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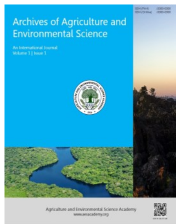

ORIGINAL RESEARCH ARTICLE

Comparative assessment of phytoremediation feasibility of water caltrop (*Trapa natans* L.) and water hyacinth (*Eichhornia crassipes* Solms.) using pulp and paper mill effluent

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ABSTRACT

Experiments for the comparative assessment of phytoremediation feasibility of water caltrop (*Trapa natans* L.) and water hyacinth (*Eichhornia crassipes* Solms.) using paper mill effluent were carried out for 60 days. The results revealed that the pulp and paper mill effluent was varied in characteristics and highly loaded with TDS, EC, BOD_5, COD, TKN, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, SPC and MPN. It was observed that and both the plant species *T. natans* and *E. crassipes* significantly ($P\leq0.05/P\leq0.01/P\leq0.001$) reduced the contents of TDS, EC, BOD, COD, TKN, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, SPC and MPN of pulp and paper mill effluent after phytoremediation experiments. Albeit, the maximum removal of these parameters were obtained at 60 days of the phytoremediation experiments but the removal rate of these parameters were gradually increased from 15 days to 45 days and it was decreased at 60 days. The most contents of Cd, Cu, Fe, Mn and Zn was translocated in the leaves of *T. natans* and *E. crassipes* during the phytoremediation experiments whereas, the least contents of Cr, Ni and Pb was translocated in the leaves of *T. natans* and *E. crassipes.* Among both the macrophytic species (i.e. *T. natans* and *E. crassipes*) used for the phytoremediation, *E. crassipes* was found to be more effective for the removal of different parameters of pulp and paper mill effluent in comparison to *T. natans*. Therefore, *T. natans* and *E. crassipes* can be used effectively to reduce the pollution load of pulp and paper mill effluent.

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INTRODUCTION

Application of aquatic macrophytes in phytoremediation of industrial effluent has become popular due to the high cost and energy intense treatment technologies (Sooknah and Wilkie, 2004; Padmapriya and Murugesan, 2012; Kumar and Chopra, 2016). Ever increasing human population, extensive industrialization and urbanization is continuously creating pressure on the water resources (Kumar and Chopra, 2013a, b; Rohit *et al.,* 2015). Industries like pulp and paper mills consume a large amount of fresh water and generate huge quantity of wastewater which is varied in characteristics with higher inorganic, organic and biological pollution load (Kumar and Chopra, 2014, 2015). The treatments of industrial effluents are quietly expensive and time consuming (Kumar and Chopra, 2011, 2012). The industrial effluents contains various nutrients like nitrogen (N), phosphorus (P), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), sulphate (SO_4^{2-}) and chlorides. Besides this the effluent also have heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb) and zinc (Zn) (Dar *et al.,* 2011;

Luqman *et al.,* 2013). Besides this, an extensive range of inorganic and organic compounds including heavy metals, hazardous wastes and chlorinated hydrocarbons present in the paper mill effluent cause contamination of potable water (Kumar *et al.,* 2010; Singh *et al.,* 2012; Kumar and Chopra, 2016).Discharge of untreated or partially treated pulp and paper mill effluent is major cause of aquatic pollution in India (Kulkarni *et al.,* 2008; Kumar and Chopra, 2012). A large number of treatment technologies are using to prevent the pollution of aquatic resources caused due to the discharge of industrial effluent (El-Gendy *et al.,* 2004; Alade and Ojoawo, 2009; Singh *et al.,* 2012). Among different methods phytoremediation is one of the most feasible methods for the treatment of industrial effluent and municipal wastewater (Alves *et al.,* 2003; Shah *et al.,* 2010; Ajibade *et al.,* 2013).

Phytoremediation is the use of aquatic plants to remediate the organic and inorganic contaminants present in the industrial effluent (Jayaweera and Kasturiarachchi, 2004; Kumar and Chopra, 2016). Phytoremediation uses plants to clean up contaminated environments. Plants can help clean up many types of contaminants including metals, pesticides, explosives, and oil. However, they work best where contaminant levels are low because high concentrations may limit plant growth and take too long to clean up (Kumar and Chopra, 2016). During phytoremediation plants eliminate, detoxify or immobilize the contaminants of wastewater (Sooknah and Wilkie, 2004). Therefore, phytoremediation technology has been receiving attention recently as an innovative, cost -effective alternative for the treatment of hazardous waste (Mahmood *et al.,* 2005; Letachowicz *et al.,* 2006; Kumar and Chopra, 2016). Likewise, phytoremediation utilizes a variety of plant biological processes and the physical characteristics of plants to remediate the various contaminants (Shah *et al.,* 2010; Kumar and Chopra, 2016).

Additionally, phytoremediation may be applicable for the remediation of metals, pesticides, solvents, explosives, crude oil, PAHs, and landfill leachates. Some plant species have the ability to store metals in their roots (Mahmood *et al.,* 2005; Shah *et al.,* 2010). They can be transplanted to sites to filter metals from wastewater. As the roots become saturated with metal contaminants, they can be harvested. Hyperaccumulator plants may be able to remove and store significant amount of metallic contaminant (Letachowicz *et al.,* 2006; Kumar and Chopra, 2016). Therefore, phytoremediation is a low cost, solar energy driven cleanup technique and most useful at sites with shallow, low levels of contamination (Liao and Chang, 2004; Singh *et al.,* 2012).

Aquatic macrophytes are accomplished to eliminate a broad range of nutrients as well as heavy metals from industrial effluent (Sooknah and Wilkie 2004; Fox *et al.,* 2008). The achievement of phytoremediation depends on the availability of plant species ideally those native to the region of interestable to tolerate and accumulate high concentrations of heavy metals (Alves *et al.,* 2003; Jayaweera and Kasturiarachchi, 2004). Moreover, certain plants are competent to eradicate or break down harmful chemicals from the ground when their roots take in water and nutrients from the contaminated soil, sediment, or groundwater. Plants can help clean up contaminants as deep as their roots can reach using natural processes to store the contaminants in the roots, stems, or leaves and also convert them to less harmful chemicals within the plant or, more commonly, the root zone (Fox *et al.,* 2008; Singh *et al.,* 2012).

Furthermore, plants used for phytoremediation must be capable to tolerate the types and concentrations of contaminants present. They also must be able to grow and survive in the local climate. A number of plants species can accumulate remarkable contents of different heavy metals such as arsenic (As), cobalt (Co), Cu, Zn, Mn, Pb, selenium (Se), Ni, and Cd, in their tissues (Alves *et al.,* 2003; Singh *et al.,* 2012) and are considered as hyper accumulating species. These species are characterized by their tolerance to toxic contents of heavy metals are often endemic to metal-rich substrates and are rare in their distribution (El-Gendy *et al.,* 2004; Jayaweera and Kasturiarachchi, 2004; Rohit *et al.,* 2015).

Water caltrop and water hyacinth are the floating aquatic macrophytes, have been of particular interest for effluent remediation (Raskin and Ensley 2000; Alves *et al.* 2003; Fox *et al.,* 2008). Although water hyacinth is considered one of the world's most noxious weeds, the characteristics that make it weedy also make it a good plant for remediation (Fox *et al.,* 2008). These plants are easily adaptable to a wide range of environmental factors including pH, electrical conductivity, and temperature (El-Gendy *et al.* 2004; Kutty *et al.,* 2009). The dense fibrous root system of these plant species provides an extensive surface area for absorption, adsorption of pollutants (Pollard *et al.,* 2002; Pilon-Smits and Pilon, 2002; Fox *et al.,* 2008). Both the macrophyte species expend the majority of their lifecycle in a vegetative state and rapidly reproduce by vegetative propagation. Increased biomass leads to increased filtering capacity (Alves *et al.,* 2003). These plants absorb excessive contents of N, P, and K along with heavy metals that requires for their luxurious growth (Zhu *et al.,* 1999; Alves *et al.,* 2003; Fox *et al.,* 2008). Although, numerous studies have evaluated the efficacy of water hyacinth; yet, results differ widely on their phytoremediation potential whereas there is no sufficient scientific reports are available on the phytoremediation efficiency of water caltrop (Mojiri, 2011; Singh *et al.,* 2012; Rohit *et al.,* 2015; Kumar and Chopra, 2016). Keeping above in view the present study was undertaken to assess the comparative phytoremediation feasibility of water caltrop (*Trapa natans* L.) and water hyacinth (*Eichhornia crassipes* Solms.) using pulp and paper mill effluent.

MATERIALS AND METHODS

Study sites and experimental design: The phytoremediation experiments were carried out in the Multipurpose Experimental Area (MEA) at Department of Zoology and Environmental Science, Gurukula Kangri University, Haridwar (Uttarakhand), India (29°55'13"N 78°7'23"E). Sagar Pulp and Paper Mills Ltd. Manglaur, Haridwar

(29°45'47"N 77°50'15"E) was selected for the collection of pulp and paper mill effluent samples. The plants species of water caltrop (*Trapa natans*) and water hyacinth (*Eichhornia crassipes* Solms.) were collected from the local pond situated at Sarai, Haridwar (Uttarakhand). For the phytoremediation experiments twelve tanks were constructed inside the MEA and arranged in two blocks, each with two parallel rows of six tanks. Each tank had dimensions of 1.5×1.5×1.0 m. Tanks were constructed with concrete and cement block. Each tank was filled with 100 liter of pulp and paper mill effluent and the water level was marked on the side wall of each tank. Ten healthy plants (known biomass) of water caltrop (*Trapa natans* L.) and water hyacinth (*Eichhornia crassipes* Solms.) were transferred in each tank having the pulp and paper mill effluent and the experiments were conducted for 60 days. The study was a randomized complete block (RCB) with six replicates of each experiment was maintained throughout the study period.

Analysis of pulp and paper mill effluent: The pulp and paper mill effluent samples were analyzed before and after phytoremediation using *T. natans* and *E. crassipes* at 0 (before phytoremediation experiments), 15, 30, 45 and 60 days, respectively. The pH, total dissolved solids (TDS) and conductivity (EC) of the effluent were determined using pH meter (pH System 362 Systronics, India), TDS meter (TDS meter 661E Systronics, India) and conductivity meter (Conductivity meter 306 Systronics, India), respectively. Biochemical oxygen demand (BOD_5) of paper mill effluent was analyzed by 5 days incubation method, while the chemical oxygen demand (COD) was determined by Open Reflux Method using potassium dichromate as oxidative agent. The total Kjeldahl nitrogen (TKN) was determined by Kjeldahl method, phosphate (PO_4^{3-}) by Olsen method. Sodium (Na^+) and potassium (K^+) were measured by Stanford and English method using the flame photometry (Flame photometer 128, Systronics, India). Calcium (Ca^{2+}) and magnesium (Mg^{2+}) were analyzed using versenate titration method. Total bacteria in pulp and paper mill effluent were counted as standard plate count (SPC) using Petri plate culture method on sterile nutrient agar media (ingredients: Beef extract 1.0g, yeast extract 2.0g, peptone 5.0g, NaCl 5.0g, agar 15.0g, distilled water 1000ml; final pH 7.40), while the coliforms bacteria as most probable number (MPN were recorded by culture tube method using McConkey's broth (ingredients: Peptone 20.0g, lactose 10.0g, bile salt 5.0g, NaCl 5.0g, neutral red 0.075g, distilled water 1000ml; final pH 7.4) following methods cited by (APHA, 2012; Chaturvedi and Sankar, 2006).

Determination of plants parameters: The plants *viz., T. natans* and *E. crassipes* used for the phytoremediation of pulp and paper mill effluent was analyzed for fresh weigh, dry weight, chlorophyll content and leaf area index (LAI) before and after phytoremediation experiments at 0, 15, 30, 45 and 60 days. Fresh weight of *T. natans* and *E. crassipes* was determined by weighing of plants using digital balance. Dry weigh was estimated by drying of plants at 105 ^oC in the oven. Chlorophyll content was analysed using acetone extraction method using spectrophotometer (Porra, 2002) whereas; LAI was determined by canopy analyzer. Biochemical parameters like crude protein was determined by acid digestion and distillation method, crude fiber of *T. natans* and *E. crassipes* was estimated by acid and alkali treatment method, total carbohydrate and total sugar were determined by anthrone reagent method while total fat was analysed by ether extraction method cited in Anonymous (1980) and Chaturvedi and Sankar (2006).

Extraction of heavy metal analysis: The contents of heavy metals Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the pulp and paper mill effluent and plant materials (root, leaves and fruit of *T. natans* and root and leaves of *E. crassipes*) were determined using acid digestion method. For digestion of samples, 10 ml samples of pulp and paper mill effluent, 1g each of *T. natans* and *E. crassipes* were taken in the digestion tubes separately. In each tube 3 ml of concentrate HNO_3 was added and it was digested in an electrically heated block for 1 hour at 145^oC. In this mixture 4 ml of $HClO_4$ was added and heated to 240^oC for 1 hour. The mixture was cooled and filtered through Whatman # 42 filter paper and the volume was made to 50 ml with double distilled water and used for metals analysis. The contents of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in the pulp and paper mill effluents, *T. natans* and *E. crassipes* was analyzed for heavy metals using AAS (Model ECIL-4129) following methods of APHA (2012) and Chaturvedi and Sankar (2006).

Quality assurance and statistical analysis: Necessary quality assurance procedures and precautions were carried out during the study. The coefficient of variation of replicate analysis was determined for the measurements to calculate analytical precision. All the reagents and standards were of analytical grade. One-way analysis of variance (ANOVA) was performed determine the significant difference of values at different days of phytoremediation experiments. Means were calculated with the help of MS Excel MS Excel (ver. 2013, Microsoft Redmond Campus, Redmond, WA). Graphs were plotted with the help of Sigma plot, 2000 (ver. 12.3, Systat Software, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Characteristics of pulp and paper mill effluent: During the present investigation, the pulp and paper mill effluent was found varied in characteristics and considerably loaded with higher values of TDS, EC, BOD_5, COD, TKN, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, SPC and MPN (Tables 1, 2). Here, it is interesting to note that TKN, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Mg^{2+} of the pulp and paper mill effluent are considered as plant nutrients and significantly contributed for eutrophication of aquatic resources if present in higher concentration. The heavy metals Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn are considered as micronutrients and associated with synthesis of different biochemical components and catalyze various biochemical processes of aquatic plants in lower contents but they produce toxicity when present in higher concentration. In the present study, TDS, BOD_5, COD, Ca^{2+}, Cd, Cu, Fe, Mn, SPC and MPN of the pulp and paper mill effluent

Table 1. Changes in characteristics of pulp and paper mill effluent after phytoremediation using *T. natans* at different days.

Parameter	**Before phytoremediation**	**After phytoremediation**				**BIS[a] for inland disposal**
		15 days	**30 days**	**45 days**	**60 days**	
TDS (mg L^{-1})	1840	1685.54ns	1420.00*	1225.80**	1150.45***	1500
EC (dS m^{-1})	2.64	2.56ns	2.28ns	1.93*	1.84*	-b
pH	7.82	7.76ns	7.68ns	7.48ns	7.32ns	5.5-9.0
BOD_5 (mg L^{-1})	475.10	425.66ns	389.20*	328.10**	290.35***	100
COD (mg L^{-1})	880.50	838.75ns	745.87*	634.12**	598.56***	250
TKN (mg L^{-1})	192.65	165.40*	132.25**	110.85**	96.55***	100
PO_4^{3-} (mg L^{-1})	145.60	120.39ns	108.65*	85.70**	76.97***	-
Na^+ (mg L^{-1})	285.44	265.82ns	225.15*	185.78**	165.30***	-
K^+ (mg L^{-1})	175.50	153.40*	132.70**	110.45**	103.65**	-
Ca^{2+} (mg L^{-1})	435.80	410.65ns	386.94*	334.82**	315.85**	200
Mg^{2+} (mg L^{-1})	148.35	134.70ns	118.90*	88.50**	74.60***	-
Cd (mg L^{-1})	2.45	2.26*	2.09**	1.75**	1.45**	2.0
Cr (mg L^{-1})	1.38	1.24ns	1.13*	0.89**	0.78**	2.00
Cu (mg L^{-1})	5.64	5.48*	4.88**	3.96**	3.75**	3.00
Fe (mg L^{-1})	8.95	8.56*	7.68**	6.35**	5.80**	1.0
Mn (mg L^{-1})	3.66	3.38ns	2.68*	1.88**	1.62**	1.00
Pb (mg L^{-1})	1.74	1.62*	1.45**	1.16**	0.85**	-
Ni (mg L^{-1})	1.02	0.95*	0.76**	0.53**	0.47**	-
Zn (mg L^{-1})	6.90	6.58*	5.58**	4.24**	3.75**	15
SPC (CFU ml^{-1})	6.66×10^{6}	$5.67\times10^{5*}$	$2.84\times10^{4**}$	$4.84\times10^{3***}$	$1.23\times10^{3***}$	10000
MPN (MPN 100 ml^{-1})	3.85×10^{8}	$4.33\times10^{7*}$	$6.92\times10^{6**}$	$5.24\times10^{5***}$	$3.74\times10^{4***}$	5000

The values are mean of six replicates; [a]BIS- Bureau of Indian standard; [b]- Not defined in standard; ns, *, ** non-significant or significantly different at P≤0.05 or P≤0.01 or P≤0.001 level of ANOVA, respectively.

Table 2. Changes in characteristics of pulp and paper mill effluent after phytoremediation using *E. crassipes* at different days.

Parameter	**Before phytoremediation**	**After phytoremediation**				**BIS[a] for inland disposal**
		15 days	**30 days**	**45 days**	**60 days**	
TDS (mg L^{-1})	1840	1640.80ns	1385.60*	1175.50**	1060.30***	1500
EC (dS m^{-1})	2.64	2.53ns	2.22ns	1.80*	1.76*	-b
pH	7.82	7.72ns	7.64ns	7.44ns	7.29ns	5.5-9.0
BOD_5 (mg L^{-1})	475.10	412.65ns	372.58*	308.94**	275.68***	100
COD (mg L^{-1})	880.50	827.50ns	735.64*	612.40**	570.40***	250
TKN (mg L^{-1})	192.65	156.84*	124.36**	104.84**	82.50***	100
PO_4^{3-} (mg L^{-1})	145.60	116.30ns	96.38*	72.75**	64.57***	-
Na^+ (mg L^{-1})	285.44	258.64ns	216.60*	170.49**	150.33***	-
K^+ (mg L^{-1})	175.50	148.95*	127.45**	102.37**	96.37**	-
Ca^{2+} (mg L^{-1})	435.80	405.64ns	369.80*	314.50**	305.80**	200
Mg^{2+} (mg L^{-1})	148.35	127.66ns	109.64*	73.60**	66.40***	-
Cd (mg L^{-1})	2.45	2.18*	1.92**	168**	1.34**	2.0
Cr (mg L^{-1})	1.38	1.21ns	1.08*	0.74**	0.69**	2.00
Cu (mg L^{-1})	5.64	5.32*	4.42**	3.55**	2.94**	3.00
Fe (mg L^{-1})	8.95	8.22*	6.85**	5.75**	4.86**	1.0
Mn (mg L^{-1})	3.66	3.32ns	2.54*	1.74**	1.42**	1.00
Ni (mg L^{-1})	1.74	1.57*	1.36**	1.07**	0.73**	-
Pb (mg L^{-1})	1.02	0.92*	0.70**	0.42**	0.36**	
Zn (mg L^{-1})	6.90	6.22*	5.14**	4.05**	3.10**	15
SPC (CFU ml^{-1})	6.66×10^{6}	$4.36\times10^{5*}$	$3.95\times10^{4**}$	$2.85\times10^{3***}$	$4.65\times10^{3***}$	10000
MPN (MPN 100 ml^{-1})	3.85×10^{8}	$3.77\times10^{7*}$	$2.74\times10^{5**}$	$4.69\times10^{4***}$	$4.12\times10^{3***}$	5000

The values are mean of six replicates; [a]BIS- Bureau of Indian standard; [b]- Not defined in standard; ns, *, ** non-significant or significantly different at P≤0.05 or P≤0.01 or P≤0.001 level of ANOVA, respectively.

were recorded beyond the permissible limit of inland disposal for wastewater prescribed by BIS (2010). The higher EC of the pulp and paper mill effluent are associated with the presence of more ionic species in the pulp and paper mill effluent. Higher values of TDS, BOD_5 and COD indicates higher organic and inorganic pollution load of the pulp and paper mill effluent. The more values of total bacteria as SPC and MPN are concerned with higher biological activities in the pulp and paper mill effluent. The higher contents of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn of the pulp and paper mill effluent are likely due the use of various dyes to manufacture coloured papers in the pulp and paper mill. The findings of the present study are in the conformity of Kumar and Chopra (2016) who reported the higher values of various plant macro and micro nutrients in the agro-residue based paper mill effluent. The findings of the present study are according to Kumar and Chopra (2012) who reported the higher values of TDS, BOD_5 and COD in the paper mill effluent. Therefore, the characteristics of pulp and paper mill effluent clearly indicated the presence of various macro and micro plant nutrients required for the luxurious growth of aquatic macrophytes.

Changes in characteristics of pulp and paper mill effluent after phytoremediation using *T. natans* and *E. crassipes*: The changes in characteristics of pulp and paper mill effluent after phytoremediation using *T. natans* and *E. crassipes* are presented in Tables 1 and 2. The ANOVA analysis on data indicated that *T. natans* and *E. crassipes* significantly ($P<0.05/P<0.01/P<0.001$) decreased the contents of TDS, EC, BOD_5, COD, TKN, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, SPC and MPN of the pulp and paper mill effluent. Here it is interesting to note that the removal rate of different parameters of the pulp and paper mill effluent was progressively increased from 15 days to 45 days of phytoremediation experiments, and there was a slight decline in the removal rate at 60 days of the phytoremediation experiments using *T. natans* and *E. crassipes*. The most reduction of the pulp and paper mill effluent parameters was noted at 60 days of phytoremediation experiments using *T. natans* and *E. crassipes*. The reduction of TKN, PO_4^{3-}, Na^+, K^+, Ca^{2+} and Mg^{2+} Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, SPC and MPN of the pulp and paper mill effluent is might be due to the uptake of these nutrients by *T. natans* and *E. crassipes* required for their growth during phytoremediation experiments. The findings of the present study were in accordance with Alade and Ojoawo (2009) who also reported that *E. crassipes* significantly reduced the BOD, COD, TKN and PO_4^{3-} of sewage effluent. Dar *et al.* (2011) reported that water hyacinth (*Eichhornia crassipes*) have the potential for the treatment of sewage wastewater and it can used for the removal of nitrogen, phosphorus, calcium, magnesium, cadmium, chromium, iron and zinc of the sewage wastewater. Kumar and Chopra (2016) also reported that *T. natans* significantly reduced the contents of Cd, Cr, Cu, Fe, Mn and Zn of the paper mill effluent. Ajibade *et al.* (2013) also reported that water hyacinth was found to be effective for the removal of heavy metals in domestic sewage in the University of Ilorin, Nigeria.

Changes in biometric parameters of *T. natans* and *E. crassipes* after phytoremediation: The values of different growth parameters of *T. natans* and *E. crassipes* during phytoremediation experiment of the pulp and paper mill effluent at different days are presented in Tables 3 and 4. The fresh weigh, dry weight, chlorophyll content and leaf area index (LAI) of *T. natans* and *E. crassipes* was increased from 15 days to 60 days of phytoremediation experiments (Tables 3, 4). The most values of fresh weigh (296.37g and 312.65g), dry weight (74.68g and 78.94g), chlorophyll content (4.78 mg/g fwt and 4.86 mg/g fwt) and LAI (3.52 and 3.57) of *T. natans* and *E. crassipes* was recorded at 60 days of phytoremediation experiments using pulp and paper mill effluent, respectively (Tables 3 and 4). The fresh weight, dry weight, chlorophyll content and LAI of *T. natans* and *E. crassipes* was recorded to be significantly ($P<0.05/P<0.01$) different after phytoremediation experiments at different days (Tables 3, 4). Among both the macrophyte species used for the phytoremediation, *E. crassipes* greatly achieved their vegetative growth luxuriously in comparison to *T. natans* and it might be due to the presence of plenty of nutrients in the pulp and paper mill effluent. The increase in fresh weigh, dry weight, chlorophyll content and LAI of *T. natans* and *E. crassipes* are in the conformity of the presence of various plant nutrients like TKN, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn in the pulp and paper mill effluent needed to achieve the maximum vegetative growth as also earlier reported by Sooknah and Wilkie (2004). These findings are in the agreement of a phytoremediation experiments carried out by Kumar and Chopra (2016) who reported that fresh weigh, dry weight, chlorophyll content and LAI of *T. natans* was increased due to the uptake of different nutrients present in the paper mill effluent.

Changes in biochemical parameters of *T. natans* and *E. crassipes* after phytoremediation: The values of biochemical parameters viz., crude protein, crude fiber, total carbohydrates, total ash and total fat of *T. natans* and *E. crassipes* during the phytoremediation experiments are shown in Figures 1, 2. The results revealed that the contents of crude protein, crude fiber, total carbohydrates, total ash and total fat of *T. natans* and *E. crassipes* was increased significantly ($P<0.05/P<0.01$) at different days of phytoremediation experiments using pulp and paper mill effluent (Figures 1, 2). The contents of crude protein, crude fiber, total carbohydrates, total ash and total fat of *T. natans* and *E. crassipes* were observed in the order of total carbohydrates > crude protein > crude fiber > total ash > total fat after phytoremediation experiments using pulp and paper mill effluent. The increase in crude protein, crude fiber, total carbohydrates, total ash and total fat of *T. natans* and *E. crassipes* during the phytoremediation experiments of the pulp and paper mill effluent are in the conformity of the presence of various macro and micro nutrients like TKN, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn in the pulp and paper mill effluent required for the synthesis of these biochemical components as earlier reported by Sooknah and Wilkie (2004). In a phytoremediation study, Alves *et al.* (2003) also reported that water hyacinth (*Eichhornia crassipes*) was found to be effective for the removal of different nutrients from wastewater and increased their biomass, crude fiber, crude protein, total fat and total ash, and it can be cultivated for thereduction of pollution load of excessively nutrients rich wastewater. Moreover, Kumar and Chopra (2016) also reported the synthesis of various biochemical parameters like crude protein, crude fiber, total sugar and total carbohydrates of *T. natans* during the phytoremediation of paper mill effluent.

Translocation of heavy metals in various parts of *T. natans* and *E. crassipes* after phytoremediation: During the study, the translocation of Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn in different parts of *T. natans* (root, leaves and fruit) and *E. crassipes* (root and leaves) was recorded after phytoremediation experiments (Figures 3-10). It was observed that the most contents of Cd, Cu, Fe, Mn and Zn was translocated in the leaves of *T. natans* and *E. crassipes* during the phytoremediation experiments and it is likely due to that these metals are associated with synthesis of chloroplast and photosynthesis processes (Porra, 2002; Sooknah and Wilkie, 2004). Least contents of Cr, Ni and Pb was translocated in the leaves of *T. natans* and *E. crassipes* and this might be due to that these metals produce toxicity in plants and reduced the growth attributes (Figures 3-10). Thus, root act as a barrier for the translocation of Cr and Pb in aerial plant parts. However, the observed differences in the metals accumulation in the different parts of the plant suggest different cellular mechanisms of metal bioaccumulation, which may control their translocation and partitioning in the plant. The Cd, Cu, Fe, Mn and Zn are essential for the survival and proliferation of all plants. There is more demand for Cu, Fe, Mn and Zn in the photosynthetic apparatus as a result plants accumulates and translocates these metals in their photosynthetic parts (Porra 2002; Sooknah and Wilkie, 2004; Kumar and Chopra, 2014).

It should be renowned that Cr, Ni and Pb are toxic and non -essential element to plants and hence the plants may not possess any specific mechanism to transport the Cr and Pb (Rohit *et al.,* 2015). Poor translocation of Cr, Ni and Pb to the leaves might be due to the sequesterization of most of

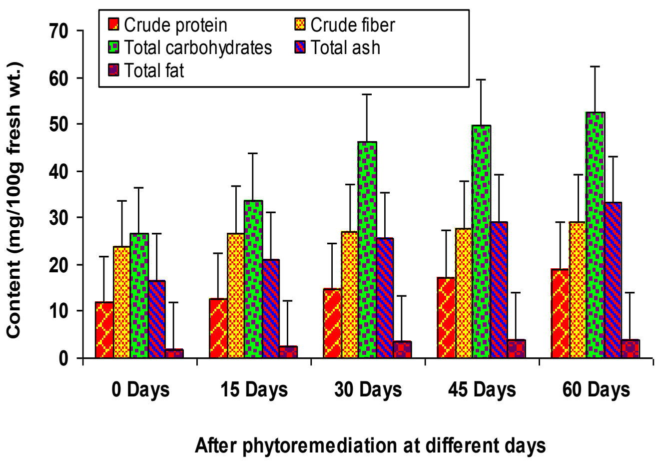

Figure 1. *Contents of biochemical components of E. crassipes after phytoremediation of pulp and paper mill effluent at different days. Error bars are the standard error of the mean.*

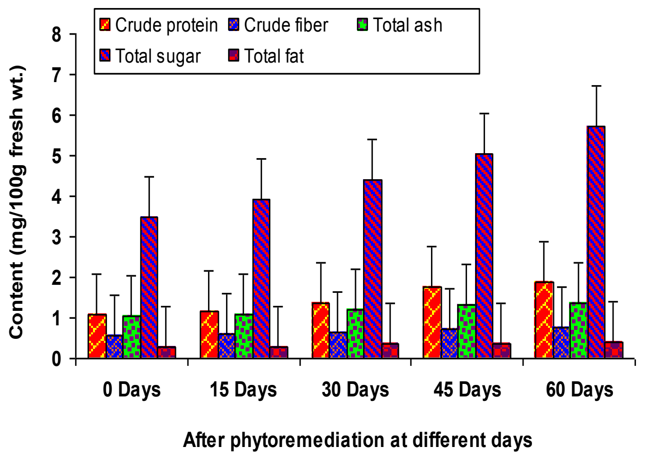

Figure 2. *Contents of biochemical components of T. natans after phytoremediation of pulp and paper mill effluent at different days. Error bars are the standard error of the mean.*

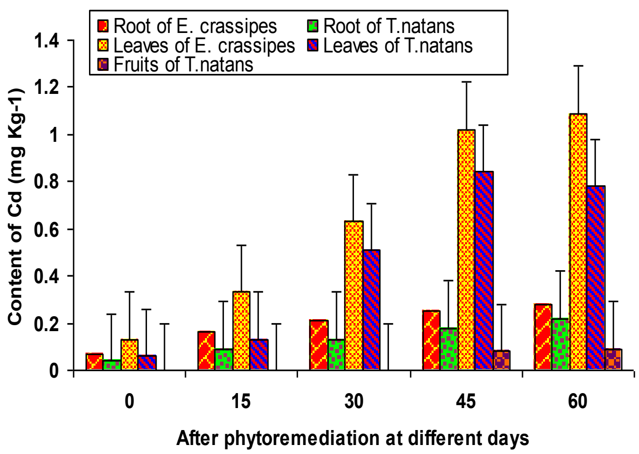

Figure 3. *Translocation of Cd in different parts of E. crassipes and T. natans after phytoremediation of pulp and paper mill effluent at different days. Error bars are the standard error of the mean.*

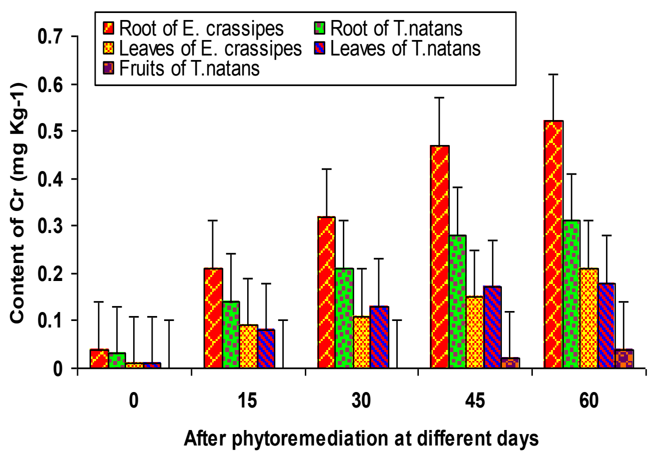

Figure 4. *Translocation of Cr in different parts of E. crassipes and T. natans after phytoremediation of pulp and paper mill effluent at different days. Error bars are the standard error of the mean.*

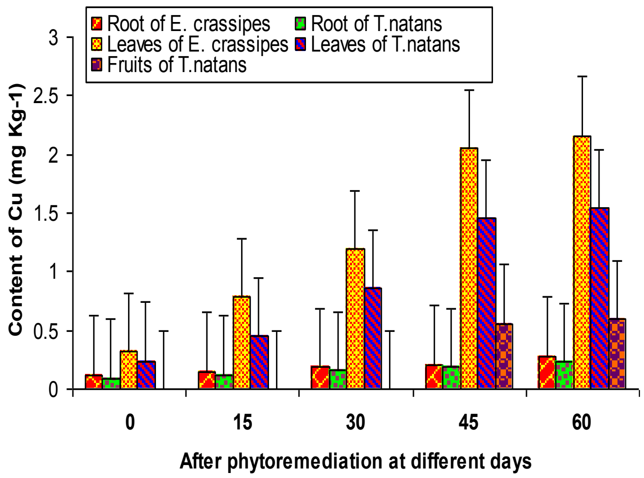

Figure 5. *Translocation of Cu in different parts of E. crassipes and T. natans after phytoremediation of pulp and paper mill effluent at different days. Error bars are the standard error of the mean.*

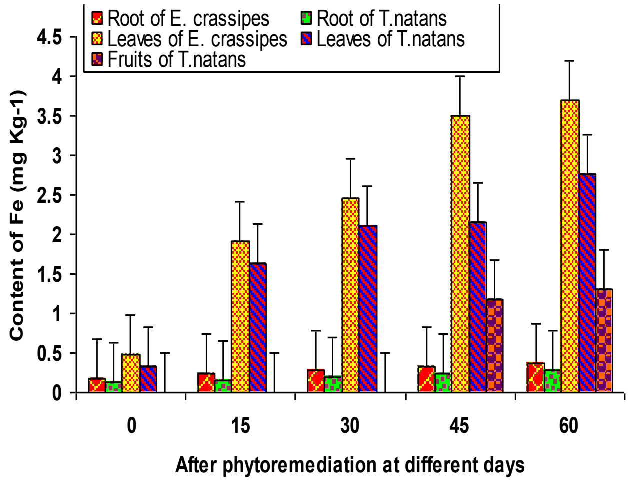

Figure 6. *Translocation of Fe in different parts of E. crassipes and T. natans after phytoremediation of pulp and paper mill effluent at different days. Error bars are the standard error of the mean.*

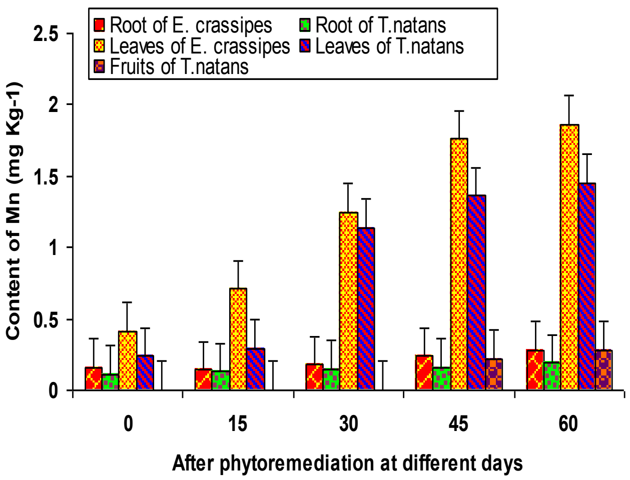

Figure 7. *Translocation of Mn in different parts of E. crassipes T. natans after phytoremediation of pulp and paper mill effluent at different days. Error bars are the standard error of the mean.*

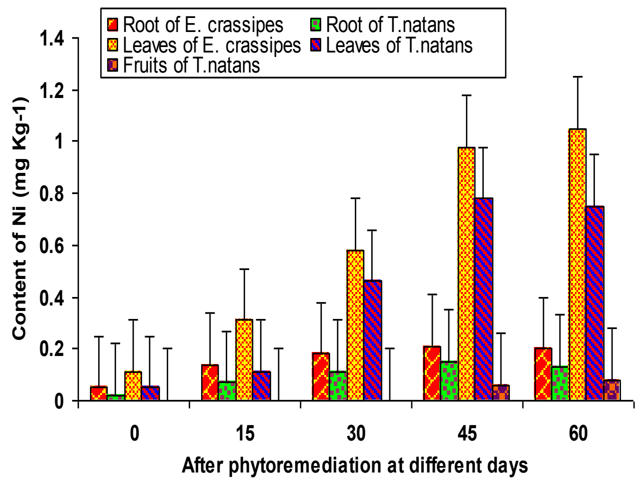

Figure 8. *Translocation of Ni in different parts of E. crassipes and T. natans after phytoremediation of pulp and paper mill effluent at different days. Error bars are the standard error of the mean.*

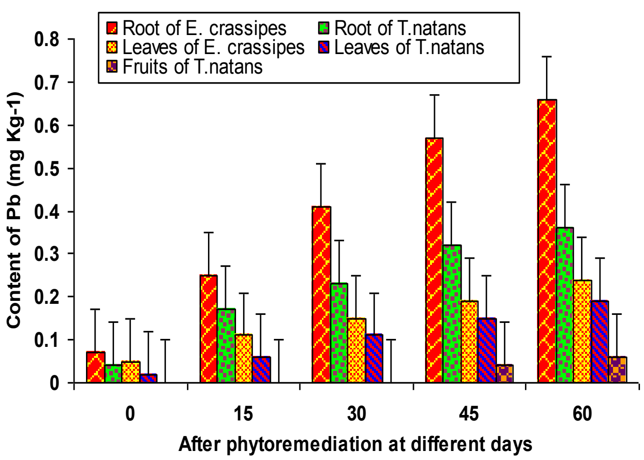

Figure 9. *Translocation of Pb in different parts of E. crassipes and T. natans after phytoremediation of pulp and paper mill effluent at different days. Error bars are the standard error of the mean.*

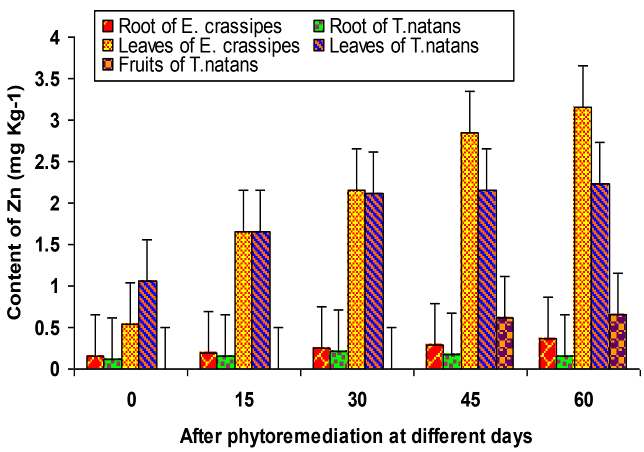

Figure 10. *Translocation of Zn in different parts of E. crassipes and T. natans after phytoremediation of pulp and paper mill effluent at different days. Error bars are the standard error of the mean.*

the Cr, Ni and Pb in the vacuoles of the root cells to render it non-toxic, which may be a natural protective response of this plant (Shah *et al.,* 2010; Kumar and Chopra, 2014). Therefore, the tolerant mechanism of plants appears to be compartmentalization of metals ions, i.e. sequestration in the vacuolar compartment, which excludes them from celular sites where processes such as cell division and respiration occur, thus proving to be as effective protective mechanism (Singh *et al.,* 2012; Kumar and Chopra, 2014). The findings are in accordance with Kumar and Chopra (2014) who reported the translocation of Cd, Cr, Cu, Fe, Mn, Pb and Zn in the root, shoot, leaves and fruits of French bean (*Phaseolus vulgaris* L.) cultivated in sewage sludge amended soil. Zhu *et al.* (1999) also reported that water hyacinth showed significant accumulation of various trace metals Cd, Cr, Cu, Fe, Mn and Zn from a wetland of China.

Conclusions

The present study concluded that pulp and paper mill effluent collected from ASP based STP was considerably loaded with TDS, EC, BOD, COD, TKN, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, SPC and MPN. Phytoremediation treatments of the pulp and paper mill effluent using *T. natans* and *E. crassipes* significantly ($P\leq0.05/P\leq0.01/P\leq0.001$) removed the contents of TDS, EC, BOD, COD, TKN, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn, SPC and MPN of the pulp and paper mill effluent. The fresh weight, dry weight, chlorophyll content and LAI of *T. natans* and *E. crassipes* was recorded to be significantly ($P<0.05/P<0.01$) different after phytoremediation experiments at different days. The most contents of Cd, Cu, Fe, Mn and Zn was translocated in the leaves of *T. natans* and *E. crassipes* during the phytoremediation experiments whereas, the least contents of Cr, Ni and Pb was translocated in the leaves of *T. natans* and *E. crassipes.* The contents of crude protein, crude fiber, total carbohydrates, total ash and total fat of *T. natans* and *E. crassipes* were observed in the order of total carbohydrates > crude protein > crude fiber > total ash > total fat after phytoremediation experiments using pulp and paper mill effluent which is in the conformity of the presence of various macro and micro nutrients in the pulp and paper mill effluent. Therefore *T. natans* and *E. crassipes* can be used for the effective treatment of the paper mill effluent.

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REFERENCES

Ajibade, F.O., Adeniran, K.A., and Egbuna, C.K. (2013). Phytoremediation efficiencies of water hyacinth in removing heavy metals in domestic sewage (A Case Study of University of Ilorin, Nigeria). *The International Journal of Engineering and Science,* 2(12): 16-27.

Alade, G.A., and Ojoawo, S.O. (2009). Purification of domestic sewage by water hyacinth (*Eichhornia crassipes*). *International Journal of Environmental Technology and Management,* 10(3): 286-294.

Alves, E., Cardoso, L.R., Scavroni, J., Ferreira, L.C., Boaro, C.S.F., and Cataneo, A.C. (2003). Physiological and biochemical evaluations of water hyacinth (*Eichhornia crassipes*), cultivated with excessive nutrient levels. *Planta Daninha, Vicosa-MG,* 21: 27-35.

Anonymous (1980). Official methods of analysis. Association of Official Analytical Communities. Washington D.C.

APHA (2012). Standard methods for the examination of water and waste water, 21st Edn. American Public Health Association, Washington pp 2462.

Chaturvedi, R.K., and Sankar, K. (2006). Laboratory manual for the physico-chemical analysis of soil, water and plant. Wildlife Institute of India, Dehradun.

Dar, S.H., Kumawat, D.M., Singh, N., and Wani, K.A. (2011). Sewage treatment potential of water hyacinth (*Eichhornia crassipes*). *Research Journal Environmental Science,* 5(4): 377-385.

El-Gendy, A.S., Biswas, N., and Bewtra, J.K. (2004). Growth of water hyacinth in municipal landfill leachate with different pH. *Environment & Technology,* 25: 833-840.

Fox, L.J., Struik, P.C., Appleton, B.L., Rule, J.H. (2008). Nitrogen phytoremediation by water hyacinth (*Eichhornia crassipes* (Mart.) Solms). *Water Air and Soil Pollution,* 194: 199 -207.

Jayaweera, M.W., and Kasturiarachchi, J.C. (2004). Removal of nitrogen and phosphorus from industrial wastewaters by phytoremediation using water hyacinth (*Eichhornia crassipes* (Mart.) Solms). *Water Science and Technology,* 50: 217-225.

Kulkarni, B.V., Ranade, S.V., Wasif, A.L. (2008). Phytoremediation of textile effluent by using water hyacinth: A polishing treatment. *International Journal of Environment Management and Technology,* 5(1): 18-27.

Kumar, V., Chopra A.K., Pathak, C., and Pathak, S. (2010). Agro-potentiality of paper mill effluent on the characteristics of *Trigonella foenum-graecum* L. (Fenugreek) *New York Science Journal,* 3(5): 68-77

Kumar, V., and Chopra, A.K. (2011). Alterations in physico-chemical characteristics of soil after irrigation with paper mill effluent. *Journal of Chemical and Pharmaceutical Research,* 3(6):7-22

Kumar, V., and Chopra, A.K. (2012). Effects of paper mill effluent irrigation on agronomical characteristics of *Vigna radiata* (L.) in two different seasons. *Communications in Soil Science and Plant Analysis,* 43(16):2142-2166.

Kumar, V., and Chopra, A.K. (2013a). Ferti-irrigational effect of paper mill effluent on agronomical characteristics of *Abelmoschus esculentus* L. (Okra). *Pakistan Journal of Biological Sciences*, 16 (22): 1426-1437.

Kumar, V., and Chopra, A.K. (2013b). Distribution, enrichment and accumulation of heavy metals in soil and *Trigonella foenum-graecum* L. (Fenugreek) after fertigation with paper mill effluent, *Open Journal of Metals*, 3: 8-20.

Kumar, V., and Chopra, A.K. (2014). Ferti-irrigation effect of paper mill effluent on agronomical practices of *Phaseolus vulgaris* (L.) in two different seasons. *Communications in Soil Science and Plant Analysis,* 45: 2151–217

Kumar, V., and Chopra, A.K. (2015). Fertigation with agro-residue based paper mill effluent on a high yield spinach variety. *International Journal of Vegetable Science*, 21(1): 69-97.

Kumar, V., Chopra, A.K. (2016). Reduction of pollution load of paper mill effluent by phytoremediation technique using water caltrop (*Trapa natans* L.). *Cogent Environmental Science,* 2: 1153216.

Kutty, S.R.M., Ngatenah, S.N.I., Isa, M.H., and Malakahmad, A. (2009). Nutrients removal from municipal wastewater treatment plant effluent using *Eichhorina crassipes*. *World Academic Science Engineering and Technology,* 60: 826-831.

Letachowicz, B., Krawczyk, J., and Klink, A. (2006). Accumulation of heavy metals in organs of *Typha latifolia*. *Polish Journal of Environmental Studies,* 15(2a): 407-409.

Liao, S.W., and Chang, W.L. (2004). Heavy metal phytoremediation by water hyacinth at constructed wetlands in Taiwan. *Journal of Aquatic Plant Management,* 42: 60-68.

Luqman, M., Butt, T., Tanvir, A., Atiq, M., Hussan, M.Z.Y., and Yaseen, M. (2013). Phytoremediation of polluted water by trees: A review. *International Journal of Agricultural Research and Reviews,* 1(2): 022-025.

Mahmood, Q., Zheng, P., Islam, E., Hayat, Y., Hassan, M.J., Jilani, G., and Jin, R.C. (2005). Lab scale studies on water hyacinth (*Eichhornia crassipes* Marts Solms) for biotreatment of textile wastewater. *Caspian Journal of Environmental Sciences,* 3(2): 83-88.

Mojiri, A. (2011). Phytoremediation of heavy metals from municipal wastewater by *Typha domingensis*. *African Journal of Microbiology Research,* 6(3): 643-647.

Padmapriya, G., and Murugesan, A.G. (2012). Phytoremediation of various heavy metals (Cu, Pb and Hg) from aqueous solution using water hyacinth and its toxicity on plants. *International Journal of Environmental Biology,* 2(3): 97-103.

Pilon-Smits, E.A.H., and Pilon, M. (2002). Phytoremediation of metals using transgenic plants. *Critical Reviews in Plant Sciences,* 21: 439-456.

Pollard, A.J., Powell, K.D., Harper, F.A., and Smith, J.A.C. (2002). The genetic basis for metal hyper accumulation in plants. *Critical Reviews in Plant Sciences,* 21: 539-566.

Porra, R.J. (2002). The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls *a* and *b*. *Photosynthesis Research,* 73: 149-156.

Raskin, I., and Ensley, B.D. (2000). Phytoremediation of toxic metals: Using plants to clean up the environment. John Wiley & Sons, Inc., New York. pp 53-70.

Rohit, A., Kale, Vinayak, Lokhande, H., and Avinash, B. (2015). Investigation of chromium phytoremediation and tolerance capacity of a weed, *Portulaca oleracea* L. in a hydroponic system. *Water and Environment Journal,* 10: 1111-12106.

Shah, R.A., Kumawat, D.M., Singh, N., Wani, K.A. (2010). Water hyacinth (*Eichhornia crassipes*) as a remediation tool for dye effluent pollution. *International Journal Science and Nature,* 1(2):172-178.

Singh, D., Tiwari, A., and Gupta, R. (2012). Phytoremediation of lead from wastewater using aquatic plants. *Journal of Agricultural Technology,* 8(1): 1-11.

Sooknah, R.D., and Wilkie, A.C. (2004). Nutrient removal by floating aquatic macrophytes cultured in anaerobically digested flushed dairy manure wastewater. *Ecological Engineering,* (22): 27- 42.

Zhu, Y.L., Zayed, A.M., Quian, J.H., DeSouza, M., Terry, N. (1999). Phytoaccumulation of trace elements by wetland plants: II. water hyacinth. *Journal of Environmental Quality,* 28: 339-344.