E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(4): 390-393

www.fisheriesjournal.com
Received: 06-05-2019
Accepted: 10-06-2019

V Venkateswarlu
Department of Marine Biology, Vikrama Simhapuri University, Nellore, Andhra Pradesh, India

CH Venkatrayulu
Department of Marine Biology, Vikrama Simhapuri University, Nellore, Andhra Pradesh, India

TCH Jhansi Lakshmi Bai
Department of Aquaculture, College of Fishery Science, Muthukur, Nellore, Andhra Pradesh, India

Correspondence
V Venkateswarlu
Department of Marine Biology, Vikrama Simhapuri University, Nellore, Andhra Pradesh, India

Phytoremediation of heavy metal Copper (II) from aqueous environment by using aquatic macrophytes *Hydrilla verticillata* and *Pistia stratiotes*

V Venkateswarlu, CH Venkatrayulu and TCH Jhansi Lakshmi Bai

Abstract
The objective of this study was to determine the ability of *Hydrilla* and *Pistia* in terms of tolerance and degradation of copper pollution in aquatic environment. The plants were released into 3 different tanks containing copper solutions of 2ppm, 5ppm and 7ppm respectively and observed for metal extraction for 28 days. After 28 days, heavy metal analysis was done with inductively coupled plasma quadruple mass spectrometry (ICP-Q-MS). The results showed that percentage of metal extractions by weeds were observed i.e., 69% in 2ppm concentration, 43.8% in 5ppm, and 33.35% in 7ppm concentration. Better growth of weeds was observed in 5ppm concentration only.

Keywords: *Hydrilla, Pistia*, copper pollution, phytoremediation, aquatic environment

1. Introduction
In the world with ever increasing population and progressive adoption of an industrial- based lifestyle has inevitably led to an increased anthropogenic impact on the biosphere [1] since the beginning of the industrial revolution, water pollution by toxic metals has accelerated dramatically. According to Nriagu [2] about 90% of the anthropogenic emissions of heavy metals have occurred since 1900 AD; it is now well recognized that human activities lead to a substantial accumulation of heavy metals in water on a global scale. Further, it results in excess release of heavy metals such as cadmium, copper, lead, nickel, zinc etc., into natural resources like soil and aquatic environments, which have deleterious health effects on human life and aquatic biota. Copper is an essential trace nutrient that is required in small amounts (5-20 micrograms per gram (μg/g)) by all animals. It is also needed for the formation of hemoglobin and Hemocyanin, the oxygen transporting pigments in the blood of vertebrates and shellfish respectively. However, copper concentrations that exceed 20 micrograms per gram (μg/g) can be toxic, as explained [3, 4]. Copper being algaecide, it decreases algal growth when discharged into water bodies which further disturbs entire food chain in an aquatic ecosystem. Great efforts have been made in the last two decades to reduce pollution sources and remedy the polluted environment. So, the search for a new, simple, effective and eco-friendly technology involving the removal of toxic heavy metal from wastewater has directed attention towards phytoremediation.
Phytoremediation technology is one of the bioremediation method, uses plants as filters for accumulating, immobilizing, and transforming contaminants to a less harmful form. This experiment includes study of the efficacy of column weed *Hydrilla* and floating weed *Pistia* in removal of copper pollution in water. *Hydrilla verticillata* is a submerged aquatic weed that can grow up to the surface and form dense mats in all bodies of water. For removal of contaminants whole plant plays important role [5] observed that the reliance upon roots for heavy metal uptake was in rooted floating-leaved taxa with lesser reliance in submerged taxa. He also observed that tendency to use shoots as sites of heavy metal uptake instead of roots increases with progression towards submergence and simplicity of shoot structure [6] found that after one week of exposure to lead shown maximum uptake (98%) of Pb by *hydrilla*. *Pistia stratiotes* is an aquatic plant that grows rapidly and a high biomass crop with an extensive root system that able to enhance the heavy metals removal. This plant exhibited different patterns to removal of heavy metals, but mostly accumulated at high concentrations mainly in the root system.

2. Materials and Methods

2.1 Collection of weed plants

a. Hydrilla: *Hydrilla* weed plants were collected from the irrigation water canal, near mini bypass road, Nellore, Andhra Pradesh in two days prior to the commencement of experiment. The weed plants selected for this study are healthy fully grown and green. The collected weed plants were thoroughly washed and measured length up to 100cm and weight is up to 15-20gms.

b. Pistia: *Pistia stratiotes* weed plants were collected from the fresh water pond at V.R College campus botanical garden, Nellore, Andhra Pradesh, two days prior to the commencement of the experiment. The collected weed plants were washed and measured wet weight up to 4-5gms and length up to 4-5cm.

2.2 Preparation of Medium

Copper metal was dissolved in concentrated Nitric acid (HNO_3) solution to prepare 2ppm, 5ppm, and 7ppm concentration solutions in triplicates of 5L each.

2.3 Experimental Procedure

The collected weed plants *Hydrilla verticillata* and *Pistia stratiotes* were kept in fresh water for 2 days to acclimatize in the laboratory conditions and then transferred to each triplicates of 3 treatments containing 2ppm, 5ppm, and 7ppm concentrations. The weed plants were kept in the control and experimental tanks for observations of morphological changes, for 28 days of experimental duration.

2.4 Sampling

The water samples were collected from the experimental tanks 7 days interval and taken to the laboratory for analysis.

2.5 Heavy metal analysis through ICP- MS

The water samples was digested with nitric acid digestion method, carried out in a COOLPEX microwave chemical reactor (Shanghai, China). An amount of 1 ml of the sample solution was first added to the tube digestion, and then we added 5 ml of concentrated nitric acid and the obtained solution was heated for 1 h. The temperature of the stove was adjusted to 150ºC, after 20 min of heating until the surface turned light yellow and was then removed for cooling. Afterward, 1 ml of per chloric acid was added and the temperature was adjusted to 180ºC and heated until dark smoke was produced. After that, we steamed the liquid until the residue turned Gray and white and the surface was clean (if there was still some color, then we tried to add more nitric acid with the same amount), and then it was filtered, and the filtrate was transferred to a 50 mL bottle added with pure water. Finally, the content of Cu was analysed with Inductively Coupled Plasma Quadruple Mass Spectrometry (ICP-Q-MS). ICP-MS is a relatively new method for determining multi-element analysis and ideal for water, since the vast majority of target compounds can be detected below 0.1 mg/l [9, 10]. Therefore a Perkin Elmer ELAN DRC (e) instrument was used with a Meinhart nebulizer and silica cyclonic spray chamber and continuous nebulization.

The operating conditions are listed below: Nebulizer Gas flow rates: 0.95 l/min; Auxiliary Gas Flow: 1.2 l/min; Plasma Gas Flow: 15 l/min; Lens Voltage: 7.25 V; ICP RF Power: 1100 W; CeO/Ce = 0.031; Ba++/Ba+ = 0.016.

3. Results and Discussion

Maximum copper was efficiently removed by two different plants i.e., *Hydrilla* and *Pistia* within 28 days. Copper was effectively removed in 2ppm concentration followed by 5ppm and 7 ppm (Fig: 1). The adsorption of heavy metal by *Hydrilla* and *Pistia* in the exposure of 7days interval was 33.5% and 46.5%, 59.0%, 69.0% in 14, 21,28 days respectively (Fig: 1). These results indicate that the weeds can remove the heavy metals by bio-accumulation of the metals. However, some mortality was observed after 2 weeks of exposure from the initial day of experiment.

Researchers were reported that the removal is dependent both on the contact time and the initial metal concentration [7] observed a rapid initial uptake up to 48 hours and gradual attainment of equilibrium after 120 hours. Such concentration and duration dependent removal were also obtained for cadmium using water hyacinth and water lettuce [8, 9, 10] obviously the results of the present study are in consonance with these observations.

Table 1: Average Per cent Removal of Heavy metal (Cu++) by Aquatic weeds after 28 days.

Sl. No	Initial Heavy Metal Concentration	Concentration of Heavy metal After 07 Days	Concentration of Heavy metal After 14 Days	Concentration of Heavy metal After 21 days	Concentration of Heavy metal After 28 Days
1	2 ppm	1.33±0.12	1.07±0.08	0.82±0.10	0.62±0.10
2	5 ppm	4.02±0.11	3.97±0.09	3.11±0.06	2.81±0.09
3	7 ppm	6.37±0.14	5.71±0.13	5.08±0.17	4.63±0.12

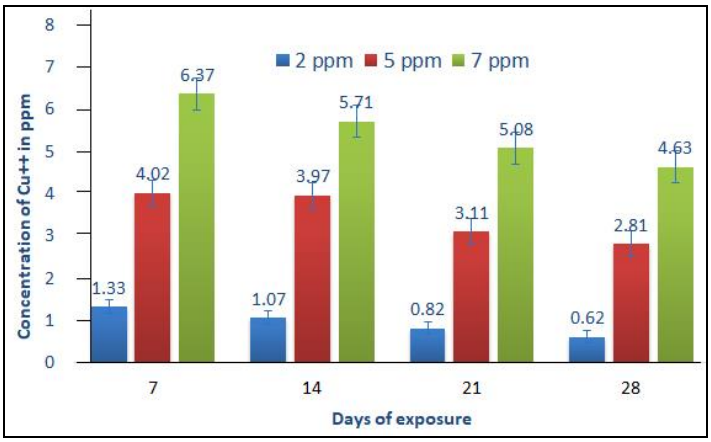

Fig 1: Mean Per cent Removal of Heavy metal (Cu++) by Aquatic weeds.

Table 2: Morphological changes Observed in *Hydrilla* and *Pistia stratiotes* in experimental tanks.

Days	Hydrilla	Pistia stratiotes
1	Weed was active and in normal condition	Weed was active and in normal condition
7	Colour of the weed was turned to dark colour from the light green	Leaf tips changed from green to pale green colour.
14	Stem parts of the weed was changed in to dark green and black colour. New leaves formed	leaves are shedding from the weed
28	Maximum plants were found dead and remaining are becomes fully pale in colour.	Maximum plants were found dead and remaining are becomes fully pale in colour.

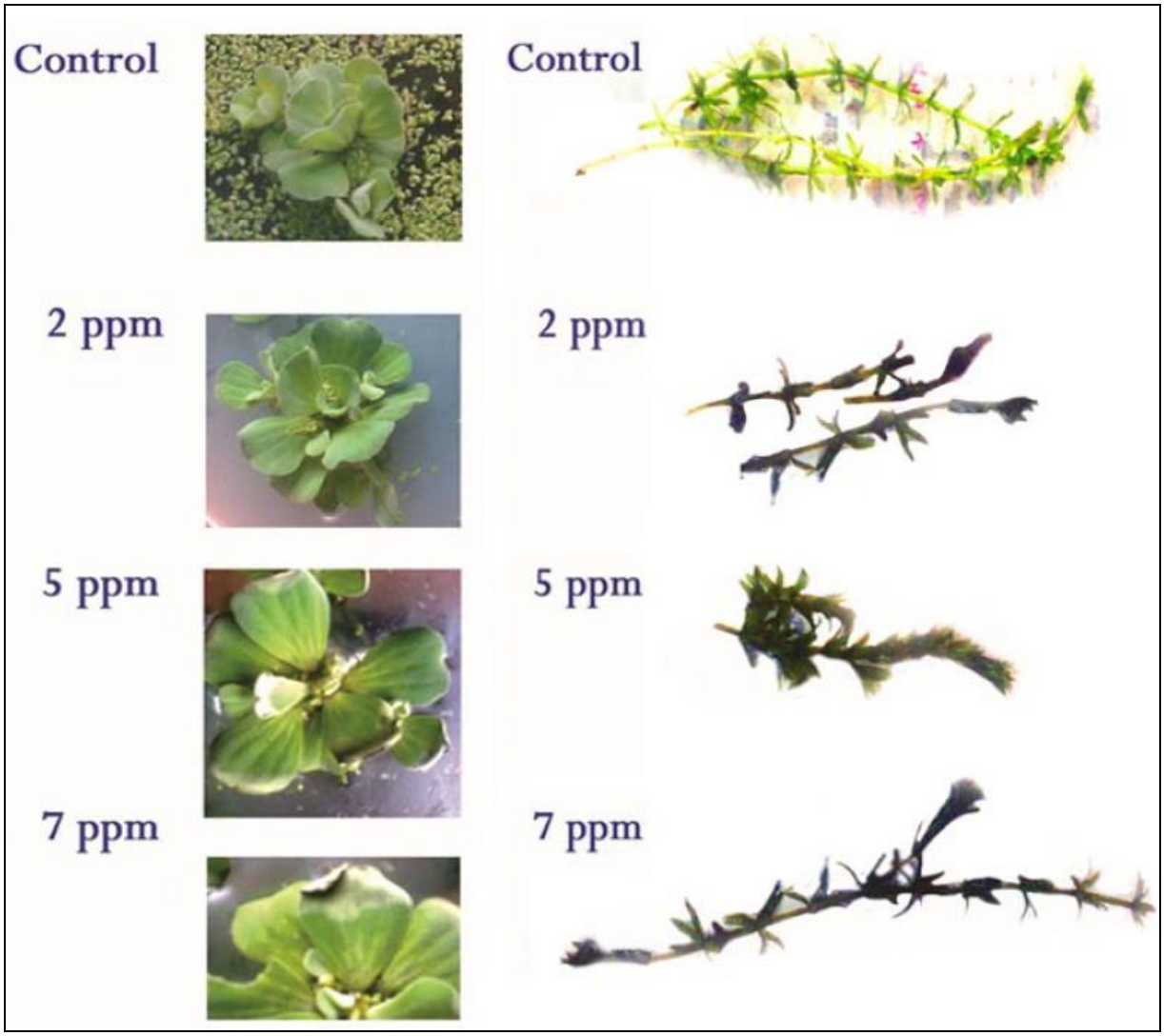

Fig 2: Morphological changes observed in *Pistia* and *Hydrilla* after 28 days.

The same results were also observed some other aquatic macrophytes such as water *Hyacinth* (*Eichornia crassips*) [11, 12] butterfly fern (*Salvinianatons* and *salvinia minima*) [13, 14], Lesser weed *L. minor* [15, 16], greater duck weed *Spirodela polyrhiza* and *Spirodela intermedia* [17, 18].

4. Conclusion

Freshwater as well as seawater resources are being contaminated by various toxic elements through anthropogenic activities and from natural sources. Therefore, remediation of contaminated aquatic environment is important as it is for terrestrial environment. Phytoremediation of the toxic contaminants can be readily achieved by aquatic macrophytes or by other floating weed plants, since the process involves bio-sorption and bioaccumulation of the soluble and bio-available contaminants from water. Increased use of copper contaminated ground water for agricultural purposes (irrigation in rice and other crop fields) results in the increased concentration of copper in freshwater systems, further these copper contaminated freshwater systems poses health threat, not only to the aquatic organisms and also to the humans. Phytoremediation of contaminated water by aquatic macrophytes would be a good option in long term method. The present study shows that *Hydrilla verticillata* and *Pistia stratiotes* can be use effectively in the removal of heavy metals from the waste water, thereby reducing the toxicity on the flora and fauna.

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