNA was amplified and sequenced by PCR using conserve primers of 5.8 S ITS region (Boysen et al., 1996) from the genomic DNA of isolated fungal strain F12 (Paria et al., 2018). All 5 fungal isolates were closely interrelated to *Aspergillus sp*. F12 (Deposition No. MN210327) (Figure 5.12a, 5.12b). Phylogenetic analysis of this obtained sequences showed close relatedness of *Aspergillus penicillioides* with 95% homology presenting in Figure 5.13. It has been perceived from previous studies that *Aspergillus penicillioides*, a benthic fungus had been isolated from soil sediment of Talsari (Paria et al., 2018).

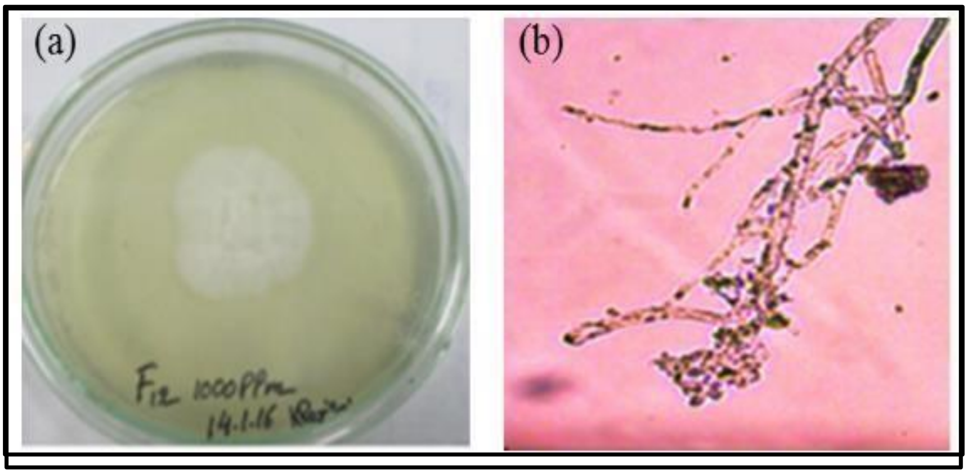


Figure 5.12: F12 strain in PDA media (a), morphological structure of F12 strain under light microscope (b)

CAAAGGGTTTGGGGCGCCCTTCTCGGGGTGTCCGTCCTCCCATCCGTGTCTATTTGT
ACCCTGTTGCTTCGGCGGGCCCGCCCTCTGTGGCCGCCGGGGGGCTTCCCTTGCCCC
CGGGCCCGTGCCCGCCGGAGACCTCAACCATGAACACTGTCTGAAGGTTGCAGTCT
GATACCGATAATAAAAATCGCCCCACTTTCAACAACGGATCTCTTGGTTCCGGCATC
GATGAAGAACGCACGAATGCGATACGTAATGTGAATTGCAAATCATGAATCATCAG
TCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGACGTCT
TGCTGCCCTCAAGCACGGTTGTGTGTGGGCCCGTCCCCTGTTCTAGGGGGGACGGG
CCCAAAGGCAGGGCGGCCCGTGTCCGGCCTCGAGCGTATGGAAGAATTTTTGTACC
GCTCTGTAGGCCGGCCACGCCTGCCACCCCATCATCTTTTTACAGTGACTCGACAGT
AGGTACCGTGACTTAACTATAAAAGCGAGGAA

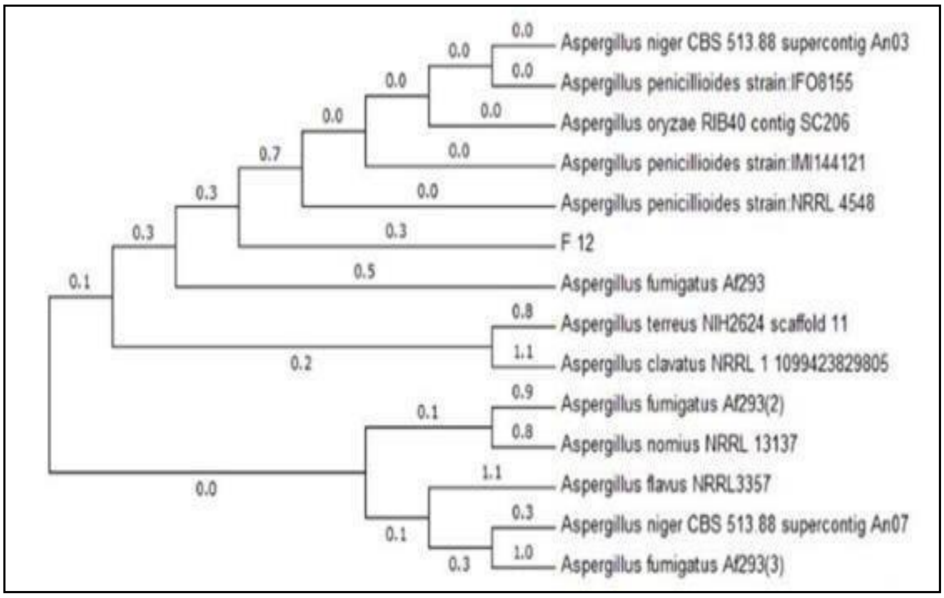


Figure 5.13: Gene sequence (a) and Phylogenetic analysis (b) of identified F12 strain (*Aspergillus penicillioides)*

5.9. Tolerance index of *Aspergillus penicillioides*

Heavy metals tolerance index found in *Aspergillus flavus*, *A. niger* (El Hameed, 2015) and *Fusarium* sp. isolated from contaminated soil (Zafar et al., 2007). By TI reduction indicates inhibitory effect of heavy metal against fungal growth (Subin and Francis, 2013).

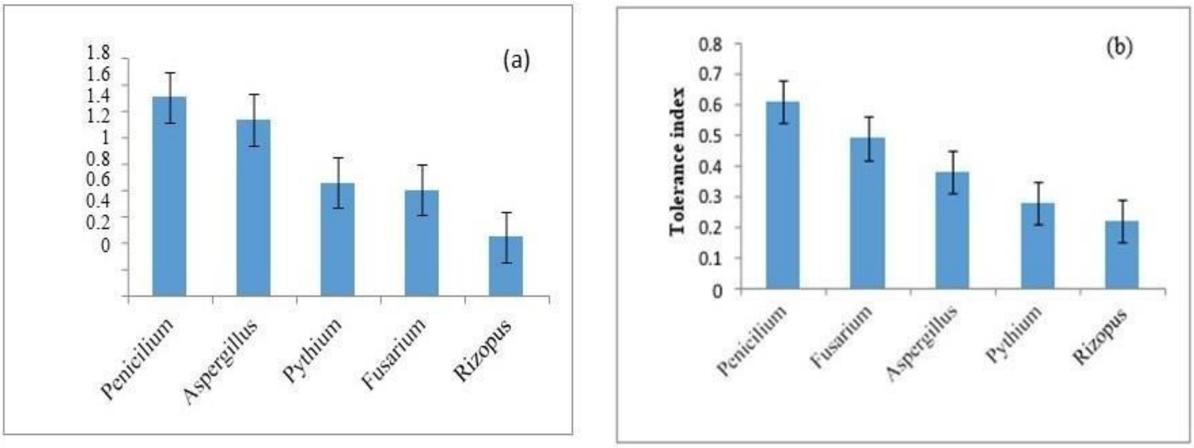


Figure 5.14: Tolerance indices of fungi against Pb (II) (500 mg/l) (a), Tolerance index of fungus against Cd (II) (500 mg/l) (b)

Fungal tolerance level was measured by T_I and MIC assays. In this study T_I of each fungus (Figure 5.14a) exhibited different orders of tolerance of Pb(II), Cd (II) and Hg (II). *Penicillium sp*. and *Aspergillus sp*. have altered up to first level of tolerance of lead followed by *Pythium sp.*, *Fusariumsp.* and *Rizopus sp*. But T_I of *Penicillium sp*. was higher than other with respect to Cd (II) followed by Pb (II) and Cd (II) (Figure 5.14b).

5.10. Fungus with potential heavy metal removing activity

Among three study sites, total fungal colony count was recorded to be higher at S-III mostly due to its eco-tonic nature considered by mixing sandy and silty sediments. The microbial growth and subsequent colony count was observed to be high in humic soil. Interestingly the fungal contamination was maximum during monsoon period having to the extreme volume stratification and mixing pollutants of water. A total of 112 fungal isolates have been effectively isolated and cultured from the water collected from three sampling sites of Subarnarekha river. Fungal isolates were successfully isolated and were grown in the culture media having lead, cadmium and mercury in proper concentration. From the preliminary study, there were 16 fungi which showed different resistance patterns against at least five of the three selected heavy metals.

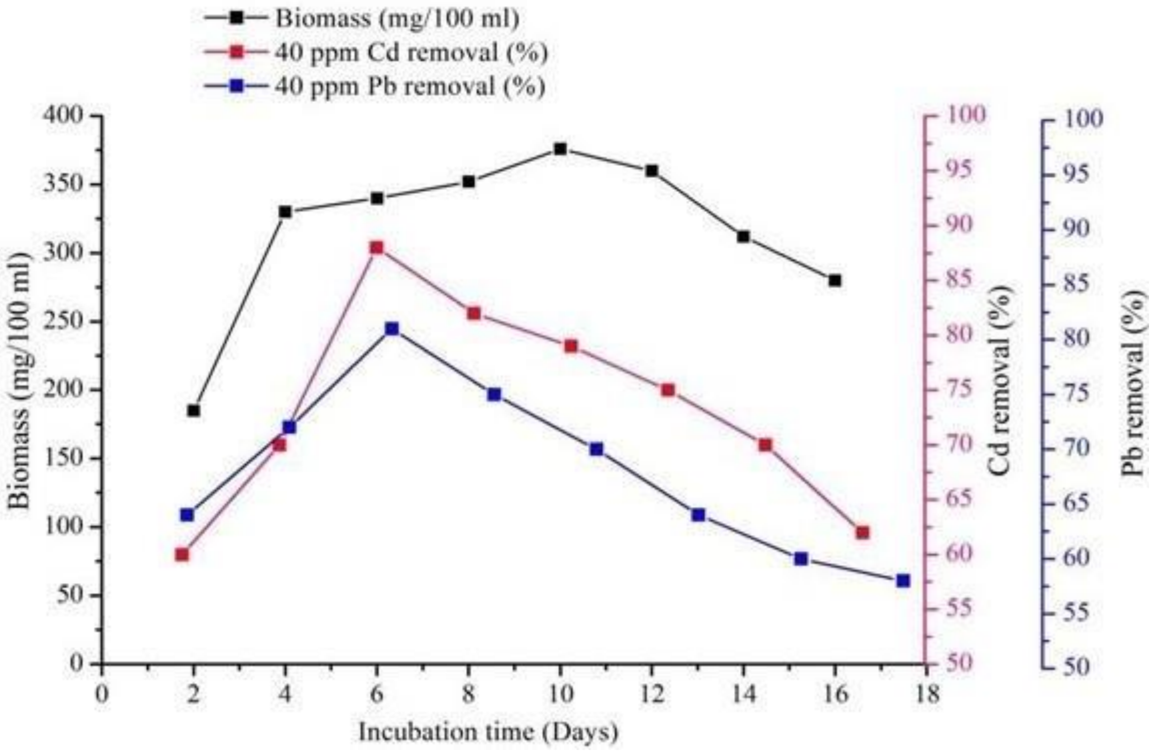


Figure 5.15: Heavy metal removal percentage with biomass concentration

All 5 isolates showed resistance against Pb (II) and Cd (II) up to 1000 ppm (Figure 5.15). Cadmium is considered as lethal since of the increasing risk after disclosure (Friberg et al., 2019). Higher concentration of Cd (II) was discharged from effluents of steel industries as well as household discharges. Lead is a neurotoxin and nephrotoxic pollutant and comes into the Subarnarekha river water by the waste of industrial effluents (Paria and Chakraborty, 2019). Among all isolates, *Aspergillus sp., Fusarium sp., Penicillium sp., Rizopous sp., Pythium sp.* have revealed heavy metal scavenging activity. Out of them, *Aspergillus penicillioides* F12 (Deposition No. MN210327) has highest heavy metals (Pb, Cd) scavenging ability in an optimum P^{H}, temperature, and time (Paria et al., 2018). The present study has also exposed that mixed solution of Pb (II), Cd (II) and Hg (II) gave the maximum scavenging activity by *Aspergillus penicillioides* F12 (Deposition No. MN210327) (Figure 5.16).

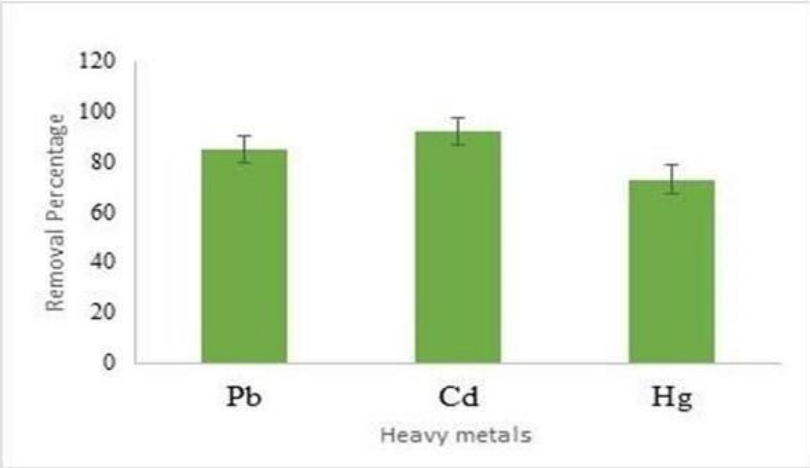


Figure 5.16: Heavy metal removal percentage by fungal EPS

5.11. Minimum Inhibitory Concentration (MIC) assay of heavy metals

Aspergillus sp. possessed marked resistance to cadmium than lead. In comparison to the other fungi exhibited more resistance to lead. Heavy metal tolerance activity of *Penicillium sp*. is higher than other fungi. MIC value of *Aspergillus sp*. is higher than other fungi against Cd (II) and other Pb (II) (Figure 5.17).

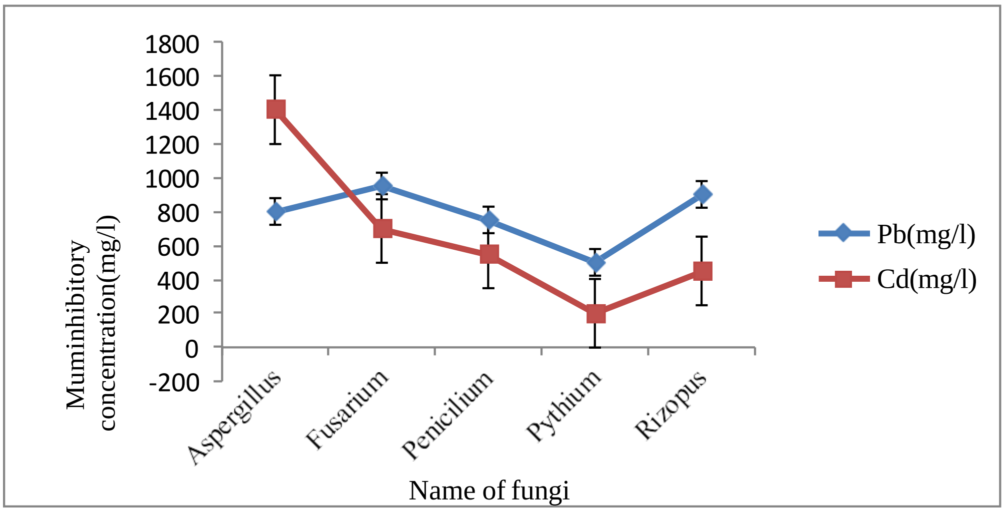


Figure 5.17: Minimum inhibitory concentrations of different fungi

5.12. Optimized process parameters for Cd (II) and Pb (II) absorption by fungal biomass and Exo polysaccharide (EPS)

In aqueous solution both Cd (II) and Pb (II) absorption were studied by dry biomass and EPS using different parameters like pH, temperature, and incubation period and biomass concentration. Maximum metal removal ability by both dry biomass and EPS against Pb(II) and Cd (II) were recorded at optimum pH of 6.0.

The outcomes from temperature variation experiments showed no significant changes in the range of 25^0C- 35^0C. Further experiments were executed at 30^0C temperature at pH of 6.0. At 30^0C higher percentage of metal removal was observed by the EPS in comparison to dry biomass at only 2 hrs of incubation period (Figure 5.18). Interestingly 90% metal removal was observed in optimized condition at a concentration of 2 mg/ml of biomass and EPS.

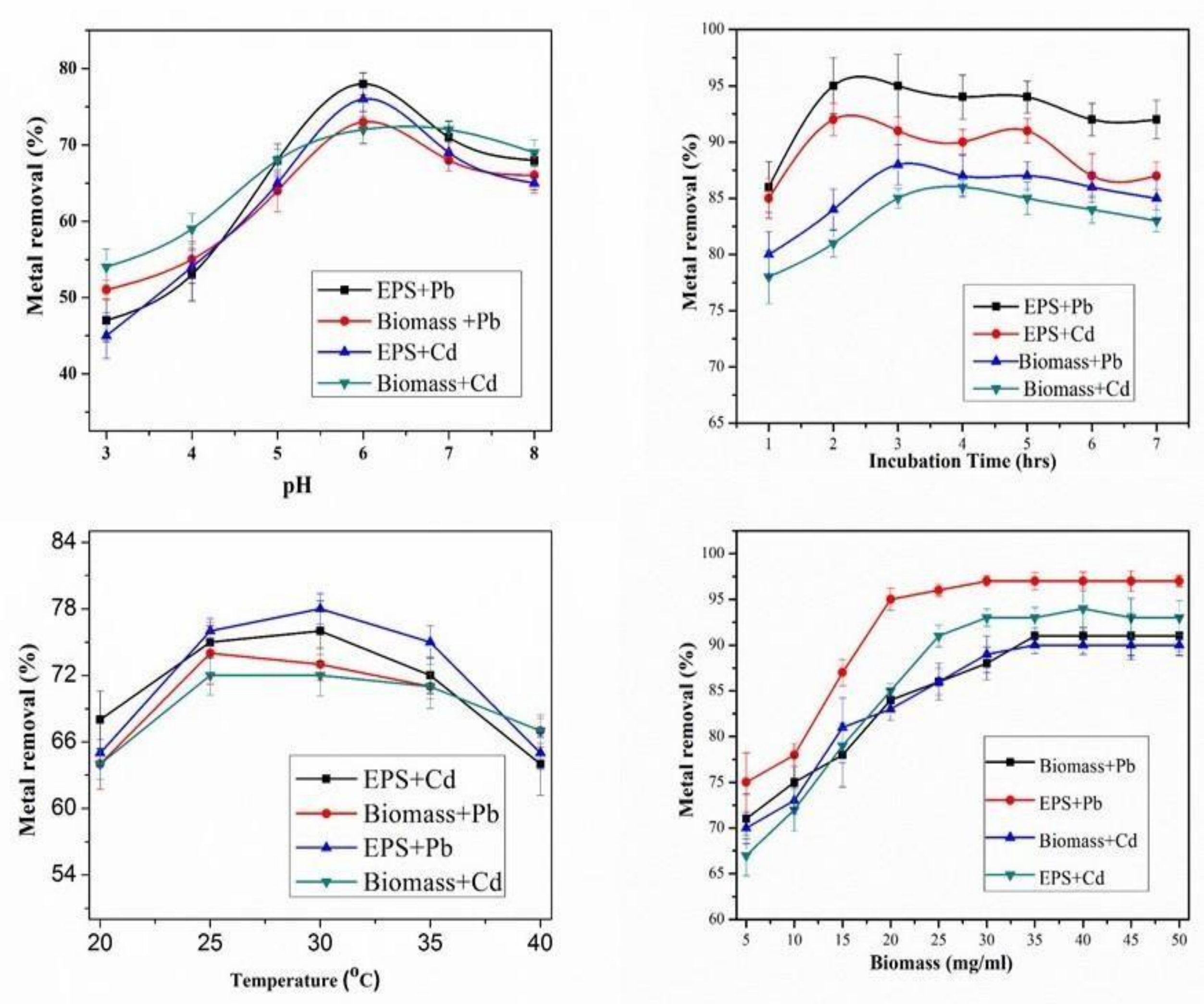


Figure 5.18: Effect of pH (a), incubation time (b), temperature (c) and biomass concentration (d) on the bio-sorption of Pb (II) and Cd (II) in respect of biomass and exo-polysacharides (EPS)

5.13. Optimization of Pb (II) absorption percentage of EPS

Generation of response curves and regression modeling

Based on a designed set of experimental data by using response surface methodology the model parameters of equation 1 was statistically determined. The BBD matrix of the three variables (pH, time and temperature) were presented in the Table 5.8 with the experimental values of Pb(II) absorption (%) of EPS. The regression equation could be obtained by applying multiple regression analysis from equation 1 and given as:

Pb (II) Absorption (%) $=+60.16-1.34A-2.27B+1.43C+0.09AB+0.08AC+0.12BC-0.102A^2-0.199B^2-0.04C^2$

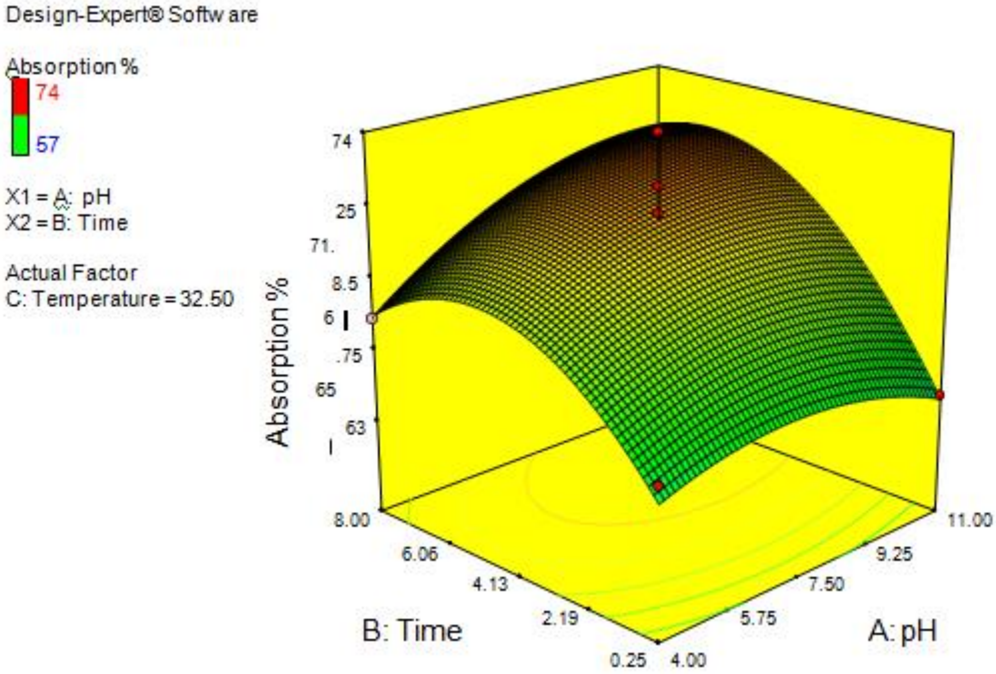
The effects of the above parameters and their interactions were evaluated (Figure 5.19). The ANOVA table (Table 5.9) was used to study the effect of each independent variable constructing a model that maximized the absorption percentage. The significance of each variable was determined by its respective p-value and F-value at a specified level of confidence. The p-value for each response was greater than 0.5, which suggests that the effect of independent variables on the response model was not statistically significant at 95% confidence level. The fit of the model was checked by the coefficient of determination (R^2) which was 0.92 and the p-value (>0.05) for the lack of fit analysis was 0.9. The value of lack of fit was non-significant, indicating that the model equations were adequate for predicting absorption percentage (%) under any combinations of variable factors.

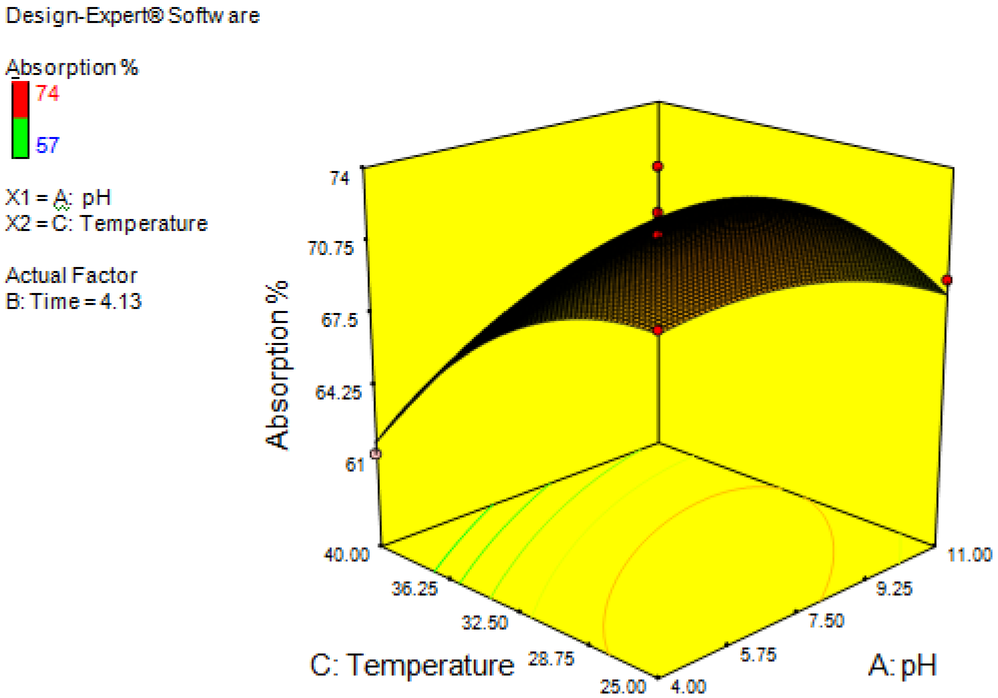
Table 5.8: Variables and their levels employed in a BB design for optimization of heavy metal absorption percentage by fungal exo-polysaccharide (EPS)

	Coded Levels		
Variables	-1	0	+1
pH	4	7.50	11
Time (hours)	0.25	4.13	8.00
Temperature(^{o}C)	25	32.50	40

Table 5.9: Analysis of variance (ANOVA) for a fitted response surface quadratic model of absorption percentage

Source	Sum of square	df	Mean square	F-value	p-value
Model	261.72	9	29.08	8.95	0.004
A-pH	8.00	1	8.00	2.46	0.16
B- Time	55.13	1	55.13	16.96	0.004
C-Temperature	45.13	1	45.13	13.88	0.007
AB	6.25	1	6.25	1.92	0.208
AC	20.25	1	20.25	6.23	0.041
BC	49.00	1	49.00	15.08	0.006
A2	6.58	1	6.58	2.02	0.198
B2	37.89	1	37.89	11.66	0.011
C2	26.32	1	26.32	8.10	0.024
Residual	22.75	7	3.25		
Lack of fit	2.75	3	0.92	0.18	0.902
Pure error	20.00	4	5.00		
Corrected Total	284.47	16			
R^2	0.920				
Adj. R^2	0.817				


Design-Expert® Software
Absorption %
74
57
X1 = A: pH
X2 = B: Time
Actual Factor
C: Temperature = 32.50
Absorption %
B: Time
A: pH
8.00
6.06
4.13
2.19
0.25
4.00
5.75
7.50
9.25
11.00


Design-Expert® Software
Absorption %
74
57
X1 = A: pH
X2 = C: Temperature
Actual Factor
B: Time = 4.13
Absorption %
74
70.75
67.5
64.25
61
C: Temperature
A: pH
40.00
36.25
32.50
28.75
25.00
4.00
5.75
7.50
9.25
11.00

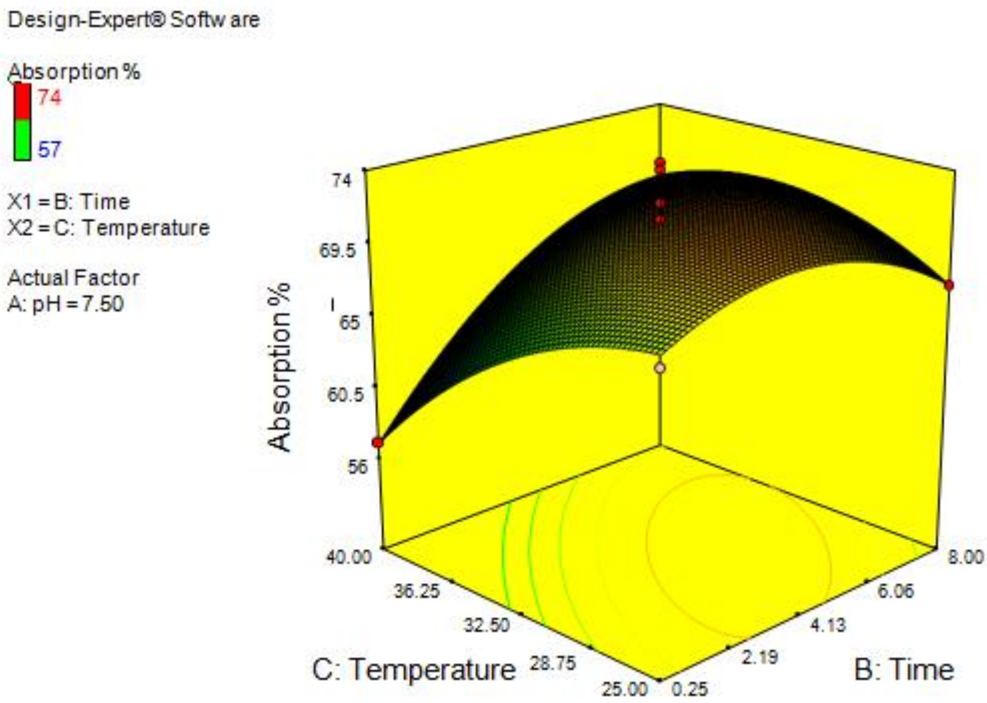


Figure 5.19: The 3D-plots showing the most important interactions, (a) between pH and time; (b) between pH and temperature; (c) between time and temperature

Table 5.10: The set of experimental variables based on Box–Behnken design and observed response of fungus absorption percentages

Run	**pH**	**Time (hours)**	**Temperature (oC)**	**Absorption %**
1	7.50	0.25	40.00	57
2	7.50	4.13	32.50	74
3	7.50	4.13	32.50	72
4	7.50	8.00	40.00	70
5	4.00	8.00	32.50	67
6	11.00	8.00	32.50	71
7	7.50	0.25	25.00	68
8	7.50	8.00	25.00	67
9	7.50	4.13	32.50	70
10	4.00	0.25	32.50	65
11	4.00	4.13	25.00	71
12	11.00	4.13	40.00	68
13	7.50	4.13	32.50	71
14	11.00	4.13	25.00	69
15	11.00	0.25	32.50	64
16	4.00	4.13	40.00	61
17	7.50	4.13	32.50	68

5.14. Light microscope and Phase contrast microscopic view of *Aspergillus penicillioides* (F12)

Bright-field microscopy is satisfactory for the direct examination of specimens if the light intensity is decreased. However, phase-contrast microscopy is better because structures are more clearly delineated without loss of light.

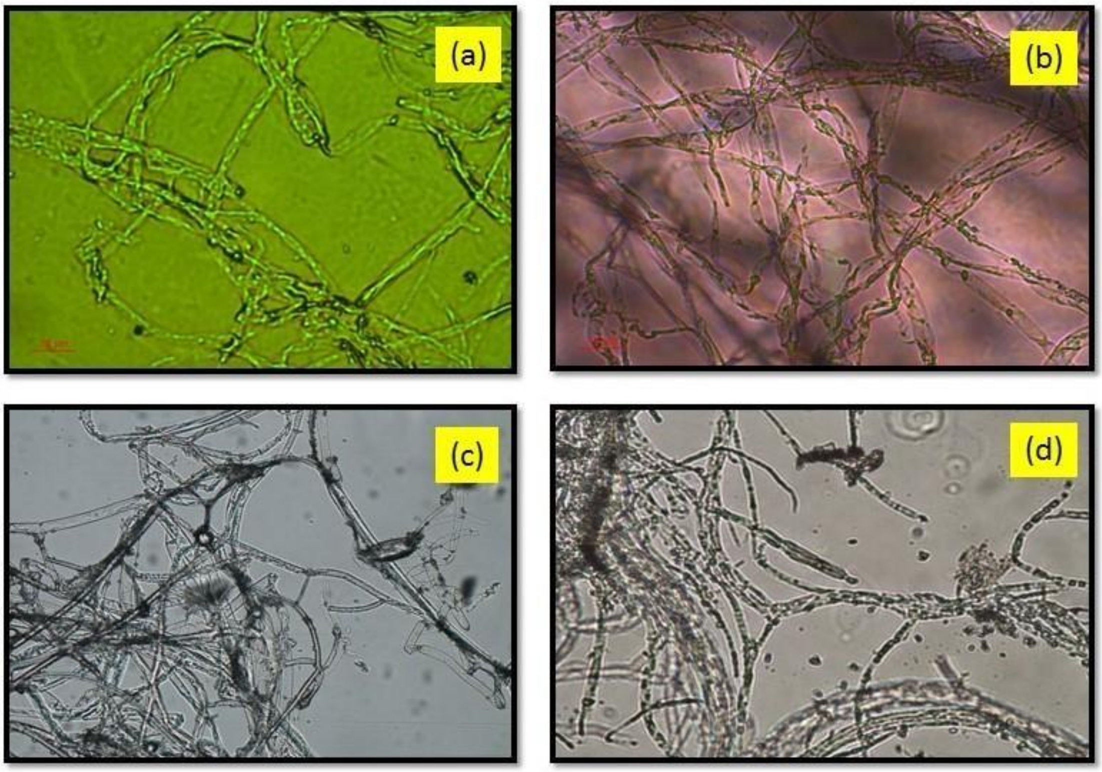


Figure 5.20: Identification by Light Microscope (a), (b); phase contrast microscope (c), (d) of *Aspergillus penicillioides*

All specimens are examined under a phase-contrast microscope with an x40 Phaco objective. The certain homogeneous morphological structures including cytoplasm, regular and defined septa, and various organelles were found (Figure 5.20).

5.15. Scanning electron microscopic (SEM) view and energy-dispersive X-ray (EDEX) analysis

Fungi sample was developed in equal concentration of heavy metal (Pb, Cd, and Hg) containing media for SEM study. The SEM study has shown that the characteristic of the genus *Aspergillus* sp. (F12) (Figure 5.21a 5.21b). The *Aspergillus* sp. (F12) (Deposition No. MN210327) is the spore like behavior structure that produce extracellular polymeric substances (Figure 5.22a, 5.22b). The conidia are formed on conidiophores getting up from the foot cells of the hyaline and septate somatic hyphae. The hypha is bifurcated and multinucleate.

The EDEX study of the dry mass also exposed the accumulation of target metals in the surfaceof fungal mycelia cell surface (Figure 5.23a, 5.23b, 5.23c and 5.23d) and fungal EPS (Figure 5.23e, 5.23f, 5.23g and 5.23h).

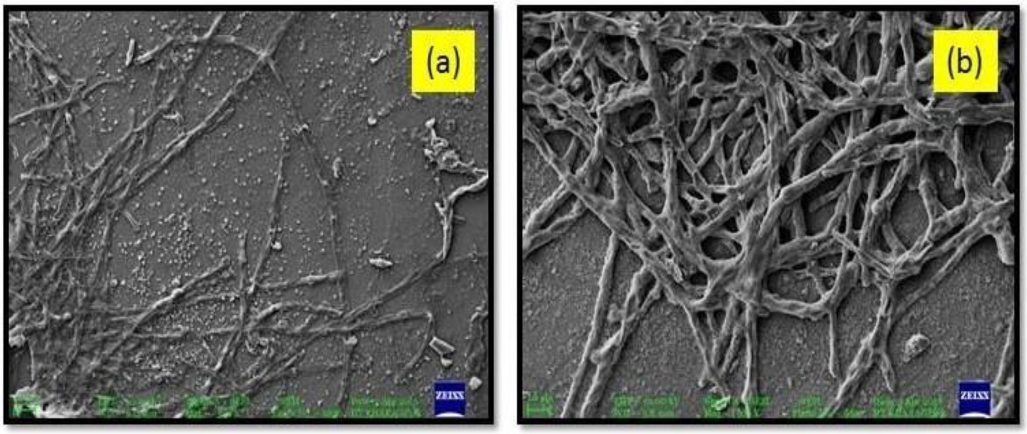


Figure 5.21: Electron microscopic view of cultured fungal mycelium (a, b)

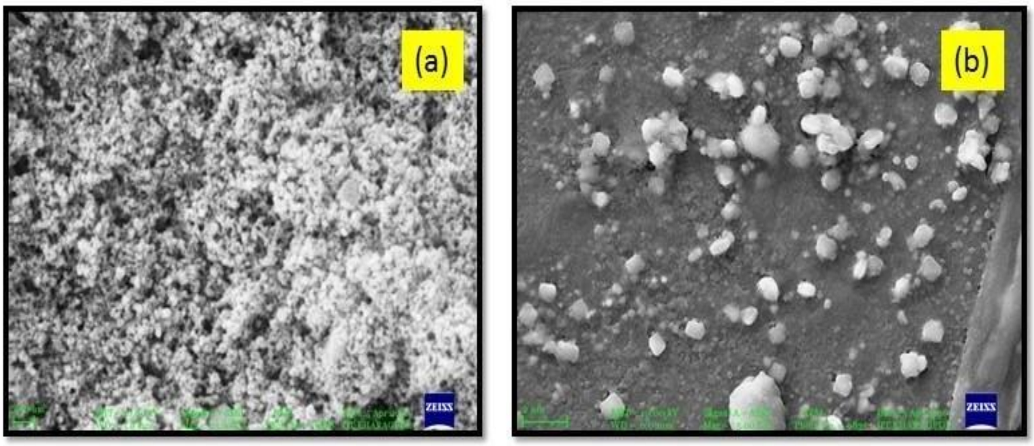
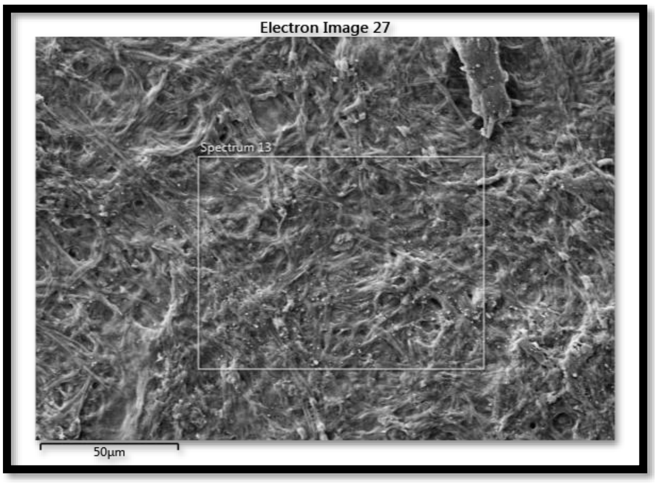
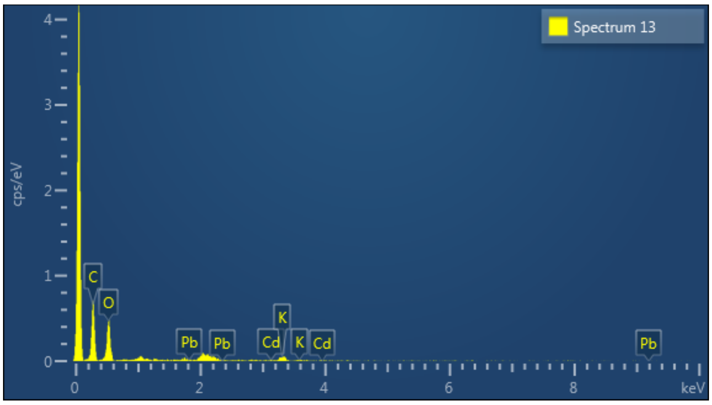


Figure 5.22: Electron microscopic view of fungal EPS (a), and heavy metal treated fungal(EPS) (b)



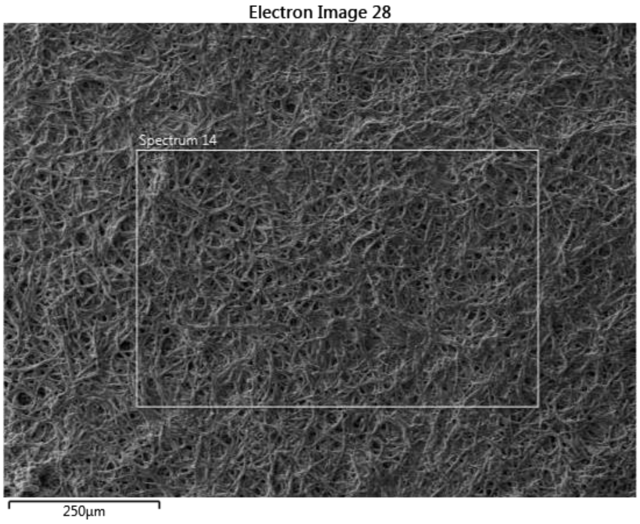
5.23a: Electron microscopic view of heavy metals (Pb and Cd) treated fungal mycelium



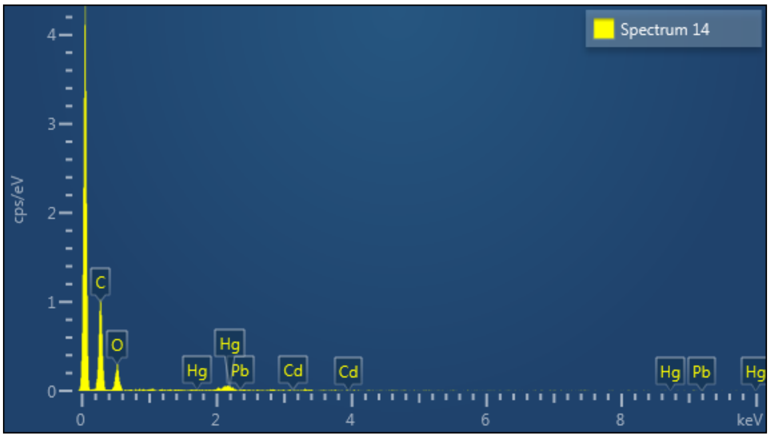
5.23b: EDEX image of heavy metals (Pb and Cd) absorption by fungal mycelium

Table 5.11: EDEX data of heavy metals (Pb and Cd) absorption mycelium

Element	Line Type	Apparent Concentration	k Ratio	Wt%	Wt% Sigma	Standard Label	Factory Standard
C	K series	0.06	0.00057	55.38	2.12	C Vit	Yes
O	K series	0.07	0.00024	38.80	2.01	SiO2	Yes
K	K series	0.01	0.00010	5.79	0.83	KBr	Yes
Cd	L series	0.00	0.00000	0.03	1.46	Cd	Yes
Pb	M series	0.00	0.00000	0.00	0.00	PbTe	Yes
Total:				100.00			



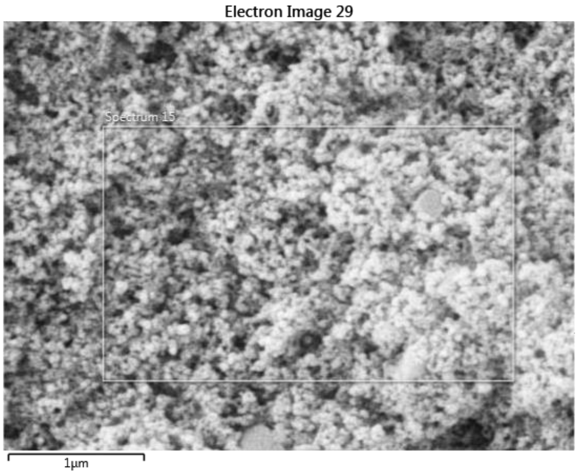
5.23c: Electron microscopic view of heavy metals (Pb, Cd and Hg) absorption by mycelium



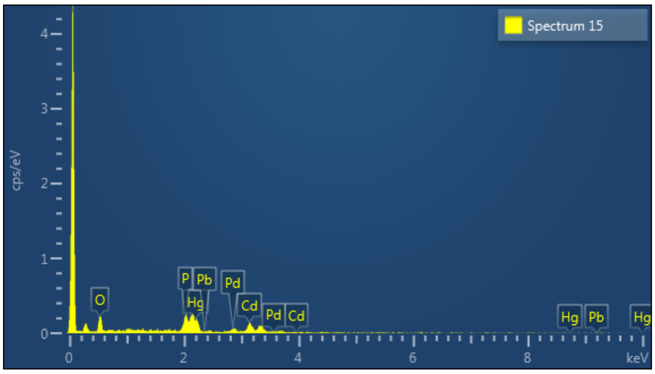
5.23d: EDEX image of heavy metals (Pb and Cd) absorption by fungal mycelium

Table 5.12: EDEX data of heavy metals (Pb, Cd and Hg) absorption by mycelium

Element	Line Type	Apparent Concentration	k Ratio	Wt%	Wt% Sigma	Standard Label	Factory Standard
C	K series	0.07	0.00072	70.77	2.96	C Vit	Yes
O	K series	0.04	0.00014	24.43	1.93	SiO2	Yes
Cd	L series	0.00	0.00002	1.22	1.16	Cd	Yes
Hg	M series	0.00	0.00003	2.15	2.36	HgTe	Yes
Pb	M series	0.00	0.00002	1.43	2.08	PbTe	Yes
Total:				100.00			



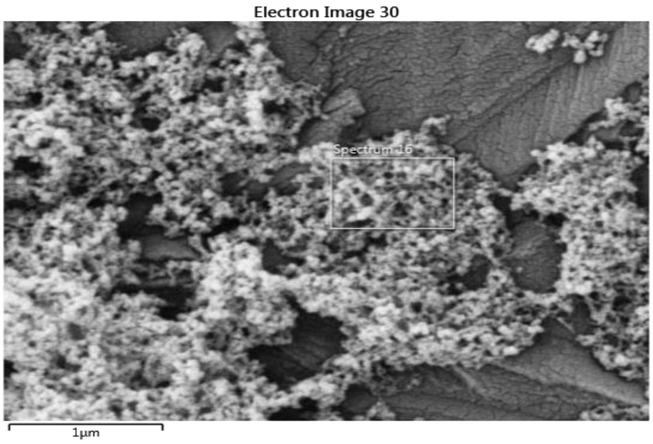
5. 23e: Electron microscopic view of heavy metals (Pb II, Cd II and Hg II) absorption fungal EPS



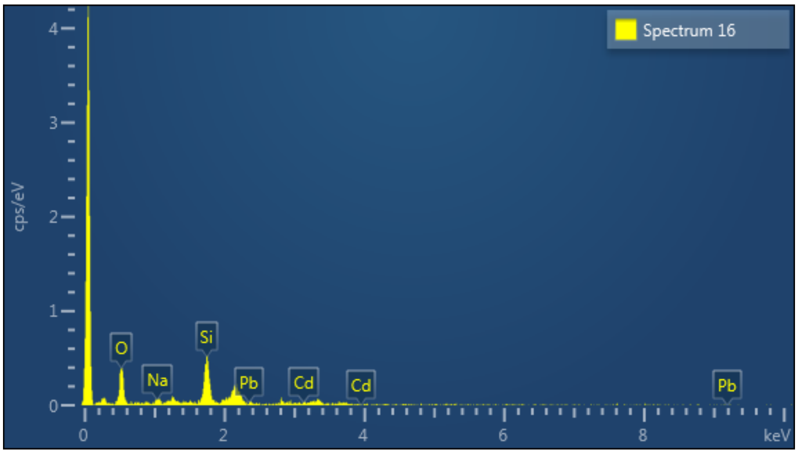
5.23f: EDEX image of heavy metals (Pb, Cd and Hg) absorption by fungal EPS

Table 5.13: EDEX data of heavy metals (Pb, Cd and Hg) absorption by fungal EPS

Element	Line Type	Apparent Concentration	k Ratio	Wt%	Wt% Sigma	Standard Label	Factory Standard
O	K series	0.03	0.00012	30.63	3.36	SiO2	Yes
P	K series	0.03	0.00018	12.99	1.79	GaP	Yes
Pd	L series	0.01	0.00012	10.49	2.86	Pd	Yes
Cd	L series	0.05	0.00050	45.66	4.18	Cd	Yes
Hg	M series	0.00	0.00000	0.23	5.87	HgTe	Yes
Pb	M series	0.00	0.00000	0.00	0.00	PbTe	Yes
Total:				100.00			



5.23g. Electron microscopic view of heavy metals (Pb, Cd) absorption by fungal EPS



5.23h. EDEX image of heavy metals (Pb, Cd) absorption by fungal exopolysaccharide

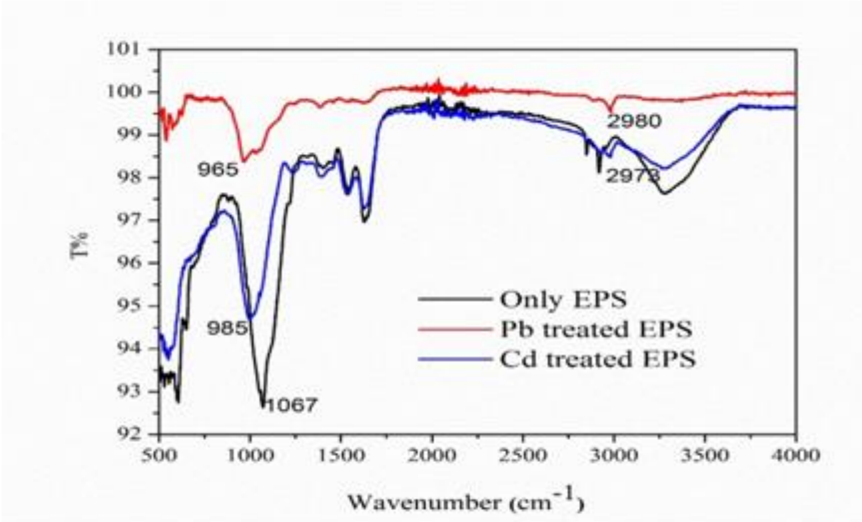
Table 5.14: EDEX data of heavy metal (Pb, Cd) absorption by fungal EPS

Element	Line Type	Apparent Concentration	k Ratio	Wt%	Wt% Sigma	Standard Label	Factory Standard
O	K series	0.06	0.00021	46.28	5.52	SiO2	Yes
Na	K series	0.01	0.00003	4.11	1.23	Albite	Yes
Si	K series	0.04	0.00035	37.08	4.50	SiO2	Yes
Cd	L series	0.00	0.00004	5.21	6.14	Cd	Yes
Pb	M series	0.00	0.00004	7.31	7.33	PbTe	Yes
Total:				100.00			

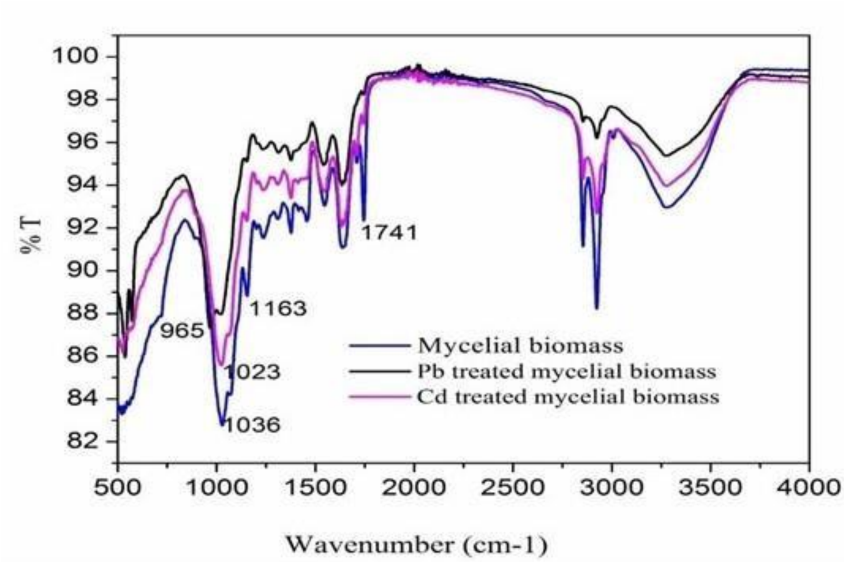
5.16. Fourier transforms infrared spectroscopy (FTIR) Study

Biomaterial surfaces can be viewed as a mosaic of different functional groups which are responsible for binding of metal ions, including amide (-NH2), carboxylate (-COO-), thiols (—SH), phosphate (PO 3−) and hydroxide (—OH) (Chain et al., 2010; Guo et al., 2019). Their intensity depends mainly on the nature, number, affinity and distribution of biopolymers. Therefore, the identification of functional groups is very important for understanding the mechanisms for binding of certain metal ions.

In the present study, the appeared peak at 1036 cm^{-1} (Figure 5. 24a) corresponded to the interaction of C- O-C symmetric stretching mode. Further, it is also recorded that the number 1036 cm^{-1} appears to be due to the vibration connections to (-CN) extending including carbon of free structure and the amino group of nitrogen (Bartness et al., 2014). Interestingly when fungal cells were treated with Cd II metal, the FTIR spectrum gained from fungal mycelia biomass displayed the value shift from 1036 cm^{-1} peak to 1023 cm^{-1} confirming that Cd (II) ion inclines to affect the C-O-C bond (Figure 5. 24b). Concurrently 965 cm^{-1} for Pb (II) treated biomass relate to C-O stretching beating. The absorption strip at 965 cm^{-1} is assigned to C-O stretching to ribose sugar and phosphodiester bond of DNA (Cugia, 2011). Hence, it appears that both metal ions of Pb (II) and Cd (II) directly target to DNA and binds to C-O stretching of phosphodiester bond ribose sugar. In the following spectra, the frequency region at 1140 to1185 cm^{-1} is mainly responsible for cellular protein and carbohydrate (Berti and Burley 2008). The peak at 1163 cm^{-1} characteristics to the vibration of the C-O bond of the ribose sugar. It is only observed for the control biomass samples whereas disappeared in Cd (II) or Pb (II) treated biomass which suggests their strong interaction. Further, the peak at 1741 cm^{-1} is responsible for the vibration of C=O ester which also disappears after metals (Pb II & Cd II) added biomass.



(a)



(b)

Figure 5.24: Fourier transform infrared spectrum of untreated EPS, Pb-loaded and Cd-loaded fungal EPS (a), and mycelial biomass, Pb treated mycelial biomass and Cd treated mycelial biomass (b)

Then, mycelial biomass associated metal removal occurs for the vibration of C-O-C and C=O ester bond. The vibration frequency observed at 1067 cm^{-1} is responsible for C-O stretching which have been shifted to 985cm^{-1} and 965 cm^{-1}for Cd (II) and Pb (II) treated EPSsample, respectively. The chief shift has been observed for Pb treated EPS individual stronginteraction of Pb with C-O bond.

5.17. Emulsifying activity of fungal EPS

In the present context, the emulsifications were evaluated by the effects of the EPS concentration. The emulsions were prepared with different concentrations of EPS and n-hexane. Then the emulsification indexes (E_{24}) were calculated according to the method of Mouafi et al., 2016. The major differences in the emulsification indexes were determined based on different emulsions having different EPS concentrations from 0.15 to 0.3 %. However, no significant variances in the emulsification index were found when the EPS concentration extended from 0.3 to 1%, although at higher concentrations emulsion activity was to some extent increased.

Emulsions were prepared with fungal EPS as well as xanthan gum by means of different hydrocarbons i.e. n-hexane, n-hexadecane and oils i.e. soybean oil, and peanut oil, liquid paraffin. The experimental results were presented in Table 16. It was revealed that the emulsifying activity of EPS of *A. penicillioides* with the hydrocarbons and oils was better than that of xanthan gum at a concentration of 0.45%. Thus the xanthan gum has less emulsifying activity than EPS of *A. penicillioides*. The EPS emulsion-stabilizing capacity of n-hexadecane, liquid paraffin, and peanut oil, was revealed by emulsification indexes higher than 50 %. Many hydrocarbons tested, such as, soybean oil and n-hexane, although have lesser emulsification indexes (44.9) produced established emulsions that were stable for several days after preparation.

Table 5.15: Emulsification activities of EPS and xanthan gum (0.45 %, w/v) with hydrocarbons and oils

Hydrocarbon or oil	*E_{24} of EPS	*$E_{24}$of Xantham gum
n-Hexane	25.3±1.3	28.3±1.9
n-Hexadecane	52.9±4.9	49.7±0.2
Liquid paraffin	70.8±3.4	60.6±3.7
Soybean oil	44.9±0.6	32.4±3.2
Peanut oil	69.8±1.4	19.6±2.5

*E_{24} Emulsification index after 24 h

Table 5.16: Emulsification activities of EPS at different concentrations with n-hexane

Concentration of EPS (%)	*E_{24}
0.15	21.4±1.8
0.30	26.6±2.6
0.45	26.4±1.3
0.60	26.2±1.6
0.75	22.8±3.7
0.90	24.8±1.9
1	23.4±0.9

*E_{24} Emulsification index after 24 h

5.18. Flocculation activity of fungal EPS

The flocculation reactions were carried out at different EPS concentrations of 0.05–50 mg/l. It was shown that the flocculating activity with kaolin clay suspension was improved with increasing EPS concentrations of 0.05 to 0.5 mg/l and thereafter decreased. The flocculating activity peak of 9.4 occurred at an EPS concentration of 0.5 mg/l corresponding to the flocculating rate of 88.4%. The flocculating activity changes using MgO suspension showeda similar trend. Whereas the highest flocculating activity reached 29.4 and the flocculating rate was 82.2% with an EPS concentration of 5 mg/l. The flocculating activity of EPS in the kaolin suspension (Figure 5.25a) and MgO suspension (Figure 5.25b) was compared with other commercial flocculants such as $Al_2 (SO_4)_3$ and xanthan gum. These preliminary tests were implemented at normal room temperature and neutral pH, for flocculants concentration of 0.5 mg/l in kaolin suspension and 5 mg/l in MgO suspension.

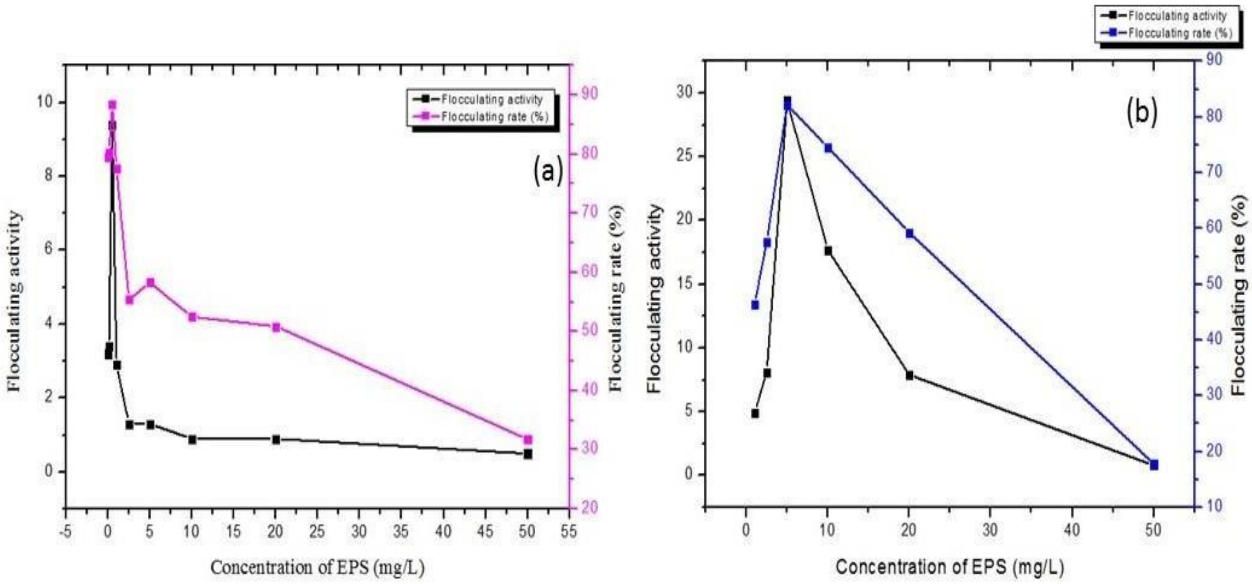


Figure 5.25: Flocculating activity and flocculating rate of EPS concentration with kaolin suspension (a) and MgO suspension (b)

Table 5.17: The effects of EPS concentrations on flocculating activity and flocculation rateusing kaolin suspension and MgO suspension at pH 7.0

Concentration of EPS(mg/L)	Flocculating activity	Flocculating rate (%)
Flocculation with kaolin clay		
0.05	3.2	79.6
0.1	3.4	80.3
0.5	9.4	88.4
01	2.9	77.6
2.5	1.3	55.4
5	1.3	58.3
10	0.9	52.5
20	0.9	50.9
50	0.5	31.8
Flocculation with MgO		
1	4.9	46.4
2.5	8.1	57.6
5	29.4	82.2
10	17.7	74.6
20	7.9	59.3
50	0.8	17.8

5.19. Antibacterial activity of F-12 fungal extract

The crude extract of fungal isolates with Hexane, Ethyl acetate and Methanol were screened for the measurement of their antimicrobial potential. The present study has evaluated the antimicrobial activity of metabolites produced by fungal strain (F12) against four references human pathogenic microorganisms such as *E.coli, Vibrio cholera, Bacillus subtilis,* and *Staphylococcus aureus*. The results on the in vitro antimicrobial activities of several fungi against four different bacterial strains were tabulated in Table 5.18. The results validated that Aspergillus sp. showed antibacterial activity followed by *Pythium sp.* ***.*** *Fusarium sp. Rizopous sp.* and *Penicillium sp.* (Figure 5.26).

Table 5.18: Antimicrobial activity of fungus (F12) against both Gram negative and Gram positive bacteria

Fungal name	*E.coli*	*Staphylococcus aureus*	*Bacillus subtilis*	*Vibrio cholerae*
Aspergillus penicillioides	+	++	++	++
Fusarium sp.	+	+	+	+
Penillium sp.	+	+	+	+
Rizopous sp.	+	+	+	+
Pythium sp.	++	+	++	++

+ Zone of inhibition ranged between 7-12mm; +Zone of inhibition ranged between 13-19mm; +++Zone of inhibition ranged >20mm; - No Zone of inhibition

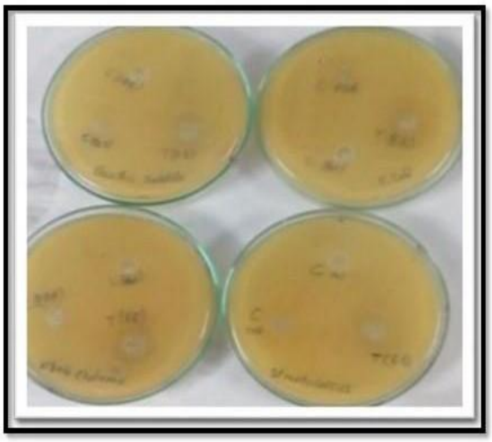


Figure 5.26: Antimicrobial sensitivity test of *Aspergillus Penicillioides* (F12) extract

5.20. Statistical analysis

5.20.1. Correlations between physicochemical parameters of water with total fungal count

The correlation coefficients are considered on the basis of a number of pairs of the results considering the level of significance. The correlation between water qualities parameters were indicated that total fungal count is also dependent upon the several factors of any season.

At premonsoon the value of the correlation coefficient is 0.975 for TDS with temperature in water. The total hardness (TH) was positively correlated with TDS by 0.904. TSS directly positive correlated with total coliform and fecal coliform by 0.913 and 0.905 respectively that depicted in Table 5.20.

At monsoon correlation coefficient value of water is 0.943for the pair TDS with TSS and 0.987 for DO with total suspended solid (TSS). On the other hand DO was positively correlated with Total coliform by 0.864 (Table 5.21). Generally, the relation between TN with BOD and COD are inversely proportional; whereas DO is negatively correlated with BOD and COD for microbial growth. In benthic soil, organic carbon and potassium concentration should be increased with pH and temperature.

During post the monsoon season, chloride content inversely correlated with total and fecal coliform. The correlation coefficient value of TN inversely correlated with BOD with COD and with TP (Table 5.22). COD describes total organic matter content, which is oxidized by a strong oxidant (Almeida et al. 2007). During increase of temperature COD was increased.

Table 5.19: Total fungi at different selected sites

Study sites	Fungus Count	Pre-monsoon (March- June) (Mean±SD)	Monsoon (July-October) (Mean±SD)	Post-monsoon (November-February)(Mean±SD)	*P value
Muri(S-I)	Total count	20.00 ± 4.00	46 ± 6.00	30.33 ± 2.51	0.02
	F12 Strain	1.67 ± 0.577	3.67 ± 0.577	2.00 ± 1.00	0.05
Sonakonia (S-II)	Total count	27.33 ± 4.16	63.33 ± 4.16	35.67 ± 5.50	0.02
	F12 Strain	2.33 ± 0.577	5.66 ± 2.08	3.33 ± 1.52	0.09
Talsari(S-III)	Total count	33.33 ± 5.03	61.33 ± 12.05	43.33 ± 4.16	0.01
	F12 Strain	1.00 ± 2.00	16.67 ± 3.05	8.00 ± 2.00	0.24

Table 5.20: Results of correlation co-efficient of physicochemical parameters and fungal count of water at pre-monsoon

	pH	TEM	ALKA	CaH	MgH	TH	TDS	TSS	BOD	COD	DO	TKN	CHL	TPP	FCOL	TCOL
pH	1															
TEM	.652	1														
ALKA	.065	.616	1													
CaH	.798	.880*	.188	1												
MgH	.398	.668	.064	.812*	1											
TH	.682	.813*	.082	.973**	.914*	1										
TDS	.660	.975**	.484	.944**	.783	.904*	1									
TSS	.758	.898*	.227	.996**	.804	.967**	.959**	1								
BOD	.194	-.270	-.617	-.065	-.077	-.009	-.292	-.117	1							
COD	.080	-.364	-.943**	.068	.195	.194	-.230	.032	.706	1						
DO	-094	-.699	-.485	-.504	-.621	-.568	-.647	-.535	-.015	.186	1					
TKN	-203	-.698	-.978**	-.315	-.205	-.216	-.582	-.340	.576	.895*	.507	1				
CHL	.202	-.105	-.823*	.332	.403	.445	.059	.315	.488	.933**	.056	.773	1			
TPP	-19*	-.832*	-.276	-909*	-.792	-.880*	-.870*	-.877*	.032	.055	.452	.451	-.114	1		
FCOL	.797	.692	-.103	.907*	.620	.873*	.762	.905*	.183	.342	-.348	.015	.578	-.704	1	
TCOL	.548	.910*	.466	.875*	.662	.820*	.946**	.913*	-.443	-.251	-.547	-.513	.091	-.691	.756	1

***. Correlation is significant at the 0.05 level (2-tailed).**
****. Correlation is significant at the 0.01 level (2-tailed).**

Table 5.21: Results of correlation co-efficient of physico-chemical parameters and fungal count of water at Monsoon

	pH	TEM	ALKA	CaH	MgH	TH	TDS	TSS	BOD	COD	DO	TKN	CHL	TPP	FCOL	TCOL
pH	1															
TEM	.848*	1														
ALKA	.662	.287	1													
CaH	.803	.572	.897*	1												
MgH	-.672	-.273	-.991**	-.887*	1											
TH	-.617	-.190	-.980**	-.829*	.994**	1										
TDS	.761	.986**	.201	.489	-.176	-.093	1									
TSS	.901*	.971**	.486	.745	-.465	-.380	.943**	1								
BOD	.906*	.729	.852*	.945**	-.825*	-.768	.662	.862*	1							
COD	.789	.985**	.205	.525	-.177	-.085	.979**	.951**	.684	1						
DO	.930**	.944**	.573	.773	-.546	-.471	.909*	.987**	.910*	.914*	1					
TKN	-.557	-.086	-.941**	-.754	.969**	.988**	.022	-.269	-.678	.026	-.365	1				
CHL	.363	.589	.248	.273	-.168	-.137	.680	.590	.487	.570	.626	-.013	1			
TPP	.038	-.031	.370	.097	-.276	-.311	.024	.042	.273	-.044	.170	-.261	.667	1		
FCOL	.486	.647	.149	.458	-.069	.029	.640	.678	.545	.757	.648	.139	.337	.040	1	
TCOL	.723	.777	.601	.859*	-.556	-.463	.759	.887*	.865*	.782	.864*	-.334	.535	.077	.723	1

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

Table 5.22: Results of correlation co-efficient of physicochemical parameters and fungal count of water at post-monsoon

	pH	TEM	ALKA	CaH	MgH	TH	TDS	TSS	BOD	COD	DO	TKN	CHL	TPP	FCOL	TCOL
pH	1															
TEM	-.360	1														
ALKA	-.234	-.724	1													
CaH	.497	.583	-.946**	1												
MgH	.827*	-.794	.262	-.012	1											
TH	.830*	.147	-.718	.884*	.457	1										
TSS	-.481	.985**	-.649	.467	-.866*	.010	.927**	1								
BOD	-.753	-.139	.754	-.876*	-.400	-.966**	-.203	-.008	1							
COD	-.367	.972**	-.649	.539	-.747	.130	.807	.947**	-.096	1						
DO	.870*	-.298	-.312	.525	.794	.839*	-.341	-.415	-.776	-.237	1					
TKN	.611	.400	-.870*	.960**	.199	.947**	.375	.275	-.933**	.394	.724	1				
CHL	.357	-.954**	.636	-.505	.733	-.106	-.833*	-.955**	.038	-.962**	.239	-.359	1			
TPP	.103	.848*	-.976**	.910*	-.409	.618	.837*	.780	-.630	.796	.186	.805	-.777	1		
FCOL	-.740	.751	-.260	.015	-.865*	-.392	.676	.843*	.423	.742	-.592	-.127	-.836*	.406	1	
TCOL	-.516	.800	-.277	.185	-.711	-.168	.549	.808	.267	.895*	-.352	.077	-.890*	.472	.807	1

*** Correlation is significant at the 0.05 level (2-tailed).**

**** Correlation is significant at the 0.01 level (2-tailed).**

5.20.2. Cluster analysis (CA)

CA, a chemo metric method of data exploration was performed in order to have visual presentation of season wise fungal strain with water quality parameters of study sites.

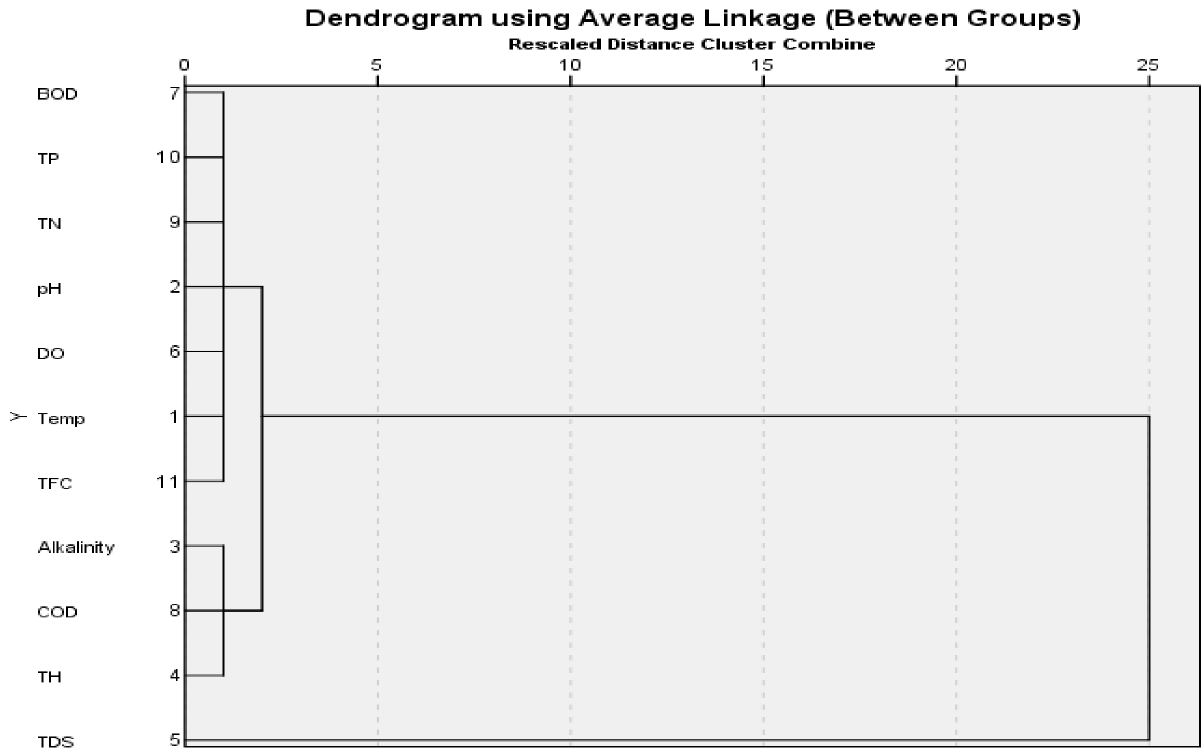


Figure 5.27a: Dendogram of water quality parameters with fungal count in pre-monsoonseason

In premonsoon (Figure 5.27a), it was observed that less than 5 % of the maximum distance D_{max} was noticed for the two pairs of variables: alkalinity, chemical oxygen demand (COD), total hardness with biological oxygen demand (BOD), total phosphate phosphorous (TP), total nitrogen (TN), pH, dissolved oxygen (DO), temperature, and total fecal coliform (TFC). It was also reflected in the values of their linear correlation coefficients that except TDS all parameters were grouped in a common cluster at a distance less than 10% of the maximum. This means that the total fungal count also depends upon TN and TP.

DO are usually associated with low values of COD and BOD in case of clean natural waters, the opposite situation for polluted waters occurs (Cieszynska, et al., 2012) The fact that TN, TP, BOD, PH, DO, Temperature, and TFC were included in a common cluster on the one side of the dendrogram may be implied in the processes of production and mineralization of organic matter.

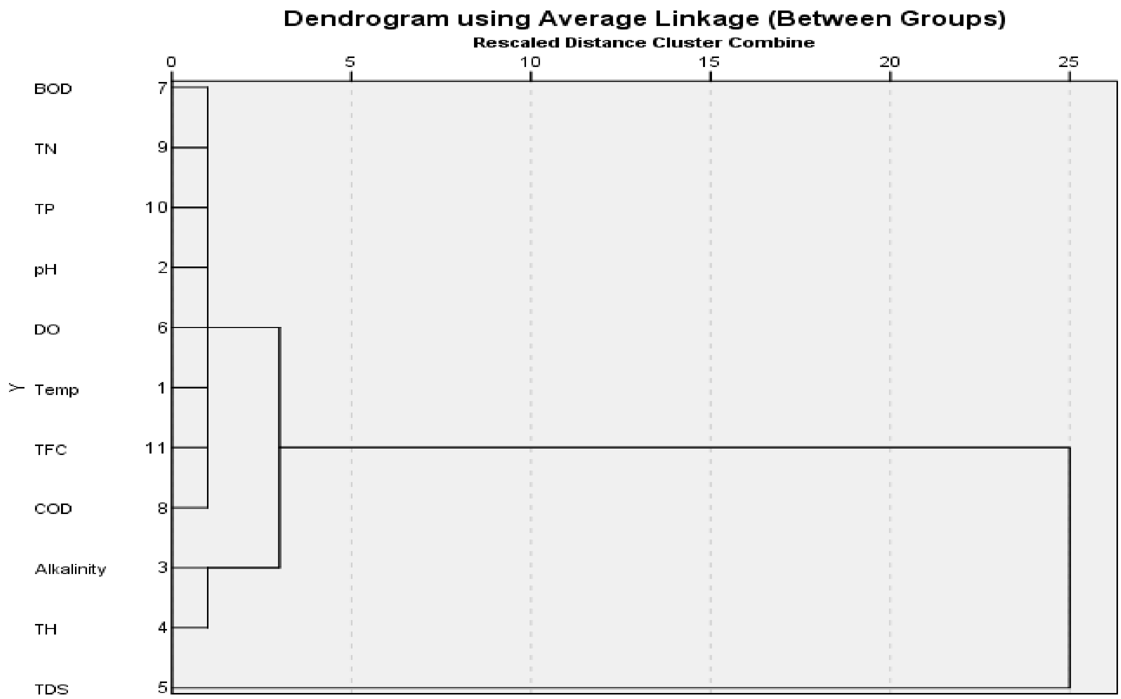


Figure 5.27b: Dendogram of Water quality parameters with fungal count in monsoon season

At monsoon in Figure 5.27b it can be observed that the lowest agglomeration distance, less than 5 % of the maximum distance (D_{max}) is noted for the two pairs of variables: alkalinit y and TH, with COD, TFC, temperature, DO, pH, TP, TN and BOD. For these two pairs of the results, the highest positive values of correlation coefficients were noted. Different results variation was observed for the pair of total hardness (TH) and alkalinity. Those physicochemical variables attachthe TDS of water at the maximum distance D_{max}. Alkalinity as well as TH are negatively correlated to all other variables except TDS and form a separate cluster on one side of the dendrogram. The remaining variables are founded on the opposite side of the dendrogram. The high values of DO are usually associated with low values of COD (Cieszynska, et al. 2012). In Figure 5.31b all physico-chemical parameters of soil are found on and

BOD in case of clean natural waters, the opposite situation for polluted water occurs one side of the dendrogram except temperature and sand belongs to less than 5% of maximum distance. Soil temperature and total fungal count acquire the same distance on the opposite side. Both groups are attached to each other by >10% of maximum distance.

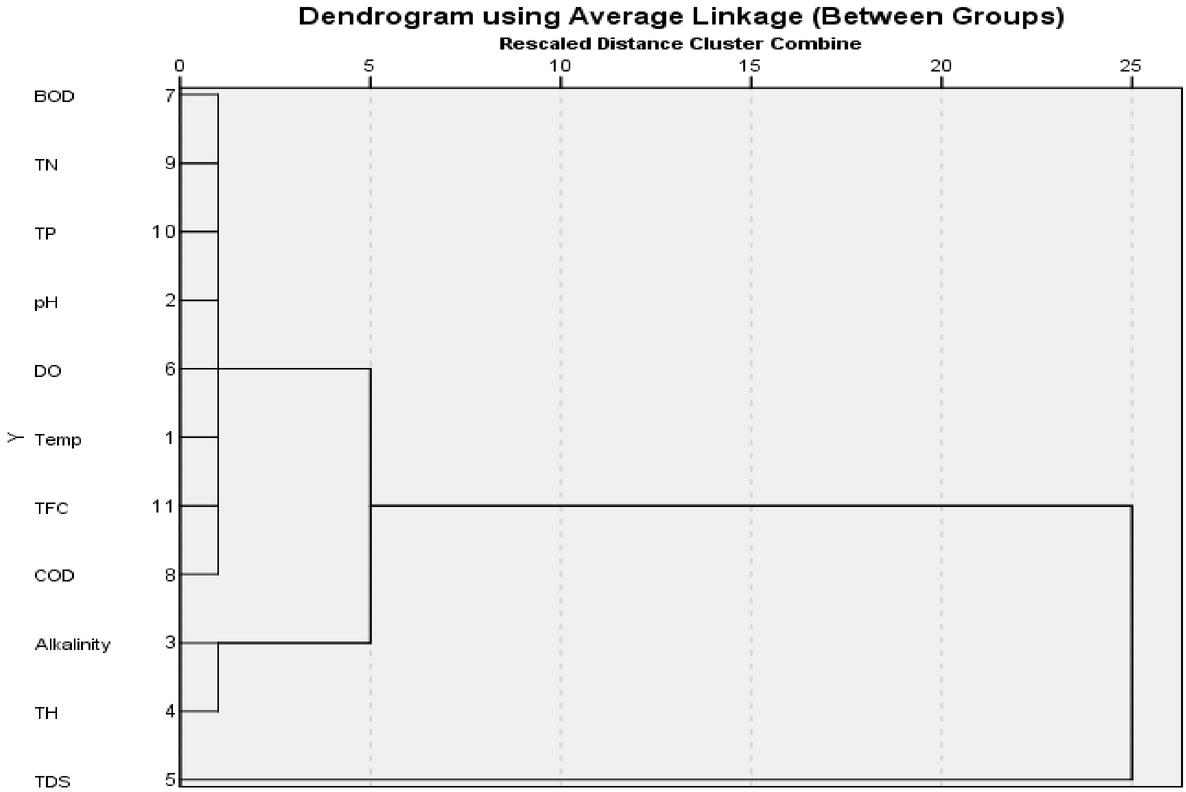


Figure 5.27c: Dendogram of water quality parameters with fungal count in post-monsoon

In post-monsoon in Figure 5.27c it can be observed that the lowest agglomeration distance, about 5% of the maximum distance D $_{max}$ is noticed for the two pairs of variables: TH with alkalinity, and all others except TDS. Two groups of variables attach with the TDS of water at the 25% maximum distance D max. Alkalinity as well as TH is negatively correlated to all other variables, except TDS and they form a separate cluster on one side of the dendrogram. The remaining variables are founded on the opposite side of the dendrogram. Seasonal patterns were detected mainly for DO with maximal values in the cool season, accompanied by minimal values of COD and BOD. From Figure, we showed that total fungal count depends upon all parameters except alkalinity, the total hardness of water. But all other parameters indirectly involve the fungal growth. For that reason, TFC, temperature and salinity belong to the same group at <5% of maximum distance.