CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com Research Article

# PHYTOVOLATILIZATION POTENTIAL OF *Bacopa monnieri* (L.) Pennell :-A PHYTOREMEDIANT FOR ENVIRONMENTAL ASSESSMENT

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**Article Received:** October 2021 **Accepted:** October 2021 **Published:** November 2021

**Abstract:**

*Plants in general and aquatic plants in particular absorb and accumulate minerals and metals from soil/aquatic environment. Bacopa monnieri is highly medicinal, a creeping emergent herb growing naturally in wet soil and shallow waters. In addition to the medicinal use, recently this plant has been recommended as an agent for phytoremediation due to the capacity of absorption and accumulation potential. Earlier studies on the effect of Hg and Cd proved that bioaccumulation of these metals taken place in all parts of plant body, but during prolonged growth, the accumulated quantity is found to be reduced. It has already been reported that phytovolatilization is one of the mode of removal of Hg and Cd from the plant body. In the present study an attempt is made to confirm the process of phytovolatilization by cultivating the root cuttings of Bacopa monnieri in Hoagland solution artificially contaminated with known quantities of Hg and Cd. The "PHYTOVOL-EXTRACTOR" was fabricated to measure the quantity of Hg and Cd liberated from the plant to atmosphere. In this system, plants growing in Hoagland solution were placed inside a transparent glass chamber fitted with provisions for intake of water-filtered air and exhaust for transpired-water providing estimation of Hg and Cd content liberated from the plant through the stomata. It is inferred that in Bacopa monnieri, phytovolatilization is the main mode of sequestration to make its possibility in environmental assessment of environmental pollution.key words: Bacopa monnieri, Phytovolatilization, Heavy metals, Detoxification, Phytovol-extractor, Sequestration, Phytoremediation, Environmental Assessment*

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*Please cite this article in press Hussain. K et al,* ***Phytovolatilization Potential Of Bacopa Monnieri (l.) Pennell :-A Phytoremediant For Environmental Assessment.,*** *Indo Am. J. P. Sci, 2021; 08(11).*

## 1. INTRODUCTION:

Phytoremediation is a new in-expensive, eco-friendly restoration technology using plants to reduce soil and water pollution due to soluble minerals and metals. In aquatic environment many species accumulate heavy metals and serve as bio-indicators of heavy metal pollution. Aquatic plants absorb and accumulate comparatively more quantity of heavy metals from polluted soil and water compared to terrestrial plants.

*Bacopa monnieri* (L.) Pennell commonly known as water hyssop and 'Brahmi', a member of Scrophulariaceae family, is a small creeping emergent herb growing naturally in wet soil, shallow water and marshes. This plant has been used in Ayurvedic system of medicine for centuries. Traditionally it is used as a brain tonic to enhance memory development, learning and concentration. [1,2]. In modern medicine, the Brahmi is well known as a 'nerve tonic' [1] and is an ayurvedic herb currently enjoying the popularity as 'brain herb' due to its effects on cognitive functions [3]. The 'Brahmin' content of the plant body is an important drug in Ayurveda for improvement of intelligence, memory and revitalisation of sensory organs [4].

*Bacopa monnieri* plant has been recommended as an agent for phytoremediation also [5-7]. According to those authors, this plant is capable of absorbing and accumulating $Hg^{2+}$ from Hoagland nutrient medium artificially contaminated with $HgCl_2$.

Studies on the effect of Hg and Cd on the eco-physiological aspects of *B.monnieri* revealed the sensitivity of the plant to the heavy metal contamination and bioaccumulation potential of the plant in naturally growing environment [8] and artificially contaminated with heavy metals as well [9]. Phytovolatilization has been reported as a method of removal of Hg from *Chromolaena odorata* [10]. The main objective of the study is to elucidate the phytovolatilization as a mean of mitigation process and the mechanism of removal of Hg and Cd by using *Bacopa monnieri* cultivated in Hoagland solution artificially contaminated with Hg and Cd.

## 2. MATERIALS AND METHODS:

### 2.1 PLANT MATERIAL

*Bacopa monnieri* (L.) Pennell cuttings were collected from different regions of Malappuram District. Cement pots half filled with potting mixture (soil: sand: cow dung 1:1:1) and flooded with tap water were used for cultivation. Ten cuttings were planted in each pot and maintained under greenhouse condition. Growth performances were observed and most profusely growing plants were selected for experiments. Healthy cuttings of length 7-8 cms consisting of 5-6 nodes were selected for experiments.

Hoagland solution (1950) modified after Epstein (1972) as described by Taiz and Zeiger (1991) was employed in the present study [11,12,13]. The cuttings selected as described above were placed in distilled water and kept under open air condition for rooting. Rooted propagules were used for experiments of heavy metal treatments.

After standardization, 5 and 10 µM of $HgCl_2$ and 20 and 30µM of $CdCl_2$ were added to Hoagland medium and cuttings of *Bacopa monnieri* were cultivated under laboratory condition for a period of 12 days and collected samples at 2 days intervals and a period of 50 days long duration and collected samples at 10 days of intervals [14].

### 2.2 BIOACCUMULATION STUDIES

#### 2.2.1 ESTIMATION OF MERCURY AND CADMIUM

Accumulation of Hg and Cd was also estimated in the samples collected at 10 days of intervals each upto 50 days of growth in nutrient medium to which repeated doses of $HgCl_2$/ $CdCl_2$ were added after each sample cultivation.

Mercury and Cadmium contents in root, stem and leaf tissue were analyzed using Atomic Absorption Spectrophotometer (AAS). Samples were prepared according to the method of Allan (1969). Oven dried plant materials were used. Known weight of the sample 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 volume was made up to 50 ml [15]. Mercury and Cadmium content of the residual nutrient medium after treatment also was estimated. Atomic Absorption Spectrophotometer (PERKIN ELMER A Analyst 300) available at Cashew Export and Promotion Council (CEPC), Kollam, was used for estimating Mercury and Cadmium.

The primary data obtained on the distribution pattern of Hg and Cd in the plant body when compared with the quantities applied to the growth medium showed significant difference in the quantity due to some loss from the plant body during growth. Since phytovolatilization is known as a method of removal of metals from the plant body as reported in *Bacopa monnieri* the present author fabricated a system device for the confirmation of the

mechanism of loss of metals from the plant during growth as diagrammatically shown in the Fig.A

**Fig. A Descriptions**.

1. Power supply
2. Aerator
3. Atmospheric Air Passage
4. Inlet Channel
5. Large Glass Chamber
6. Water - The Air Filter with Double Dist. $H_2O$
7. Plant Growing in Chemically Treated Medium
8. Nutrient Solution Contaminated with Hg & Cd
9. Transpiration Water
10. Filtered Air Pressure
11. Outlet Channel
12. Closed Collection Bottle

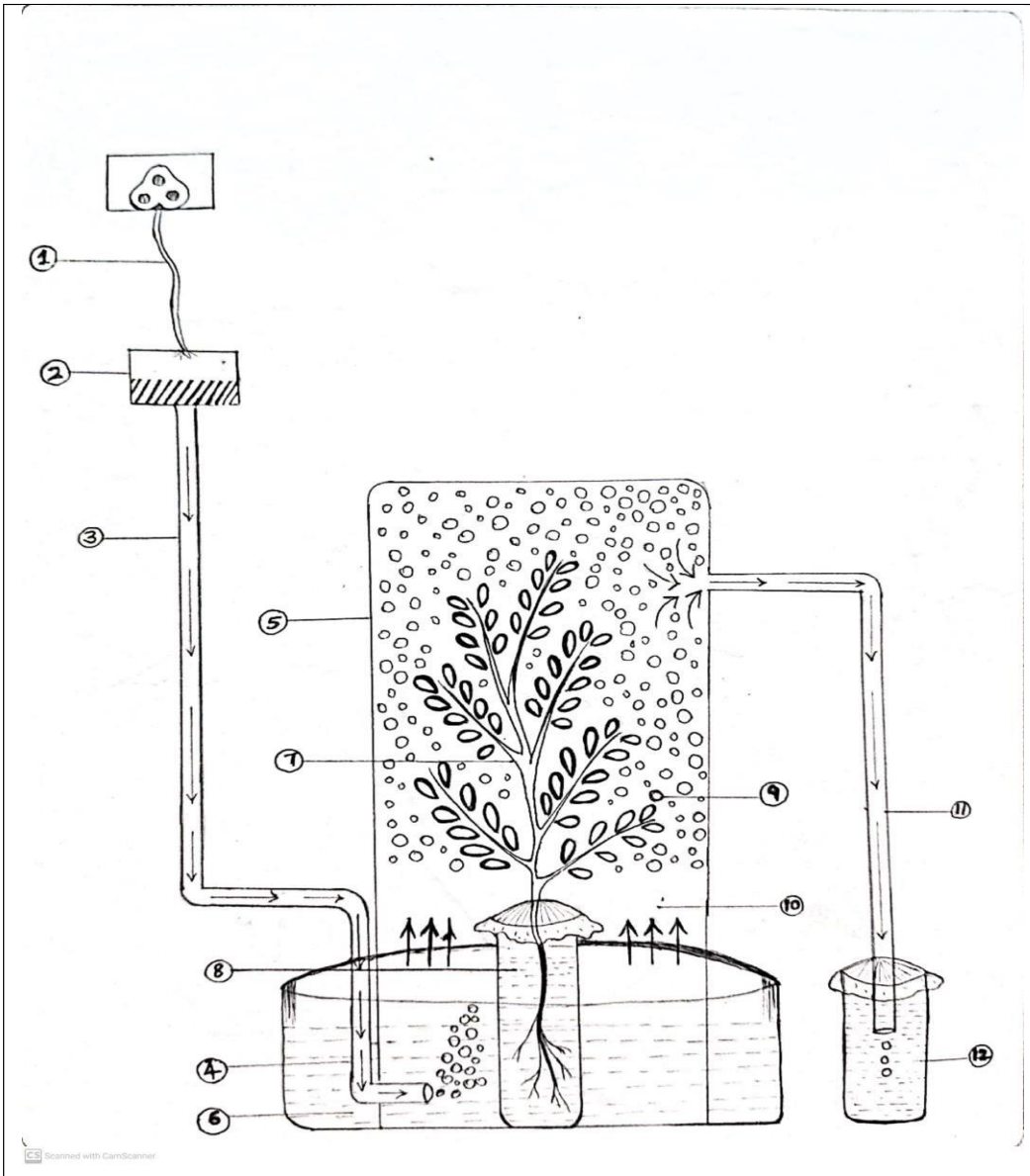


Fig.A PHYTOVOL-EXTRACTOR - Self explanatory fabricated system

## 3. RESULTS:

### 3.1. STUDIES ON STOMATA INDICES

Upper and lower epidermis of *Bacopa monnieri* leaves showed the presence of stomata, more or less uniformly. Treatment with 2 □M $HgCl_2$ showed only negligible variations in stomata index in both upper and lower epidermis. But due to the treatment of 5 □M $HgCl_2$ the values of stomata index of upper epidermis were reduced up to 6$^{th}$ day and thereafter increased insignificantly ($P<0.05$). These values were higher in upper epidermis than the lower epidermis during 10–12 days (Table 1). In the lower epidermis, treatment with 5 □M $HgCl_2$ resulted in significant increase ($P<0.02$) compared to the control throughout the experimental period. Similarly, 10 □M $HgCl_2$ also showed enhanced values of stomata index in upper and lower epidermis compared to their respective controls as well as that of the treatments with 2 □M and 5 □M $HgCl_2$. The upper epidermis showed more stomata index values in comparison with that of the lower epidermis during 8$^{th}$ day onwards (Table 1).

**Table-1 Effect of Mercury on stomata index in *Bacopa monnieri* during growth.**

| Interval-Days | Control | | Treatment concentrations ($HgCl_2$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Upper epidermis | Lower epidermis | Upper epidermis | | | Lower epidermis | | |
| | - | - | 2 □ M | 5 □ M | 10□ M | 2 □ M | 5 □ M | 10□ M |
| 0 | 22.01 □1.46 | 20.00 □1.06 | 21.41 □ 1.98 | 22.61 □ 1.04 | 21.25 □ 1.46 | 23.00 □ 1.02 | 24.12 □ 1.10 | 26.12 □ 1.05 |
| 2 | 22.04 □0.04 | 20.98 □1.04 | 21.01 □ 1.46 | 20.00 □ 1.33 | 20.98 □ 1.77 | 23.02 □ 1.33 | 24.01 □1.2 | 25.03 □ 1.02 |
| 4 | 22.12 □0.05 | 21.68 □0.98 | 21.06 □ 1.74 | 19.76 □1.20 | 20.93 □ 1.66 | 24.03 □ 0.96 | 25.12 □1.2 | 26.10 □ 2.00 |
| 6 | 22.96 □0.16 | 22.73 □0.16 | 22.01 □ 1.28 | 20.43 □ 1.02 | 24.48 □ 1.54 | 23.00 □ 1.34 | 26.31 □1.1 | 28.11 □ 2.01 |
| 8 | 22.81 0.81 | 20.65 □1.12 | 22.15 □ 1.50 | 26.12 □ 1.10 | 30.00 □ 1.62 | 24.02 □1.2 | 26.21 □1.1 | 26.30 □ 1.06 |
| 10 | 22.61 □1.00 | 21.42 □1.00 | 23.50 □ 1.44 | 29.26 □ 1.04 | 32.82 □ 1.71 | 25.01 □ 1.21 | 27.23 □1.3 | 27.31 □ 1.09 |
| 12 | 23.48 □1.10 | 21.95 □0.96 | 23.70 □ 1.30 | 35.50 □ 1.31 | 38.00 □ 1.73 | 23.03 □ 0.96 | 27.15 □1.4 | 29.23 □ 1.20 |

Stomata index value of *Bacopa monnieri* treated with 10 □M $CdCl_2$ showed only negligible increase in upper epidermis but significant increase in stomata index value was shown by lower epidermis during 10–12 days of growth (Table 2). Treatment with 20 □M resulted in significant increase ($P<0.02$) of stomata index value of upper epidermis compared to that of the lower epidermis after 8th day. Similarly, during 8th, 10th and 12th days stomata index value of both upper and lower epidermis of plants treated with 30 □M $CdCl_2$ exhibited significant increase compared to that of the other treatments and control (Table 2).

**Table-2 Effect of Cadmium on stomata index percentage in *Bacopa monnieri* during growth.**

| Interval-Days | Control | | Treatment concentrations ($CdCl_2$) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Upper epidermis | Lower epidermis | Upper epidermis | | | Lower epidermis | | |
| | - | - | 10 □M | 20 □M | 30 □M | 10 □M | 20 □M | 30 □M |
| 0 | 22.01 □ 1.46 | 20.00 □ 1.06 | 20.04 □ 0.16 | 21.79 □ 1.40 | 20.03 □ 1.00 | 24.03 □ 1.78 | 24.12 □ 2.01 | 27.20 □ 1.00 |
| 2 | 22.04 □ 0.94 | 20.98 □ 1.04 | 21.01 □ 0.91 | 20.98 □ 1.43 | 22.89 □ 1.91 | 21.14 □ 1.03 | 26.11 □ 2.00 | 26.20 □ 1.02 |
| 4 | 22.12 □ 0.85 | 21.68 □ 0.98 | 21.90 □ 0.81 | 20.48 □ 1.38 | 21.68 □ 1.90 | 20.01 □ 1.00 | 25.21 □ 1.81 | 27.23 □ 1.02 |
| 6 | 22.96 □ 0.16 | 22.73 □ 0.16 | 23.40 □ 0.23 | 20.21 □ 1.79 | 25.00 □ 1.91 | 22.00 □ 1.04 | 25.22 □ 1.70 | 26.19 □ 1.30 |
| 8 | 22.81 □ 0.81 | 20.65 □ 1.12 | 23.61 □ 0.16 | 27.92 □ 1.84 | 31.96 □ 1.92 | 24.00 □ 1.3 | 26.23 □ 1.82 | 28.12 □ 1.31 |
| 10 | 22.61 □ 1.00 | 21.42 □ 1.00 | 22.02 □ 0.18 | 32.82 □ 1.78 | 33.58 □ 1.89 | 23.03 □ 1.00 | 28.20 □ 1.81 | 38.09 □ 1.20 |
| 12 | 23.48 □ 1.10 | 21.95 □ 0.96 | 24.96 □ 0.31 | 38.06 □ 1.96 | 39.75 □ 1.94 | 28.05 □ 0.99 | 30.19 □ 1.90 | 37.19 □ 1.19 |

### 3.2. STUDIES ON BIOACCUMULATION OF MERCURY

(During 12 days treatments)

For bioaccumulation studies of Hg and Cd, *Bacopa monnieri* plants were grown in Hoagland solution containing a known quantity of these metals. Hence, a comparison between accumulation (Content/tissue cultivated) and quantity of metal retained (residual) inthe medium during 12 days of growth was calculated in order to ascertain the patterns of distribution of these elements. Mercury given in the two concentrations showed only marginal increase between the contents of accumulation in plants at each interval, whereas Hg content left behind during the intervals showed gradual reduction and almost exhausted on 12$^{th}$day. When the loss of Hg calculated as the difference between amount given and the sum of accumulation and residual showed significant amount of Hg lost during 12 days and the loss was increased proportional to the quantity given in Table.3

**Table: 3. Percentage distribution of Mercury in *Bacopa monnieri* in relation to the availabilityand loss during growth.**

**[µg/whole plants (Content)** ]

| Treatment | Quantity given | | 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 (400µgHg) | 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 + residueand total Mercury content given).

### 3.3. STUDIES ON BIOACCUMULATION OF MERCURY

(During 50 days treatments)

When plants were exposed to repeated dozes of $HgCl_2$ at an interval of 10 days ($20^{th}$, $30^{th}$, $40^{th}$, $50^{th}$) during a period of 50 days of growth, Mercury accumulation in the plant was increased proportional to the concentration. But residual amount remained unchanged and slight increase in the loss of Hg was observed. The percentage distribution of accumulation was almost uniform irrespective of the period and concentration.

Ten □M concentrations of $HgCl_2$ during 50 days of growth at an interval of 10 days also showed more accumulation but percentage distribution was lower than that of 5 □M. Proportional increase in residual Hg was shown but percentage did not change. Loss of Hg showed slight increase but the percentage distribution slightly reduced (Table 4).

**Table: 4. Bioaccumulation of Mercury in *Bacopa monnieri* during repeated exposure of $HgCl_2$ up to 50 days. [µg/whole plants (content) ]**

| Treatment | Concen-tration | | Interval-Days | | | |
|---|---|---|---|---|---|---|
| | | | 20 | 30 | 40 | 50 |
| | | | Quantity given | | | |
| | | | 250 µg | 300 µg | 350 µg | 400 µg |
| | | A | 98 (39.2) | 112 (37.3) | 148 (42.2) | 174 (43.5) |
| | 5 µM | R | 43 (12.2) | 74 (24.6) | 76 (21.7) | 92 (23.0) |
| | | L | 109 (43.6) | 114 (38.0) | 126 (36.0) | 134 (33.5) |
| $HgCl_2$ | | | Quantity given | | | |
| | | | 500 µg | 600 µg | 700 µg | 800 µg |
| | | A | 121 (24.2) | 164 (27.3) | 189 (27.0) | 204 (25.5) |
| | 10 µM | 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 + residueand total Mercury content given).

### 3.4. STUDIES ON BIOACCUMULATION OF CADMIUM

(During 12 days treatments)

Cadmium showed very high accumulation in roots but the residual amount was considerably high showing gradual reduction as growth progressed. The quantity of Cd present in the residual medium was not much reduced during growth and hence the loss was not much elaborated (Table 5). When a comparison is made between Hg and Cd, absorption of Hg was more and hence lesser amounts were retained in residual medium but loss was significantly high. The Cd accumulation was significantly increasing but considerable amount was retained in the medium resulting in a reduced rate of loss during growth up to 12days.

**Table: 5. Percentage distribution of Cadmium in *Bacopa monnieri* in relation to theavailability and loss during growth. [µg/ whole plants (content) ]**

| Treatment | Quantitygiven | | Interval-Days | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 2 | 4 | 6 | 8 | 10 | 12 |
| $CdCl_2$ | 20 **µM (448µg Cd)** | A | 13 (2.9) | 127 (28.3) | 174 (38.8) | 290 (64.7) | 309 (68.9) | 310 (69.1) |
| | | R | 415 (92.6) | 310 (69.1) | 201 (44.8) | 109 (24.3) | 69 (15.4) | 60 (13.2) |
| | | L | 20 (4.4) | 11 (2.4) | 73 (16.2) | 49 (10.9) | 70 (15.6) | 78 (17.4) |
| | 30 **µM (672µg Cd)** | A | 25 (3.7) | 145 (21.5) | 274 (40.7) | 355 (52.8) | 396 (58.9) | 458 (68.1) |
| | | R | 606 (90.1) | 457 (68.0) | 328 (48.8) | 234 (34.8) | 168 (25.0) | 94 (13.9) |
| | | L | 41 (6.1) | 70 (10.4) | 70 (10.4) | 83 (12.3) | 108 (16.0) | 120 (17.8) |

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 + residueand total Cadmium content given

### 3.5. BIOACCUMULATION OF CADMIUM

(During 50 days treatment)

In 20 □M concentration of $CdCl_2$ treatment, bioaccumulation of Cd during 50 days growth, exhibited proportional increase to the concentration applied. But the percentage distribution values did not change significantly. Residual amount of Cd also was proportional to the concentration and percentage distribution did not show much variation. Loss of Cd exhibited slight increase during growth and percentage distribution remained unchanged (Table 6).

Thirty □M concentration of $CdCl_2$, resulted in slight increase in the bioaccumulation pattern but distribution percentage did not vary. Residual amount of Cd also was proportional to the concentration and period of growth, but slight increase was observed in the percentage distribution. Loss of Cd showed only slight enhancement retaining the percentage distribution unchanged.

**Table: 6. Bioaccumulation of Cadmium in *Bacopa monnieri* during repeated exposure of $CdCl_2$ up to 50 days**

**[ µg/whole plants (content) ]**

| | | | | | | |
|---|---|---|---|---|---|---|
| **$CdCl_2$** | 20µM | *Interval-Days* | | | | |
| | | | 20 | 30 | 40 | 50 |
| | | *Quantity given* | | | | |
| | | | 560 µg | 672 µg | 784 µg | 896 µg |
| | | A | 364 (65.0) | 404 (60.1) | 432 (55.1) | 508 (56.6) |
| | | R | 112 (20.0) | 161 (23.9) | 238 (30.3) | 262 (29.2) |
| | | L | 84 (15.0) | 107 (15.9) | 114 (14.0) | 126 (14.0) |
| | Quantity given | | | | | |
| | 30µM | | 840 µg | 1008 µg | 1176 µg | 1344 µg |
| | | A | 409 (48.6) | 491 (48.7) | 508 (50.3) | 565 (49.9) |
| | | R | 275 (32.7) | 353 (35.0) | 480 (47.6) | 574 (42.7) |
| | | L | 156 (18.5) | 164 (16.2) | 188 (18.6) | 206 (15.3) |

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 of growth
L - Quantity (µg) lost during 50 days growth (difference between accumulation + residueand total Cadmium content given).

## 4. DISCUSSION:

Treatment of $HgCl_2$ at 5 and 10 µM concentrations resulted in significant increase of stomata index in the upper epidermis (Table 1). Plants treated with $CdCl_2$ also showed almost the same pattern of stomata index (Table 2). But more increase in stomata index values are shown by the upper epidermal cells. Effect of heavy metals in general, Cadmium in particular on plants like *Arabidopsis thaliana* has been shown to render stomata conductance by osmoregulation of guard cell-water relations [16]. According to those authors, an important aspect of Cd toxicity is perturbation of plant-water relationship. Contradictory to this, Cadmium treatment has been reported to cause stomata closure in *Brassica rapens* [17] and increased stomata resistance in *Brassica juncea* [18]. *Bacopa monnieri* plants showed increased stomata index on both upper and lower epidermis due to the exposal of Hg and Cd. This character may cause enhanced transpiration rate and resultant water stress. A significant role of increased stomata index in the detoxification of Hg and Cd is apparent as it is related to the bioaccumulation pattern of these metals in *B. monnieri* .

More or less uniform content of Hg is accumulated in the roots of *Bacopa monnieri* in both 5 and 10 µM $HgCl_2$ treatments. About 40 µg Hg $g^{-1}$ dry tissue of roots appear to be a threshold level of accumulation to which the plants are tolerant and above this level, accumulation of Hg may cause toxicity [19]. Studies on bioaccumulation of Hg in plants either in natural soil or artificially contaminated media are very scanty. Similarly no plant has yet been identified as natural hyper accumulator of Hg [20]. 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 [21].

Cadmium when present in the growth medium, it is reported to be easily taken up by the roots and transported to the leaves [22]. According to Sersen *et al*., (2005) Maize plants grown in nutrient medium containing Cd are able to absorb it and translocate to shoot and leaves and the accumulation is proportional to the availability of the metal [23]. The accumulation pattern of Cd in the roots of *Bacopa monnieri* is almost in consistent with the views of Sersen *et al.*, (2005) since Cd accumulation is proportional to the increase of $CdCl_2$ concentration in the nutrient medium [23] (Table 3,4). According to Sanita-di-Toppi and Gabbrielli (1999) immobilization of Cd by binding to the cell wall is one of the causes of Cd hyperaccumulation in plants [24]. Enhanced accumulation of Cd in root tissues compared to shoot/leaves has been reported in many plants [25]. Linger *et al.*, (2005) reported that *Cannabis sativa* cultivated in soil artificially contaminated with Cd, resulted in a significant reduction of biomass and roots showed hyperaccumulation potential to absorb more than 100 mg $kg^{-1}$ Cd in dry tissue [26]. In *Bacopa monnieri*, inspite of the reduction of biomass cadmium accumulation was 458 µg $g^{-1}$ dry tissues after 12 days of growth (Table 5).

Hussain-koorimannil *et al*., (2011) reported that transverse sections of *Bacopa monnieri* root and stem stained with safranin show coloured deposits filling the entire lumen of the xylem cells [27]. Pilon-Smits (2005) opined 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 [28]. As per this concept, the distribution of comparatively reduced contents of Hg and Cd is found to be due to release of these ions through stomata because bulk flow of water ions driven by transpiration pull may enable the escape of the ions through stomata maintaining very low metal concentration in leaves.

By comparing the total Hg accumulated in the plant tissue (Tables 3&4) and quantity present in the medium, it was found that obvious loss of substantial amount of the metals 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 may occur presumably through stomata because a corresponding increase of stomata index is shown by the plants treated with $HgCl_2$. [29].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 [30].

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 [28]. Since *Bacopa monnieri* grows profusely in aquatic environment transpiration rate may be very high which can maximise loss of Mercury or Cadmium 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 stomata index may play an additional role in the transpiration rate of *Bacopa monnieri*.

In the present study, loss of mercury in plants 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% . This loss can be correlated to the release of the metal either through stomata as described earlier or through trichome like appendages present all over the stem as reported in *Vigna mungo* treated with 10 µM $HgCl_2$ [31] and in *Chromolaena odorata* treated with Hg $(NO_3)_2$ at 1 and 2 µM concentrations [10]. The possibility of loss or release of Hg through stomata cannot be ruled out in *Bacopa monnieri*, because in plants treated with $HgCl_2$ both at 5 and 10 µM concentrations, stomata index values were significantly higher than that of thecontrol. Ali *et al*., (2000) suggested that in *Bacopa monnieri* regenerants, Cd and Zn treatment resulted in increase of stomata conductance. As the stomata conductance is increased, the efflux of water through stomata also may be increased, facilitating the escape or diffusion of contaminant Mercury [32]. Earlier, release of Hg as volatile form wasreported 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 [33].

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. In order to assess the accumulation potential of *Bacopa monnieri*, plants were allowed to grow 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 thereby confirming continuous absorption as well as release of Hg from the plants as long as the metal is present in the medium. An indirect evidence of loss of Hg from the leaves through stomata is the distribution and accumulation in the plant which range between 46% and 44% for 12 and 50 days respectively.

As mentioned earlier, bioaccumulation of Cd has been reported in many plants. Sensitivity towards accumulation of Cd in nutrient culture studies have been reported in *Typha latifolia* [34] and in pea seedlings [35]. According to Reid *et al*., (2003) Cd absorbed by basal roots of potato is translocated to phloem and ultimately enters and accumulates in phloem tubes [36]. Kruger *et al*., (2001) suggested that divalent metals are transported as complexes of metal binding proteins within the phloem. Those authors further speculatedthat physical chemistry of Cd reveals the role of Cd in forming strong complexes with sulfhydryl groups of proteins as well as $Cl^-$ ions both of which are usually abundant in the phloem [37].

Cadmium ions are fast mobile in plants. Many plants such as *Potamogeton pectinatus* [38], *Arabidopsis thaliana* [16], *Phragmites australis* [39], *Brassica juncea* [40] are reportedas hyperaccumulators of Cd. Eventhough most of the Cd accumulators are recommended for phytoremediation [28], translocation of $Cd^{2+}$ to rice grains causing health hazards have been reported recently by Tanaka *et al*., (2007). According to those authors, $Cd^{2+}$ are transported to *Oryza sativa* grains through phloem and about 90-100% of the Cd is present in phloem sap[41].

In *B. monnieri* storage tissues/organs are not present as it is a vegetative propagated herb and hence the phytovolatilization of accumulated Hg and Cd is the only mode of detoxification which is essential for absorption /translocation of these metals continuously from the medium.

Loss of both Hg and Cd from the leaves to the atmosphere is another method to reduce the bioaccumulation of these elements. Hence it is interesting to note that some sort of 'cycling' of Mercury and Cadmium occur from the nutrient medium to the plant and from the plant to the atmosphere. So *Bacopa monnieri* plant is neither an excluder [42,43,44,28,] nor an accumulator of Mercury [43,45]. Similarly, this plant never shows strategies like avoidance, internal detoxification or biochemical tolerance [46], because both Hg and Cd

enter the plant and internal detoxification or biochemical tolerance are not apparent. However, the strategies of response shown by *Bacopa monnieri* towards Mercury and Cadmium may be considered as amelioration. According to Fitter and Hay (1983), amelioration means plant absorb the toxic ions and act upon it in such a way as to minimize the effect variously and this may involve chelation, dilution, localization or excretion [47]. The 'cycling' of Mercury and Cadmium between growth media and atmosphere involves absorption, chelation to some extent by phytochelatins formation, localization in roots,translocation to the shoot and finally 'excretion' through stomata. If the growth medium is soil contaminated with Hg/Cd (heavy metals) the metals may enter the plant, translocates to the leaves and gets returned to the atmosphere, and finally reach the soil from the atmosphere. So the 'cycling'of Mercury and Cadmium here can be considered as Soil-Plant- Atmosphere-Continuum, comparable to the SPAC concept of Water Relations in plants.

## 5. CONCLUSION:

Treatment of $HgCl_2$ at 5 and 10 µM concentrations resulted in significant increase ofstomata index in the upper epidermis. Plants treated with $CdCl_2$ also showed almost the samepattern of stomata index. But more increase in stomata index values are shown by the upper epidermal cells. Bioaccumulation study was conducted by growing *Bacopa monnieri* plants in nutrient medium containing known quantities of $HgCl_2$ and $CdCl_2$ for a short period of 12 days at intervals of 2 days and a long period of 50 days at 10 days of intervals showed translocation of the metals to the entire plant body. Distribution pattern of both metals in different plant parts and metals retained in the medium after 12 days of growth, revealed the loss of significant amount of metals from the plant. The same pattern of absorption, translocation and phytovolatilization was confirmed by growing *Bacopa monnieri* plants in nutrient medium containing $HgCl_2$ and $CdCl_2$ for a period of 50 days. Comparison between distribution and accumulation of Hg and Cd present in the residual medium and loss occurred during growth for 12 days, show more or less uniform quantity of Hg and Cd 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 both Hg and Cd accumulated in the plant body maintained more or less uniform quantity and loss was proportionally increased as growth advanced. The distribution pattern of Hg and Cd in the plant, nutrient medium and loss occurred from the leaves exhibits some sort of 'cycling' of these metals andhence a continuum is established between soil, plant and atmosphere comparable to the SPAC concept of water relations in plants.

## 5. DECLARATION OF COMPETING INTEREST

The author reports no conflicts of interest. The author alone is responsible for the content andwriting of this article. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## 6. ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude to my teacher, Prof. Dr. Nabeesa Salim, Professor and Head (Retd.), Department of Botany, Calicut University, for the interest in the investigation.

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