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BRYOPHYTES: A USEFUL BIOMONITORING TOOL FOR HEAVY METAL POLLUTION CAUSED BY INDUSTRIAL WASTE.

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The species diversity of bryophytes growing under the influence of heavy metal industries of Rajasthan were studied to determine the absorption and accumulation of heavy metals potential and use them as biomonitoring tool. Seven bryophytes were collected from different localities of the surrounding industrial areas from the Hindustan Zinc Smelter Debari (HZS) Udaipur; and Hindustan Copper Smelter (HCS) Khetri, Jhunjhunu. The collected bryophytes belonged to two important taxonomic groups: (1) mosses, such as *Funaria hygrometrica* Hedw. (Pottiaceae), *Hyophila involuta* (Hook.) A. Jaeger (Pottiaceae), *Barbula constricta* Mitt. (Pottiaceae), *Hydrogonium consenguineum* (Thwaites & Mitt.) Hilp. (Pottiaceae), *Bryum recurvulum* Mitt. (Bryaceae); and (2) liverworts such as *Plagiochasma appendiculatum* Lehm & Lindenb. (Aytoniaceae) and *Riccia glauca* L. (Ricciaceae). The heavy metal absorption and deposition efficiency were determined by using Atomic absorption Spectrophotomer from digested plant samples in nitric and hydrochloric acid (HNO_3-HCl, 1-3 ratio) mixture. Results showed that high absorption and accumulation of Zn and Cu were observed in moss *F. hygrometrica* (54.26 and 55.96 ppm) followed by *H. involuta* (51.33 and 46.12) and *B. constricta* (50.98 and 51.97). A liverwort *P. appendiculatum* accumulated comparatively less amount of Zn and Cu (48.78 and 44.88 ppm) respectively suggesting that mosses are more efficient to absorb and accumulate heavy metals than liverworts very less amount of Pb and Cd was also reported in all analyzed samples. *In vitro* studies of selected bryophytes showed that *F. hygrometrica* and *B. constricta* survived upto 50 ppm concentrations of Zn and Cu contaminated culture medium whereas *P. appendiculatum* only upto 40 ppm concentrations of both the heavy metals with high percentage of regenerants and gametophores formation. The present study revealed that mosses and liverworts have a significant potential to absorb and accumulate heavy metals, making them useful biomonitoring tool. Further this study will also unlock the naturally occurring bryophytes may be used as pollution monitoring agents due to their high efficiency of absorption and accumulation of heavy metals growing in contaminated environment.

Keywords: Accumulation, Bryophytes, Biomonitors, Metal pollution, Industrial waste.

Introduction

Since bryophytes were the first green land plants to settle on land[1], they had to develop defenses against the much higher levels of heavy metals found on land compared to water[2]. Due to these processes, many bryophytes have the capacity to regularly colonize environments contaminated with metals[3] or to accumulate significant amounts of heavy metals in highly polluted areas without exhibiting any discernible detrimental effects on their growth and development[4] and this is one of the prerequisites for their usage as biomonitors[5]. The fact that these plants lack root systems suggests that they may take heavy metals throughout their whole surface[2,6]. A necessary result of contemporary society is industrial waste. The kind of procedure used and the industry determine the amount and quality of different wastes, or effluents. Despite the fact that copper and zinc are key trace elements needed for plant development and metabolism[7,8] but higher quantities however, make them toxic[9-11]. Industrial wastes have a negative impact on the environment's biological circumstances, which changes the local flora and fauna[12]. Increased levels of Zn and Cu were found in the wastes or effluents of the Hindustan Zinc Smelter and Hindustan Copper Smelter industries, which adversely affect the local flora and fauna of their surrounding areas[13], in addition to this, adverse effect had also been seen on the growth, development, gametophores formation, and survival rates of bryophytes tested in the various concentrations of these two heavy metals under controlled laboratory conditions[14].

The industries of Hindustan Copper Smelter (HCS) Khetri, Jhunjhunu, and Hindustan Zinc Smelter (HZS) Debari, Udaipur, in the Indian state of Rajasthan, continuously release wastes in the form of effluents into the surrounding soil and water bodies, which have a harmful impact on the local flora and fauna. Despite the establishment of waste water treatment facilities as a result of government law, HCS and HZS have shown that the treated waste and effluent have not improved the environment to the extent that was intended. The current study's goals were to collect, examine, and evaluate the potential of heavy metal accumulation in bryophytes growing close to these industries, as well as to observe how different Zn and Cu concentrations affected the formation of gametophores, regeneration, and survival rate in a controlled laboratory conditions. Given the aforementioned perspectives, using bryophytes as biomonitoring agents of pollution may be a convenient method to assess the amount of heavy metal deposition in adjacent water bodies or soil substrata.

Material and Methods

Plant collection and processing:

The plants were collected from various localities of the Hindustan Copper Smelter Khetri in Jhunjhunu (Rajasthan) and the Hindustan Zinc Smelter Debari in Udaipur. Seven bryophytes in all were gathered from various locations in the surrounding industrial regions. These belonged to two important taxonomic groups: (1) mosses, such as *Funaria hygrometrica*, *Hyophila involuta, Hydrogonium consenguineaum, Barbula constricta, and Bryum recurvulum*; and (2) liverworts, such as *Plagiochasma appendiculatum* and *Riccia glauca.* After being taken out from their soil substratum and placed in polythene bags, these plants were regularly cleaned with tap water and then again with double-distilled water to get rid of any remaining debris in the laboratory. Some fresh cleaned plants were stored in as such condition for further use of laboratory culture whereas remaining plants were air dried at 80 ^{o}C in an oven for further use for analysis and to determine heavy metal accumulation potential.

Plant sample digestion and analysis:

One gm cleaned oven dried plant material of each sample was digested in nitric and hydrochloric acid (HNO3-HCl, 1:3 ratios) mixture[15]. Atomic absorption spectroscopy technique was used to determine the presence of heavy metals such as Zn, Cu, Pb and Cd in the digested plant samples.

Laboratory culture of certain bryophytes:

To determine uptake, accumulation and survival potential of bryophytes contaminated with heavy metals like Zn and Cu in culture medium under controlled laboratory conditions, three bryophytes were selected for experiments viz. *P. appendiculatum* (liverwort), *F. hygrometrica* and *B. constricta* (moss). The criteria for selection of these plants were their dominance and maximum diversity in the study area. In case of liverwort *P. appendiculatum,* thoroughly washed thalli were cut into small segments whereas in case of mosses leaves were detached from the main axis. The detached leaves and defoliated axis were again thoroughly washed in double distilled water. These detached leaves and defoliated axis further used for culture.

The petri-dishes used for culture of explants were lined with Whatman's filter paper no. 1, wrapped in aluminium foil and sterilized in an autoclave for 20 minutes at 15 PSI. M.S. (Murashige and Skoog) culture (solid) medium and various dilutions of Zn and Cu concentrations (1 to 50 ppm) contained in corning glass flasks were also sterilized. Ph of the medium was adjusted to 5.7 before autoclaving.

5 ml of M.S.culture medium solution containing required concentrations metal of metal (1 to 50 ppm) were poured on filter papers in each petri-dish and allowed the medium to solidify. After that explants of *P. appendiculatum* thallus segments and ten segments of detached leaves and defoliated axis of both mosses *F. hygrometrica* and *B. constricta* were placed in each petri-dish on solid culture medium. In all the treatments culture medium without metal concentrations constituted as control. All the petri-dishes were kept under controlled aseptic conditions in laboratory maintaining temperature at about 25^0C and humidity about 80 to 85. Observations were taken at regular periodic intervals to find out percentage regeneration, development and survival of gametophores in each treatment.

Results and Discussion

Heavy metal uptake and accumulation potential of bryophytes: In vivo:

Bryophytes are helpful for monitoring pollution because they have the ability to absorb and accumulate heavy metals like Zn Cu Pb and Cd from the polluted soil substrata. The whole surface of these plants, which is coated in a layer of cells that provide surface area for collecting water and minerals in addition to heavy metals. Heavy metal analysis results of the present study showed that the existence and accumulation of Zn was 54.26 ppm in moss *F. hygrometrica* followed by *H. involuta* and *B. constricta* (51.33 and 50.98 ppm) respectively. Liverwort *P. appendiculatum* accumulated comparatively less amount (48.78 ppm) of Zn concentration in comparison to the tested mosses, very less amount of other heavy metals like Pd and Cd had also been reported in all analyzed samples collected from Hindustan Zinc Smelter Debari, Udaipur (Fig.1).

High accumulation of Cu concentration was observed in again in *F. hygrometrica* (55.96 ppm) followed by *B. constricta* (51.97 ppm) in the mosses whereas, comparatively less concentration (44.88) was observed in liverwort *P. appendiculatum* (44.88), very less amount of other heavy metals like Pb and Cd had also been noticed in every sample (Fig.2) collected from Hindustan Copper Smelter Khetri, Jhunjhunu suggesting that mosses have more uptake and accumulation of heavy metal potential than liverworts from their surrounding soil substrata.

Due to their morphological properties mosses[5,16] and liverwort can absorb and accumulate heavy metal concentrations from their surroundings[17]. Further it has also been reported in earlier work that great capacity of bryophytes to absorb and retain heavy metals in high concentrations and there are few ever contamination problems and it also easy to perform chemical analysis of these plants[18] Mosses and liverworts have the potential to tolerate excessively high concentrations of heavy metals as compared to vascular plants. *Milichoferia elongata* accumulated as much as 450 ppm of Cu on ash weight basis[19, 20]. Some mosses have been proved to be universal indicator of copper[21]. A moss *Merceya ligulata* is known as copper moss indicating high concentration of copper upto 770 ppm[22].

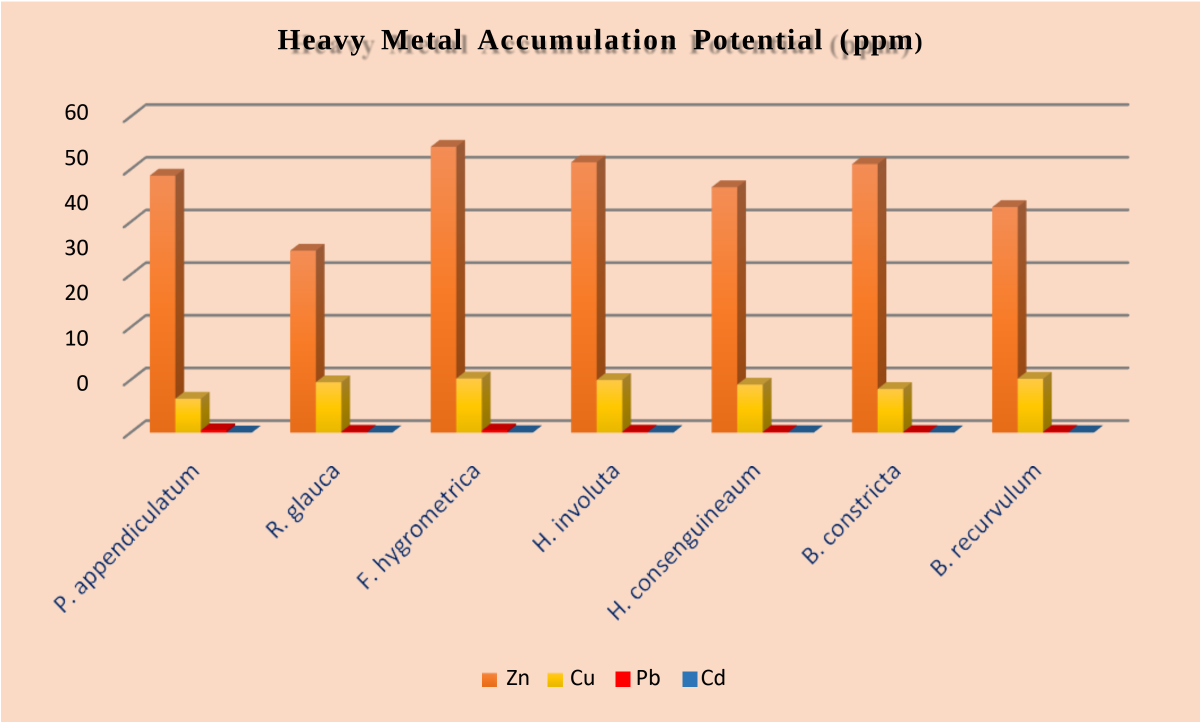

Figure-1. Heavy metal accumulation potential of certain bryophytes collected from Hindustan Zinc Smelter area Debari, Udaipur (Rajasthan).

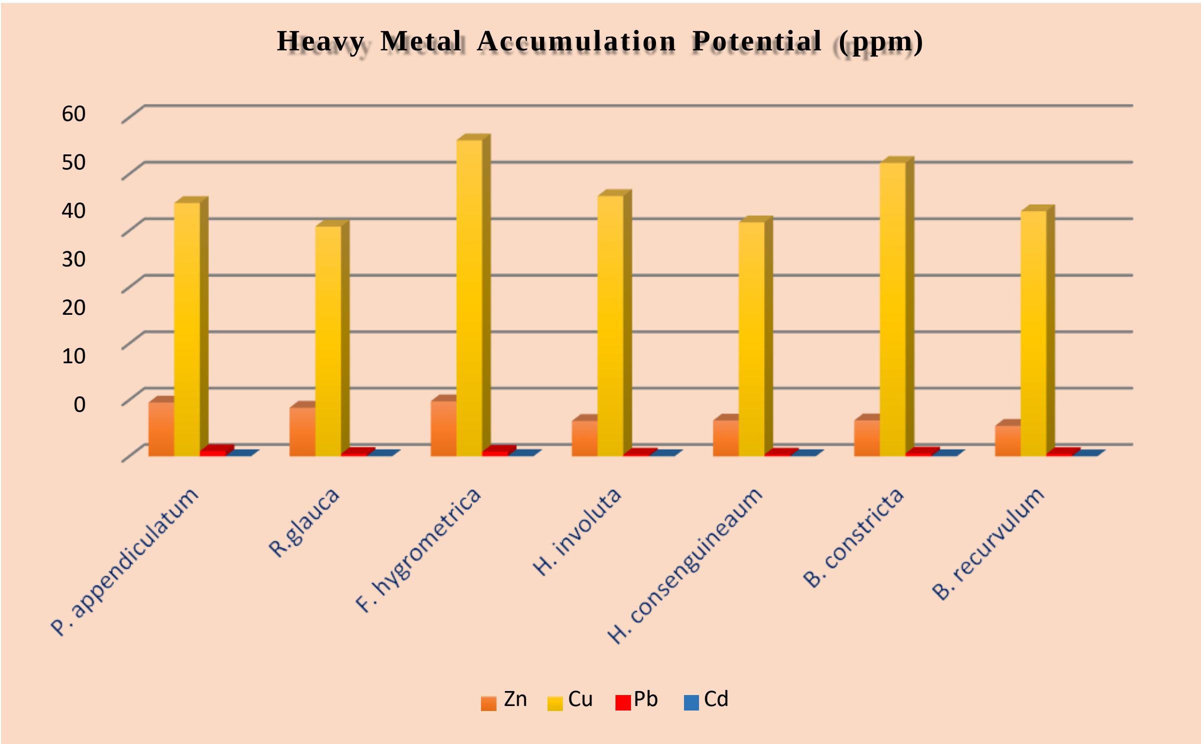

Figure- 2. Heavy metal accumulation potential of certain bryophytes collected from HindustanCopper Smelting Khetri Jhunjhunu, (Rajasthan).

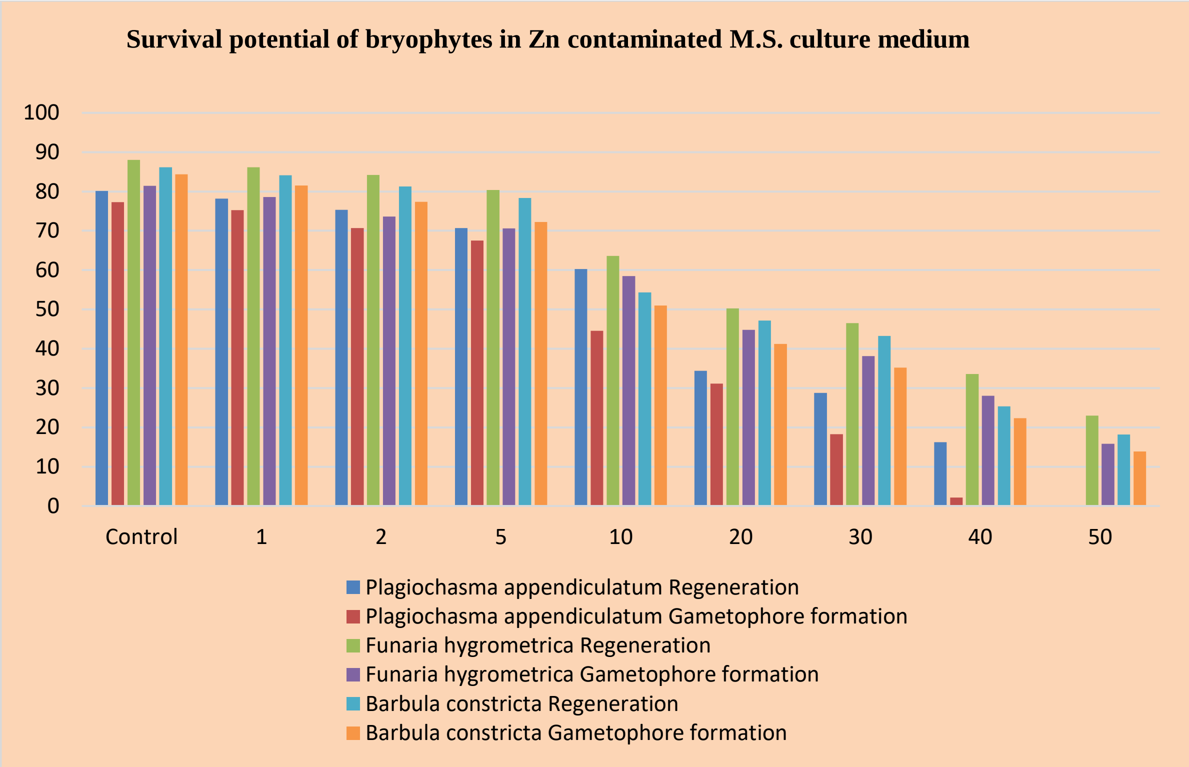

Figure-3. Showing regeneration, gametophores formation and survival potential of bryophytes in Zinc contaminated M.S. culture medium.

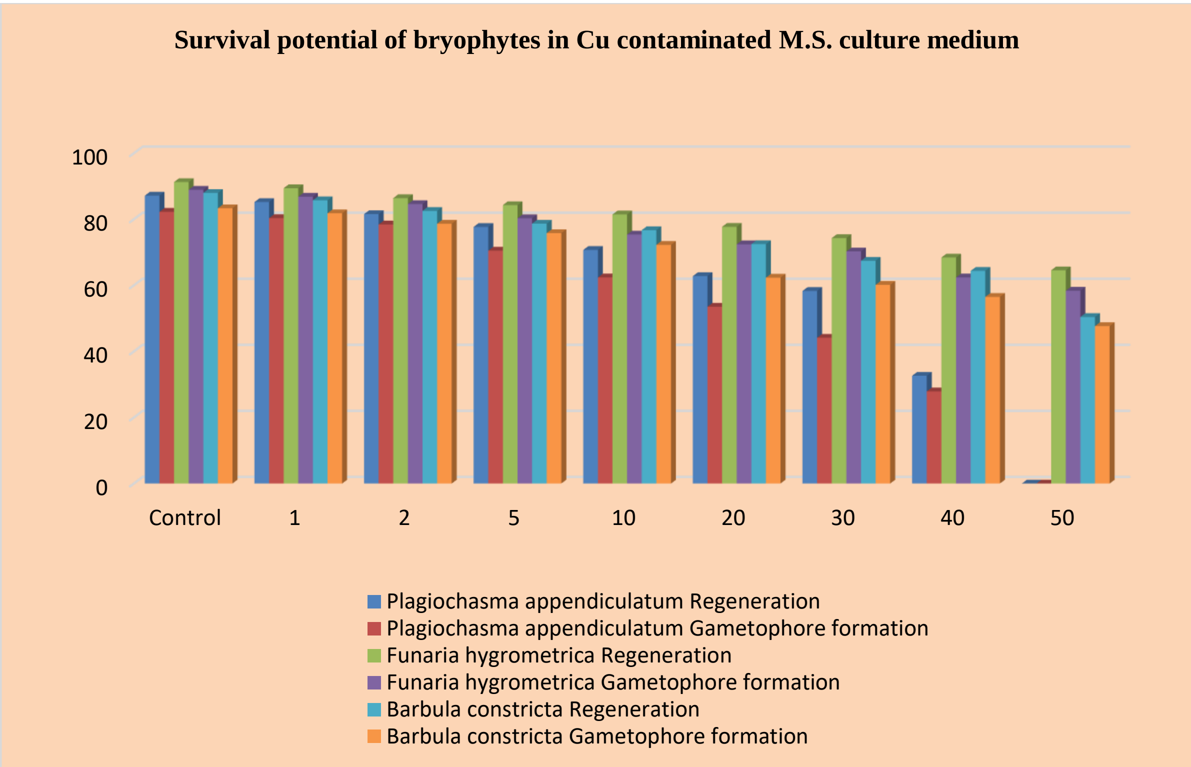

Figure-4. Showing regeneration, gametophores formation and survival potential of bryophytes in Copper contaminated M.S. culture medium.

Survival potential of bryophytes under the influence of heavy metals: In vitro

Several experiments have been carried out to study the sensitivity of bryophytes to various concentrations of Zn and Cu contaminated culture medium in controlled aseptic laboratory conditions. The results were presented in percent regeneration potential of explants and survival of gametophores in different concentrations of Zn and Cu contaminated M.S. culture medium.

The mosses *F. hygrometrica* and *B. constricta* were shown to have the capacity to regenerate up to 50 ppm of zinc, and the percentage of regenerants declined with increasing concentrations of Zn. However, *F. hygrometrica* was found to have the highest number of renerants and gametophores. A liverwort *P. appendiculatum* explants were regenerated upto 40 ppm concentrations only and the number of regenerants and gametophores were very less, 16.23 and 2.13 respectively (Fig.3).The mosses *F. hygrometrica* and *B. constricta* exhibited the highest number of regenerants and gametophores in Cu-contaminated culture media with 64.56 and 58.38 regenerants and 50.42 and 47.67 gametophores, respectively at 50 ppm concentrations. At 40 ppm concentration of Cu, liverwort *P. appendiculatum* showed the lowest number of regenerants (32.62) and gametophores (27.89). At 50 ppm concentration, no regenerants and gametophores formation was seen (Fig.4). The mosses have got efficient uptake mechanism of absorbing metals from their surroundings hence mosses serve as a sink for heavy metals and provide an indicator of the level of environmental contamination because they absorb them extracellularly in their cell[23, 24]. Because of their sensitivity to heavy metals, bryophytes are referred to be bioindicator of heavy metal pollution[25]. The availability of metals in soil is depends not only on their concentration in soil solution but also on their shape. The moss *Hygrohypnum ochraceum* is also is an excellent collector of environmental heavy metal pollution[26]. Due to their great capacity of heavy metal absorption and accumulation, the current study's findings indicated that *P. appendiculatum*, in the case of liverwort, and *F. hygrometrica*, in the case of moss, might be used as heavy metal biomonitoring tool. Additionally, this research will unlock naturally existing bryophytes that may be used as pollution monitoring agents due to their capacity to collect heavy metals when grown in contaminated environments.

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