International Journal of Botany

ISSN: 1811-9700

science alert

ANSInet
an open access publisher
http://ansinet.com

International Journal of Botany 7 (2): 170-176, 2011
ISSN 1811-9700 / DOI: 10.3923/ijb.2011.170.176

Metal Content in Water and in Green Filamentous Algae *Microspora quadrata* Hazen from Coal Mine Impacted Streams of Jaintia Hills District, Meghalaya, India

M. Das and P. Ramanujam
Algal Ecology Laboratory, Department of Botany, School of Life Sciences,
North-Eastern Hill University, Shillong 793022, Meghalaya, India

Abstract: *Microspora quadrata* Hazen, a green filamentous alga has been found to grow luxuriantly throughout the year, in the streams affected by Acid Mine Drainage (AMD) caused by unscientific coal mining in Jaintia Hills, Meghalaya. The AMD effluents contain metals which are known to be toxic to the aquatic organisms. Filamentous green algae are capable of accumulating and thereby help in removing metals from the affected water bodies. The present study was undertaken to find the seasonal variations in the metal content in the AMD impacted streams and the efficiency of *Microspora quadrata* to remove the toxic metals by accumulation of the metals in its biomass. Water and filamentous algal mats were collected seasonally from streams located in active mining, coal storage and abandoned areas and were analyzed for iron, lead, zinc and manganese by atomic absorption spectrophotometer. Significant seasonal changes in concentrations of toxic metals were observed in water and in *Microspora quadrata* Hazen. Accumulation of metals like iron and lead were higher in algal mat compared to water medium in all seasons. Maximum accumulation of iron was observed in autumn whereas lead and manganese were more in spring. Zinc content in water was significantly higher than that of algal mat indicating very low rate of absorption or adsorption. Thus, from the present study *Microspora quadrata* could be suggested as a useful plant material to remove toxic metals like iron lead and manganese from the AMD streams of the region.

Key words: Coal mining, Jaintia Hills, metal content, *Microspora quadrata*, streams

INTRODUCTION

Water discharge from mine drainage is habitually acidic in nature, often containing high concentrations of heavy metal ions. This discharge commonly referred as AMD (Acid Mine Drainage) is considered as one of the biggest environmental dilemma causing severe damage to aquatic ecosystems. Singh (2005) reported extremely acidic habitats from anthropogenic sources associated with massive burden of spoil and heavy metals. In most cases, low pH and high metal concentrations were known to effect aquatic organism adversely but green algae are known for the removal of metals from their surrounding waters. Presence of conspicuous bright green algal mats composed of filamentous taxa such as *Klebosormidium*, *Microspora*, *Mougetia*, *Ulothrix* and *Stigeoclonium* species were often observed from AMD streams. (Verb and Vis, 2001). Kaonga *et al*. (2008) reported higher concentration of metals in green filamentous algae *Spirogyra aequinoctialis* than in the corresponding water in relation to pH in dry and rainy seasons. Bishnoi *et al*. (2004) reported adsorption capacity, quantitative uptake and accumulation of copper by *Spirogyra* sp. Predominance of Chlorophytic filamentous green algae namely *Klebsormidium rivulare* and *Microspora tumidula* in AMD impacted water bodies and their capacity to remove heavy metals from coal mine areas have been reported by Verb and Vis (2001). Chlorophyte like *Microspora* sp. dominate AMD streams and it is a common feature in severely affected streams (Verb and Vis, 2005).

Meghalaya state, an important component of North East India, Hot spots of the globe represents a rich floristic wealth. The state is equally rich in its mineral resources. Coal is one of the most exploited mineral of the region. Primitive “rat hole” method of coal mining and its huge storage in the Jaintia Hills, situated in the Eastern part of the state Meghalaya (lying in between 25° 02’N to 25° 45’N latitudes) and between (91° 58’ E to 92° 50’E longitudes) has resulted in serious acid mine drainage of the aquatic system (Nath and Ahmed, 2005). Water bodies of the areas showed low pH, high conductivity, high

Corresponding Author: P. Ramanujam, Department of Botany, Algal Ecology Laboratory, School of Life Sciences, North-Eastern Hill University, Shillong 793022, Meghalaya, India
Tel: +91 0364 2722235 Fax: +91 0364 2550108

concentration of sulphates, iron and many toxic metals, low dissolved oxygen and high Biological Oxygen Demand (Singh, 2005).

The present work deals with seasonal changes in concentrations of toxic metals in the AMD impacted streams and accumulation pattern of these toxic metals in dominant algal taxa *Microspora quadrata* in coal mine areas of Jaintia Hills.

MATERIALS AND METHODS

Site selection: This study was carried out in four seasons namely Spring (March, April, May), Monsoon (June, July, August), Autumn (September, October, November) and Winter (December, January, February) in the year 2008-2009. The location of each site was determined by a Garmin 12 GPS receiver. Streams affected by coal mining processes at different levels were selected for the study in Jaintia Hills of Meghalaya and were categorized in three groups.

- Stream receiving wastes from active coal mining areas lying between 25° 22.462'N to 092° 23.381' E
- Stream receiving acid water through seepage from huge coal storage (mined coal is stored in huge lumps on the road side for transportation to different places) lying between 25°23.271 'N to 092° 19.471' E
- Stream in abandoned site (left abandoned for 5-7 years after active coal mining) lying between 25° 24.512'N to 092° 18.941' E

Sampling procedure: Physico-chemical parameters like water temperature, average current velocity, depth of the water bodies, pH and conductivity were measured in the field. Conductivity, pH and water temperature were measured using Deluxe water and soil analysis kit (Model 191E). The mean current velocity was calculated by timing a bobbler as it moved over a distance of one meter. The mean depth was calculated from 10 randomly selected points.

Water sample were collected from 5 different points of each stream in different seasons. At each sampling point, stream water was collected separately for heavy metal analysis and other physico-chemical analysis of water. Water samples were stored in 1 L polyethylene bottles and 1.5 mL concentrated nitric acid (AR) was added to those samples where heavy metals would be determined. Small portion of the algal mat sample was preserved in 4% formalin for determination of algal taxa using Olympus microscope (BX41) photographed with the help of digital camera directly fitted with the microscope. The remaining algal samples collected from different points of the stream were rinsed with water to remove the debris and collected in specimen bottles and brought to the laboratory.

Laboratory analysis: Cleaned algal samples were blotted to remove the excess water and dried in oven at 80°C for 24 h. Dried tissue (1 g) and 500 mL of water sample was taken for digestation using concentrated nitric acid in a hot plate. Digestion was done following the procedure given in APHA (2005). The concentration of heavy metals was determined for iron, lead, zinc and manganese by running samples on Atomic Absorption Spectrophotometer (Perkin Elmer, Analyst 700).

Statistical analysis: Significance among seasonal sampling, different sites and two media (Algae and Water) were tested by Analysis Of Variance (ANOVA). In addition, scatter graphs were plotted using STATISTICA to correlate the concentration of metals in water to that of algae with seasonal changes.

RESULTS

Algal specimens preserved in 4% formalin were examined to identify the mat composition. Mat samples were mainly composed of *Microspora quadrata* Hazen. which contributed almost 98-100% of the algal biomass measured by quadrat method. Another filamentous green alga, a species of *Klebosormedium* was also found attached to the mat in small proportion in some samples. Some other taxa found in the mats were few diatom species like *Navicula cuspidata*, *Navicula cryptocephala* etc. Diatom species were removed by washing but the filamentous algae *Klebosormedium* wherever present in small proportions remained attached with the mat.

Physical and chemical characteristics mainly the depth, current velocity, pH, conductivity varied between sites and seasons. Maximum depth was recorded in streams from coal storage site during autumn followed by active and abandoned mining site in summer-monsoon. The pH ranged from 3.13 in abandoned mining site to 4.35 in coal storage site. Lowest pH was recorded during spring from Active coal mining stream site and the highest was recorded during winter from coal storage stream site. Water from all the sites and in all the seasons was acidic throughout the study period. Conductivity ranged from 0.23 mS cm^{-1} in abandoned mining site to 0.81 mS cm^{-1} in active mining site. Highest conductivity was measured during spring from active coal mining stream site and the lowest was measured during monsoon from abandoned mining stream site. As expected, temperature varied seasonally when compared among sites and seasons it was higher in streams receiving seepage of coal storage and only in Spring. Maximum current velocity was recorded during summer -monsoon in abandoned mining site and the lowest was recorded during winter in active mining site. Physical and chemical characteristics like depth, current velocity, pH, conductivity and temperature varied significantly between seasons ($p<0.05$, df = 4) (Table 1).

Significant seasonal differences were found in metal content in both algal mat and water collected from different sites. Total accumulation of iron and lead in algal mat was higher than in water in all the seasons and in all the sites and the maximum accumulation of iron was from coal storage site (21.44 ppm) during autumn and lead accumulation was maximum in active mining site during spring (0.88 ppm). Accumulation of manganese in algal mat was significantly higher in spring and summer-monsoon in storage and active mining site. Maximum manganese accumulation by the mat was observed in active mining site (0.96 ppm) during spring. In case of zinc it was different from other metals. Zinc accumulation in algal mat was significantly lower than that of water in all the seasons and the concentration of zinc was maximum in water in coal storage site during spring (1.01 ppm). Among the sites, metal concentration was least in both water and also in algal mat from abandoned sites compared to active mine and coal storage sites (Table 2). Results of two way Analysis of Variance (ANOVA) in four seasons showed significant difference between two medium (algae and water) in different seasons (Table 3). Iron and lead concentration in algae was significantly higher in all the seasons studied ($p<0.00001$). Manganese concentration was significantly higher in algal mat during spring and summer-monsoon ($p<0.05$) whereas zinc concentration in water was significantly higher in all the seasons ($p<0.001$). In general, iron content was maximum

Table 1: Physical and chemical characteristics of sites sampled in four seasons

Seasons	Locations	pH	Conductivity (mS cm^{-1})	Temperature (°C)	Depth (cm)	Current velocity (m sec^{-1})
Spring	Abandoned site	3.62±0.01	0.39±0.04	22.6±0.48	13.4±1.35	0.35±0.08
	Active mining site	3.13±0.02	0.81±0.01	23.4±1.01	15.8±2.58	0.61±0.05
	Coal storage site	3.52±0.02	0.69±0.01	28.1±3.19	14.4±17.05	0.28±0.06
Monsoon	Abandoned site	3.85±0.01	0.32±0.04	23.7±0.80	25.0±9.78	1.02±1.02
	Active mining site	3.99±0.03	0.71±0.01	23.6±1.73	25.0±3.84	0.86±0.38
	Coal storage site	3.78±0.02	0.69±0.01	21.4±0.98	17.6±7.33	0.67±0.31
Autumn	Abandoned site	3.47±0.01	0.23±0.01	26.0±0.69	20.8±2.31	0.33±0.33
	Active mining site	3.18±0.04	0.52±0.32	26.1±0.89	23.8±2.31	0.29±0.04
	Coal storage site	3.25±0.04	0.35±0.01	22.5±1.67	30.6±2.87	0.28±0.10
Winter	Abandoned site	3.53±0.01	0.64±0.03	19.1±1.01	19.4±0.80	0.19±0.04
	Active mining site	3.60±0.01	0.70±0.01	19.4±1.41	19.0±0.80	0.24±0.02
	Coal storage site	4.35±0.01	0.43±0.03	19.4±0.81	19.0±0.38	0.21±0.04

Table 2: Metal concentration in water and algae form each site sampled seasonally

		Fe (ppm)		Pb (ppm)		Zn (ppm)		Mn (ppm)	
Season	Location	Water	Algae	Water	Algae	Water	Algae	Water	Algae
Spring	Abandoned site	0.45	7.90	0.07	0.51	0.68	0.31	0.09	0.54
		0.41-0.48	7.14-8.19	0.03-0.12	0.06-1.12	0.64-0.75	0.23-0.42	0.03-0.15	0.06-1.18
	Active mining site	0.25	10.5	0.11	0.88	0.18	0.296	0.11	0.96
		0.20-0.31	10.29-11.11	0.08-0.15	0.14-1.19	0.11-0.28	0.16-0.43	0.04-0.19	0.19-1.15
	Coal storage site	2.61	17.87	0.25	0.52	1.01	0.13	0.18	0.85
		2.90-3.50	17.69-18.12	0.02-1.11	0.09-2.11	0.94-1.12	0.09-0.21	0.18-0.20	0.32-2.12
Monsoon	Abandoned site	0.71	3.77	0.04	0.04	0.38	0.39	0.07	0.07
		0.14-0.20	3.32-4.12	0.02-0.06	0.02-0.07	0.32-0.38	0.36-0.39	0.06-0.07	0.07-0.09
	Active mining site	2.06	9.54	0.03	0.06	0.34	0.31	0.07	0.14
		0.21-0.29	9.21-10.11	0.02-0.05	0.02-0.38	0.32-0.39	0.21-0.39	0.05-0.09	0.12-0.20
	Coal storage site	0.77	4.99	0.02	0.02	0.27	0.11	0.16	0.24
		0.70-0.82	4.87-5.12	0.02-0.03	0.02-0.03	0.26-0.28	0.07-0.16	0.14-0.18	0.20-0.29
Autumn	Abandoned site	0.58	17.83	0.08	0.24	0.23	0.14	0.18	0.02
		0.52-0.62	17.62-17.99	0.07-0.11	0.15-0.32	0.01-0.59	0.02-0.3	0.10-0.30	0.01-0.04
	Active mining site	0.31	18.39	0.17	0.24	0.57	0.15	0.13	0.25
		0.24-0.38	18.10-18.45	0.15-0.32	0.19-0.28	0.55-0.60	0.05-0.4	0.09-0.30	0.22-0.29
	Coal storage site	1.49	21.44	0.06	0. 16	0.65	0.86	0.07	0.07
		0.44-1.82	21.22-21.68	0.05-0.08	0. 13-0.22	0.82-0.89	0.32-0.42	0.07-0.08	0.07-0.08
Winter	Abandoned site	0.14	9.81	0.09	0.23	0.62	0.57	0.18	0.51
		0.10-0.20	9.12-10.15	0.03-0.13	0.18-0.28	0.50-0.70	0.50-0.70	0.16-0.20	0.07-0.90
	Active mining site	0.13	7.89	0.09	0.27	0.93	0.45	0.61	0.14
		0.11-0.15	7.58-7.99	0.04-0.15	0.22-0.32	0.78-0.98	0.42-0.70	0.07-0.90	0.11-0.15
	Coal storage site	0.04	18.45	0.06	0.23	0.41	0.43	0.14	0.08
		0.02-0.06	18.26-18.99	0.03-0.11	0.18-0.28	0.20-0.60	0.20-0.60	0.12-0.18	0.05-0.15

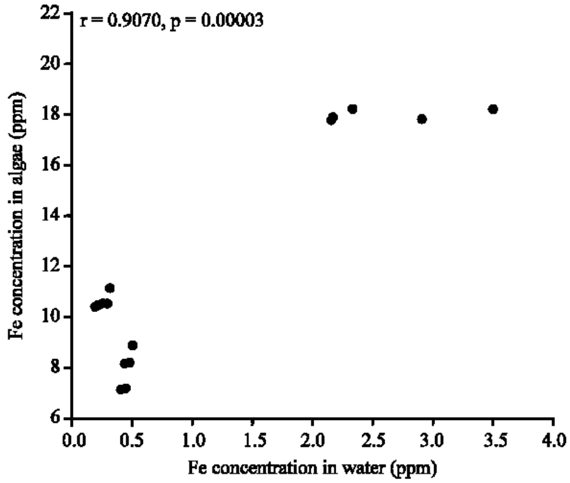

Fig. 1: Iron concentration in algae and water during spring

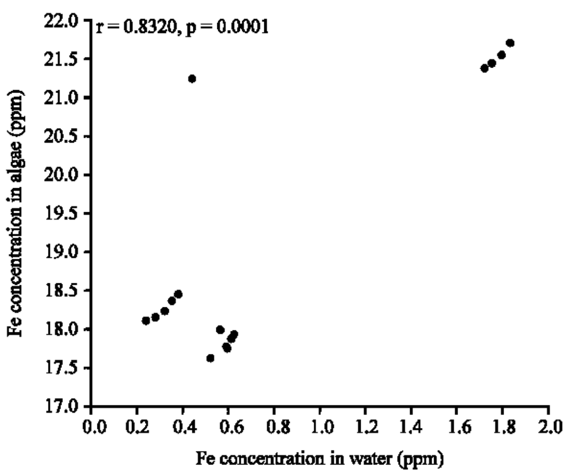

Fig. 2: Iron concentration in algae and water during autumn

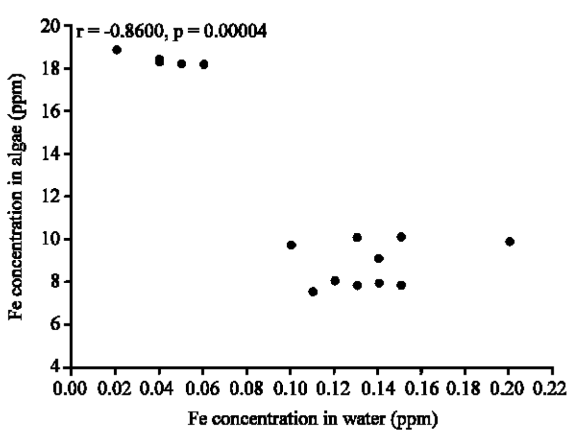

Fig. 3: Iron concentration in algae and water during winter

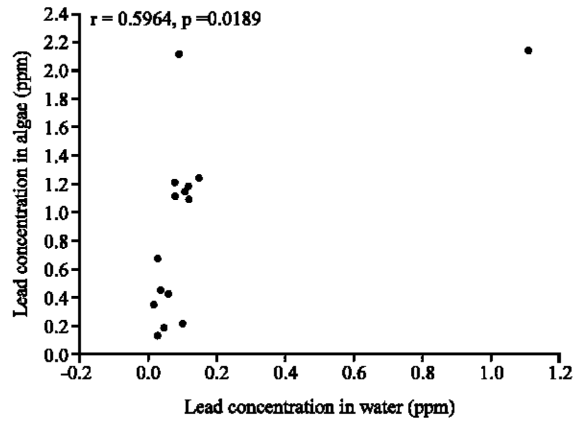

Fig. 4: Lead concentration in algae and water during spring

Table 3: Results of two way analysis of variance (ANOVA) in four seasons

Season	Metals	DF	MS	F-value	p-value
Spring	Fe	1	910.14	5283.28	<0.05
	Pb	1	4.46	21.04	<0.05
	Zn	1	1.07	178.98	<0.05
	Mn	1	3.24	17.64	<0.05
Monsoon	Fe	1	195.38	69.70	<0.05
	Pb	1	0.00	4.78	<0.05
	Zn	1	0.03	19.32	<0.05
	Mn	1	0.01	31.22	<0.05
Autumn	Fe	1	2533.31	3546.23	<0.05
	Pb	1	0.0885	55.75	<0.05
	Zn	1	0.85	50.54	<0.05
	Mn	1	0.001	0.42	>0.05
Winter	Fe	1	1071.01	21073.37	<0.05
	Pb	1	0.26	133.55	<0.05
	Zn	1	0.23	0.79	<0.05
	Mn	1	0.03	0.42	>0.05

followed by zinc, manganese and lead in water whereas accumulation was maximum for iron followed by lead, manganese and zinc in the algal mat from both active mine and coal storage sites.

Significant correlations were observed in metal content in algal mat and water. Iron concentration in water and algal mat was positively correlated ($r = 0.90$ $p<0.05$) during spring ($r = 0.83$ $p<0.05$) and in autumn (Fig. 1, 2) but was negatively correlated ($r = -0.86$ $p<0.05$) during winter showing more accumulation in algal mat (Fig. 3). Lead concentration in water and algal mat was positively correlated ($r = 0.59$ $p<0.05$) during spring (Fig. 4) and in autumn ($r = 0.70$ $p<0.05$) (Fig. 5). In addition, lead concentration was positively correlated ($r = 0.59$, $p<0.05$) even in summer-monsoon (Fig. 6). No significant correlations could be observed in case of zinc and manganese content in water and algal mat.

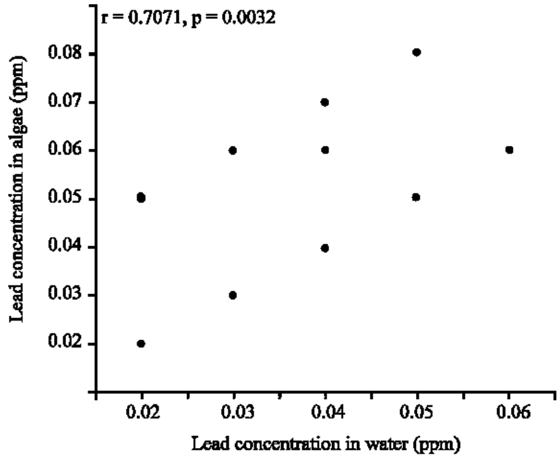

Fig. 5: Lead concentration in algae and water during monsoon

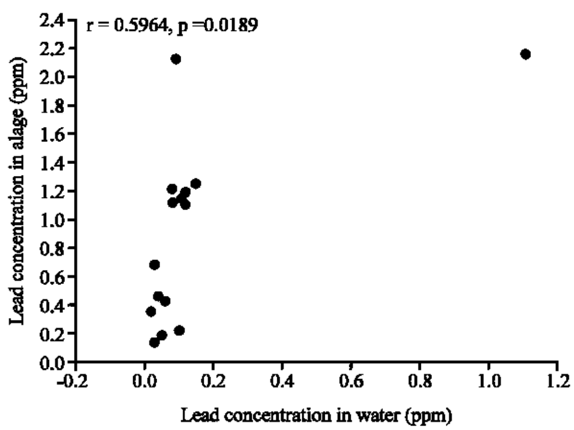

Fig. 6: Lead concentration in algae and water during autumn

DISCUSSION

The present field study revealed that coal mine impacted streams of all the categories of Jaintia Hills were highly acidic and low in nutrients in all the seasons. Heavy rainfall in the monsoon resulted in high current velocities which lead to poor growth of the filamentous algal mat. Except monsoon, in all other seasons mainly from Autumn to Spring, the streams remained covered with dark green filamentous *Microspora quardata* mats which showed very high efficiency to accumulate metals like iron, lead and manganese from the surrounding water. Iron precipitations as deep brown colour in the streams and also in the algal mat could be observed as prominent feature in winter and spring when the level of water was very low and these systems are saturated with iron (Stevens *et al*., 2001). Low pH, high conductivity and high metal concentrations in the water bodies of the region have already been reported by Singh (2005). In the present study, *Microspora quadrata* mats growing in coal mine affected streams have significantly greater amount of iron, lead, manganese in comparison with the water samples which suggested accumulation of metals. Likewise, Foster (1982) reported several times higher accumulation of iron and zinc by algae from rivers impacted by copper and lead mines. Rai *et al*. (1981) reported metal accumulation by algae in greater amount than the surrounding water. Similarly, in a study of metal polluted streams in southeastern Ohio Stevens *et al*. (2001) found a similar relationship between algal metal uptake and water concentrations for iron, manganese in *Klebosormedium* dominated algal mats. In addition, a number of researchers have reported algae sequestering metals in field as well as in laboratory conditions. For example, Okuo *et al*. (2006) studied the adsorption capacity of *Spirogyra* to remove Cr^{6+} and Cu^{2+} ions from aqueous solutions. Rezaee *et al*. (2006) also studied the biosorption capacity of *Spirogyra* to remove mercury from waste water. Fereshteh *et al*. (2007) studied the arsenic concentration in surface water in different seasons and the efficiency of *Chara vulgaris* in removing this metal. Similarly metal accumulation was also studied in marine algae. Uptake capacities of lead (II) and cadmium (II) by *Sargassum* sp. biomass was studied from aqueous solutions by Nabizadeh *et al*. (2006). Khorramabadi and Cheshmeh Soltani (2008) studied the adsorption capacity of two marine algae (*Gracilaria salicornia* and *Sargassum* sp.) to sequester Cr (VI) from aqueous solutions. Lamai *et al*. (2005) reported accumulation of cadmium and lead by filamentous green algae *Cladophora fracta* at different concentrations and exposure times. All these reports were in support of the present study where different groups of algae were capable of uptaking metals from natural as well as laboratory conditions.

Many times higher accumulation of iron followed by lead and manganese could be observed in the present study but the same was in contradiction for zinc. Whitton *et al*. (1989) demonstrated significant increase of iron and zinc in algal mass in filamentous green *cladophora glomerata*. Lawrence *et al*. (1998) also found similar metal sequestration by algal mat.

In the present case, marked seasonal variations were observed in accumulation pattern of different metals. Iron accumulation was maximum in autumn; lead and manganese were in spring. This could be due to the fact that the *Microspora* mat grew luxuriantly in all seasons except monsoon where high current velocity affected the growth of those mat. Karadede-Akin and Unlu (2007) reported similar results of accumulation pattern of iron, zinc and manganese in Spring by *spirogyra sp.* from Tigris River, Turkey. Zinc concentration was lower in algal mat compared to water. Say *et al.* (1977) reported that increased in phosphate reduce zinc toxicity in the alga. This can be due to some intracellular mechanism that binds zinc with phosphate in order to reduce its toxic effects. Low accumulation of zinc by *Microspora quadrata* in the present study might be due to similar reasons.

Metal concentrations in the water bodies receiving water from different categories of mining and metal accumulation in algal mat clearly indicate that storage of coal and active mining in these regions have caused serious damage to water bodies and the reclamation process is very slow as reflected by the more or less equal concentration of metals in streams of abandoned sites. Luxuriant growth of *Microspora*, its high accumulation efficiency and huge biomass growing in AMD streams could be a useful plant material to reduce the toxic effect of those metals to certain level.

CONCLUSION

It is evident from the present work that primitive method of coal mining in Jaintia Hills district of Meghalaya has caused serious damage to water bodies of that region. The ability of *Microspora* mat (growing in all the seasons) to remove toxic metals could be utilized as an important plant material in AMD streams. This study has shown that *Microspora quadrata* accumulate metals like iron, lead and manganese. Further studies are required to determine whether these metals are absorbed or adsorbed except zinc. In addition, behaviour of this alga in other metal polluted water bodies are needed to establish the efficiency of this alga as a bio-monitoring agent in metal polluted aquatic systems.

ACKNOWLEDGMENTS

The authors would like to thank University Grants Commission for the financial assistance and Botany Department, North Eastern Hill University, Shillong, for providing all the facilities.

REFERENCES

APHA, 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edn., American Public Health Association, Washington, DC., USA.

Bishnoi, N.R., A. Pant and Garima, 2004. Biosorption of copper from aqueous solution using algal biomass. J. Sci. Indus. Res., 63: 813-816.

Fereshteh, G., B. Yassaman, A.M.M. Reza, A. Zavar and M. Hossein, 2007. Phytoremediation of arsenic by macroalga: Implication in natural contaminated water, Northeast Iran. J. Applied Sci., 7: 1614-1619.

Foster, P.L., 1982. Species associations and metal contents of algae from rivers polluted by heavy metals. Freshwater Biol., 12: 17-39.

Khorramabadi, Gh.S. and R.D. Cheshmeh Soltani, 2008. Evaluation of the marine algae *Gracilaria salicornia* and *Sargassum* sp. for the biosorption of Cr (VI) from aqueous solutions. J. Applied Sci., 8: 2163-2167.

Kaonga, C.C., S. Chiotha, M. Monjerezi, E. Fabiano and E.M. Henry, 2008. Levels of cadmium, manganese and lead in water and algae, *Spirogyra aequinoctialis*. Int. J. Sci. Technol., 5: 471-478.

Karadede-Akin, H. and E. Unlu, 2007. Heavy metal concentrations in water, sediment and fish and some benthic organisms from Tigris River, Turkey. Environ.Monit. Assess., 131: 323-337.

Lamai, C., M. Kruatrachue, P. Pokethitiyook, S. Upatham and V. Soonthornsarathool, 2005. Toxicity and accumulation of lead and cadmium in the filamentous green alga *Cladophora fracta* (O.F.Muller ex Vahl) kutzing: A laboratory study. Sci. Asia, 31: 121-127.

Lawrence, J.R., G.D.W. Swerhone and Y.T.J. Kwong, 1998. Natural attenuation of aqueous metal contamination by an algal mat. Can. J. Microbiol., 44: 825-832.

Nabizadeh, R., N. Kazem and S. Reza, 2006. Biosorption of lead (II) and cadmium (II) from aqueous solutions by protonated *Sargassum* sp. Biomass. Biotechnology, 5: 21-26.

Nath, M. and M. Ahmed, 2005. Environmental Impact of Coal Mining of Bapung Coalfield, Jaintia Hills, Meghalaya. In: Mining Environment: Problems and Remedies. Regency Publications, New Delhi, India.

Okuo, J.M., S.B. Sanni and S.U. Aigbedion, 2006. Selective biosorption of heavy metal ions from aqueous solutions by pre-treated Nigerian fresh water algae. Trends Applied Sci. Res., 1: 83-90.

Rai, L.C., J.P. Gaur and H.D. Kumar, 1981. Phycology and heavy metal pollution. Biol. Rev., 56: 99-151.

Rezaee, A., B. Ramavandi, F. Ganati, M. Ansari and A. Solimanian, 2006. Biosorption of mercury by biomass of filamentous algae *Spirogyra* species. J. Boil. Sci., 6: 695-700.

Say, P.J., B.M. Diaz and B.A. Whitton, 1977. Influence of zinc on lotic plants. I. Tolerance of *Hormidium* species to zinc. Freshwater Biol., 7: 357-376.

Singh, O.P., 2005. Mining Environment: Problems and Remedies. Regency Publications, New Delhi.

Stevens, A.E. B.C. McCarthy and M.L. Vis, 2001. Metal content of *Klebosormedium*-dominated (chlorophyta) algal mats from acid mine drainage waters in Southeastern Ohio. J. Torr. Bot. Soc., 123: 226-233.

Verb, R.G. and M.L. Vis, 2001. Macroalgal communities from an acid mine drainage impacted watershed. Aquatic Bot., 71: 93-107.

Verb, R.G. and M.L. Vis, 2005. Periphyton assemblages as bioindicators of mine-drainage inunglaciated Western Allegheny Plateau lotic systems. Water Air Soil Pollut., 161: 227-265.

Whitton, B.A., I.G. Burrows and M.G. Kelly, 1989. Use of *Cladophora glomerata* to monitor heavy metals in rivers. J. Applied Phycol., 1: 293-299.