



Potential of *Mimulus glabratus* in removal of Fe and Cu from the aqueous solutions containing Nitrate and Phosphate and its growth responses

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ABSTRACT: The metal bioabsorption potential and survival efficiency of aquatic macrophyte *M. glabratus* was examined for the removal of Fe and Cu in presence of nitrate and Phosphates. *M. glabratus* removes Fe 10% more than Cu in case of bio-chemical and physical responses the increment in fresh weight found 0.74% more in Fe treated plants than Cu treated plants and in photosynthetic pigments there was 10% more increment was noted in the plants treated with Fe. Bioabsorption of Fe was noted 18.9% more than Cu by *M. glabratus*. The results demonstrate that *M. glabratus* can be utilized in the remediation operations of aquatic systems © JASEM

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Overload of heavy metals in environment is outcome of industrializations, urbanization and advance technique of agriculture (Chemical, fertilizers, herbicides, insecticides etc), as they are non-biodegradable and have the accumulation efficiency, so they cause severe threads to nature. Pollution of heavy metal is worldwide problem, while risk and intensity of pollution vary from place to place. Term heavy metals used by toxicologist, researchers for those metals that have the tendency to degrade the environment quality and causes trouble. In today's scenario Cd, Hg, Zn, Co, V, Ti, Mn, are studied comprehensively along with Fe and Cu due to their toxic properties and effect on environment. Some metals are necessary for living organisms like Fe in hemoglobin activity, Cu respiratory pigments system, Co as Vitamin B 12, Mo and Mn in certain enzymetic activities. It is also reported that transition metals like Fe, Cu, Co and Mn are important for human and plants, but they are toxic at high concentration. Intake of copper through drinking water or supplements cause threat to cognition (Brew and Macn, 2009) Copper concentrations more than 20 µg g⁻¹ leads to lethal, human and environment (Thilakar *et al.*, 2012). High concentration of iron deeply influences the organization and function of aquatic ecosystems. Fe causes necrosis or death and colonies disintegration as well as roots abscission by exposure to plants, while notable variation in, synthesis of chlorophyll, protein and carbohydrate is also observed. Fe (iron) also inhibits the uptake of nitrogen and phosphate due to which plants growth are affected (Xing and Liu, 2011). Copper is very toxic to algae, it act as algaeicide, and imbalanced the

ecology of aquatic system by distorting the balance of aquatic system (USEPA, 2008). Heavy metal contamination in fresh water resources is a very severe problem in the modern world (Brankovic *et al.*, 2011). Metal ions characterize group of pollutants mix up into aquatic system considerably as a result of several industrial and agricultural process (Ghani *et al.*, 2009). Aquatic plants are reported to be used as the natural catalysts to take in and accumulate heavy metals in their tissues from water (Afrous *et al.*, 2011). Phytoremediation structure of aquatic plants can be floating on surface of water or submerged into the water. The floating plants take up or amass contaminants by their roots even as the submerged plants amass metals by their whole body (Rahman and Hasegawa, 2011). Anning *et al.*, (2013), Mishra *et al.*, (2013) and Zohar *et al.*, (2014) reported macrophytes like *Ecornia Crassipa*, *Salvina Molesata*, *Pistia stratiote*, *Phragmites australis*, *Reeds and Thypha* are capable in deletion of, Fe, Cu, Mn, Pb, Ar, Cd, Al and Cr by the polluted aquatic system. In the present study the potential of macrophyte *M. glabratus* was tested for the removal of Fe and Cu. *M. glabratus* was selected as it is a common and foremost plant in this part of world at most of the aquatic systems. The species is almost found in industrial wastewater lakes, ponds, polluted rivers sites, etc which indicate that the plant is resistant to contamination. The influence of pollutant (metals and excess nutrient) on physio chemical properties of macrophyte was also examine in the given study

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MATERIAL AND METHOD

Experiment design: *M. glabratus* plants were collected from the Gomti river (Lucknow city) and cultivated in the 10 liter plastic tubs (n=5) containing definite amount of Fe (0.90 mgL^{-1}), Cu (0.15 mgL^{-1}) which is three times higher than recommended limit of (ISI, 1993) in presence of nitrate and phosphate and their concentration was mentioned five times more than permissible limit (MOEF, 1993 and WHO, 1984) respectively. The culture was monitored for 21 days; samples are taken at every 7th day for analysis, ahead of sampling deionized water was added to the tubs to replenish the water lost by evaporation and to recover the maintained level

Sample Analysis: water and plant samples were collected from the treatment tubs on every 7th day and

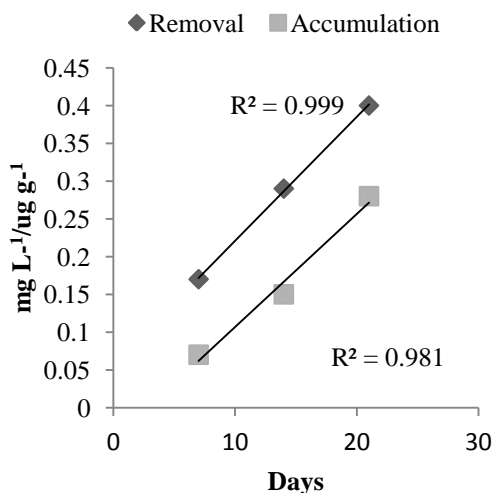


Fig.1 Removal/ Accumulation ratio of Fe in presence of nitrate and phosphate by *M.glabratus*

From figure 1 it is observed that at 21st day *M.glabratus* remove 44.4% of Fe and accumulates 32.2% in presence of nitrate and phosphates, whereas 12.2% of Fe is lost in environment, by reduce in initial concentration 0.90 (maintained) to 0.50 mg L^{-1} . Increment of Fe in tissues of *M.glabratus* was increased up to the concentration of 31 % on the last observed day. It has been also observed that the rate of the accretion and the bio-removal was steadily increased during observation time from the initial day to the last day (fig 1). *M.glabratus* eliminate total 33.3 % Cu metal ion from the water on the 21st day and accretion of 13.3 % was noted (fig. 2). Here also 14% environmental loss of Cu metal ion is noted and it was also observed that there 10% less bio-removal was done then that in case of Fe. Chandra *et al.*, (2014) also observed that *M.Glabratus* remove less

analyzed for Fe and Cu levels. Simultaneously protein and chlorophyll (Photo synthetic pigments) estimation in the plant was also carried out. Metals was estimated by the (Fritioff and Greger, 2007) Protein was analyzed by the method as described by (Lowry *et al.*, 1951) whereas chlorophyll was assayed using the method of (Arnon, 1949)

Data Analysis: The data was analyzed using one way ANOVA (Analysis of Variance). The difference between treatments was considered significant at $p \leq 0.05$.

RESULT AND DISCUSSION

Removal and accumulation of Fe and Cu by *M.glabratus* and its responses in presence of Nitrate and Phosphate:

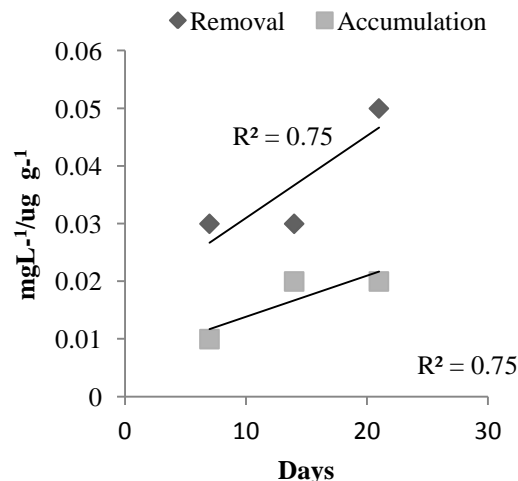


Fig. 2 Removal/Accumulation ratio of Cu in presence of nitrate and phosphate by *M. glabratus*

Cu than Fe in natural condition by set up a phytoremediation experiment at a river site (Gomti river, Lucknow city, India).

Biomass: In the term of biomass of the *M.glabratus* for both the metals Fe and Cu. Biomass increase from 141.08 - 149.55 at 21st day was noted which is increased up to 6 % in Fe treated plants (table 1 and fig.3). Biomass of *M.glabratus* treated with Cu was increase from 149.03 - 156.8 on 21st day which is increased up to 5.26% (table 2 and fig.4), while the final difference was observer between both the conditions (Fe and Cu) was resulted that macrophytes treated with Fe have biomass increased 0.74 % more than Cu' Dry weight of macrophyte treated with Cu was observed 7.8 % less than macrophyte treated with Fe

Table 1 Biochemical responses of *M. glabratus* against Fe (iron) cultivated in plastic tubs for 21 days in presence nitrate and phosphate

Parameters	0d	7d	14d	21d	
Biomass (g)	146.01±1.01	146.81±1.06	150.02±1.05	153.03±2.08	
Dry weight (g)	Nd	Nd	Nd	62.19±1.22	
Protein	Leaf	0.63±0.02	0.69±0.09	0.74±0.14	0.79±0.011
	Root	0.043±0.001	0.045±0.007	0.048±0.05	0.052±0.06
	Stem	0.019±0.10	0.020±0.93	0.021±0.19	0.022±0.18
Chl- a+b (mg g ⁻¹ fr. wt.)	1.08±0.10	1.10±0.13	1.16±0.12	1.19±0.17	

Values shown are the mean S.D. (n=5).

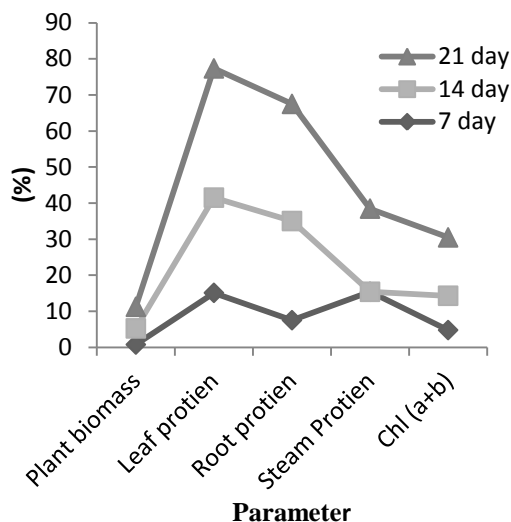


Fig. 3 Biochemical responses in (%) of *M. glabratus* against Fe in presence of nitrate and phosphate

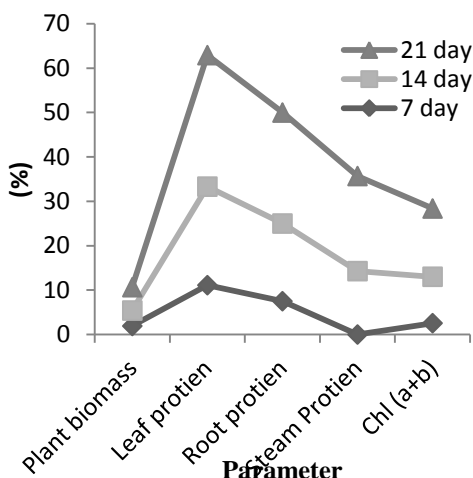


Fig. 4 Biochemical responses in (%) of *M. glabratus* against Cu in presence of nitrate and phosphate.

Table 2 Biochemical responses of *M. glabratus* against Cu (copper) cultivated in plastic tubs for 21 days in presence nitrate and phosphate.

Parameters	0d	7d	14d	21d	
Biomass (g)	149.03±3.21	151.90±2.01	154.22±3.10	156.85±1.03	
Dry weight (g)	Nd	Nd	Nd	75.03±3.07	
Protein	Leaf	0.27±0.08	0.30±0.6	0.33±0.03	0.35±0.01
	Root	0.04±0.07	0.043±0.02	0.047±0.04	0.050±0.03
	Stem	0.014±0.08	0.014±0.11	0.016±0.13	0.017±0.09
Chl- a+b (mg g ⁻¹ fr. wt.)	1.17±0.08	1.20±0.18	1.29±0.12	1.35±0.02	

Values shown are the mean S.D. (n=5).

Protein content: Concentration of protein was also estimated in the some parts of plants i.e. leaf, root, and stem of *M. glabratus* for both the metals (Fe and Cu). It was observed at the 7th, 14th and 21st day during the

experiment. *M. glabratus* is able to tolerate the Fe stress more than the Cu and it is observed that 6.22 %

(fig. 3 & 4) increment in leaf protein in Fe treated plant than Cu. Similar outcome is discovered in

case of root protein, the percentage increment in root protein was noted 7.5 % more in Fe treated plant than Cu (fig. 3 & 4). The protein concentration in stem of *M. glabratus* was found 1.62 % more in the presence of Fe then that of Cu.

Photosynthetic pigments: The concentration of Chl. a+b was increased from to 1.08 -1.19 mg g⁻¹ on 21st day in case of Fe and in the case of Cu it was increased from 1.17 -1.35 mg g⁻¹ on 21th day (tab. 2 & 3) which confirm that there is 0.81 % more photosynthetic pigments are present in the macrophyte treated with Fe (fig. 3 & 4). From the results it is concluded that *M. glabratus* is comfortably survive and remove Fe more stress-free than Cu in presence of nitrate and phosphate Chandra *et al.*, (2013) concluded same result during the observation of stress responses of Fe and Cu in presence of nitrate and phosphate on macrophyte *T. natans*

Conclusion: This study reveals the *M. glabratus* remove Fe and Cu both from solution, but the removal of Fe found more significantly than Cu, and the stress tolerance performance against Fe was also observed much better than Cu in relating to this conclusion Chandra *et al.*, (2015) reported that *M. glabratus* remove Fe more significantly than Cu and tolerate considerably saline stress. *M. glabratus* is easy to harvested in water systems to remediate heavy metals pollution effectively, it also played vital role as bio indicator of given pollutant in aquatic system

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