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Removal of Copper by *Eichhornia crassipes* and the Characterization of Associated Bacteria of the Rhizosphere System

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Abstract

Excess doses of trace element contamination make conventional water treatment methods less effective and more expensive, where in alternative biotechnological applications open up new opportunities with their reduced cost and lesser impacts to the environment. In the present investigation, effectiveness of aquatic macrophyte *Eichhornia crassipes* was tested for the removal of copper in laboratory conditions. Water samples were collected from macrophytes natural habitat and water tubs used for growing *E. crassipes* and analysed along with plant tissues for Cu content. The work also characterized the associated microbiota of the rhizosphere system of the *E. crassipes* as well as the wetland system of its occurrence. Copper concentration of the wetland water samples ranged from 0.009 to 0.03 ppm. Six bacterial genera (*Acinetobacter, Alcaligenes, Bacillus, Kurthia, Listeria* and *Chromobacterium*) were represented in rhizosphere of *E.crassipes* and 4 bacterial genera (*Acinetobacter, Bacillus, Listeria* and *Chromobacterium*) were recorded in wetland water samples. Copper resistance studies of the bacterial isolates showed that out of 26 isolates from rhizosphere and 19 strains from water samples,12 of them showed low resistance (<100 µg/ml) and 5 isolates showed high resistance to copper at concentration of 400-500 µg/ml. Two high copper-resistant bacteria from the rhizosphere were selected for 16S rDNA analysis. The results found that one isolate showed 99% similarity with *Bacillus altitudinis* strain- Y118 16S ribosomal RNA gene, partial sequence (Accession No: JX134625.1) and the other showed 99% similarity with *Bacillus altitudinis* strain SH164 16S ribosomal RNA gene, partial sequence (Accession No: KC172059.1). Results of copper removal revealed high removal (>80%) of copper during 15 days experiment. Copper accumulation was found to be high in the root followed by leaf and petiole. Results of the present study concluded that *E. crassipes* is an efficient plant for the removal of copper.

Keywords: aquatic macrophytes; copper; rhizosphere bacteria; phytoremediation

1. Introduction

Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the Industrial Revolution (Nriagu, 1979; Sayyed and Sayadi, 2011). The heavy metal load from domestic wastewater and sewage alone (Nriagu and Pacyna, 1988) ensures that this will be a continuing problem for science and humankind. The effects of metal pollution on local environments and organisms may therefore be substantial and long lasting in spite of extensive remediation efforts (Mahimairaja, 2000). These heavy metals are highly toxic to the aquatic plants and animals as well as do not vanish easily from the environment. The technologies used for their treatment are reverse-osmosis, ion-exchange, electrodialysis, adsorption, etc. Most of these technologies are quite costly, energy intensive and metal specific.

Phytoremediation, the removal of pollutants by the use of plants offers a promising technology for heavy metal removal from waste water (Miretzky *et al*., 2004). Aquatic macrophytes have great potential to accumulate heavy metals inside their plant body. These plants can accumulate heavy metals 100,000 times greater than in the associated water. Therefore, these macrophytes have been used for heavy metal removal from a variety of sources (Mishra and Tripathi, 2008). Copper (Cu) is essential micronutrient for plants, but it can be toxic at higher concentrations. Industrial releases are only a fraction of the total environmental releases of copper and copper compounds. Other sources of Cu release into the environment originate from domestic waste water, combustion processes, wood production, phosphate fertilizer production, and natural sources (e.g., windblown dust, volcanoes, decaying vegetation, forest fires, sea spray, etc.) (Georgopoulos *et al*., 2001; Harrison, 1998). Exposure to copper is toxic to fish growth, and it affects the repair capability of aquatic ecosystems (Hoyle *et al*., 2007; Roussel *et al*., 2007).

Eichhornia crassipes (Water hyacinth) is a member of pickerelweed family (Pontederiaceae) that has proven to be a significant economic and ecological burden to

many sub-tropical and tropical regions of the world. It is a monocotyledonous, perennial, free floating (except when stranded in the mud) aquatic plant (Wolverton and McDonald, 1979). The plant has been used successfully in wastewater treatment systems to improve water quality by reducing the levels of organic and inorganic nutrients (Delgado *et al*., 1995). In phytoremediation, the root zone is of special interest. The contaminants can be absorbed by the root to be subsequently stored or metabolized by the plant (Merkl *et al*., 2005). The removal of pollutants and the consequent wastewater purification are the results of a series of processes, which involve reaction and interaction among substratum, microorganisms and plants, and hence the process is aptly termed as 'rhizosphere treatment' as the root zone micro flora plays a chief role in pollutant removal. Aquatic macrophytes are generally not considered as the main mode of remediation in 'rhizosphere treatment' technique. Rather, the plant creates a niche for rhizosphere microorganisms to carry out the degradation. Rhizospheric microorganisms are well known for their coexistence with plants and for providing nutrition to plants (Uroz *et al*., 2007). *E.crassipes* showed increased removal efficiency of heavy metals through the activity of its rhizospheric bacteria (So *et al*., 2003). By characterizing the microbial communities of the rhizosphere, a significant contribution is made to clarifying the process mechanism that contributes to the microbial removal.

The main aim of the present study was to evaluate the effectiveness of *E. crassipes* for removing copper from solution as well as the potential of the plants to accumulate copper and to determine the suitability of these plants for their large scale utilization. For this water samples were collected from the selected *Eichhornia* beds of Kuttanad wetland (Part of Ramsar siteVembanadu-coal wetland) for the basic understanding of copper contamination and microbial diversity of the system. The study also aims to identify the root zone associated bacteria of *E. crassipes* and to evaluate copper resistance of the bacterial isolates along with its molecular characterization and phylogeny.

2. Methodology

2.1. Experimental plant

The aquatic macrophyte *Eichhornia crassipes* (Water hyacinth) was selected to assess copper removal capacity from water under laboratory conditions. Water hyacinth is a perennial aquatic weed spread all over the world, considered noxious and extremely invasive for freshwater environments. These species carry out their entire life cycle as free-floating plant, only the root system is completely submerged. Kuttanad has good diversity of *E. crassipes* which is growing gregariously in this wetland causing ecological and economic threat. The remnants of the fertilizer and pesticides and other agrochemical waste pollutants are accumulating in the water and sediment which helps the growth of this nuisant weed.

2.2. Collection of E. crassipes and water samples

E. crassipes, of uniform size (30-40 days old) were collected from the water bodies of selected *Eichhornia* bed (Kainady, Poovam, and Nedumudi) of Kuttanad wetland ecosystem. The *E. crassipes* were transferred to a sterile polythene cover for copper analysis. The root region of *E. crassipes* was washed in to a sterile polypropylene bottles using sterilized water for the isolation of rhizosphere associated bacteria. Water samples were also collected from the selected *Eichhornia* bed. The plant, water and rhizosphere samples were transported to the laboratory in an ice box and stored at 4ºC until analysis.

2.3. Analysis of copper

The collected plants were first washed with tap water and then with deionised water, allowed to drain off excess water and the plants were divided in to three parts: petioles, leaves and roots. Subsequently, the plant parts were dried in the oven for 24 hours at 70°C, for preparation to ascertain the accumulation of heavy metal (Hamizah *et al*., 2011). A 0.2 g of dried ground plant samples were taken in digestion tubes and digested by Nitric- Perchloric Acid Digestion method as described by USEPA, 1995 (APHA, 1995). The collected water samples were also digested by the same method. The digested plant and water samples were analysed for Cu content by Voltametric trace metal analyzer (Metrohm 797 VA Computrace) using HMDE (Hanging Mercury Drop Electrode) method.

2.4. Isolation and identification of bacteria

Bacterial strains were isolated from rhizosphere and growing water bed of *E. crassipes*. Isolation and enumeration of bacteria were carried by standard serial dilution plate technique. Serially diluted samples were sow in Nutrient Agar and incubated at 37°C for 24-48 hours. Bacterial colonies from Nutrient agar were isolated, purified and maintained as a pure culture which were characterized and identified up to genus level by morphological tests as per Bergey's Manual of Determinative Bacteriology: 9th edition and 8th edition (Buchanan and Gibbons, 1974). Morphological

tests carried out for the identification of the isolates are Gram's staining, cell shape and arrangement, pigment production, O/F glucose tests, endospore staining, motility, catalase, oxidase etc.

2.5. Bacterial copper resistance test

Resistance of the bacterial isolates (from water and rhizosphere) to varying concentrations of copper was determined by agar dilution method (Lui *et al*., 1983). Fresh overnight cultures of the isolates grown in peptone water were aseptically inoculated into nutrient agar plates, which were supplemented with increasing concentration of copper ions (100 µg/ml to 600 µg/ml). The plates were incubated at room temperature and observed for bacterial growth. The lowest concentration of Cu at which no growth occurred when compared with the control plates was considered as the Minimal Inhibitory Concentration (MIC). Metal salts was added to the medium after autoclaving and cooling to 45-50°C, from filter sterilized stock solutions. The copper salt used for the study was Copper sulphate ($CuSO_4.5H_2O$)

2.6. Genomic DNA isolation from culture cell

Two copper resistance isolates which showed high resistance were selected for molecular characterisation. The pure bacterial culture was inoculated in Luria Bertani broth and incubated at 37ºC for 24 h. After incubation 1ml of the broth culture was transferred to sterile micro centrifuge tube and the cells were harvested by centrifugation at 12000 rpm for 10 minutes at room temperature. Supernatant was discarded and the pellet was re suspended in 1 ml of 0.85% (w/v) NaCl solution and centrifuged as above and the supernatant was discarded and added 600 µl lysis buffer along with 7 µl of proteinase-K, vortexed the mixture and incubated at 65ºC for 1 hour. Eequal volume of chloroform and isoamyl alcohol (24:1) were added followed with gentle mixing by inverting the tube for 2-5 minutes. Then the sample centrifuged for 15 minutes at 12000 rpm at room temperature. Aqueous phase was collected in another micro centrifuge tube without disturbing the interface and lower phase. Chloroform: isoamyl alcohol extraction step was repeated. Again the aqueous phase was collected and added 50 µl volume of 3M Sodium Acetate (pH 5.2) followed by equal quantity of ice cold isopropanol, so that the DNA gets precipitated and centrifuged it again at room temperature for 5 minutes at 12000 rpm. The supernatant was discarded and rinsed the pellet twice with 70% ethanol, followed by maintaining the tubes for 1hour in vacuum desiccators. The desiccated DNA samples were completely re suspended in 50 µl of DNA dissolving buffer (TE buffer) and stored at -20ºC. The Ultra violet (UV) absorbance was checked at 260 and 280 nm for determination of DNA concentration and purity. Concentration of DNA was estimated using the formula. Concentration of DNA (mg/ml) = OD 260 x 50 x Dilution factor.

2.7. 16S rDNA gene amplification

The bacterial 16S rDNA fragment was amplified from the extracted genomic DNA by using 16S rDNA universal primer, 8F (5'-AGAGTTTGATCMTGG-3') and reverse primer 1492r (5'-ACCTTGTTAC-GACTT-3'). PCR was performed in a final reaction volume of 25 µl in 200 µl capacity thin wall PCR tube. After the initial denaturation of 5 minutes at 95ºC there were 29 cycles consists of denaturation at 94ºC for 30 seconds, annealing at 58ºC for 30 seconds, extension at 72ºC for 45 seconds and final extension at 72ºC for 10 min. PCR was carried out in ABI3730xl Genetic Analyzer (Applied Biosystems, USA). PCR product were analysed by 1.5% (w/v) agarose gel electrophoresis in 1 X TBE (Tris-Borate-EDTA; electrophoresis buffer) with Bromophenol blue loading dye. Viewed the gels on UV transilluminator and photograph of the gel was taken.

2.8. 16S rDNA Sequencing

The PCR product was sequenced by ABI3730xl Genetic Analyzer (Applied Biosystems USA). Sequences were matched with previously published bacterial 16S rDNA sequence in the NCBI database using ADVACED BLAST (www.ncbi.nlm.nih.gov/BLAST).

2.9. Phylogenetic analysis

A phylogenetic tree was constructed using the neighbor-joining (NJ) distance method with the MEGA4 software and the reliability of the bootstrap consensus inferred from 1000 replicates. Some reference sequence from the Gene Bank of most closely related to that of Bacillus were used in generating phylogenetic tree. The evolutionary distance was computed using the maximum composite likelihood method. The 16S rDNA sequence of heavy metal resistant bacteria (P9 and K12) from the rhizosphere have been deposited in the NCBI GeneBank.

2.10. Phytoremediation study

2.10.1. Preliminary study

As a preliminary assessment for determining the

survival of *E. crassipes* in different copper concentrations, the plant was grown in three different copper concentrations of 1mg/ml, 5 mg/ml, and 10 mg/ml,. In high copper concentration (10 mg/ml,) plant get wilted within 3 days. From the preliminary study and the literature collected, it was understood that *E. crassipes* showed comparatively high survival at 5 mg/ml,. Hence the working standard fixed as 5 mg/ml,.

2.10.2. Experimental setup

Fiber tanks of 150 liters capacity filled with the water from selected wetland were used for removal studies. Copper was added in an amount which makes their concentration in the experimental tanks as 5 ppm. The plants with uniform size were put in experiment tank and control tank was maintained without any plants. Experimental setup was maintained in duplicate for 15 days. The volume of water in each tank was kept constant and the change in volume due to evapo transpiration was compensated by the addition of deionised water. Every 3 days interval, the water samples and plant were collected from the experimental set up and were analyzed for copper content. Different plant parts like root, petiole and leaves of the plant were analyzed separately for the determination of copper.

2.11. Bioconcentration factor and translocation ability

The bioconcentration factor (BCF) provides an index of the capacity of the plant to accumulate the metal with respect to the metal concentration in the substrate. It is calculated as the ratio of the trace element concentration in the plant tissues at harvest to the concentration of the element in the external environment and is dimensionless (Zayed *et al*., 1998).

BCF is given by:

$$BCF = (P/E)i$$

Where i denote the heavy metal, P represents the trace element concentration in plant tissues (mg/kg dry wt.) and E represents the trace element concentration in the water (mg/l). A larger ratio implies better phytoaccumulation capability.

Translocation ability (TA) was calculated by dividing the concentration of a trace element accumulated in the root tissues by that accumulated in shoot tissues and is dimensionless (Wu and Sun, 1998).

TA is given by:

$$TA = (Ar/As)i$$

Where i denotes the heavy metal, Ar represents the amount of trace element accumulated in the roots (mg/kg dry wt.) and As represents the amount of trace element accumulated in the shoots (mg/kg dry wt.). A larger ratio implies poorer translocation capability.

3. Results and Discussion

3.1. Copper content of E. crassipes plant and growing water bed

The copper concentration of the water samples collected from Vembanad region ranged from 0.009 to 0.03 mg/ml, (Table 1). Canal systems in Kuttanad are interconnected and they receive run off from paddy fields and different types of agriculture systems. They also receive the domestic and municipal sewage from the nearby town ship in Kuttanad. That may be the reason for the presence of heavy metals in canal systems.

The copper content in different parts of *Eichhornia crassipes* collected from different sampling stations were studied (Table 1). Copper accumulation ranged from 8.7 to 14.7 mg/kg, from 4.11 to 23.67 mg/kg and from 7.6 to 10.87 mg/kg in root, petiole and leaf respectively. Metal accumulation in wetland plants are affected by many factors. In general, variations in plant species, the growth stage of the plants and the element characteristics control absorption, accumulation, and translocation of metals. Furthermore, physiological adaptations also control toxic metal accumulations by sequestering metals in the roots (Guilizzoni,1991). During cultivation, the excess application of synthetic chemical fertilizers, herbicides and fungicides are common in Kuttanad (Thampatti and Padmakumar, 1999). Copper sulphate is the most ingredients of all fungicide and herbicide (Hoffman, 2001). The remnants of these may be the reason for high occurrence of copper in the macrophytes. Floating plants have the ability to accumulate the heavy metals from surrounding water

Table 1. Copper content (mg/kg) in plant parts of *E. crassipes* and water samples of sampling locations (mg/ml)

Sampling locations	Copper concentration			
	Water samples (mg/ml)	Plant parts (mg/kg)		
		Root	Petiole	Leaf
Kainadi	0.032	11.7	11.4	7.67
Poovam	0.019	8.7	4.1	10.8
Nedumudi	0.009	14.7	23.6	9.2

Table 2. Bacterial genera isolated from rhizosphere of *E. crassipes* and water

Serial No:	Rhizosphere	Water
1	*Acinetobacter*	*Acinetobacter*
2	*Alcaligenes*	*Bacillus*
3	*Bacillus*	*Chromobacterium*
4	*Kurthia*	*Listeria*
5	*Listeria*	
6	*Chromobacterium*	

and it mainly accumulates in the aerial parts (Valittuto *et al*., 2006).

3.2. Isolation of rhizosphere bacteria and their copper resistant ability

In the present study culturable bacteria from the rhizosphere of *Eichhornia crassipes* and water samples were isolated, enumerated and identified up to genus level. The isolates were also checked for their resistance to copper. The microbial load in rhizosphere ranged from log 5.3 CFU/ml to log 7.6 CFU/ml and the same associated water system was log 4.3 CFU/ml to 4.9 CFU/ml (Fig. 1). Microbial load in rhizosphere was higher than that of growing water body and these results in tune with the reports of Zhan *et al*. (1993). Six bacterial genera from the rhizosphere of *E. crassipes* were identified which belong to Acinetobacter, *Alcaligenes, Bacillus, Kurthia, Listeria* and *Chromobacterium* (Table 2). Zhan *et al*. (1993) also reported most of these genera in the root zone of water hyacinth. Four bacterial genera, identified from the water samples belong to *Acinetobacter, Bacillus, Listeria* and *Chromobacterium* (Table 2). Maya *et al*. (2011) reported the same in the water samples of Kuttanad wetland, which belongs to *Bacillus, Listeria, Kurthia, Carnobacterium* and *Staphylococcus*.

Out of the 26 bacteria isolated from rhizosphere of *E. crassipes* (Table 3), five bacterial strains were showed high resistance to copper (400-500 µg/ml). Four strains showed copper resistance between 300 and 400 µg/ml. Only one bacterial strain showed the resistance between 200-300 µg/ml. Four strains showed the resistance between 100 and 200 µg/ml. Rest of the 12 strains showed the copper resistance below 100 µg/ml. Out of the 19 bacteria isolated from water samples (Table 3), six bacterial isolates showed high bacterial resistance of 400-500 µg/ml. Two strains showed the copper resistance between 300 and 400 µg/ml. Only one showed the resistance of 200-300 µg/ml. One showed copper resistance of 100-200 µg/ml. Nine strains showed the copper resistance below 100 µg/ml. So *et al*. (2003) reported that bacteria isolated from *E. crassipes* resisted high copper concentrations and could increase the copper removal capacity of the roots of *E. crassipes*. The high levels of resistance found among the isolates are probably attributed to past or present copper contamination in the growing environment (Abou-Shanab *et al*., 2003b). Berg *et al*. (2010) also reported that microbes isolated from copper amended systems were more resistant to copper than strains isolated from control plots.

Based on the 16S rDNA analysis, the highest sequence similarity of the highly copper resistant bacteria from the rhizosphere of *Eichhornia crassipes* are: Culture K12 showed 99% similarity with *Bacillus altitudinis* strain- Y118 16S ribosomal RNA gene, partial sequence (Accession No: JX134625.1) and the Culture P9 showed 99% similarity with *Bacillus altitudinis* strain SH164 16S ribosomal RNA

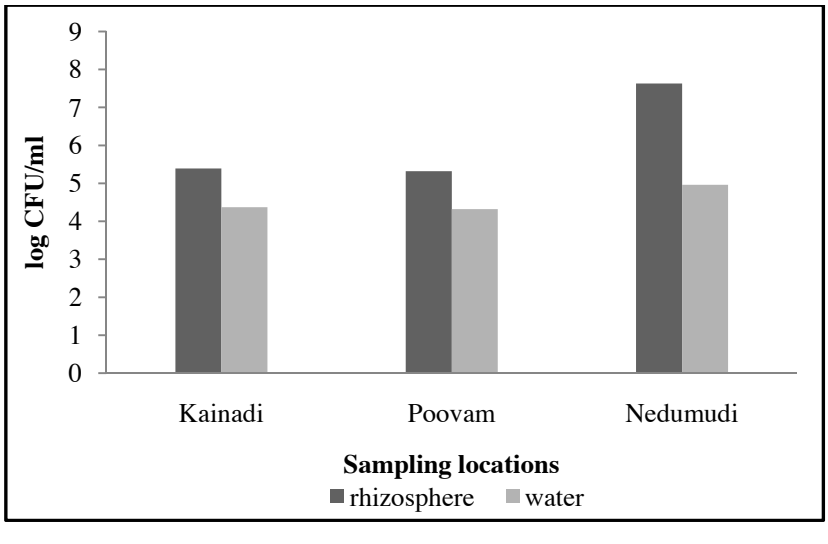

Figure 1. Load of heterotrophic plate count of rhizosphere associated bacteria and water of *E. crassipes*

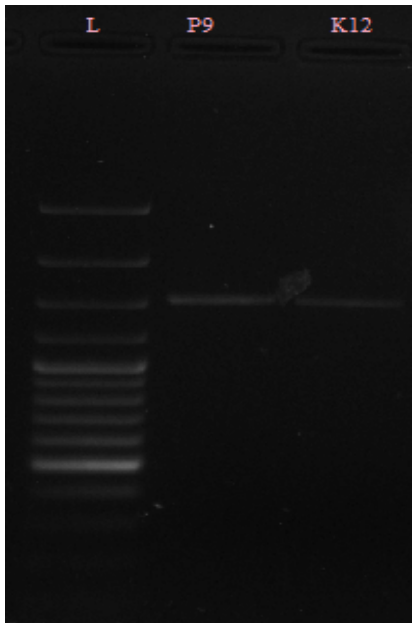

Figure 2. PCR amplification of partial fragment of 16S rDNA gene obtained from isolate K12 and P9

gene, partial sequence (Accession No: KC172059.1) (Figs. 2 and 3). Microorganisms isolated from the rhizosphere may be better adapted to plants and provide better conditions for plant growth than organisms isolated from the other sources as these are already closely associated with the plant system as well as adapted to the local environment. *Bacillus altitudinis* was previously isolated from the rhizosphere of rice from South India by Gopalakrisnan *et al*. (2010) and there was no much report on the presence of these strains from wetlands and rhizosphere. It was the first report on the occurrence of *Bacillus altitudinis* in the rhizosphere of *E. crassipes*.

3.3. Phytoremediation of copper using E. crassipes:

In phytoremediation studies, copper accumulation in different parts of *E. crassipes* and percentage removal of copper from the water in an experimental set up were studied (Figs. 4 and 5). Eighty four percentage of copper content were removed from the experimental tank compared to 21 percentages in control tank during the experimental period. Hamizah *et al*. (2011) and Mishra (2008) reported the copper removal of 61.4% and 86%, respectively using *E. crassipes* which was in tune with the present study. Bioaccumulation studies revealed that there was a high accumulation of copper in the different parts of *E. crassipes*. Copper accumulation was found to be high in root followed by leaf and petiole. Hyperaccumulation of heavy metals is said to occur when the plants are able to accumulate more than 1000 mg/l of the heavy metal in to the plants system, either by accumulating in the roots or shoots (Baker and Brooks, 1989). *E. crassipes* can be considered as hyper accumulators since the amount of copper that can be accumulated are more than 1000 mg/kg. In the present study, copper accumulation was higher in root systems compared to petiole and leaf.

3.4. Plant growth assessment

In this study, the growth of the plants was assessed by monitoring the wet weight of the plants at the start

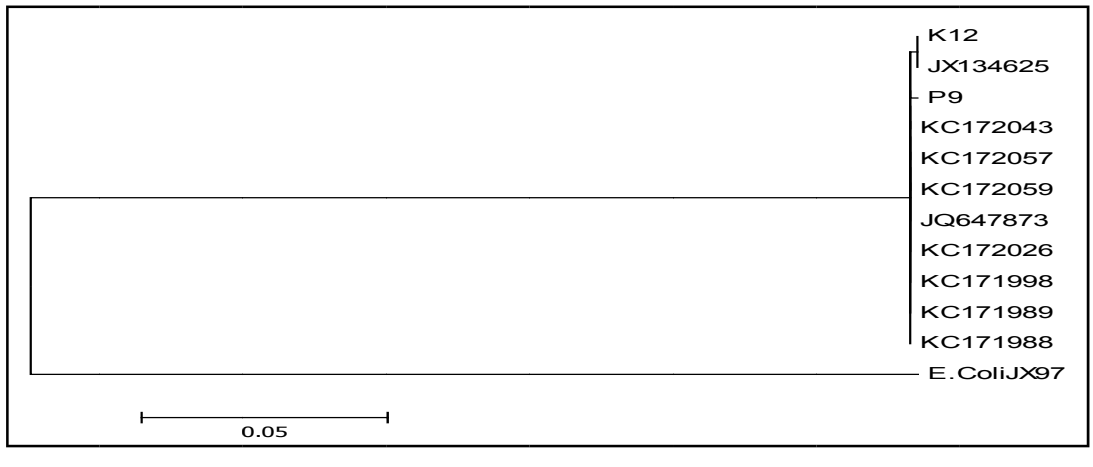

Figure 3. Phylogenetic analysis based on 16S rDNA sequence

E.ColiJX97: Outgroup
K12: Bacillus altitudinis strain K12 (Sample)
P9: Bacillus altitudinis strain P9 (Sample)
KC172043, KC172057, KC172059, KC172026, KC171998, KC171989, KC171988, JX134625, JQ647873: Bacillus altitudinis strains of NCBI database

Table 3. Copper resistance patterns/ MIC of bacterial strains isolated from rhizosphere of *E. crassipes* and water from the sampling locations

Culture code (Rhizosphere samples)	Cu Concentration (µg/ml)	Culture code (Water samples)	Cu Concentration (µg/ml)
K1	400-500	K1	300-400
K2	<100	K2	<100
K3	<100	N1	<100
K5	<100	N2	400-500
K6	400-500	N3	400-500
K7	300-400	N5	400-500
K9	100-200	N6	<100
K10	200-300	N7	<100
K11	<100	N8	<100
K12	400-500	N9	<100
N1	300-400	P1	400-500
N2	<100	P2	200-300
N3	400-500	P3	100-200
N4	100-200	P4	<100
N5	100-200	P5	<100
N6	<100	P7	400-500
N7	<100	P8	400-500
P1	100-200	P9	<100
P3	300-400	P10	300-400
P4	<100		
P5	<100		
P6	<100		
P7	300-400		
P8	<100		
P9	400-500		
P10	<100		

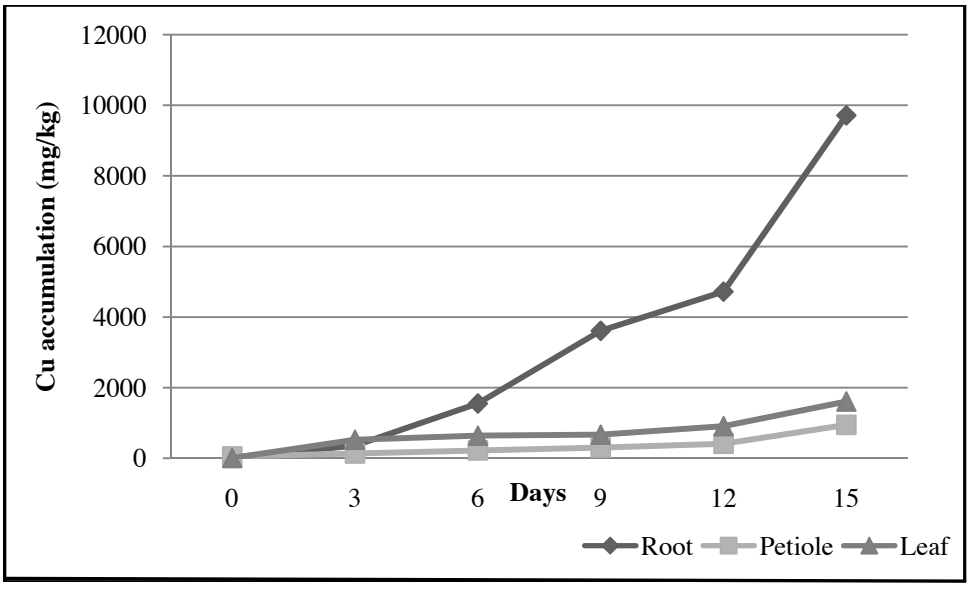

Figure 4. Copper accumulation in plant parts of *E. crassipes*

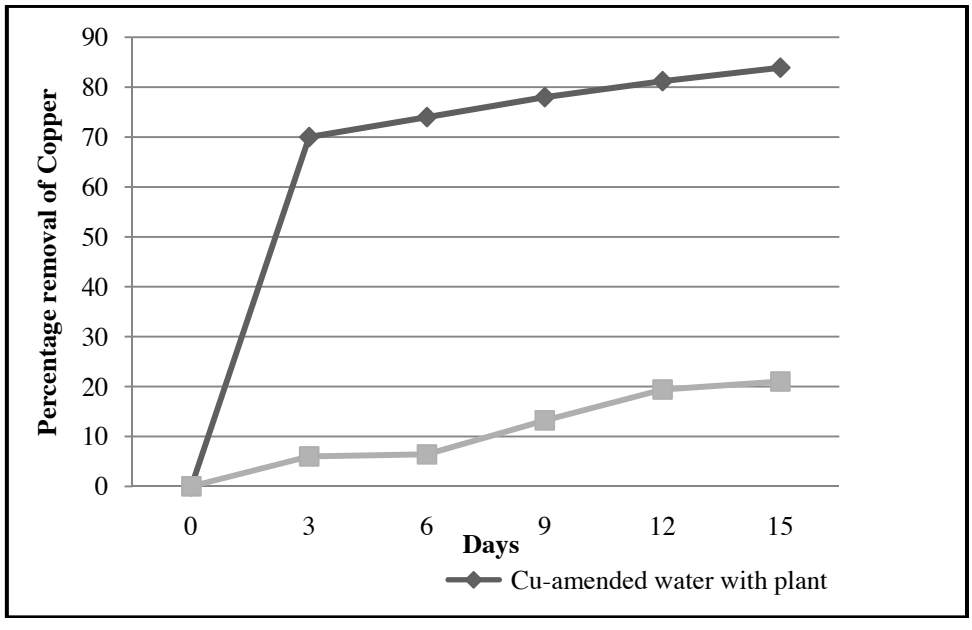

Figure 5. Percentage removal of copper from water

and at every three-day intervals of the experiment. The relative growth of the plants was calculated as Wf/Wi, where Wf is the final wet weight of plants after exposure to contaminant and Wi is the initial weight of the plants (Lamai *et al*., 2005). An increase of wet weight of the *E. crassipes* after the exposure to contaminant (Table 4) was noted. The wet weight increased from 32.1 g to 49.6 g for plant control whereas the wet weight of *E. crassipes* increased from 31.2 g to 40.5 g at copper contaminant of 5 mg/L. This result indicates that concentration of copper contaminant did not significantly affect the growth of *E. crassipes*. The plants seemed healthy and produced new shoots. The results showed that the plants to be able to accumulate acceptable amount of metals and also survive in the contaminated condition.

3.5. Bioconcentration factor and translocation ability

According to (Zhu *et al*., 1999; Abd-Elmoniem, 2003), the ratio between plant metal concentration and that of the growth media expresses the bioconcentration factor (BCF) which reflects the affinity of aquatic macrophytes to a specific heavy element or pollutant. A good accumulator is recognized by two criteria in experimental conditions (a) its ability to take up concentration more than 5,000 mg /kg dry wt. of a given element, and (b) its ability to bioconcentrate the element in its tissues; for example, the BCF value exceeds 1,000.

Table 4. Wet weight of plant before and after exposure to contaminant

Exposure time (days) *Eichhornia crassipes*	Wet weight of plant	
	Plant control	5mg/l
0	32.16	31.21
3	33.1	31.1
6	40.2	31.4
9	57.6	36.4
12	49.8	30.93
15	49.61	40.52

In this study, water hyacinth absorbs copper in concentrations greater than 5,000 mg/kg dry wt. BCF in plant roots, petiole and leaf was considered to evaluate the effectiveness of water hyacinth as a phytoremediator for the copper. The BCF for copper in root, petiole and leaf were 11295.6 mg/kg dry wt, 1098.32 mg/kg dry wt and 1870 mg/kg dry wt respectively. Since the water hyacinth and it's plant parts met the criteria of a good accumulator. Based on the BCF values copper plant parts, water hyacinth can be primarily used as a good phytoaccumulator of copper. Translocation ability (TA) is the ratio between the concentrations of a trace element accumulated in the root tissues by that accumulated in shoot tissues, a larger ratio implies poorer translocation capability. Present study reveals that the translocation ratios between root/leaves and root/petiole were found to be 6.03 and 10.28. So the results showed that water hyacinth has poor translocation capability for copper and the water hyacinth concentrate copper on the roots. Brun *et al*. (2001) recorded that in most of the species studied to date, it has been found that there is a strong barrier to translocation of Cu; hence Cu tends to be largely accumulated in fibrous plant roots rather than in other plant parts which are more usually consumed.

Copper is more localized in the aquatic plant roots; it indicates that rhizofiltration may be the predominant mechanism for accumulation of copper (Zhu *et al*., 1999). Plants may accumulate higher concentration of metals in the roots since roots are usually at the base of the plant and removed from photosynthetic process for their own tolerance (Qian *et al*., 1999). Chandra and Kulshreshtha (2004) reported that the accumulation of heavy metals was higher in roots compared to shoots in aquatic plants and the least accumulation of metals in the shoots were due to the slow mobility of metal transport from root to shoot. The results showed that upon addition of heavy metal the solution pH was greatly modified which is in tune with the result of (Erzsebet *et al*., 2011).The root system of water hyacinth has pH dependency, which are responsible for the absorption and accumulation of large amounts of cations by roots (Yahya, 1990).

4. Conclusions

Aquatic macrophyte, *E. crassipes* was tested for the removal of heavy metal Cu. The macrophyte proved highly effective in the uptake of copper at the concentration of 5 mg/ml. This plant has removed the metal successfully without any indications of toxicity. The high correlation between the final copper concentration in the water and the copper concentration in *E. crassipes* indicates that these plants can be effectively used for the removal of copper from water systems. The microbiota isolated from this plant with copper resistance has high potential for the further research in the area of bioremediation.

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