CHAPTER 8
GENERAL DISCUSSION

Chemistry of any water body depends on its physical, chemical and biological processes involving regional geology, biological characteristics, climate and human activity (Pinol et al., 1992; Fukushima et al., 2000; Holloway & Dahlgren, 2001; Sanchez & Ayuso, 2003; Ohta et al., 2005). Acid mine drainage (AMD) is formed when pyritic minerals are exposed to atmospheric, hydrological or biological weathering (oxygen, water and chaemoautotrophic bacteria) and get oxidised and result in sulphuric acid formation with low pH, dissolved metal ions, elevated sulphate contents (Skousen et al., 1994), high acidity and conductivity. Arsenic, copper, iron, manganese, lead, zinc and sulphates are frequently found in high concentrations, and their solubility increases with acidity (Dickson, 1975, Dickson et al., 1978; Stokes & Hutchinson, 1975; Beamish & Vanloon, 1977; Van Dam et al., 1994; Harding & Boothroyd 2004). Water pollution by heavy metals due to mining has attracted considerable attention since it is a worldwide problem with serious environmental consequences. Essential metals are needed for the growth and metabolism of organisms. However, both essential and non essential metals can be toxic when present above certain threshold levels (Lyngdoh & Kayang, 2012).

In the present study, analysis of various water parameters clearly indicated the adverse effect of extensive coal mining on the streams of Jaintia hills district. Low pH was obtained throughout the study from all the three coal mine impacted streams which ranged from 4.28 to 2.88 depending on the sites and seasons.
Low pH in coal mine impacted streams of the region has been reported (Swer & Singh, 2005; Das & Ramanujam, 2010). Another important factor reflecting the health of an aquatic system is the dissolved oxygen. Dissolved oxygen in the impacted streams varied from 7.26 to 2.85 mg/l, conductivity varied from 233 to 954.35 µS/cm, turbidity varied from 1.16 to 10.77 NTU and sulphate content varied from 42.01 to 188.12 mg/l from the entire three coal mine impacted streams depending on the sites and seasons. Low level of dissolved oxygen in mine affected creeks or streams in a mining community in Ghana were reported by Debrah et al., (2010). Lee et al., (2005) demonstrated the effect of dissolved oxygen in the water bodies of the abandoned zinc mines in and around of Korea. Presence of higher conductivity, turbidity and sulphate content in the impacted streams have been reported (Adekunle et al., 2007; Gray, 1998; Kennedy et al., 2003; Soucek et al., 2000). But, the stream away from the mining area showed comparatively neutral environment with high dissolved oxygen (5.72 to 13.53 mg/l) nearly neutral pH (6.02 to 6.69), low conductivity (19.87 to 79.75 µS/cm) and sulphate content (4.60 to 31.12 mg/l) (Das & Ramanujam, 2010, 2012). The values were below the guidelines prescribed by WHO and BIS. Analysis of impacted stream water for various metals - iron, manganese, lead, nickel, and chromium indicated the exceeded limit of these metals from the limit prescribed by BIS (2009) and WHO (2011). Only, zinc has been found to be within permissible limits. Low pH, dissolved oxygen, along with elevated metal concentrations clearly indicated the AMD (Acid mine drainage) nature of the coal mine impacted streams. Swer and Singh (2005) have already reported the AMD nature of the streams from Jaintia hills district. PCA of different water parameters confirmed that the physico-chemical parameters like high metal
concentrations, conductivity and low pH in the mining streams (sites SII, SIII and SIV) characterised the streams in mining regions and differed significantly from that of clean, unimpacted stream (SI). The spatial gradient was the main driving force for characterization of the streams (Luis et al., 2009). Hierarchical cluster analysis further established the fact that low pH and high conductivity of water played a major role in grouping the streams. Similar clustering of streams based on pH, water conductivity and metal concentrations has been reported across the AMD gradient by Verb and Vis (2005) in Southeastern Ohio.

Periphytonic and benthic communities that usually grow attached to different substrata for a long time have long been widely used in assessment of any physical and chemical changes in the aquatic environment as they are very sensitive and responsive (Horne & Goldman, 1994; Allan, 1995; Stevenson & Pan, 1999). Significant reduction in algal species number to varied 18 -36 in coal mine impacted streams from 234 algal species recorded in the unimpacted stream indicated the severity of the problem. Algal assemblages belonging to 8 different groups viz. Chlorophyceae, Bacillariophyceae, Cyanophyceae, Euglenophyceae, Xanthophyceae, Chrysophyceae, Dinophyceae and Rhodophyceae indicated the diverse and rich assemblage of algal species in unimpacted streams, whereas presence of only 36 to18 species in impacted streams, represented by members of four different algal groups, Chlorophyceae, Bacillariophyceae, Cyanophyceae, Euglenophyceae represented the highly unfavourable habitats for the growth of diverse algal groups. Quality of water played a major role in the composition and biomass production of algal communities. Species were evenly distributed in unimpacted stream with high evenness index in all the seasons whereas dominance index was high in all three AMD streams in different
seasons, indicating the dominance of only a few tolerant species. Similar findings have been reported from mine affected water bodies (Biggs & Gerbeaux 1993; Biggs 1996; Verb & Vis 2001; Biggs & Smith, 2002; Hayward, 2003; Bray, 2008). Moreover, the physicochemical conditions of Acid mine drainage constrained the number of species capable of growth, resulting in changes in community composition and function within affected streams along a temporal gradient. Verb and Vis (2001, 2005) reported that the harsh change in chemical and physical conditions of moderately and severely impacted streams constrained the number of taxa to grow and thus gradually lowered and significantly changed the taxonomic groups in streams with increase in AMD severity. Many other studies from different AMD systems supported these results where species richness was low (Bray, 2008; Sabater et al., 2003; Luis et al., 2009; Pena & Barreiro, 2009). In the present study, communities in AMD impacted streams were found to be dominated throughout the year with Microspora quadrata and to a lesser extent by Klebosormedium acidophilum, with minimal seasonal changes. Similar findings were also reported by others (Bray, 2005; Verb & Vis, 2001; 2006; Stevens et al., 2001). In addition to these two green algae, a few tolerant diatom species like Frustulia rhomboideis, Kutzing., Navicula cryptocephala Kutzing., Navicula microspora Kant and Gupta., Navicula viridis Kutz., Pinnularia viridis (Nitz.) Ehrenberg., Eunotia exigua (Brebisson ex Kuetzing), Euglena mutabilis Schmidtz, a member from Euglenophyceae also dominated the AMD streams with high cell density. Tolerance of these taxa in extreme stress conditions were reported by many (Joseph,1953; Bennett, 1969; Rai et al., 1981; Whitton & Kelly,1995; DeNicola, 2000; Sabater et al., 2003; Gerhardt et al., 2008). The dominance of Bacillariophyceae and
Chlorophyceae as observed in all the streams were also reported elsewhere by Temel (2006); Saadet and Sahin (2009); Spackova et al., (2009); Sahin et al., (2010); Baba et al., (2011) and Hussein and Gharib (2012). Structure of algal communities in AMD was governed by a wide range of abiotic factors mainly physicochemical variables (Biggs 1990; Biggs & Gerbeaux 1993; Biggs et al., 1998). CCA again established the fact that pH, conductivity and dissolved metals were the important aspects governing the distributional pattern of algal communities in unimpacted and AMD impacted streams (Fjellheim & Raddum, 1990; Planas 1990; Stormer & Smol, 1999; Peterson 2007; Luis et al., 2009). Based on algal composition, the sites were separated into clusters of unimpacted and impacted streams category separately.

In any AMD system, communities are restricted to a few tolerant organisms with their different functional aspects. A few diatom species with high abundance dominated the AMD streams along with two members from Chlorophyceae group. Disappearance of many sensitive species was compensated by tolerant periphytonic and benthic algal species which dominated the AMD systems by increasing their productivity and biomass. These dominant species adopted structurally and functionally to the altered and adverse physico chemical conditions of the surroundings ((Niyogi et al., 1999; 2002). An important character observed in AMD streams of this region was formation of huge biomass by Microspora quadrata and Klebosormedium acidophilum as the main component. Algal biomass was significantly greater in the AMD impacted, low pH streams, which is in accordance with several studies (Mulholland et al., 1986; Verb & Vis, 2001; Sabater et al., 2003) but according to others, the biomass in the AMD streams were low (Kinross et al., 1993; Anthony, 1999; Hill et al., 2000; Verb & Vis, 2005). According to Verb and
Vis (2005), and Novis (2006), the growth of tolerant species might be stimulated when physicochemical conditions such as pH remain optimised for the particular species. Alternatively, removal of other taxa by the extreme physicochemical conditions release the tolerant species from interspecific competition (Niyogi et al., 1999). Furthermore, altered physicochemical conditions exclude grazers, thus, release algae from any top-down control that might normally be occurring (Rosemond et al., 1993; Niyogi et al., 2002). Phosphorus content in the present AMD streams were found in elevated quantity which in turn enhanced the growth of the dominant taxa. Bray, (2008) reported that nutrient concentrations in acidic streams were high, inorganic phosphorus exceeded 1mg/l and inorganic nitrogen (mainly nitrate and nitrite) reached over 1mg/l. High nutrient concentrations, in conjunction with other factors, such as stable flow and limited grazing, probably accounted for the high biomass in the acidic streams. High productivity of the algal assemblages from AMD streams showed significant positive correlation ship with N: P ratio. Phosphorus loading to these streams (above 1mg/l) could increase the biomass of periphytons and macro algae as measured by chlorophyll a content (Welch et al., 1998). Low pH of the impacted streams could be the reason for phosphorus availability as phosphorus was highly soluble in low pH (Lessmann et al., 2003; Lund & McCullough, 2009). High conductivity in AMD streams was also another important factor responsible for enhanced productivity (Pena & Barreiro, 2009). Productivity varied significantly with the addition of different nutrients in Microspora quadrata, the green tolerant alga, isolated from the AMD impacted systems. The cultured alga when exposed to different concentrations of metals for different time periods, productivity showed a differential growth response in the form of chlorophyll a content in correspondence
with the metals and its concentrations. These differences were directly linked to the respective sensitiveness of the alga to acidity and elevated metal concentrations. Olaveson and Nalewajko (2000) also demonstrated similar results by using two strains of *Euglena* species, isolated from AMD conditions. Lamai et al., (2005) studied the toxicity of different metals on *Cladophora fracta* under laboratory conditions. Chlorophyll *a* content of the alga decreased after a specific time period and with higher doses of metals. This could be due to the fact that metals present in high concentrations disorganized the thylakoids membrane. Consequently the photosynthetic activity was severely affected causing growth inhibition or complete death of the cells. Further, it was found in the study that *Microspora* filamenst when grown on different pH range, preferred pH 2.0 to 4.0 which confirmed the acidophilic nature of the alga. Leavitt (1999) studied high abundance of alga at low pH. Hargreaves and Whitton (1976) studied the effect of pH on the growth of five algal species isolated from AMD systems and grew them in laboratory under different pH conditions. They reported that these species could grow even at lower pH than the range from where they were isolated.

Stress-tolerant algae may exhibit unique responses when exposed to multiple stress factors (Roleda et al., 2010). Toxicity from metals play an important role in structuring algal communities of AMD streams. The dominant algal species from AMD like *Microspora quadrata* and dominant diatom species showed mineral depositions on its cell wall. When found in natural conditions, the alga was growing well as the metals were not toxic to the alga at the concentrations found in the environment. Ultra structural studies in this case showed the adsorption capacity of the alga by depositing minerals on its outer cell surface. Similar results were
demonstrated by Roleda et al., (2010) when a green macroalga *Urospora penicilliformis* from Arctic Spitsbergen was stressed by UV radiation. Disorganized thylakoid membrane was also observed in some filaments which were also demonstrated in *Synechocystis* sp. at higher (6 and 8 mg/l lead) exposure by Arunakumara and Xuecheng (2009). Another important strategy of algal communities when exposed to stress was their capability to accumulate metals either by absorption or adsorption. Green algae are well known for the removal of metals from their surrounding water which was proved by many studies from AMD systems and was also supported by laboratory studies. Kaonga et al., (2008) reported higher concentration of metals in *Spirogyra aequinoctialis* than in the corresponding water in relation to pH in dry and rainy seasons. In this case, metals like iron, lead, manganese, nickel, chromium and zinc were found in elevated quantity from different AMD streams in different seasons. Concentrations of most of the metals studied were significantly higher in algal mat except zinc which was more in water than in algal biomass. Similar high accumulation capacity of different algal taxa have been reported from several AMD systems worldwide (Whitton & Kelly, 1989, 1995; Lawrence et al., 1998; Stevens et al., Akin & Unlu, 2007; Das & Ramanujam, 2011). Zinc accumulation was less in algal mat compared to water. Say et al (1977) reported that increase in phosphate reduced zinc toxicity in the alga. Stevens et al., (2001) too reported low zinc accumulation in *Klebsormedium* mat and explained that this reduced toxicity is an indicative of an intracellular mechanism that bound zinc with phosphate in order to reduce its toxic effect. Low accumulation of zinc by *Microspora quadrata* in the present study might be due to similar reasons. This metal uptake and accumulation pattern by filamentous green algae (*Microspora quadrata*)
has been supported by laboratory studies. Similar results were observed where there was a gradual increase in iron and lead concentrations in the alga and the maximum accumulation was recorded on the 8\textsuperscript{th} day of exposure in all the given concentrations. Zinc accumulation by the alga was maximum on the 1\textsuperscript{st} exposure, i.e., 2\textsuperscript{nd} day and no trend was followed in accumulation pattern thereafter. 95-98\% of iron and lead removal was observed by the alga. 93-95\% removal of zinc was observed till 4\textsuperscript{th} day exposure, thereafter reducing the removal potential (60-75\%), manganese removing potential of the alga was also recorded during different days of exposure. Similar pattern of uptake and accumulation by filamentous algae for different metals has been reported (Pagnelli \textit{et al.}, 2000; Lamai \textit{et al.}, 2005; Okuo \textit{et al.}, 2006; Paula \textit{et al.}, 2011; Kumar, 2012).