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ORIGINAL ARTICLE

Bioaccumulation Pattern of Mercury in *Bacopa monnieri* (L.) Pennell

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Bioaccumulation pattern of mercury was studied in *Bacopa monnieri* plants cultivated in Hoagland nutrient medium artificially contaminated with 5 and 10µM $HgCl_2$. Mercury content of roots, stem and leaves were analysed using Atomic Absorption Spectrophotometry (AAS). During a period 12 days of growth, more accumulation was noticed in roots followed by stem and leaves. Repeated addition of $HgCl_2$ and enhanced growth period up to 50 days showed only negligible increase in accumulation maintaining a threshold level of mercury in the root. When a comparison was done between the quantities of $HgCl_2$ added to the growth medium and the sum of total accumulation of the plant and content present in the residual medium, a significant quantity of mercury is found to be lost presumably through the process of phytovolatilization from the plant. Studies on the effect of pH on bioaccumulation of mercury showed that acidic pH enhanced accumulation rate and hence for phytoremediation technology 'chlorination' is recommended whereas for medicinal purpose, *Bacopa monnieri* plants can be harvested after 'liming' to increase the pH and thereby reducing accumulation rate of mercury.

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Plants growing/cultivated in soil contaminated with heavy metals like mercury are under toxicity stress. Nevertheless, heavy metal ions are absorbed by roots and translocated to the entire plant body and the pattern and the gravity of absorption and translocation depend on the chemistry of the metal, habit, habitat and genetic make up of the plant (Pilon-Smits, 2005). Many plants are hyperaccumulators can accumulate unusually high content of mercury from the environment in the root system and translocate the metal to the aerial parts and most of these are recommended for phytoremediation technique (Pilon-Smits, 2005). Plants like *Chloris barbata* and *Cyperus rotundus* (Lenka *et al*., 1993) and *Chromolaena odorata* (Velasco-Alinsug *et al*., 2005) have been reported as accumulators of mercury.

In addition, a number of marshy, aquatic or submerged herbaceous plans such as *Hydrilla verticillata* and *Bacopa monnieri* have received attention for their role as biological detoxification systems for phytoremediation of Hg, Cd, Cr, Cu, Fe, and Mn contamination (Sinha *et al*., 1993; 1997; Sinha 1999; Sinha & Pandey, 2003; Sinha *et al*., 2003; Askari *et al*., 2007).

Studies on bioaccumulation of Hg in plants either in natural soil or artificially contaminated media are very scanty. As mentioned earlier, even though some plants have been reported as accumulators of Hg, no plant has yet been identified as natural hyperaccumulator of Hg (Henry, 2000; Raskin & Ensley, 2000). However, transgenic plants such as *Arabidopsis thaliana, Liriodendron tulipifera,* and *Nicotiana tabacum* are capable of converting methyl mercury to Hg^{2+} and are having the potential of phytoremediation in alleviating Hg polluted areas (Bizily *et al*., 1997; Rugh *et al*., 1996; 1998).

Bacopa monnieri has been used in Ayurvedic systems of medicine and traditionally it is used as a 'brain tonic' to enhance memory development (Nair, 1987). According to Wohlmuth (2001), Nathan *et al*., (2001) and Stough *et al.,* (2001) *Bacopa monnieri* is an Ayurvedic herb and currently enjoying the popularity as 'brain herb'. Many products derived from *B. monnieri* are available in nutraceutical market with content of bacoside A and B (Deepak & Amit, 2004).

In addition to the well-established multipurpose medicinal use, *Bacopa monnieri* has been recommended for phytoremediation due to the hyperaccumulation potential (Sinha *et al*., 1996; Sinha, 1999; Yadav *et al.*, 2005). In this context the fate of the plant for medicinal uses should be determined and considered as part of risk assessment because many Ayurvedic preparations containing *B. monnieri* are available in the market and are profusely consumed by humans. Therefore, the dual uses or qualities of *B. monnieri i.e.,* phytoremediant on one hand and medicinal on the other do pose a threat to mankind.

Bacopa monnieri is found to be highly sensitive to soil/water pollution (Hussain-koorimannil *et al*., 2010) and hence it is an ideal plant for monitoring contamination of water or soil. This plant is a vegetatively propagated plant, and hence cultivation in nutrient solution artificially contaminated with heavy metals can effectively be done. Since the entire plant is used for medicinal purpose, accumulation of toxic metals in root, stem and leaf may highlight the gravity of health hazard due to the entry of the toxic metals to human body.

The objective of this investigation is the elucidation of accumulation pattern of mercury in the root, stem and leaf tissues during different intervals of growth in Hoagland nutrient medium artificially contaminated with different concentrations of $HgCl_2$. The feasibility of *Bacopa monnieri* for phytoremediation technology and its medicinal uses are seemed to be paradoxical each other and hence a systematic study on the bioaccumulation strategy of *Bacopa monnieri* towards mercury is essentially warranted.

MATERIALS AND METHODS

Bacopa monnieri collected from cultivated plants (medicinal plant collection) of Calicut University Botanical Garden. Cuttings of 7-8 cms were placed in distilled water and kept for rooting in water and these propagules were used for experiments of heavy metal treatments. Two concentrations of $HgCl_2$ (5 μM and 10 μM) were selected and based on a preliminary study conducted, in which almost 50% growth retardation was taken as the criterion for determining the of toxicity level. Five μM and 10 μM solutions of $HgCl_2$ were prepared in Hoagland nutrient medium

and 200 mls of each solution was taken in separate containers. Eight well rooted propagules were planted in each container and at least a minimum of five containers was used for each treatment. Propagules cultured in Hoagland nutrient solution without $HgCl_2$ served as the control.

Random sampling was done by collecting plants from all replicate containers at an interval of 2 days up to 12 days and plants were cut into root, stem and leaf for estimation of mercury.

In order to assess the accumulation pattern of Hg in the entire plant body of *Bacopa monnieri*, the quantity of Hg accumulated during each interval in relation to the availability of Hg in the growth medium, the quantities of metal accumulated in root, stem and leaves estimated as described above were added together to get the total accumulation after sampling at each interval. The residual nutrient medium was analysed to estimate the quantity of Hg left behind in the medium after sampling at each interval.

Mercury contents in root stem and leaf tissue were analyzed using AAS (PERKIN ELMER A Analyst 300). Samples were prepared according to the method of Allan (1969). Five hundred mg dried tissues was wet digested by refluxing in 10ml of nitric acid, and perchloric acid mixed in the ratio of 10:4 until the solution became colourless by using Kjeldahls flasks heated in a sand bath and the digest was used for AAS. Mercury content of the residual nutrient medium after each sampling also was estimated.

In order to assess the effect of prolonged duration on bioaccumulation pattern, another experiment was conducted by growing a known quantity of rooted propagules in the nutrient medium containing a known quantity of Hg, during a period of 50 days. The sampling and harvesting of entire plant was done at 10 days (20th 30^{th} 40^{th} and 50^{th} day) intervals. Analyses of Hg accumulated in the cultured plants and residual medium were done using samples of all intervals.

Effect of pH on the rate of accumulation of mercury in *B.monnieri* was done by growing the rooted propagules in distilled water containing $HgCl_2$. Acidic and alkaline pHs were adjusted by adding dilute solutions of NH_4Cl and Ca $(OH)_2$ respectively. Ten µM $HgCl_2$ each in distilled water and Hoagland solution without adjusting the pH served as the controls. All experiments were repeated a minimum of 5 times keeping duplicates at each experiment and the data was analyzed to calculate SD and SE. These values are incorporated in the tables/figures. Test of significance was done following student't' test and 'p' values are given in appropriate places.

RESULTS

During growth for 12 days, accumulation of Mercury was maximum in roots followed by stem and leaves. The accumulation of Hg in stem and leaves was increasing proportional to the concentration and period of growth (Table 1, Fig. 1). When a comparison was made between 5 µM and 10 µM concentrations, Hg accumulation of the root remained almost unchanged and hence was not proportional to the concentration ($p<0.01$) of $HgCl_2$ supplied.

Since the *Bacopa monnieri* plant was grown in Hoagland solution containing a known quantity of mercury, a comparison between accumulation of mercury (content/tissue) and quantity retained (residual) in the medium during 12 days of growth was calculated in order to ascertain the patterns of distribution of Hg (Table 2).The accumulation showed only marginal and gradual increase ($p<0.01$) during growth whereas Hg content left behind in the medium showed gradual reduction ($p<0.02$) and was almost exhausted on 12^{th} day. A comparison between the quantity of Hg supplied in

the medium and the sum of accumulation and residual showed significant differences indicating loss of some quantity of Hg during 12 days and the loss was increased($p<0.01$) proportional to the quantity given (Table 2).

When plants were exposed to repeated dozes of $HgCl_2$ at an interval of 10 days during a period of 50 days, mercury accumulation of the plant was increased ($p<0.02$) proportional to the concentration of $HgCl_2$ supplied (Table 3). But residual amount remained unchanged ($p<0.05$) and slight increase in the loss ($p<0.01$) of Hg was observed. The percentage distribution of accumulation was almost uniform irrespective of the period and concentration.

Ten µM concentrations of $HgCl_2$ during 50 days of growth at an interval of 10 days also showed more accumulation. Proportional increase in residual Hg was shown ($p<0.02$) but percentage did not change. Loss of Hg showed slight increase ($p<0.001$) but the percentage distribution showed only slight variation. But the percentage distribution of mercury loss remained unchanged ($p<0.002$) irrespective of the mercury content supplied (Table 3).

At acidic pH (5.5) maximum quantity of Hg was accumulated whereas in the alkaline pH (7.5) the quantity was only one half. In the controls, more accumulation was observed in Hoagland medium ($p<0.001$) compared to the distilled water, which showed high pH than the Hoagland solution.

Table 1: Accumulation of Mercury contents in different parts of *Bacopa monnieri* treated with $HgCl_2$ during growth

Treatment concentrations		Plant parts	µg g^{-1} dry tissue (concentration)					
			Interval-days					
			2	**4**	**6**	**8**	**10**	**12**
$HgCl_2$	5 µM	Root	39.1 ± 2.1	41.9 ± 2.6	45.5 ± 2.7	44.5 ± 2.4	43.3 ± 2.3	43.2 ± 2.4
		Stem	16.2 ± 0.9	17.3 ± 0.9	20.1 ± 1.1	24.3 ± 1.2	26.1 ± 1.2	28.4 ± 1.3
		Leaf	5.7 ± 0.03	6.8 ± 0.04	8.4 ± 0.05	10.8 ± 0.05	16.0 ± 0.1	20.8 ± 0.21
	10 µM	Root	40.1 ± 2.6	41.0 ± 2.7	40.8 ± 2.6	40.1 ± 2.6	42.4 ± 2.3	50.1 ± 3.1
		Stem	16.5 ± 0.5	17.9 ± 0.4	33.7 ± 1.2	40.2 ± 2.2	44.7 ± 2.4	68.0 ± 2.5
		Leaf	6.4 ± 0.02	7.6 ± 0.02	14.9 ± 0.1	17.6 ± 0.1	26.3 ± 1.1	38.4 ± 2.1

Table 2. Percentage distribution of mercury in *Bacopa monnieri* in relation to the availability and loss during growth.

Treatment	Quantity given		µg/whole plants (Content)					
			Interval-days					
			2	**4**	**6**	**8**	**10**	**12**
$HgCl_2$	5 µM (200µg Hg)	A	61 (30.5)	66 (32.2)	74 (37.0)	79 (39.5)	85 (42.5)	92 (46.0)
		R	58 (29.0)	42 (21.0)	30 (15.0)	22 (11.0)	13 (6.5)	4 (2.0)
		L	81 (40.5)	93 (46.5)	96 (48.0)	99 (49.5)	102 (51.0)	104 (52.0)
	10 µM (400µg Hg)	A	63 (15.5)	66 (16.5)	89 (23.2)	98 (24.5)	113 (28.0)	156 (39.0)
		R	205 (51.2)	190 (47.5)	146 (36.5)	118 (29.5)	81 (20.2)	5 (1.3)
		L	133 (33.2)	144 (36.0)	161 (40.2)	184 (46.0)	207 (51.7)	239 (59.7)

Values in parenthesis are percentage distributions
A= Total accumulation in plants (µg/whole tissue)
R= Residual content (µg) present in the medium, during 12 days of growth
L= Quantity (µg) lost during 12 days growth (difference between accumulation + residue and total mercury content given)

Table 3. Bioaccumulation of Mercury in *Bacopa monnieri* during repeated exposure of $HgCl_2$ up to 50 days.

Treatment	Concentration		µg/whole plants (content)			
			Interval-days			
			20	30	40	50
			Quantity given			
			250 µg	300 µg	350 µg	400 µg
$HgCl_2$	5 µM	A	98 (39.2)	112 (37.3)	148 (42.2)	174 (43.5)
		R	43 (17.2)	74 (24.6)	76 (21.7)	92 (23.0)
		L	109 (43.6)	114 (38.0)	126 (36.0)	134 (33.5)
			Quantity given			
			500 µg	600 µg	700 µg	800 µg
	10 µM	A	121 (24.2)	164 (27.3)	189 (27.0)	204 (25.5)
		R	160 (32.0)	197 (39.4)	247 (35.2)	290 (36.2)
		L	219 (43.8)	239 (47.8)	264 (37.7)	306 (38.2)

Values in parenthesis are percentage distributions
A= Total accumulation in plants (µg/whole tissue)
R= Residual content (µg) present in the medium, during 50 days growth
L= Quantity (µg) lost during 50 days growth (difference between accumulation + residue and total Mercury content given)

Table 4. Effect of pH on heavy metal (Hg) uptake in *Bacopa monnieri* during 4 days of growth.

Treatment $HgCl_2$ concentrations	(µg g^{-1} dry tissue)			
	Hoagland solution pH 6.2	Control (distilled water) pH 6.8	Acidic medium pH 5.5	Basic medium pH 7.5
400 µg	66±4	54 ± 3	69 ± 4	35 ± 2

Table 5. Relationship between BCF* and TF* in the bioaccumulation pattern of Hg in *Bacopa monnieri*.

Treatments & concentrations			Interval -days					
			2	4	6	8	10	12
$HgCl_2$	BCF	5 µM	0.7	**1.0**	**1.5**	**2.0**	**3.3**	**10.8**
		10 µM	0.19	0.21	0.28	0.3	0.5	**10.0**
	TF	5 µM	0.5	0.6	0.6	0.8	**1.0**	**1.1**
		10 µM	0.5	0.7	**1.2**	**1.4**	**1.7**	**2.1**

Ref *: Yoon *et al.,* (2006)
BCF= Bioconcentration factor (Accumulation ratio of growth medium to root)
TF= Translocation factor (Accumulation ratio of root to shoot)
Calculations based on the values of Tables 1 and 2
Values >1 are in bold front

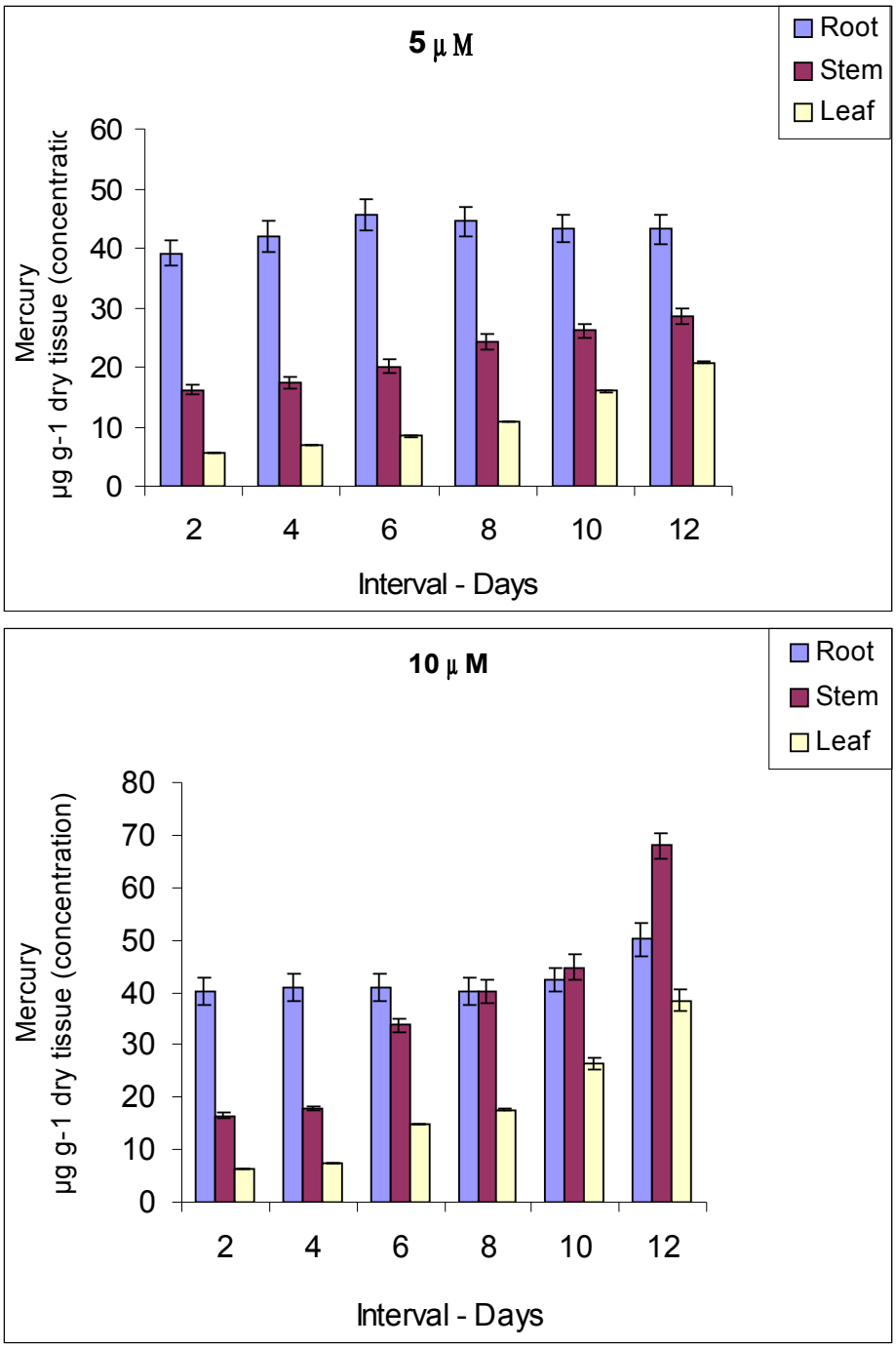

Figure 1. Accumulation of Mercury contents in different parts of *Bacopa monnieri* treated with $HgCl_2$ during growth. µg g^{-1} dry tissue (concentration).

DISCUSSION

Accumulation of Hg in the roots of *Bacopa monnieri* grown in Hoagland medium artificially contaminated with $HgCl_2$ shows the accumulation about 40 µg g^{-1} dry tissue and this level is maintained right from the beginning (2days) to50 days (Tables 1&2) of growth irrespective of the quantity available in the growth medium as well as the duration of growth. More or less same quantity of Hg is accumulated in the roots of both 5 and 10 µM $HgCl_2$ concentrations. About 40 µg Hg g^{-1} dry root tissue appear to be a threshold level of

accumulation to which the plants are tolerant and above this level, accumulation of Hg may cause toxicity as suggested by Beauford *et al.,* (1977). However, accumulation never exceeds this quantity.

In the treatment of 5 and 10 µM $HgCl_2$, Hg was gradually decreasing during 12 days and only negligible quantities were present in the residual medium. By comparing the total Hg accumulated in the plant tissue and quantity present in the medium, it was found that obviously loss of substantial amount of the metal was occurred. The percentage distribution of Hg accumulated in plants, present in the residual medium and the calculated loss enabled to presume the release of Hg from the plant to the atmosphere. The loss (Table 2) may occur presumably through stomata because a corresponding increase of stomatal index is shown by the plants treated with $HgCl_2$. Therefore *Bacopa monnieri* can be effectively used for phytoremediation to remove Hg from contaminated soil or water. According to Sinha (1999) metal accumulation property of *Bacopa monnieri* may be used for amelioration of polluted wetlands and water.

Pilon-Smits (2005) opines that the bulk flow of the metal ions from root to shoot and leaf is driven by transpiration which creates negative pressure potential in the xylem that pulls up water and solutes. As per this concept, the distribution of comparatively reduced contents of Hg in the plant body is found to be due to release of the ions through stomata because bulk flow of water ions driven by transpiration pull may enhance the escape of Hg ions through stomata maintaining very low metal concentration in leaves.

Aquatic plants are reported hyperaccumulators of several heavy metals and they are quiet effective in separating metals from their surrounding water (Lenka *et al*., 1993; Velasco-Alinsug *et al*., 2005; Yadav *et al.,* 2005). *Bacopa monnieri* also has been recommended as a plant for phytoremediation of metal contaminated water and wetlands (Sinha *et al*., 1996; Sinha, 1999). Nevertheless, this plant is not a hyperaccumulator of heavy metals.

The loss/release of Hg can be considered as one of the methods of phytoremediation designated as phytovolatilization in accordance with the view of Pilon-Smits (2005), according to whom phytovolatilization is the release of pollutants by plants in volatile form. This process completely removes the pollutants from the site as gas without any need for plant harvesting and disposal. The process of volatilization can be maximised by promoting transpiration rate through sufficient irrigation. Since *Bacopa monnieri* grows profusely in aquatic environment transpiration rate may be very high which can maximise loss of Mercury from the leaves. In nutrient culture also water deficit do not occur so the volatilization may be at an enhanced rate due to maximum availability of water in the medium and increased stomatal index may play an additional role in enhancing the transpiration rate. Volatilization of mercury by transgenic plants has been achieved by introducing a bacterial mercury reductase and this plant can volatilize the elemental mercury to the atmosphere (Rugh *et al*., 1996).

In the present study, loss of mercury in *Bacopa monnieri* treated with 5 µM concentration of $HgCl_2$ is found to be increased proportional to the period of growth and on 12^{th} day, more than 50% of the total quantity given is lost and at 10 µM, the corresponding loss is 59% (Table 2). This loss can be correlated to the release of the metal either through stomata as described earlier or through trichome like appendages developed on the stem as reported in *Vigna mungo* treated with 10 µM $HgCl_2$ and in *Chromolaena odorata* treated with Hg $(NO_3)_2$ at 1 and 2 µM concentrations (Velasco-Alinsug *et al.*, 2005). Ali *et al*., (2000) suggested that in *Bacopa monnieri* regenerants, Cd and Zn treatment

resulted in increase of stomatal conductance. As the stomatal conductance is increased, the efflux of water through stomata also may be increased, facilitating the escape or diffusion of contaminant Mercury. Earlier, release of Hg as volatile form was reported by Siegel *et al*., (1974) according to whom certain vascular plants accumulate Hg from soil and release as volatile form of the element from their leaves.

Another important aspect of phytoaccumulation of Hg in *Bacopa monnieri* is that the quantity of Hg given initially in nutrient medium was almost exhausted since only small quantity was retained in the residual medium after 12 days (Table 2). In order to assess the accumulation potential of *Bacopa monnieri,* plants were allowed to grow beyond 12 days (up to 50 days) under additional dozes of $HgCl_2$. It was found that accumulation as well as loss of Hg followed the same pattern as that of 10 µM during 12 days of growth (Table 3), thereby confirming continuous absorption as well as release of Hg from the plants as long as the metal is present in the medium. According to Velasco-Alinsug *et al.,* (2005) even-though *Chromolaena odorata* plants accumulates only 1.26–2.23 µg g^{-1}, 21-23 µg g^{-1} and 30 µg g^{-1} Hg in root, stem and leaves respectively, these plants are recommended for phytoremediation because the product of Hg+Sulphur content of cysteine called 'cinnabar' is quite stable and non-degradable. Hence even after death and decay of the plant, the Hg in the form of cinnabar is stable and unavailable for further leaching into water resulting pollution. So *Chromolaena odorata* is an agent for phytoremediation by phytoextraction/ phytostabilization. On the contrary, *Bacopa monnieri* plants are capable of accumulating about 90 µg g^{-1} and 150 µg g^{-1} Hg in 5 and 10 µM $HgCl_2$ treatments respectively and the percentage distribution pattern of Hg between the plant and residual contents indicates considerable loss of Hg through stomata. So the phytoremediation efficacy of *Bacopa monnieri* is manifested through phytovolatilization in accordance with the view of Pilon-Smits (2005).

Accumulation of Hg in *Bacopa monnieri* cultivated in nutrient solution is found to be dependent on the pH of the growth medium. Acidic pH encourages Hg accumulation while alkaline pH results in reduced accumulation. These qualities are beneficial for the two economic importance of *Bacopa monnieri* in such a way that for phytoremediation technology reduction of pH can be done by adding NH_4Cl, which acts also as nutrient for profused growth (Sharif & Khan, 2009). On the other hand alkaline pH can be maintained by liming before harvesting for medicinal purpose because accumulation of Hg significantly reduced at high pH.

Bioaccumulation strategy of plants can also be interpreted according to Yoon *et al*., (2006) who proposed a specific scheme to assess the ability of plants to accumulate metals from soil/water. The bioaccumulation factor (BCF) is the ratio of metal concentration in the root to that of the soil and translocation factor (TF) is defined as the ratio of metal concentration in the shoot to root. In *B.monnieri* at 5 µM of Hg BCF values are above one during 6^{th} day onwards where as at 10 µM concentration BCF value is less than one up to 10^{th} day (Table 5). The reduced BCF value in 10 µM indicates the limited absorption during the period of ten days. The TF ratio is reverse in the case of 10 µM, which shows more TF from 6^{th} day onwards because the accumulation potential of root is almost constant irrespective of the amount translocate to the shoot, which is getting increased progressively during 12 days. Another reason for reduced TF ratio is seemed to be significant loss of Hg through stomata by phytovolatilization as described earlier.

CONCLUSION

Bacopa monnieri shows bioaccumulation potential of Hg in root, stem and leaf in the pattern of R>S>L. Distribution and accumulation of Hg present in the residual medium and loss occurred during growth for 12 days, show more or less uniform quantity of Hg is retained in the plant. But in the medium, the metals are almost exhausted as growth proceeded and loss is proportionally increased. When additional doze of heavy metals is given and growth proceeded up to 50 days also, the quantity of Hg accumulated in the plant body maintained more or less uniform quantity and loss was proportionally increased as growth advanced. This loss is presumed to be due to phytovolatilization from the leaves through stomata. Absorption and accumulation of Hg is maximum at acidic pH and very low at alkaline pH. So, for medicinal purpose, cultivation in alkaline soil/water and for phytoremediation purpose, cultivation in acidic soil/water is recommended. When Bioconcentration factor (BCF) and Translocation factor (TF) of Hg are considered, the values are less than one though the shoot system accumulates more Hg. The loss of the metal from the shoot system by phytovolatilization results in the lowering of the ratios and hence *Bacopa monnieri* can not be included under hyperaccumulator of Hg. The medicinal property and wide use of *Bacopa monnieri* as an ingredient of many Ayurvedic medicines and food supplements on one hand and bioaccumulation potential and phytoremediation efficacy on the other are paradoxical.

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