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A STUDY ON STRESS IMPOSED BY FE^{3+} AND CU^{2+} ON AN AQUATIC MACROPHYTE (*TRAPA NATANS)* IN PRESENCE OF NITRATE AND PHOSPHATE UNDER SIMULATED NET HOUSE CONDITION

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ABSTRACT

The experiment was design and conduct in control environmental condition for the purpose to investigate the impact of given metals Fe^{3+} and Cu^{2+} in the presence of nitrate and phosphate on the growth, protein level (root, leaf, fruit) production of photosynthetic pigments in macrophyte and its metal accumulation and removal capacity in the provided condition. *T. natans* survive very well with Fe^{3+} in presence of nitrate and phosphate by increasing in biomass 1.17% more than the Cu^{2+}. In the protein level there is increment of 10.11%, 5%, 13.16% observed in leaf, root and fruit respectively. Photosynthetic pigments was observed 4.88% more in Fe^{3+} treated plant than that in Cu^{2+} treated plant and it was also noticed that bio accretion of Fe^{3+} was 8.8% more than of Cu^{2+}. Its resulted that given plant also played an important role in the elimination or purging of pollutant/metal ions (Fe^{3+}, Cu^{2+}) in the maintenance of water quality, whereas in case of Fe^{3+} *T. natans* also tolerated the biochemical and physiological stress and have a positive characteristic for the remedial process of polluted water.

KEY WORD: ***T. natans*, Biomass, Protein, Photosynthetic pigment, Metal ions.**

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INTRODUCTION

The organic/inorganic pollutants create unfavourable condition for the survival of aquatic ecosystems by effecting their naturally growing flora and fauna. Heavy metals are special group of contaminants of water reservoirs they have high ecological consequence since it cannot be removed from water as a result of self-purification, but accumulate in reservoirs and enter into the food chain so it also concern to human health issue [1,2]. Heavy metal contamination is one of the major quality issues in fast growing cities because maintenance of water quality and sanitation infra-structure does not increase along with population and urban growth especially in developing countries. Studies on heavy metals pollution in rivers, lakes, fish and sediments have been a major environmental focus especially during the last some years[3,4] Fresh water bodies have a great facility to break down some organic waste materials with the help of habitual action of micro organisms The energy from sunlight drives the process of photosynthesis in aquatic macrophytes for the production of oxygen which inturn is used by the micro-organisms to break down the organic and inorganic substances and animal waste. The decomposition produces carbon dioxide, nutrients and other substances needed by macrophytes and animals living in the water. The purification cycle continues when these macrophytes and animals die and the micro organism decompose them, providing new generations of organisms with nourishment. These cycles are adversely effected by the metals involve meant. Self-cleaning capacity of water bodies and the overload results on the extinct of water bodies and at a time water bodies are not seems to have any dissolved oxygen and no wonder they fail to support the growth of desired aquatic flora and fauna or inhibit the growth and productivity. On the other hand nutrients like nitrate and phosphate are in large concerns pollutant and their main sources are the agriculture waste (by using in fertilizers). Overload of this nutrient has caused many problems in entire aquatic ecosystems such as harmful phytoplankton blooms, closed mats of floating macrophytes, hypoxia and loss of biodiversity of flora and fauna[5,6] Fe^{3+} and Cu^{2+} are selected for study because both the metals are found naturally as well as both the metals are commonly found in all type of industrial waste i.e. motor and vehicle industry, electronic, and electroplating industries, synthetic fertilizers, pesticides, paint industry etc. in the light of given above facts experiment was designed and conduct on the stress response of *T. natans* an aquatic macrophyte of the fresh water system.

MATERIALS AND METHODS

EXPERIMENT DESIGN

To evaluate the stress response of aquatic macrophyte *T. natans*. Fresh plants were picked from their original habitat, brought to the laboratory and washed with tap water. The experiment was conducted in the simulated net house condition in $10L^{-1}$ plastic tubs, containing water and plants in which known concentration of Fe^{3+} and Cu^{2+} i.e. 0.09 mg L^{-1} and 0.15 mg L^{-1} respectively was maintained which was three fold of maximum acceptable limit for surface water given by[6] were as nitrate and phosphates concentrations was maintained fivefold of their acceptable limit as given by [7,8]. The experiment was set up for 21 days and the samples were collected at every 7^{th} day for the analysis.

ANALYSIS OF SAMPLES

(A) Estimation of protein was done as per the method described[9].

(B) Estimation of photosynthetic pigments was done by the method given [10].

(C) Nitrate was estimated by the method of [11]

(D) Phosphate in water was estimated by the stannous chloride method [12].

(E) Metal in the aquatic macrophytes in the water was estimated as per the method of using Varian ModelSpectra AA-250 plus Atomic Absorption Spectrophotometer.[13]

Metal concentration (µg g^{-1} dry wt.) = $\frac{XV}{W}$

Where:
X = Reading in ppm on AAS,
V = Final volume of digested samples (ml)
W = Dry weight of the sample (g)

RESULTS AND DISCUSSION

(i) Bio-elimination and accretion of Fe^{3+} and Cu^{2+} in aquatic Macrophyte T. natans

As substantiation from table and figure 1 it has been observed that at 21st day, *T. natans* remove total 55% of Fe^{3+} in presence of nitrate and phosphates. Level of iron was also reported high by[13,] among some observed metals i.e. Fe, Cu, Cr, Mn and Pb in the plant *T. natans*.

Table 1

Bio-elimination and accretion of Fe3+[(mg L-1) & (µg g-1)] from water by T. natans grown in the plastic tubs for 21 days in presence of nitrate and phosphate (Initial conc.= 0.90 mg L-1).

T. natans	0thd	7thd	14thd	21std
Bio elimination	0.90±0.0	0.71±0.05	0.52±0.03	0.40±0.03
Accretion	219.14±0.02	219.31±2.31	219.44±3.65	219.58±1.02

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

There was decrease in initial maintained level of Fe^{3+} in water was up to 0.50 mg L^{-1} and the increment of Fe^{3+} in tissues of macrophyte was noted up to the level of 48.8% on the last observed day (21th day) by the initial day (0th day). It has been also observed that the rate of the accretion and the bio-elimination was steadily increased during observation time from the initial day to the last day. However, it was noted that there was difference of 6.7% in bio elimination and accretion which was considered as environmental loss of Fe^{3+}.

Table 2

Bio-elimination and accretion of Cu2+ [(mg L-1) & (µg g-1)] from water by T. natans grown in the plastic tubs (capacity = 10L) for 21 days in presence of nitrate and phosphate (Initial conc. = 0.15 mg L-1).

T. natans	0thd	7thd	14thd	21std
Bio elimination	0.15±0.0	0.12±0.002	0.10±0.003	0.07±0.001
Accretion	4.05±0.05	4.07±0.05	4.09±0.03	4.11±0.01

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

In the term of Cu^{2+} *T. natans* eliminate total 53% metal ion from the water on the 21st day and accretion of 40% was noted (Table 2 and Fig. 2).

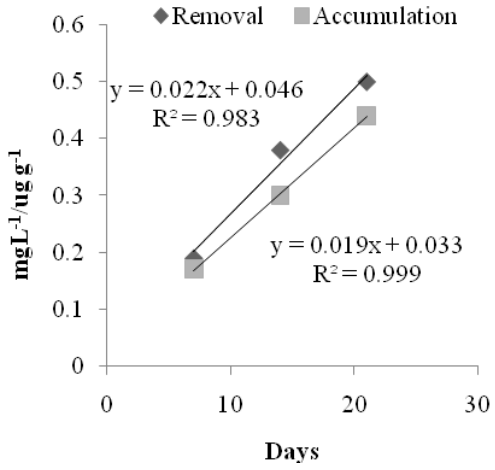

Figure 1
Removal/accumulation of Fe^{3+} by T. natans

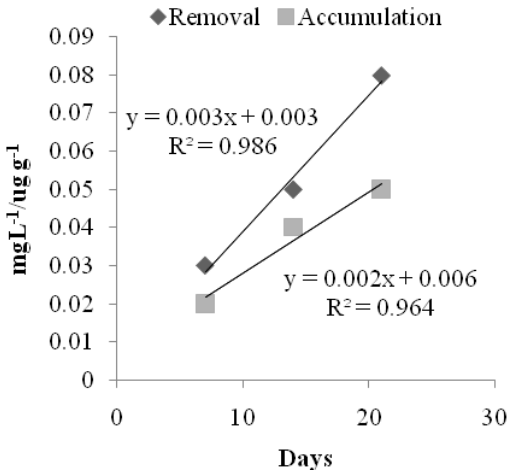

Figure 2
Removal/Accumulation ratio of Cu^{2+} by T. Natans

Now here also 14% environmental loss of metal (Cu^{2+}) is noted and it was also observed that there was 2.5% less bio-elimination was done than that in case of Fe^{3+} and a remarkable difference was observed in term of accretion of metal ions that was 8.8% less Fe^{3+} its resulted that *T. natans* bio elimination and accretion Fe^{3+} more efficiently (Table and Fig. 1) in presence of nitrate and phosphate.

(ii) Biochemical responses of T. natans against the Fe^{3+} and Cu^{2+} in presence of nitrate and phosphate

There are so many studies by several researchers time to time on the macrophyte *T. natans* for various reasons like role in water quality management, metals hyper accumulation, role and response in removal of pollutant (organic and inorganic) etc. (A) Biomass: In the term of biomass, the macrophyte showed potential increase i.e. 7.37% at 21st day when treated with Iron (Table 3 and Figure 1). Biomass of macrophyte treated with Cu^{2+} was increased by 6.21% on 21st day and the total difference was observed between both the conditions (Fe^{3+} and Cu^{2+}) was found to be 1.16% (Table 3, 4 and Figure 1, 2). Baldisserotto[15] also observed positive increment in *T. natans* biomass when treated with manganese.

Table 3
Biochemical responses of T. natans against iron (Fe^{3+}) cultivated in plastic tubs for 21 days in the presence nitrate and phosphate

Parameters		0d	7th d	14th d	21st d
Biomass (g)		130.05±3.93	133.06±4.32	137.67±5.01	139.65±3.21
Dry weight (g)		Nd	Nd	Nd	47.07±3.98
Protein	Leaf	1.09±0.11	1.30±0.13	1.43±0.02	1.56±0.05
	Root	0.06±0.03	0.07±0.02	0.073±0.03	0.081±0.05
	Fruit	1.63±0.13	2.07±0.15	2.32±0.11	2.63±0.13
Chl a+b (mg g^{-1} fr.wt.)		1.57±0.11	1.61±0.11	1.75±0.16	1.89±0.11

Values shown are mean ± s.d. of 5 replicates.

Table 4
Biochemical responses of T. natans against copper (Cu^{2+}) cultivated in plastic tubs for 21 days in the presence nitrate and phosphate

Parameters		0d	7th d	14th d	21st d
Biomass (g)		130.02±2.43	131.78±3.03	136.07±8.10	138.09±1.07
Dry weight (g)		Nd	Nd	Nd	40.32±3.05
Protein	Leaf	1.06±0.02	1.24±0.11	1.31±0.06	1.78±0.04
	Root	0.06±0.01	0.065±0.03	0.071±0.01	0.083±0.04
	Fruit	1.08±0.12	1.31±0.12	1.48±0.13	1.55±0.19
Chl a+b (mg g^{-1} fr.wt.)		1.48±0.13	1.51±0.11	1.61±0.14	1.71±0.17

Values shown are mean ± s.d. of 5 replicates

(B) Protein level: In the biochemical response in the protein level was estimated in the different parts of macrophyte i.e. leaf, root, and fruit and photosynthetic pigments observed at the different time of interval during the experiment. Increment in protein level in leaves of the experimental macrophyte shown that the *T. natan*s was growing, glowing and tolerating the Fe^{3+} stress more than the Cu^{2+} and the difference of about 10.11% is observed between the macrophyte leaf protein of Fe^{3+} and Cu^{2+} . Similar results are shown in case of root protein, the percentage increment in root protein was noted 5% more in Fe^{3+} treated macrophyte than Cu^{2+} (Figure 3 and 4). The protein level in fruit of *T. natans* was found 13.16 % more in the presence of Fe^{3+} than that of Cu^{2+}.

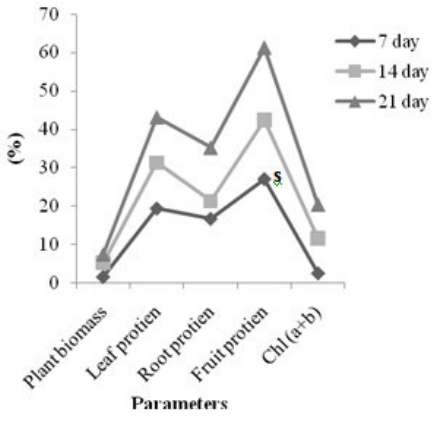

Figure 3 Biochemical responses in (%) of *T. natans* against Fe^{3+} in presence of nitrate and phosphate.

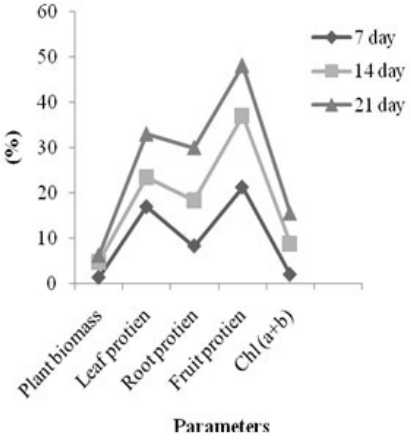

Figure 4 Biochemical responses in (%) of *T. natans* against Cu^{2+} in presence of nitrate and phosphate.

(C) Photosynthetic pigments**:** In the macrophyte *T. natans* the level of Chl. a+b was noted 1.89 mg g^{-1} on 21st day in case of Fe^{3+} and in the case of Cu^{2+} it was noted 1.62 mg g^{-1} on 21th day (Table 3and 4) which showed that the 4.5% more photosynthetic pigments are present in the macrophyte (Figure 1 and 2) treated with Fe^{3+} analogous result was also obtained by[16] in presence of nitrate and phosphate. The concluded results from the study state that *T. natans* can survive very well and accumulate /remove Fe^{3+} more efficiently than Cu^{2+} load from water even in presence of nitrate and phosphate. Kousar and Puttaiah[17] also acknowledged *T. natans* as an efficient macrophyte in treating the pulp and paper industry effluent and also pose an outstanding ability for assimilating nutrients and heavy metals.

REFERENCES

1. Hoseinizadeh G. R, Azarpour E, Ziaeidoustan H, Moradi M and Amiri E, Phytoremediation of heavy metals by hydrophytes of Anzali Wetland (Iran). World Appl. Sci. Jr., 12 (11): 2078-2081, (2011).
2. Subhalakshmi A, Rajagopal K, Nithiyanandhan G, Jayanandi D and Karthikeyan , Chromium phytoaccumulation *AlternantheraSessilis In Vitro* – A First Report. Int Jr. Pharm Bio Sci., 4(3): 618 – 624,(2013)
3. Ideriah T.J.K, David O.S. and Ogbonna D.N, Distribution of heavy metals in water and sediment along Abonnema Shoreline, Nigeria. Resources and Env.,(2): 133-40, (2009).
4. Mitra N, Rezvan Z, Mazaher S.A , Mahmudy G and Hosein M, Studies of water arsenic and boron pollutants and algae phytoremediation in three springs, Iran. International Journal of Eco., 2 (3): 32-37, (2012).
5. Seralathan K, Bprabhu, D. B, Kui J. L, Assessment of heavy metals (Cd, Cr and Pb) in water, sediment and seaweed (Ulva lactuca) in the Pulicat Lake, South East India. Chemosphere, 71(7): 1233–1240,(2008).
6. Annelies J, Veraart Wilco J.J, De Bruijne Jeroen, J.M., De Klein Edwin T.H.M and Scheffer P.M. Effects of aquatic vegetation type on de nitrification. Bio-geochem., (10): 267-274, (2011).
7. ISI specification for drinking water, 10,500 1993 ISI, New Delhi (1993).
8. WHO, 1984. World Health Organization, Technical Report.
9. Lowry O.H, Rosebrough, N. J , Farr A.L and Randall R.J. Protein measurement with the folin phenol reagent. Jr. Biol. Chem. (193): 265-275, (1951).
10. Arnon D.L, A Copper enzyme is isolated chloroplast polyphenol oxidase in *Beta vulgaris*. Plant Physiol., (24): 1-15, (1949).
11. Cataldu D.A, Haroon M, Schrader L E, and Young V. L, Rapid colorimetric determination of nitrate in plant tissue by nitrification of salicylic acid. Commun. Soil Sci. Plant Anal. 6(1):2095-2097, (1973).
12. APHA, Standard methods for examination of water and wastewater 20th ed. American Public Health Association. New York, (1998).
13. Fritioff and Greger M, Functions as a proton-coupled symporter for phytosiderophore and nicotianamine chelated metals. Journal of Biology and Chem., (279):9091-9096, (2007).
14. Rai U.N and Sinha S Distribution of metals in aquatic edible plants: *Trapa natans* (roxb.) makino and ipomoea aquatic forsk. Environmental monitoring and assessment (70): 241–252, (2007).
15. Baldisserotto C, Ferroni L , Anfuso E , Pagnoni A , Fasulo M.P. and Pancaldi S, Responses of *Trapa natans* L. floating laminae to high concentrations of manganese. Protoplasma, (231): 65-82, (2007).
16. Chandra S , Rawat S.K , Garg S.K. , Singh R.P., Responses of *Trapa natans* against the soaring concentrations of nitrate and phosphate in a tropical river Gomti in Lucknow city, Indian. Journal of recent advances in applied sciences, (28):78-81,(2013).
17. Kousar H and Puttaiah E.T. Application of Trapa bipinosa for the treatment of pulp and paper industry effluent. Journal of Environmental Biology, 30(5):659-66,(2009).