Physiological Effects of Mining Contaminants on Algae with Special Reference to Heavy Metal Toxicity

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Abstract:

The process of mining affects all the components of environment and ecology. resulting in different types of pollution problems like soil, surface and ground water pollution. Acid Mine Drainage (AMD) and mine spoil dumps may contain an elevated amount of Heavy metals. Physiological effect of heavy metal toxicity on algal species like Chlorella vulgaris, Scenedesmusbijugatus, Oscillatoriaamphibia and Lyngbyamajuscula isolated from mine waters of Mansar, Kandri,Beldongari and Gumgaon Manganese mines of MOIL, Nagpur Region were investigated. Results obtained showed significant concentrations of heavy metals in mine spoil and mine water. Observed Mn and Fe concentrations were very high (215.2 to 224.3 and 47.9 to 52.5 mg kg^{-1}, respectively in mine spoil followed by Cu, Zn, Cr, Pb and Cd concentrations. Similarly, mine waters of all sampling sites showed elevated concentrations of manganese (1.645 to 1.745 mg/l), iron (0.950 to 1.124 mg/l) followed by copper (0.029 to 0.032 mg/l) and zinc (0.011 to 0.013 mg/l) while concentrations of cadmium, chromium and lead remained below detecting limit and hence they were selected for toxicity tests. Most of the tested algal species were reported with maximum inhibitory level and TIC to iron and manganese than zinc and copper. Among all algal species cyanophycean member Oscillatoriaamphibia was reported with maximum inhibitory concentration for Mn(2.5 mg/l), Fe (2.4 mg/l), Cu (1.0 mg/l) and Zn (1.7 mg/l) and Lyngbyamajuscula with Mn (2.2 mg/l), Fe (2.3 mg/l), Cu (0.9 mg/l) and Zn (1.4 mg/l) whereas chlorophycean member Chlorella vulgaris was reported with minimum inhibitory concentrations. Tolerance Index Concentration values also revealed more tolerance to iron and manganese and least to copper and zinc. Among cyanophycean members, Oscillatoriaamphibia was reported with more TIC to zinc (0.784 mg/l) while Lyngbyamajuscula with more TIC to iron (1.811 mg/l) followed by manganese (1.574mg/l) and copper (0.665 mg/l). The chlorophycean member, Scenedesmusbijugatuswas reported with more TIC to iron (1.543 mg/l) followed by manganese (1.268 mg/l) and copper (0.227 mg/l) whereas Chlorella vulgaris with more TIC to zinc (0.271 mg/l).Overall, all the algal species were affected by heavy metal toxicity and their observed tolerance to the heavy metals especially to higher concentrations of Mn and Fe might be due to adaptation contaminated mine water in the vicinity of mines.

Keywords: *Mining pollution, AMD, Heavy metals, Inhibitory level, TIC*

Introduction:

It is known that minerals and metals are the basis of the economic development and welfare of the society. However, their exploration, excavation and mineral processing directly interferes and affects other natural resources like land, water, air, flora and fauna, which needs to be conserved and specifically utilized in a sustainable manner. The mineral sector in India is on the doorstep of expansion with more and more open cast/ underground manganese ore mines in several states including Maharashtra state especially in Vidarbha region because of sufficient amount of manganese deposits. Under such conditions, orderly and scientific exploitation of manganese ore, in accordance with the state of the environment is necessary for survival of our future generation.

The process of mining affects all the components of environment and the impacts are permanent or temporary, beneficial or harmful, repairable or irreparable, and reversible or irreversible. Open cast manganese mines, due to its own peculiarity can cause various ecological disturbances, resulting in different types of pollution problems. The environmental problems are of more concern in India, as most of the manganese ore mines located either on top of hills or plains near forest areas and human settlement areas where agricultural practices takes place.

The environmental problems linked with the manganese ore mining are varied (Singh, 1997). The removal of vegetation, top soil, overburden or waste and ore, brings about the expected natural consequences, which were apparent in many ways, land disturbances, change in land use pattern and fertility of soil (Ghosh,2004; Warhateet al. 2006; Mohapatra and Goswami 2012), destruction of floral and faunal habitats (Harding, 2005; Freitas et al. 2010), disturbances in natural watershed and drainage pattern of the area, affects underground water level, deforestation (Juwarkar et al. 2009 and 2010), climatic change, erosion, air pollution due to dust and noxious fumes, water pollution due to surface runoff (Lambert et al. 2004; Harding,2005; Singh et al.2008) and health hazards (Roy et al. 2003).

The scale and consequences of these impacts on environment and ecology due to mining will depend on the size and magnitude of mining activity with respect to the geology, topography and climatic conditions of the area (Roy et al. 2003, Serban and Balteanu, 2004; Ravengai et al. 2005; Kraft, et al. 2006).

Natural and anthropogenic influences both are the main sources of heavy metals; anthropogenic inputs are proportionately greater in some areas than those from natural sources. Some researchers (Holdgate 1979, Alloway, 1994; Ahmad, 2005) proposed that it's regularly the by-products of mining, manufacturing, disposing of industrial metals and domestic waste responsible for almost all environmental pollution.

Mining activities produce large amount of waste materials and tailings that are deposited at the surface in the form of mine spoil dumps which are nutritionally poor habitats (Gonzalez-Sangregorioet al.1991).Metal contamination is not limited to the site since considerable discharge of metal occurs from native place to surrounding environment in the form of acid mine drainage and erosion of waste spoil dumps and tailings deposits (Salomons, 1995).

The threat of soil, surface and groundwater pollution increases considerably when the waste materials from the mine contain reactive sulphide minerals or ores generating acid mine drainage (Liao et al. 2007; Robb and Robinson, 1995). Acid mine drainage typically contains a high load of heavy metals and characterized by low pH, which poses a major risk to surrounding water and soil systems (Braungardtet al. 2003; Achterberget al.2003). Chemical problems connected with surface mining, usually acid generating materials, are thus noteworthy and the geomorphic system in mine spoils is in disequilibrium (Darmodyet al. 2002; Dutta and Agarwal, 2001).

Heavy metal pollutants can concentrate and lay dormant; unlike organic pollutants they do not decay and hence require different approach for remediation. However, some plants or microorganisms are uncertainly used to remove heavy metals from soils. Plants exhibiting hyper accumulation can be used to remove metals through the process of concentrating them into their bio matter. An imminent apprehension associated with the persistence of heavy metals is the possibility for bioaccumulation and biomagnifications to become more prevalent to some organisms rather than occurring naturally (Hogan 2010).

Many heavy metals are necessary micronutrients for algal metabolisms e.g. Fe, Cu, Zn, and Mn, and they may restrict algal growth at low external concentrations while other heavy metals, e.g. Au, Ag, Pb and Cd, have no known metabolic functions but of all these elements may be highly toxic towards algae and other aquatic organisms (Gadd and Griffiths, 1978).

Almost all heavy metals are toxic to algae at high concentration. Heavy-metal toxicity resulted in the poisoning and inactivation of enzyme systems as well as many physiological and biochemical processes such as photosynthesis, respiration, protein synthesis and chlorophyll synthesis, etc.. Some algae inhabit water constantly polluted with wastes containing heavy metals from mining and smelting operations. *Nodularia* sp., *Oscillatoriaamphibia* sp., *Cladophora* sp., *Hormidium* sp., *Fucus* sp. and *Laminaria* sp., etc., occur in metal-rich waters. These algal forms are most likely to be capable of combating the toxic levels of heavy metals and this feature resulted in physiological and/or genetic adaptations in them. The sensitivity or tolerance to heavy metals varies amongst different algae and some algae exhibits phenomenon of multipletolerance and co-tolerance (Raiet al. 1981).

Inhibitory effects exhibited in algae with increasing concentration of heavy metals included depression of net growth rate, biochemical changes followed sometimes by morphological changes in cells and eventually death. The biological variables used to measure inhibition includes cell counts, net

photosynthesis, respiration, chlorophyll content, ATP, DNA, RNA, dry matter content, wet weight, carbon balance. Toxicity of heavy metals varies from species to species and metal to metal. The study of heavy metals in relation with algae has been widely investigated and reviewed by Rivkin (1979), Say et al. (1977); Abdullab and Royle (1972); Moore and Rammoorthy (1984); Whittonet al. (1981); Sultan and Fatma (1999); Fargasova (1999); Qianghuet al. (2000); Pradhan (1992) and Khapekar (2006).
The capability of algae to survive and reproduce in metal polluted habitats may be depends upon genetic adaptation by mutation, genetic exchange, selection, etc. over extended time periods or to changes in physiology resulting from metal exposure (Gadd,1990). It is still complicated to define such terms exactly and it is not possible to determine concentration ranges for heavy metals which demarcate them.

Aquatic habitats mainly contains heavy metals in dissolved forms or chelated with inorganic/organic ligands or in particulate forms and the comparative proportion of each constituent may result in the modification of the overall metal toxicity (Sunda and Guillard, 1976). Heavy metals exert their harmful effect in many ways, while the major mechanisms of toxicity are outcome of the strong coordinating properties of the heavy metals ions (Ochiai, 1987). The effect of heavy metals on algae may include an irreversible increase in plasmalemmapermeability (De Filips, 1979) and changes in cell volume (Christensen et al. 1979), inhibition of respiratory oxygen consumption (Rivkin, 1979), reduction in photosynthetic electron transport (Shioiet al.1978) and photosynthetic carbon fixation (Davies and Sleep, 1980), Enzyme inhibition, due to the displacement of essential metal ions (Rebhun and Ben-Amotz, 1984), disruption of nutrient uptake processes (Harrison and Morrel, 1983), inhibition of protein synthesis (Kremer and Markham, 1982), abnormal morphological development (Say et al. 1977, Rosko and Rachlin, 1977) and ultrastructural changes including mitrochondrial swelling (Silverberg, 1976), multinucleation (Massalskiet al. 1981), granulation (Thomas et al. 1980), and alterations in vacuolar and chloroplast size (Smith, 1983), impairment of motility and loss of flagella in certain microalgae (Nakonoet al. 1978) and degradation of photosynthetic pigments, coupled with reductions in growth (Monahan, 1976) and in extreme cases, cell mortality (Fennikohet al. 1978), reduced nitrogenase activity in blue green algae (Stratton and Corke, 1979).

The opencast and underground mining deteriorates the environment in numerous ways. One of the aspects of environment, it harms the most to the water in the form of heavy metal contamination. Thus, in present investigation estimation of mine water quality especially heavy metal content and its impact on physiology of algal species were taken in to account for proper assessment of the associated hazards with special reference to the inhibitory level and Tolerance Index Concentration (TIC) of heavy metals on collected algae from mine waters of different mining sites.

Material and Methods:

Study Area

Manganese Ore India Limited (MOIL) a Government of India undertaking having mines in Maharashtra and Madhya Pradesh, is producing about 0.6 million tonnes of mine ore per annum, mines are both underground and opencast in nature and the total lease area under soil is 2145, 89 ha. Gum gaon, Kandri, Mansar and Beldongri manganese mines of MOIL are selected for the studyand the survey of all these mines for water and algal sample collection was conducted during the year 2009. These mines are situated in Nagpur District of Vidarbha Region.

Collection of soil and water Samples

The soil samples were collected from the mine spoil dumps in mining area of Gumgaon, Kandri, Mansar and Beldongari Manganese mines during the year 2009. Depth wise soil samples were collected from the mine spoil dumps at the depths of 0-30, 30-60, and 60-90 cm. The soil samples were then air dried in shade, ground and passed through 2 mm sieve. For the determination of heavy metals, the soils were ground and passed through 80/100 mesh sieve. The screened samples were well mixed and labeled and stored for subsequent use. The soil was analyzed for its heavy metal content following the standard procedures. The method DTPA (DiethylenetriaminePenta acetic acid) (Lindsay and Norvell 1978) were used for the determination of heavy metal content from mine spoil samples with the help of atomic absorption spectrophotometer (AAS).

Water samples were collected in 1 liter polyethylene bottle from the open mining pit of all mines to assess its heavy metal contents by adding HNO_3 to pH <2 and were analyzed by AAS (Atomic Absorption Spectrophotometer) according to the standard procedures. The samples were acid digested with HNO_3, H_2O_2, and HCL before analysis by AAS. The details of the soil and water samples collected from mines are presented in table 1.

Selection of Heavy metals for algal toxicity

In present investigation, it was observed that the mine water and mine spoil of sampling sites was contaminated with Fe, Mn, Zn and Cu along with others heavy metals and hence it become prime important to study the effect of these metals on algae. The selection of heavy metals for study was based on their common occurrence in mine water and mine spoil.

Algal species *Chlorella vulgaris, Scenedesmusbijugatus, Oscillatoriaamphibia*and *Lyngbyamajuscula* were collected from different sources at mines isolated, cultured in laboratory and selected to study the toxicity effect of Mn, Fe, Zn and Cu using A.R. grade, $MnSO_4H_2O$, $FeSO_4$.7 H_2O, $ZnSO_4$.7 H_2O and $CuSO_4$. 5 H_2O.

Preparation of stock solution of metals.

The stock solution of metals were prepared by adding 4.3979 gm Zn SO_4 7 H_2O, 3.9282 gm of $CuSO_4$ 5 H_2O, 3.076 gm of $MnSO_4$ H_2O and 4.978 gm of Fe SO_4 7 H_2O to 100 ml distilled water separately. These stock solutions contained 1 ml = 10 mg of Zn, Cu, Mn and Fe, respectively. These stock solutions were prepared every month and stored in polyurethane bottle. These metal solutions were diluted to various concentration in the range of 0.001 -10 mg/l for determining inhibitory level.

Determination of Inhibitory level

An inhibitory level of each alga to Fe, Mn, Zn and Cu was determined in selecting the range of 0.001 mg/l to 10 mg/l. The heavy metals consider for study, were omitted from the micronutrient stock of BG-11 medium with respect to Cyanophycean members and Chu 10 medium with respect to Chlorophycean members while determining inhibitory level. Interaction of heavy metals was avoided by using separate glasswares. Different stock solution and BG-11 and Chu 10 medium were autoclaved separately.

The medium was poured in the test tube in the next day in laminar flow. At the time of inoculation, an inoculum was transferred in each tube followed by addition of metal stock solution. Each test was repeated in triplicate. The culture tubes were transferred to photon flux density 20-30u mol photon m^{-2} S^{-2} at 20 to 25°C with moderate shaking. The algae were grown for 20 days. The optical density for unicellular and dry weight for filamentous alga was selected as the criteria for measurement of growth of algae. A strongly inhibitory level of each alga was determined for Fe,Mn, Zn, and Cu. Inhibitory level of a toxic agent refered to that level which just permits a detectable growth, slightly higher level killed all cells unless mutation or adaptation occurs (Shehata and Whitton, 1982).

Determination of Tolerance Index Concentration (TIC)

TIC for algae is based on the procedure described by Say et al. (1977). Culture medium was prepared by omitting Fe, Mn, Cu, and Zn from composition of BG -11 and Chu -10 media, respectively. The medium poured in test tubes was autoclaved. Different dilutions of metal stock solution were made and autoclaved separately. At the time of inoculation, inoculum was transferred in each test tube followed by addition of metal solution. Each test was repeated in triplicate. The culture tube was transferred to photon flux density 20-30 µ mole photon $m-^{2}$ $s-^{2}$ at 22 to 25 ^{O}C with moderate shaking .Growth in test tubes was compared visually on 4 , 8 and 12 days, both against reserved replicate of the original inoculum and also with each tubes one against the other. Observation was recorded on each occasion as follows:

I. Maximum concentration causing no inhibition.
II. Maximum concentration causing some inhibition.
III. Maximum concentration at which alga is alive.
IV. Maximum concentration at which alga is killed.

The data from the toxicity test were further simplified by the following empirical formula given by (Say et al. 1977 and Whitton 1970a).

Just Non-inhibitory **(JNI) = (I.II) $^{0.5}$**

Just Lethal **(JL) = (III.IV) $^{0.5}$**

Tolerance Index Concentration **(TIC) = (I.II.III.IV) $^{0.25}$**

Statistical Analysis

SPSS statistical package (Window version 17), and Microsoft software Excel 2007 are used for data analysis. The analysis of the mine water heavy metal content data was carried out by using one-way ANOVA by Tukey's Honesty Significant Difference (HSD).

Results and Discussion:

Heavy metal content of mine spoil and mine water

In present study the result as depicted in Table 2 showed that the mine spoil at all sampling sites of manganese mines had adversely affected in case of its heavy metal content. Heavy metal analyses of spoil showed lower Cr, Pb, , Cd, Zn and Cu concentrations (0.026 to 0.030, 0.018 to 0.022, 0.008 to 0.012, 0.29 to 0.32 and 0.395 to 0.437 mg kg^{-1}, respectively) while, Mn and Fe concentrations were very high (215.2 to 224.3 and 47.9 to 52.5 mg kg^{-1}, respectively). Similar findings in support of present study were reported on heavy metal content by several researchers (Juwarkar and Jambhulkar, 2008; Juwarkar and Singh, 2010; Juwarkar et al. 2009; Juwarkar, et al. 1992).

Similarly, the heavy metal contents of mine waters of all sampling sites showed elevated concentrations of zinc (0.011 to 0.013 mg/l), copper (0.029 to 0.032 mg/l), iron (0.950 to 1.124 mg/l) and manganese (1.645 to 1.745 mg/l) while concentrations of cadmium, chromium and lead remained below detecting limit (Table 3). It was also evident the one –way ANOVA results that the heavy metals Fe, Cu, Zn were significantly differ ($p \leq 0.05$) in all sampling sites except Mn as the concentration remained nearly same among all sampling sites (Table 4). The present study finds support from the study carried out by Tiwary et al. (1995), Tiwary (2001) and Taranekar (1993) for manganese mines and coal mines of India whereas the findings of Van Hille et al. (1999) and Bamforth (2006), Caruso et al. (2011) also supports the results.

Heavy metal toxicity with respect to inhibitory level and TIC

Heavy metals are integral component of biosphere and occur naturally in water and soil. Almost all heavy metals are toxic to algae at higher concentration (Raiet al. 1981). They exhibit inhibitory effects with increasing concentration. Heavy metals like Fe, Mn, Zn, Mo are essential as trace nutrients for plant life including algae while others like cadmium, lead, nickel, chromium are not necessary.

In present investigation inhibitory levels of Fe, Cu, Zn and Mn to algal species were studied. It has been evident from the results (Table 5 and Fig.1) that most of the algal species were reported with maximum inhibitory concentration of iron and manganese. *Chlorella vulgaris* and *Scenedesmusbijugatus* were reported with maximum inhibitory (2.1mg/l) concentration of Fe and Mn, while *Oscillatoriaamphibia* and *Lyngbyamajuscula* were reported with 2.5 mg/l concentration of Mn and 2.3 mg/l of Fe. Among all algal species cyanophycean member *Oscillatoriaamphibia* was reported with maximum inhibitory concentration for Mn (2.5 mg/l), Fe (2.4 mg/l), Cu (1.0 mg/l) and Zn (1.7 mg/l) and *Lyngbyamajuscula* with Mn (2.2 mg/l), Fe (2.3 mg/l), Cu (0.9 mg/l) and Zn (1.4 mg/l) whereas chlorophycean member *Chlorella vulgaris* was reported with minimum inhibitory concentrations of all heavy metals under study. This suggests that cyanophycean members in present study were more tolerant species to the heavy metals.

Tolerance to heavy metals has been reported for a large number of organisms. Tolerance to the metal confers tolerance to one or more others. It was also evident from the calculated Tolerance Index Concentration values (Table 6 and Fig.2) that all algal species were reported with more values of TIC to iron and manganese and least to copper and zinc. Among cyanophycean members, *Oscillatoriaamphibia* was reported with more TIC to zinc (0.784 mg/l) while *Lyngbyamajuscula* with more TIC to iron (1.811 mg/l), manganese (1.574mg/l) and copper (0.665 mg/l). Whereas in case of chlorophycean members, *Scenedesmusbijugatus*was reported with more TIC to iron (1.543 mg/l), manganese (1.268 mg/l) and opper (0.227 mg/l) while *Chlorella vulgaris* with more TIC to zinc (0.271 mg/l).

However, algae require some major elements such as calcium, magnesium, potassium, nitrogen, phosphorus, sulphur, chloride as in case of higher plants (O'kelley, 1974) but certain trace elements like iron, silicon, zinc, cobalt, copper, manganese, molybdenum are also essential for the proper growth of algae (Round, 1973). Vanadium, cobalt and zinc are necessary for healthy growth and reproduction of some species (Noda and Horiguchi ,1971).

Similar findings were reported on inhibitory concentration and tolerance of algae to heavy metals with antagonistic action between zinc and cadmium to *Euglena gracilis* (Nakano et al. 1978, Pakalneet al. 1970; Upitiset al. 1973). However, it has been reported that high value of copper (10 mg/l) requires to inhibit growth of *Chlorella vulgaris* (Agrawal and Kumar, 1975) which is contradictory to our result for the same algal species isolated from mine water whereas nearly similar results were noted for *Lyngbyanigra* (0.45 mg/l of Cu) by Gupta and Arora (1978). Observed elevated inhibitory and tolerance index concentration to these essential heavy metals seems to be controlled by the algal species in mine pit waters as reported by Leland et al.(1973) and Andelman (1973) and might be adapted (Klerks and Weis, 1987) to contaminated aquatic habitats or ecosystems.

Also the findings of Gachter (1976), O.Kelley (1974); Thomas, et al.(1977); Gupta and Arora (1978) and Audholia and Saxena (1990) reported the same facts observed in present study.

Present study revealed that the isolated algae collected from mining sites showed more tolerance to higher level of heavy metals especially to iron and manganese which might be due to the physiological process in the tolerant algal species resulted additionally in an increase in heavy metal tolerance (Bradshaw 1975; Cox and Hutchinson 1979) as supported by various reports on tolerance (Allen and Sheppard 1971; Stokes et.al.1973; Preston and Huisingh, 1975; Tatsuyamaet.al. 1975 and Okomotoet.al. 1977).

Elevated levels of Mn, Fe, Cu and Zn in mine waters may introduce tolerance mechanism related to other physiological processes which develop in the tolerant population of algae as a result of adaptation to that particular habitat (Klerks and Weis 1987; Hall et al. 1979; Peters et al. 2011). The present study finds support from the observations on many algal species viz. *Stigeocloniumtenue* (Harding and Whitton, 1977; Kelly and Whitton, 1989) and *Homidiumrivulare* (Say and Whitton, 1977). Singh and Kashyap (1978) for *Chroococcuslimneticus* and *Plectonemaboryanum*, Blaylacket al. (1985) for *Selenastrumcapricomutum* and *Chlorella vulgaris*,Pradhan (1992) for *Phormidiumbohneri*, Khapekar (2006) for *Oscillatoriaamphibia,* Rousch and Sommerfeld (1999) for *Ulothrix sp.*

Table. 1-Soil and water samples with their respective locations and identity.

Sr.No.	Sampling sites	Location	Sample ID
Soil sample			
1.	Munsar Manganese Mine	Spoil dump	MMS
2.	Kandri Manganese Mine	Spoil dump	KMS
3.	Beldongari Manganese Mine	Spoil dump	BMS
4.	Gumgaon Manganese Mine	Spoil dump	GMS
Water sample			
1.	Munsar Manganese Mine	Mine pit	MW1
2.	Kandri Manganese Mine	Mine pit	KW1
3.	Beldongari Manganese Mine	Mine pit	BW1
4.	Gumgaon Manganese Mine	Mine pit	GW1

Table. 2- Heavy metal content of mine spoil samples of different Manganese Mines.

Sampling sites	*GMS	*KMS	*MMS	*BMS
Heavy Metals				
Chromium (Cr)	0.026±0.0008	0.028±0.001	0.030±0.0008	0.026±0.001
Lead (Pb)	0.018±0.0009	0.021±0.0009	0.022±0.0009	0.020±0.0009
Copper (Cu)	0.395±0.012	0.417±0.009	0.437±0.009	0.398±0.01
Cadmium (Cd)	0.008±0.0005	0.011±0.0008	0.012±0.001	0.01±0.0008
Zinc (Zn)	0.32±0.008	0.29±0.008	0.31±0.008	0.31±0.01
Manganese (Mn)	2152±23	2243±19	2220±15	217±1.3
Iron (Fe)	52.5±0.9	49.9±0.9	51.0±0.8	47.9±0.5

All values are in mg/kg

Table. 3- An average concentration of heavy metals in mine waters of different sampling sites.

	Sampling sites			
Heavy metals	**GW1**	**MW1**	**KW1**	**BW1**
Zinc	0.011 ±0.001[a]	0.012 ±0.001[b]	0.013 ±0.002[b]	0.013 ±0.002[b]
Cadmium	BDL	BDL	BDL	BDL
Copper	0.029 ±0.001[a]	0.031 ±0.002[b]	0.031 ±0.002[b]	0.032 ±0.002[b]
Iron	1.102 ±0.117[b]	1.124 ±0.144[b]	1.043 ±0.071[ab]	0.950 ±0.271[a]
Lead	BDL	BDL	BDL	BDL
Manganese	1.666 ±0.188[a]	1.645 ±0.299[a]	1.658 ±0.278[a]	1.745 ±0.266[a]
Chromium	BDL	BDL	BDL	BDL

All values are in mg/l , BDL= Below Detecting Limit Mean±SEM; For each column, different lowercase letter significantly differ at $p \leq 0.05$ level, as analyzed by 2-sided Tukey's HSD between different sampling sites.

Table. 4-One way ANOVA to test significant difference in average values of heavy metal content of mine water.

		Sum of Squares	df	Mean Square	F	Sig.(p≤0.05)
Zinc	Between Groups	.000	3	.000	9.038	**.000**
	Within Groups	.000	92	.000		
	Total	.001	95			
Copper	Between Groups	.000	3	.000	9.537	**.000**
	Within Groups	.001	92	.000		
	Total	.001	95			
Iron	Between Groups	.434	3	.145	5.108	**.003**
	Within Groups	2.608	92	.028		
	Total	3.043	95			
Manganese	Between Groups	.148	3	.049	.719	.543
	Within Groups	6.291	92	.068		
	Total	6.438	95			

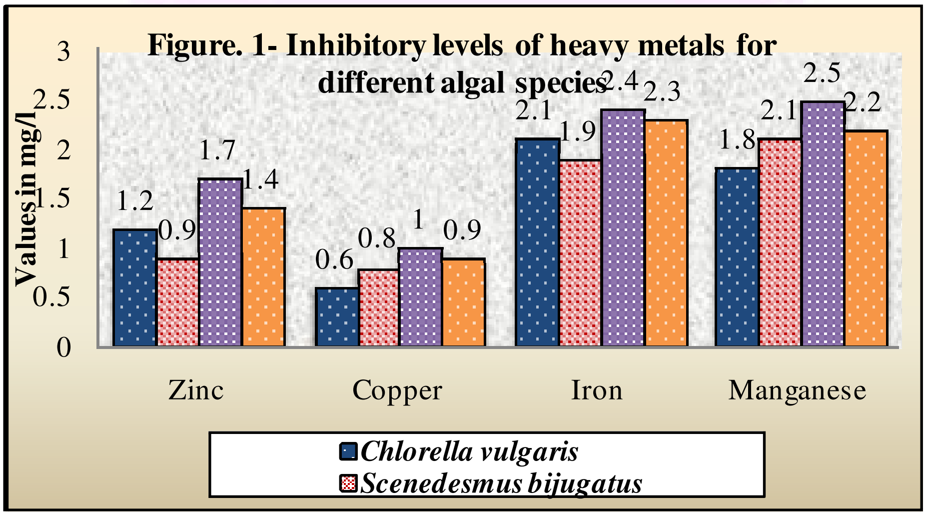
Table.5- Inhibitory levels of heavy metals for different algal species isolated from the mine water.

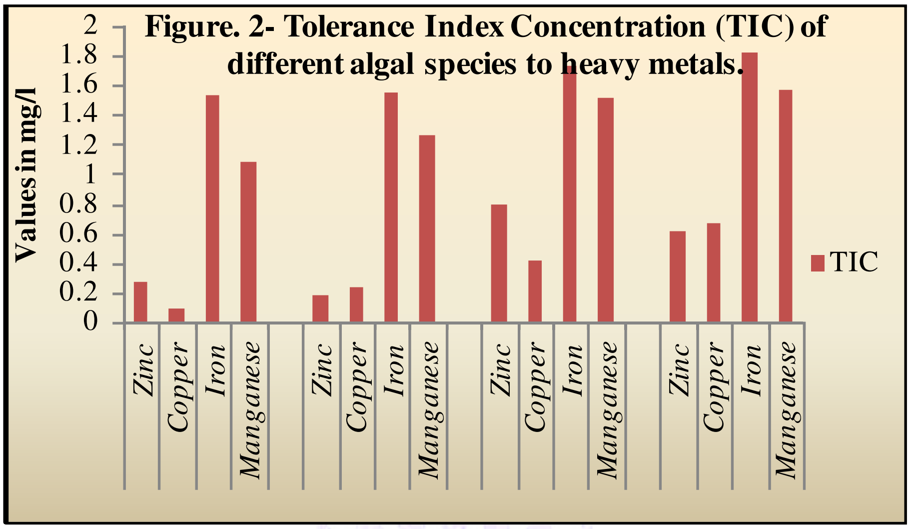
Sr. No.	Name of the algal species	Class	Zinc	Copper	Iron	Manganese
1.	*Chlorella vulgaris*	Chlorophyceae	1.2	0.6	2.1	1.8
2.	*Scenedesmusbijugatus*	Chlorophyceae	0.9	0.8	1.9	2.1
3.	*Oscillatoriaamphibia*	Cyanophyceae	1.7	1.0	2.4	2.5
4.	*Lyngbyamajuscula*	Cyanophyceae	1.4	0.9	2.3	2.2
# All values in mg/l						

Table.6-Tolerance Index Concentration (TIC) of different algal species to heavy metals.

Sr.No.	Name of the algal species	Class	Heavy metals	JNI	JL	TIC
1.	*Chlorella vulgaris*	Chlorophyceae	Zinc Copper Iron Manganese	0.173 0.074 1.341 0.894	0.424 0.094 1.732 1.303	0.271 0.084 1.524 1.079
2.	*Scenedesmusbijugatus*	Chlorophyceae	Zinc Copper Iron Manganese	0.089 0.134 1.449 1.039	0.374 0.387 1.643 1.549	0.182 0.227 1.543 1.268
3.	*Oscillatoriaamphibia*	Cyanophyceae	Zinc Copper Iron Manganese	0.458 0.282 1.549 1.296	1.341 0.591 1.918 1.754	0.784 0.409 1.723 1.508
4.	*Lyngbyamajuscula*	Cyanophyceae	Zinc Copper Iron Manganese	0.400 0.529 1.697 1.396	0.948 0.836 1.933 1.774	0.616 0.665 1.811 1.574
# All values in mg/l, **JNI**= Just Non Inhibitory, **JL** = Just Lethal.						

Figure. 1- Inhibitory levels of heavy metals for different algal species





Conclusions:

It can be concluded from the results obtained in the present investigation that the mine spoil and mine water both had affected adversely by the presence of heavy metals due to mining activities like mineral ore excavation, mineral preparation and anthropogenic activities upto certain extent which might seems to be affect the algal species in mine water as evident from heavy metal toxicity results with respect to the inhibitory level and TIC. Most of the tested algal species *Chlorella vulgaris, Scenedesmusbijugatus, Oscillatoriaamphibia*and *Lyngbyamajuscula* were reported with maximum tolerance to higher level of heavy metals especially to iron and manganese which might be due to the physiological process in the tolerant algal species resulted additionally in an increase in heavy metal tolerance. However, studied metals are essential heavy metals but high inhibitory level of these metals was essential for increasing the tolerance of alga. Therefore, elevated levels of mining contaminants like Mn , Fe, Cu and Zn in mine waters may introduce tolerance mechanism related to other physiological processes which develop in the tolerant population of algae as a result of adaptation to that particular habitat of contaminated mine water with heavy metals in the vicinity of mines.

Acknowledgements:

Manganese Ore India Limited (MOIL) Nagpur Region is highly acknowledged by the authors. We are highly obliged to Shri. V. R, Parida (Senior Mine Manager), I. A. Siddique, Assist. Manager (Survey) Gumgaon Manganese Mine, Shri K.R.Bhattacharya (Senior Mine Manager), Shri. Bhanse (Geologist) Kandri Manganese Mine, Shri. S. Pathak (Senior Mine Manager), Shri Gupta, Beldongari Manganese Mine and Shri. Imran Khan (Mine Surveyor) Mansar Manganese Mine for their kind support during mine survey and sample collection. Also thankful to UGC New Delhi for providing fellowship under SAP DRS-I programme, Department of Botany, R.T.M.Nagpur University, Nagpur.

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