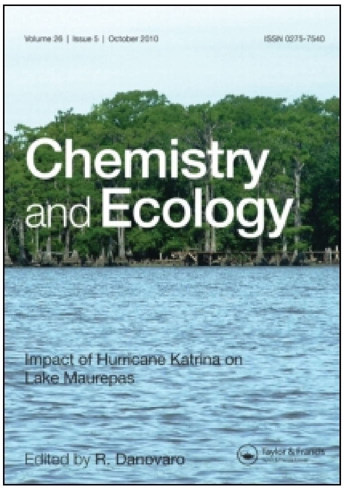

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

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Online publication date: 13 May 2010

To cite this Article Pal, Rama and Rai, J. P. N.(2010) 'The phytoextraction potential of water hyacinth (*Eichchornia crassipes*): removal of selenium and copper', Chemistry and Ecology, 26: 3, 163 — 172
To link to this Article: DOI: 10.1080/02757541003785833
URL: http://dx.doi.org/10.1080/02757541003785833

Chemistry and Ecology
Vol. 26, No. 3, June 2010, 163–172

The phytoextraction potential of water hyacinth (*Eichchornia crassipes*): removal of selenium and copper

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(*Received 5 September 2009; final version received 9 February 2010*)

The phytoextraction potential of water hyacinth (*Eichchornia crassipes*) was assessed for the removal of selenium and copper individually and from binary solutions. Plant growth, estimated on day 16 of metal treatment, decreased at all concentrations of selenium (2–12 ppm), whereas it increased at lower concentrations of copper (4–12 ppm) and decreased at higher exposure levels. Unlike copper, the rate constant for selenium uptake and its accumulation factors, calculated for both root and shoot, were lower in a binary solution than in the corresponding single-metal solution. Analysis of the elemental composition of the plant revealed that in single-metal copper treatment, the level of Mg decreased with a higher magnitude, followed by K and Ca. However, in the case of selenium, the Ca level increased, Mg remained unaffected and the K level decreased with increasing exposure. Our results revealed that a water hyacinth-based system could successfully remove selenium and copper from water/wastewater.

Keywords: water hyacinth; accumulation factor; phytoremediation; selenium and copper

1. Introduction

Rapid industrialisation in western Uttar Pradesh and the Tarai Bhabar region of Uttarakhand, India, has led to severe stress in aquatic ecosystems, created by excessive discharge of industrial effluent containing toxic heavy metals which adversely affect the living components of these systems through the food chain. Selenium and copper are trace elements required for proper growth and development in plants and animals; however, high levels can cause adverse effects [1,2]. The recommended upper limits for selenium and copper discharge are 0.01 and 0.05 $mg{\cdot}L^{-1}$, respectively (http://www.groundwatertnpwd.org.in/bis_std.pdf).

Several conventional methods are well known to remove heavy metals from wastewater, viz. ion exchange, chemical precipitation, reverse osmosis, land application, coagulation, membrane filtration, co-precipitation and electrochemical processes. However, there are major drawbacks to these methods: for example, incomplete metal removal, high energy requirements and the generation of toxic sludge, which creates a secondary pollution problem, in addition to financial constraints [3–7]. Recently, the high metal accumulation capability of aquo-vascular plants has

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ISSN 0275-7540 print/ISSN 1029-0370 online

DOI: 10.1080/02757541003785833
http://www.informaworld.com

suggested their efficient application in the removal of metals from wastewater [8,9]. Water hyacinth has been shown to possess an ability for the sorption of Pb(II), Hg(II), Cd(II), Al(III), Zn(II) and Cr(IV) [10–13]. In this study, the uptake of selenium and copper ions from single- and binary-metal solutions was investigated, employing water hyacinth to quantify uptake of the metals and determine whether they are influenced by each other.

2. Materials and methods

2.1. *Experimental set-up*

Water hyacinth plants collected from a natural pond located near Pantnagar, India were kept in a plastic tank for 10 days to acclimatise them. Prior to experimentation, plants were kept in a greenhouse receiving natural light and with a temperature range of 24 to 28 °C. A stock solution of copper and selenium, each with an initial concentration of $1000\,mg{\cdot}L^{-1}$, was prepared by dissolving the appropriate masses of AR grade $CuCl_2$ and $SeCl_4$ grade in distilled water. Experiments were conducted by maintaining individual weighed plants in 1 L of tap water containing one-fifth strength Hoagland's solution [14], a dilute nutrient solution comparable with the natural environment, at pH 7 and exposed to single metal concentrations of Se (2, 4, 5, 8 and 12 ppm) and Cu (4, 8, 12, 16 and 24 ppm), as well as a mixture of the two metals (Se 12 ppm + Cu 24 ppm). The metals were analysed in water and plant samples on days 1, 2, 4, 6, 8, 12 and 16 after the start of the experiment. The aim was to study the kinetics of test metal uptake in the whole plant and to assess the usefulness of water hyacinth in phytoremediation of Se and Cu from contaminated sites. On each analysis date, metal loss owing to earlier uptake by the plant was compensated for by adding the requisite amount of stock solution in the experimental tubs to maintain the initial concentration. A control set-up of plants, in which metal ion solutions were not added, was used to study plant growth in response to metal stress.

2.2. *Plant and metal analysis*

Plant growth variables were measured in terms of dry weight, growth (biomass – initial dry weight) and growth rate (g dry weight increase per day). Each plant was separated into shoot and root, washed, dried for 72 h at 65 °C and weighed for biomass estimation. The original dry weight of the plant was computed by multiplying the original fresh weight by the ratio of final dry weight to final fresh weight [10]. Dried and ground plant tissue samples weighing ~ 0.5 g were ashed in a muffle furnace at 450 °C for 5 h. Ashed samples were digested for ~ 3 h with 5 mL of $HNO_3/HClO_4$ (1: 1 v/v) and the volume adjusted with deionised distilled water [15]. Water and digested plant samples were analysed for Se and Cu using a Perkin–Elmer atomic absorption spectrophotometer (Model 373). In order to determine the phytoextraction potential of water hyacinth for selenium and copper, the accumulation factor (for shoot and root) and removal per cent were calculated using the following equations:

$$\text{Accumulation factor} = (\mu\text{g of metal}\cdot\text{g}^{-1}\text{ dry weight of plant tissue})/(\mu\text{g of metal}\cdot\text{g}^{-1}\text{ water}),$$

$$\text{Removal per cent} = \frac{\text{total metal accumulation in whole plant (mg)}}{\text{total metal content in solution}} \times 100.$$

2.3. *Statistical analysis*

Experimental data were analysed statistically using SPSS statistical software (v. 5.5). Regression analysis was performed to determine the correlation coefficient (r) between metal uptake and ambient metal concentration. To verify the statistical significance of any differences among treatments, data were analysed using the Student's t-test.

3. Results and discussion

3.1. *Metal accumulation in shoot and root*

The accumulation of selenium in both shoot and root of water hyacinth increased with increasing metal concentration and duration of exposure, except for 8 and 12 ppm (Table 1). After 6 days of treatment at 8 and 12 ppm, a sudden increase in uptake was observed. However, in the case of copper, accumulation tended to show a regular increase with experimental period in both the shoot and root at all concentrations (Table 2). The accumulation of selenium in both shoot and root upon exposure to a binary-metal solution (Se + Cu) was lower than in the corresponding single-metal solution at 12 ppm, whereas in the case of copper, accumulation in shoot and root was higher than in the corresponding single-metal solution (24 ppm). Similar observations of enhanced copper uptake in the presence of selenium have also been made in *Pisum sativum* and *Triticum aestivum* [16], showing that selenium promoted the uptake of copper. On a whole-plant basis, a linear dependence was observed between the uptake of selenium and copper and their concentration in solution. Regression coefficients estimated for selenium uptake as a function of ambient metal concentration were 0.821, 0.9219, 0.9874, 0.9878 on days 1, 4, 8 and 16 of treatment, respectively, indicating linearity at the higher levels of exposure (days 8 and 16), however, linearity could not be established with assurance at lower levels of exposure (days 1 and 4). By contrast, the regression coefficients for copper were 0.9754, 0.9625, 0.9967 and 0.9987 on days 1, 4, 8 and 16 of treatment, respectively, indicating a much closer linear relation at each level of exposure. Thus, the uptake process followed a monophasic isotherm with a linear increase in the concentration of both the metals.

3.2. *Effect of metals on plant growth rate*

The growth rate of water hyacinth decreased drastically after exposure to 2–5 ppm of selenium, from 70 to 90% compared with control, and became negative at higher concentrations (Figure 1(a)). However, in the case of copper, the growth rate increased by 7.5% to 66% with respect to control after exposure to 4–12 ppm and decreased slightly at higher concentrations (Figure 1(b)) due to metal toxicity, which is in accordance with previous reports in other plant species [17–20]. In the case of a binary-metal solution (Se 12 ppm + Cu 24 ppm), the plant growth rate was 4.5×10^{-2} g·day^{-1}, indicating a 32% reduction with respect to control and was lower than the growth rate (5.31×10^{-2} g·day^{-1}) corresponding to the single-metal copper solution of 24 ppm. This might be because of reduced selenium uptake and enhanced copper uptake in the presence of selenium, and consequently increased metal toxicity [16].

3.3. *Metal uptake as a function of treatment duration*

The uptake of selenium with respect to exposure time at higher concentrations of 8 and 12 ppm exhibited two stages: the first extended to 6 days and second with enhanced uptake occurred between 6 and 16 days (Figure 2). The biphasic nature was clearly evident at each stage of exposure, although at the two lower concentrations (2 and 4 ppm) only the single stage of the biphasic nature was observed. Similar observations with respect to cadmium have been reported by Hasan et al. [21]. Copper uptake was also observed to be biphasic but exhibited only one

Table 1. Concentration of selenium ($\mu g \cdot g^{-1}$ dry weight ± SE) found in water hyacinth treated with selenium alone and with a binary-metal solution.

		Days of treatment						
Treatment metal (ppm)	Part	1	2	4	6	8	12	16
2	Shoot	40.92 ± 0.52	57.72 ± 0.42	78.72 ± 0.4	124.3 ± 1.3	177.6 ± 1.0	248.6 ± 1.4	334.8 ± 2.1
	Root	109.2 ± 2.6	235.2 ± 1.7	457.2 ± 2.5	481.2 ± 2.7	589.2 ± 2.8	711.6 ± 2.6	794.4 ± 3.2
4	Shoot	100.32 ± 3.2	138.72 ± 1.3	271.92 ± 1.0	413.52 ± 1.9	469.5 ± 3.6	609.6 ± 3.2	711.6 ± 4.1
	Root	313.2 ± 5.2	466.8 ± 2.3	524.4 ± 3.9	697.2 ± 4.2	847.2 ± 4.7	1057.2 ± 4.9	1093.2 ± 5.8
5	Shoot	248.4 ± 4.6	268.32 ± 1.4	403.2 ± 2.3	533.52 ± 3.2	600 ± 5.3	633.6 ± 3.8	682.8 ± 3.8
	Root	1642.8 ± 9.8	1687.2 ± 7.2	1807.2 ± 4.5	1882.8 ± 5.9	1934 ± 7.3	2040 ± 3.7	2268 ± 5.6
8	Shoot	489.6 ± 5.7	646.32 ± 3.4	768 ± 3.2	840 ± 4.1	1128 ± 6.5	1320 ± 5.6	1664 ± 4.7
	Root	1537.2 ± 7.2	1654.8 ± 5.2	1716 ± 4.8	1908 ± 6.2	2400 ± 6.6	3000 ± 6.9	3240 ± 6.2
12	Shoot	492 ± 3.6	702 ± 4.1	874 ± 2.8	948 ± 5.7	1080 ± 4.7	1344 ± 7.1	1428 ± 4.8
	Root	1540 ± 6.8	1800 ± 7.5	2000 ± 6.2	2552 ± 6.8	3640 ± 5.8	4456 ± 8.2	4780 ± 3.2
12 ppm Se + 24 ppm Cu	Shoot	590.4 ± 3.1	612 ± 3.8	720 ± 4.8	871.2 ± 2.6	966 ± 3.3	1098 ± 3.2	1174.8 ± 4.7
	Root	1680 ± 5.9	1824 ± 5.8	1920 ± 7.1	1944 ± 4.2	2172 ± 4.8	2388 ± 4.9	2534.4 ± 6.5
Control	Shoot							1.8 ± 0.04
	Root							6.2 ± 0.5

Note: No selenium solution was added to the control plants.

Table 2. Concentration of copper (μg/g dry weight ± SE) found in water hyacinth treated with copper alone and with a binary-metal solution.

		Days of treatment						
Treatment metal (ppm)	Part	1	2	4	6	8	12	16
4	Shoot	58.32 ± 0.3	127.92 ± 0.9	355.9 ± 1.1	360 ± 1.7	500.4 ± 1.5	646.8 ± 1.8	981.2 ± 1.6
	Root	709.2 ± 3.4	721.2 ± 3.7	853.2 ± 5.4	1075.2 ± 2.3	1164 ± 2.8	1315.2 ± 2.2	1549 ± 2.3
8	Shoot	178.32 ± 1.1	382.8 ± 1.8	516 ± 3.2	648 ± 3.2	888 ± 2.2	984 ± 1.6	1188 ± 2.2
	Root	1525.2 ± 3.4	1729.2 ± 5.3	1848 ± 4.7	2460 ± 4.2	2640 ± 3.4	3144 ± 3.5	3360 ± 3.4
12	Shoot	250.32 ± 1.2	1252 ± 4.3	1828 ± 6.3	1936 ± 1.5	2076.6 ± 5.6	2825 ± 2.8	3040 ± 3.1
	Root	2149.2 ± 4.4	2400 ± 5.9	2700 ± 6.2	3030 ± 4.2	3709.6 ± 4.8	3960 ± 3.3	4080 ± 4.2
16	Shoot	421.92 ± 5.3	442.3 ± 2.1	658.3 ± 2.2	1042.3 ± 2.5	2102.3 ± 4.4	2248 ± 3.0	3440 ± 3.8
	Root	3001.2 ± 6.8	4212 ± 5.5	4921.2 ± 5.3	6019.2 ± 3.4	6369.6 ± 5.5	6589 ± 4.7	5960 ± 5.2
24	Shoot	1114.32 ± 5.8	1205.5 ± 3.3	1142.3 ± 3.6	1440 ± 2.1	1448 ± 2.2	1566 ± 2.2	1560 ± 2.1
	Root	3909.2 ± 7.3	5709.2 ± 6.7	7772.6 ± 6.7	8624 ± 7.2	9104 ± 8.3	9402 ± 7.2	9940 ± 3.2
12 ppm Se + 24 ppm Cu	Shoot	1225.4 ± 3.5	2115.5 ± 4.8	1892.8 ± 3.8	1454.5 ± 2.5	2083 ± 3.2	2993 ± 2.1	5980 ± 6.2
	Root	4820 ± 6.4	7205 ± 7.3	9330 ± 9.2	10628 ± 3.2	11022 ± 9.2	11527 ± 9.8	11000 ± 7.7
Control	Shoot							1.8 ± 0.5
	Root							8.0 ± 0.5

Note: No copper solution was added to the control plants.

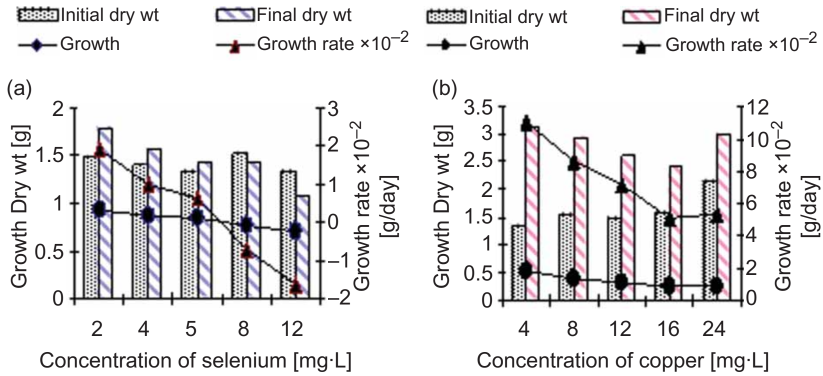

Figure 1. Effect of (a) selenium and (b) copper exposure on the growth of water hyacinth.

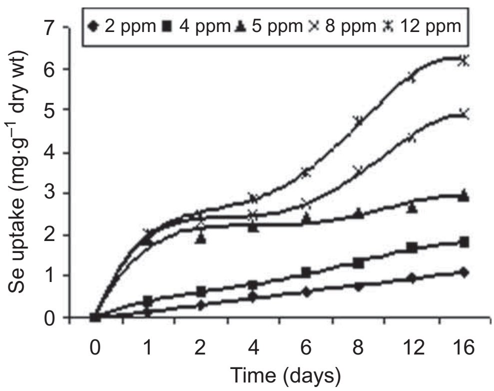

Figure 2. Selenium uptake (mg·g^{-1} dry weight ± SE) in water hyacinth as a function of treatment duration at various selenium concentrations.

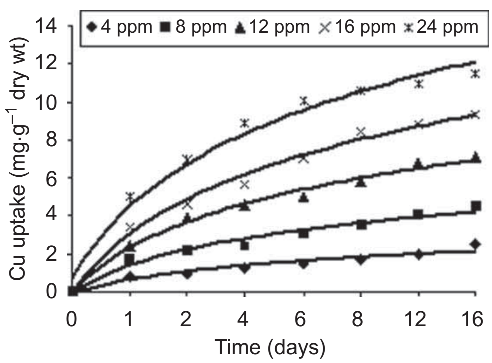

Figure 3. Copper uptake (mg·g^{-1} dry weight ± SE) in water hyacinth as a function of treatment duration at various copper concentrations.

stage, in contrast to the uptake of selenium (Figure 3). Thus, the current observations revealed that the extent of metal uptake by the plant was positively related to the concentration of metals in solution, as well as exposure time [22,23].

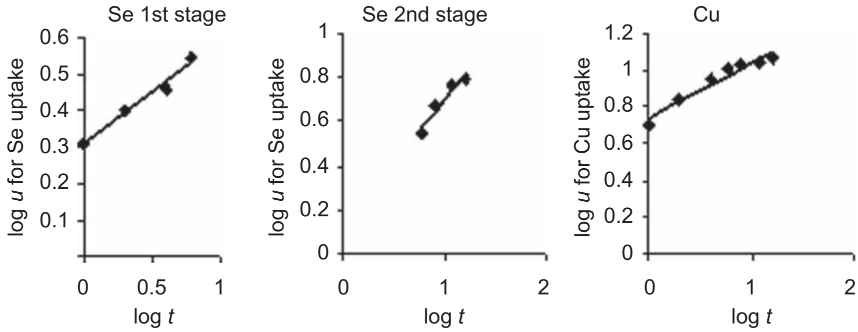

Figure 4. Effect of varying time on the uptake of metal in water hyacinth in a single-metal solution, i.e. 12 ppm for selenium and 24 ppm for copper.

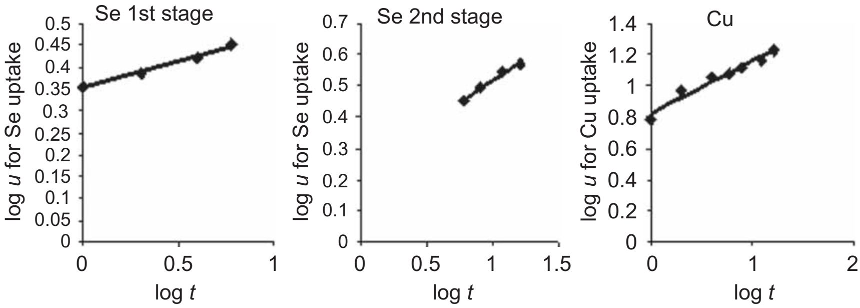

Figure 5. Effect of varying time on the uptake of metal in water hyacinth in a binary-metal solution (12 ppm Se + 24 ppm Cu).

3.4. *Kinetics of metal uptake*

A arithmic relationship was developed between metal uptake and exposure time at the highest concentrations of selenium (12 ppm) and copper (24 ppm) in order to obtain better precision in metal uptake rates for treatment with a single-metal solution (Figure 4) and a binary solution of the two metals (Figure 5). The functional relationships between metal uptake, u (ppm) and time (days) are as follows:

12 ppm Se, Stage I:

$$\log u = 0.3065 + 0.2872 \log t \tag{1}$$

12 ppm Se, Stage II:

$$\log u = 0.1255 + 0.5731 \log t \tag{2}$$

24 ppm Cu (one stage only):

$$\log u = 0.7397 + 0.2965 \log t \tag{3}$$

12 ppm Se + 24 ppm Cu, Se in stage I:

$$\log u = 0.3537 + 0.1184 \log t \tag{4}$$

12 ppm Se + 24 ppm Cu, Se in stage II:

$$\log u = 0.2389 + 0.278 \log t \tag{5}$$

Table 3. Parameters for the exponent of time, in metal uptake for Se and Cu.

Metal	u_1	n_1	u_2	n_2
Se (alone)	2025.3	0.2872 (0.993)	1335.0	0.5731 (0.996)
Cu (alone)	5491.6	0.2965 (0.995)		
Se (in a binary-metal solution)	2257.9	0.1184 (0.998)	1733.4	0.278 (0.990)
Cu (in a binary-metal solution)	6655.8	0.3349 (0.994)		

Note: Values in parentheses represent correlation coefficients for the equation between metal uptake and time.

12 ppm Se + 24 ppm Cu, Se in stage I, Cu (one stage only):

$$\log u = 0.8232 + 0.3349 \log t \tag{6}$$

The above equations can be transformed into a function with the following form:

$$u_t = k(t)^{nj}, \tag{7}$$

where

u_t, uptake of metal at any time;
nj, parameter for the power relationship for the appropriate stage or slope; and
k, constant.

The values of u_t and *nj* for the respective concentrations for individual metals and a mixture of two metals were calculated using SPSS v. 5.5. Higher values of *nj* (Table 3) indicated that the rates of uptake in the case of single-metal solutions were greater than in the binary-metal solution for selenium, whereas the reverse was true for copper. The effect of exposure to a binary-metal solution on selenium uptake alone was conducted using a t-test which showed that the difference in the rate was highly statistically significant with a 98.9% level of probability.

3.5. *Metal accumulation factor*

In general, metal accumulation factors calculated on day 16 in both single- and binary-metal solutions were higher in roots than in shoots, which may be because of their adsorption on the surface of root tissue (Table 4) [24]. Accumulation factors for both shoots and roots were shown to be dependent on the metal concentration in solution. In the case of single-metal solutions, accumulation factors for selenium in roots and shoots were substantially higher than those from mixtures of selenium and copper, whereas the reverse was true for copper.

The metal removal percentage measured on day 16 decreased with increasing concentrations of both selenium and copper (Table 4). The metal removal percentage from single-metal solutions was $> 85\%$ for concentrations between 2 and 12 ppm for selenium, whereas for copper, it was $> 90\%$ for concentrations between 4 and 24 ppm. However, the percentage of selenium removal from a binary-metal solution decreased markedly compared with the corresponding single-metal solution, whereas in the case of copper, removal was 97.8% from a binary-metal solution, reasonably higher than from the corresponding single-metal solution, thus depicting differential behaviour of the two metals with respect to their removal pattern, probably because of differences in their hydrated ionic sizes.

3.6. *Elemental composition as a result of metal exposure*

The concentration ranges calculated on day 16 day for Ca, Mg and K were 12.3–15.8, 9.5–15.4 and 10.5–16.4 mg·g^{-1} dry weight in untreated (control) plants. In the case of selenium, Ca content

Table 4. Accumulation factor and metal removal percent for selenium and copper by water hyacinth part with exposure to metal solution on day 16.

	Accumulation factor		
Metal concentration (ppm)	Shoot	Root	Removal percent
Selenium			
2	0.167	0.397	95.2
4	0.178	0.273	91.3
5	0.136	0.454	89.2
8	0.208	0.405	89.3
12	0.119	0.398	89.3
12 ppm Se + 24 ppm Cu	0.098	0.211	83.9
Copper			
4	0.245	0.387	97.5
8	0.148	0.420	97.6
12	0.253	0.340	95.0
16	0.215	0.372	93.5
24	0.065	0.414	94.5
12 ppm Se + 24 ppm Cu	0.249	0.458	97.8

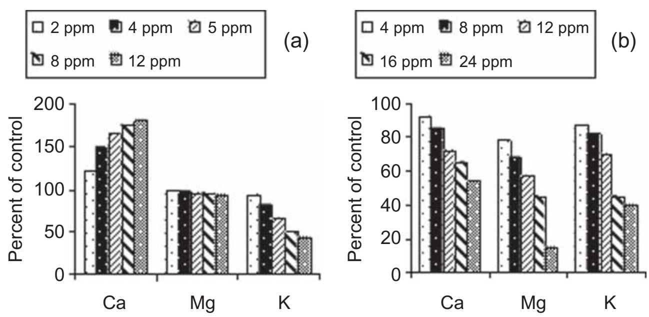

Figure 6. Effect of various concentrations of (a) selenium and (b) copper on mineral element composition in water hyacinth.

increased and K level decreased with increasing exposure (Figure 6(a)); no significant changes were found in Mg content. The results conform with observations made on selenium treatment in maize [20]. However, in the case of copper, the level of Mg decreased with a greater magnitude, followed by K and Ca (Figure 6(b)). Similarly, a decrease in Ca, Mg and K content in *Cucumis sativus* on treatment with excess copper has been observed previously [25]. In the case of treatment with a binary-metal solution, reductions in mineral element concentrations were comparatively higher than in a corresponding single-metal solution of 24 ppm.

4. Conclusion

Our experimental results showed that the rate constant for metal uptake from a binary solution of selenium and copper was quite different from that for a solution containing a single metal. Selenium accumulated more in water hyacinth than copper. The accumulation factors in shoot and root for both individual metals were higher than those from a binary solution of selenium and copper. The metal removal percentage for selenium in a binary solution was lower than in the corresponding single-metal solution, whereas the reverse was true for copper. Although laboratory experiments exclude the impact of various factors such as microclimate, soil physicochemical and biological properties, which often prevail under natural conditions and affect phytoextraction, our investigation quantifies selenium and copper removal by water hyacinth from single- and binary-metal solutions and paves way for further research.

Acknowledgements

Research facilities by G.B. Pant University of Agriculture and Technology, Pantnagar and financial assistance from Indian Council of Agricultural Research, Government of India, in the form of the research project, are deeply acknowledged.

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