Heavy Metal Absorption Efficiency of two Species of Mosses (Physcomitrellapatens and Funariahygrometrica) Studied in Mercury Treated Culture under Laboratory Condition.

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Abstract: As an important component of ecosystems, mosses have a strong influence on the cycling of water, energy and nutrient. Given their sensitivity to environmental change, mosses can be used as bioindicators of water quality, air pollution, metal accumulation and climate change. In the present study, the growth, differentiation and heavy metal (Hg) absorption of two species of mosses like Physcomitrella patens and Funariahygrometrica were studied in solid cultures under laboratory conditions. It was observed that, the number of gametophores developed from single inoculated gametophores after 45 days of growth of F. hygrometrica was 11±2.0 in control whereas it has decreased at higher concentrations, 4±1.5 in 1ppm of mercury treatment. P. patens also shows a similar trend. The heavy metal uptake of both the species of mosses
was studied. It was observed that Hg content in pseudo leaves of *P. patens* ranged from 0.98 ppm to 2.76 ppm at different Hg treatment (0.1-1 ppm), whereas in *F. hygrometrica* it ranged from 0.78 ppm to 2.43 ppm under the same treatment condition. Comparing between the Hg content in pseudo-leaves and rhizoids of *P. patens* and *F. hygrometrica*, it was observed that the Hg content was elevated about 60-64% in rhizoids than that of pseudo-leaves at 0.1% treatment level, whereas it was increased almost up to 50% in other treatment level.

Keywords: Heavy Metals, Hg Pollution, Mosses, *P. patens*, *F. hygrometrica*.

1. INTRODUCTION

The heavy industrialization, usage of modern technology, usage of chemicals in agriculture and improper waste disposal practices has collected much bio-degradable and non-biodegradable waste that has accelerated in soil and water, which spreads contamination round the globe [1]. The most common contaminants constitute herbicides, pesticides, heavy metals and hydrocarbons. Among these contaminants, heavy metal pollution owes a major issue due to their toxicity and carcinogenic nature [2],[3]. In many parts of India, the water and soil has been contaminated by heavy metals like Cd, Pb, Hg, Cr, Co, Zn, Ni and Mn which needs immediate mitigation measures. Several methods like chemical precipitation, coagulation, ion-exchange, adsorption and absorption are used for removal of metals from wastes [4],[5]. A number of literatures are also available regarding the bio-remediation and phyto-remediation of contaminated water [6]–[12]. Environmental contamination due to mercury is caused by several industries, petrochemical, mining, painting and also by agricultural sources such as fertilizer and fungicidal sprays [13]. Very often Hg enters into the human body through the food chain. It generally affects gastro-intestinal, neurological and renal organ systems [14]. Bacteria and algae contain enzymes to convert metallic mercury into soluble methyl mercury entering the waterways. Methyl mercury undergoes biomagnifications and finally enters into human through the fishes [15]. Utilization of bio-organisms for remediation of heavy metals from soil and water is an important measure adopted now a day. Bio-remediation is affected by various factors like type of species, tolerance of species, climatic conditions and other environmental factors. The demand of the situation is the need for a robust methodology which can spread as par with other technology for a quicker, cost effective, less energy consumption and economical remediation [16],[17]. Mosses have been found to be one of the best bio-indicator species for this purpose [18],[19]. They usually have efficient mechanism for absorbing metals from their environment [20],[21]. Mosses have very thin under developed cuticle which allows them to perform ion exchange from the surface through their cell membrane. They have a great capacity for trace element retention [22],[23]. Mosses have been used to monitor pollutant in terrestrial [23],[24], air [25] and aquatic [26],[27] ecosystems. *Funaria hygrometrica* is also known as bonfire moss grows on shady, damp, moist soil, moist walls and crevices of the rocks [28]. The plant body is upright, soft and green in colour. It consists of main stem bearing sessile pseudo leaves which are spirally arrange in the stem [28]. *Physcomitrella paten* is also known for its sensitiveness to toxic chemical and use as a model organism for several
research works [29],[30]. It is widely use in biotechnology and molecular biology research [28]. In the present study an attempt has been made to study the efficiency of two species of mosses (Funaria hygrometrica and Physcomitrella patens) in removing heavy metal under laboratory conditions.

2. Materials and Methods

2.1 The Model Plant

Physcomitrella patens and Funaria hygrometrica are two varieties of mosses chosen for this study because of their ubiquitous nature and also well adapted to the adverse environmental conditions.

2.2 Solid Culture

Aseptic culture of Funaria hygrometrica and Physcomitrella patens were grown under white light at 25°C on solid agar plates as described by [31]. Sub cultures of moss gametophore were carried out on routine intervals. These moss gametophore containing plates serve as the inoculum for the entire experiment. Five concentrations of Hg (Control, 0.1 ppm, 0.2 ppm, 0.5 ppm and 1 ppm) were taken for experiment. In order to maintain sterile condition, BCD and MM media were autoclaved for 15 minutes and 30 minutes respectively at 120°C. For solid culture, 15 petriplates for each of P. patens and F. hygrometrica were taken for sub-culturing. The mercury chloride solutions of different concentrations were poured into the respective petriplates containing the agar media. Single gametophore was inoculated with the forceps eventually from the mother plate to the media plates for solid culture in the laminar air flow. The plates were wrapped properly and then kept in white light incubator (16hrs light and 8hrs dark) at 25°C.

Mercury chloride stock solution was prepared by dissolving 13.576 mg of HgCl₂ in one litre of de-ionized water. Further dilutions were made to prepare 0.1 ppm, 0.2 ppm, 0.5 ppm and 1 ppm Hg solution for experimental purpose. BCD and MM agar media was prepared by following the standard method [32] for P. patens and F. hygrometrica separately. The mercury uptakes by the mosses were evaluated after 45 days of treatment of solid culture media by standard AAS, (Atomic Absorption Spectrophotometry).

3. Results and Discussions

3.1 Effect of mercury on the growth and differentiation Physcomitrella patens and Funaria hygrometrica

Effect of different Hg concentrations on the growth and differentiation of gametophore of both the species were studied. The reading was taken after 6 weeks of moss culture with known doses of Hg concentration. It was observed that, the number of gametophores developed from single inoculated gametophores after 45 days of growth of F. hygrometrica were 11±2.0 in control whereas it showed a decreasing trend at higher concentrations of
mercury treatment, the number of gametophores were 10±1.9, 9±1.5, 7±1.6 and 4±1.5 in 0.1, 0.2, 0.5 and 1ppm of Hg treatment respectively. In P. patens similar trend was also observed. In control the number of gametophores were 12±2.5, and in 0.1, 0.2, 0.5 and 1.0 ppm of Hg concentration the gametophores were 11±2.1, 10±1.8, 1.0±0.8 and 0 respectively. One way ANOVA test showed a significant different between concentration after 45 days between two species of moss (F=7.6, p<0.5). In the present study, physical characteristics of mosses, such as living form and morphology has a great influence on the heavy metal accumulation capacity of mosses, which is also follows the findings of other workers [33],[34]. So the morphological differentiation might be influenced due to inhibition of nutrient uptake with high accumulation of heavy metal in substratum.

3.2 Hg uptake by P. patens and F. hygrometrica after 45 days of solid culture

Table 1 shows that Hg content in pseudo-leaves of P. patens are in range from 0.98ppm to 2.76 ppm at different Hg treatment (0.1-1ppm), where as in F. hygrometrica it ranges from 0.78ppm to 2.43ppm under the same treatment condition. The Hg content in pseudo-leaves of P. patens was increased upto a maximum of 20% over F. hygrometrica (Fig. 1). Similarly Hg content in rhizoids increased up to a maximum of 28% at 0.1ppm Hg treated culture. But the Hg content at higher treatment level has also increased by 16.61% and 20.24% at 0.2 and 1ppm respectively.

Comparing between the Hg content in pseudo-leaves and rhizoids of P. patens, it was observed that the Hg content was accelerated of about 64% in rhizoids than that of pseudo-leaves at 0.1% treatment level. Whereas it was increased almost up to 50% in other treatment level (Table 1 and Fig. 2).

Table 1: Heavy Metal (Hg) content in Pseudo Leaves and Rhizoids of P. patens and F. hygrometrica after 45 days of Heavy Metal Treatment

| Hg treatment in PPM | P. patens (Pseudo leaves) in µg/gm dry wt. | F. hygrometrica (Pseudo leaves) in µg/gm dry wt. |
|---------------------|------------------------------------------|-----------------------------------------------|
| 0.1                 | 0.98±0.15                                | 0.78±0.12                                     |
| 0.2                 | 1.76±0.26                                | 1.48±0.43                                     |
| 0.5                 | 2.23±0.63                                | 2.01±0.32                                     |
| 1                   | 2.76±0.65                                | 2.43±0.5                                      |

| Hg treatment in PPM | P. patens (Rhizoids) in µg/gm dry wt. | F. hygrometrica (Rhizoids) in µg/gm dry wt. |
|---------------------|--------------------------------------|---------------------------------------------|
| 0.1                 | 2.75±0.37                            | 1.98±0.37                                  |
| 0.2                 | 3.31±0.56                            | 2.76±0.42                                  |
| 0.5                 | 4.31±0.76                            | 3.80±0.61                                  |
| 1                   | 5.68±0.83                            | 4.53±1.16                                  |
Fig. 1: Hg content (µg/gm dry wt.) in pseudo-leaves of *P. patens* and *F. hygrometrica* after 45 days of experiment.

In case of *F. hygrometrica* similar trend was observed i.e. an increase of about 60% in Hg content of rhizoids than that of pseudo-leaves at 0.1ppm. In other treatment level (0.2, 0.5 and 1ppm) also showed similar trend like that of *P. patens*.

From the above experiment it has been found that with increase in Hg concentration, the decreases in number of gametophores which is emerged in a colony is clearly evident after 45 days of culture. But the numbers of rhizoids significantly increased with increasing concentration of Hg. In 1ppm, auxin concentration most likely could be high, so less gametophores but more percentage of rhizoids were developed in both the species [35],[31]. Mercury might be altering auxin homeostasis [36]. In both the cases uptake of nutrient might be inhibited by heavy metal accumulation so that the growth of shoot part is reduced at higher percentage of treatment level (0.5 and 1ppm) of Hg solution. Our finding has shown similar trend with work on another moss plant (*Pleurozium schreberi*)[37]. The shoot growth was drastically reduced in both the cases and almost absent in *P. patens* at 1ppm treatment level. Some researcher also indicate that environmental characteristics such as climatic conditions, mineral composition of soil dust, soil water, natural element cycling process and vegetation zone may have a significant influence on uptake efficiency of heavy metal in moss [38],[40].

It was also observed that rhizoids are the high accumulator of heavy metals than shoot part. They have reported that the substratum of moss is a high accumulator of heavy metals than shoot part. Here *P. patens* also more sensitive and highly accumulated Hg metal than *F. hygrometrica*. There was variation in heavy metal uptake in moss species.
**Conclusion**

Mosses are now a day’s broadly used as bio- indicator for heavy metal pollution of air water and soil. In the present study, the efficacies of two species of mosses like *Physcomitrella patens* and *Funaria hygrometrica* was studied in removal of mercury from water environment. It was observed that, both the species could be used as a bio- indicator of metal pollution in aquatic environment. These observations can also be visually noticed and can serve as a parameter for detecting heavy metal pollution of environment.

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