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Comparison of arsenic uptake ability of barnyard grass and rice species for arsenic phytoremediation

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Abstract In this research, the relative performance in arsenic (As) remediation was evaluated among some barnyard grass and rice species under hydroponic conditions. To this end, four barnyard grass varieties and two rice species were selected and tested for their remediation potential of arsenic. The plants were grown for 2 weeks in As-rich solutions up to 10 mg As L^{-1} to measure their tolerance to As and their uptake capabilities. Among the varieties of plants tested in all treatment types, BR-29 rice absorbed the highest amount of As in the root, while Nipponbare translocated the maximum amount of As in the shoot. Himetainubie barnyard grass produced the highest biomass, irrespective of the quantity of As in the solution. In all As-treated solutions, the maximum uptake of As was found in BR-29 followed by Choto shama and Himetainubie. In contrast, while the bioaccumulation factor was found to be the highest in Nipponbare followed by BR-29 and Himetainubie. The results suggest that both Choto shama and Himetainubie barnyard grass varieties should exhibit a great potential for As removal, while BR-29 and Nipponbare rice species are the best option for arsenic phytoremediation.

Keywords Arsenic · Phytoremediation · Barnyard grass · Rice · Biotypes · Bioconcentration factor

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Introduction

The environmental significance of public exposure to arsenic (As) has drawn a great deal of attention due to the massive scale of its dispersion by natural and anthropogenic processes. Chronic and epidemic effects of As have been reported due to its widespread contamination in food and water resources (Smith et al. 2000; Ahmed 2000; Fazal et al. 2001; Hopenhayn 2006; Chakraborti and Das 1997; Banerjee 2000). In many developing countries, As contamination in drinking water and in agricultural fields is still a serious socioeconomic issue. For instance, approximately 28–62 % of the population in Bangladesh have been at risk for over exposure to As through drinking water (Smith et al. 2000) and as of 2009, 5.6 million were exposed to As in drinking water at above 200 μg L^{-1} (UNICEF 2009). There are many controversies regarding the actual cause and route of arsenic entering the groundwater. However, it is now widely believed that the high arsenic levels in the groundwater (e.g., in Bangladesh) are due to natural geological source. More specifically, as excessive withdrawal of underground water led to the decrease of water level in the quaternary confined and semiconfined alluvial or deltaic aquifers, it facilitated the oxidation of pyrites (FeS) and arsenopyrites (FeAsS) (Dhor et al. 1997). The use of As-contaminated ground water for agricultural activities has been the driving force of the rapid spread of its contamination in soils of Bangladesh (Zaman 2002). Under these conditions, it is desirable to establish an effective remediation technology to clean up vast areas of soils contaminated with arsenic.

A number of methods are available to remediate As-polluted soils (Lombi et al. 2000). However, most of them are generally expensive and are not free from the generation of the secondary waste. Recently, environmentally friendly, low-input technologies such as phytoremediation have been proposed for cleaning up soils contaminated with heavy metals and metalloids. Phytoremediation technology has been studied intensively in recent years as one of the main tools to treat contaminated soils (Cunningham and Lee 1995; Raskin et al. 1997; Mcgrath et al. 2002; Robinson et al. 2003b). Phytoremediation aims to remediate a contaminated system with minimal environmental disturbance after the removal of the contaminants at a low cost of implantation (Schwitzguebel 2001; Lombi et al. 2001). Weeds, in general, have a high adaptation capacity in a wide range of environmental conditions. Thus, weeds can grow better than the crop plants in adverse environments, e.g., nutrient-deficient and/or contaminated soil. The phytoremediation of As has become a subject of interest since its initial application made by the Chinese brake fern (*Pteris vittata*) (Ma et al. 2001). Recently, a number of plants have been studied for their potential for As remediation (Visoottiviseth et al. 2002; Zhang et al. 2002; Rahman et al. 2008). Nevertheless, relatively little is known about the weed's potential for the phytoremediation of arsenic. Few efforts have been made to screen and select naturally grown As-accumulating weeds from the As-contaminated areas in Bangladesh (Zaman et al. 2005). Several species of weeds have been selected and examined for their remediation capacity in different types of soils contaminated with arsenic. Accordingly, barnyard grass and water taro were found as the most suitable for such remediation (Sultana et al. 2005; Zaman et al. 2006; Sultana and Zaman 2008).

In Bangladesh, barnyard grass (*Echinochloa crus-galli* L.) is a common weed growing in rice fields in upland and paddy conditions. It is an annual weed that is found naturally and abundantly even in the As-contaminated areas. In our previous study, the As remediation potentiality of one barnyard grass variety, Himetainubie, was tested in comparison to one rice variety Nipponbare. The results indicated that both the barnyard grass and rice had strong potential for the remediation of As-contaminated soils (Sultana and Kobayashi 2011a, b). However, such evaluation has not been extended further to other barnyard grass and rice varieties (or species). The uptake and remediation capacity of As is expected to vary greatly depending on their biotype of plants. In this research, we expanded our efforts to characterize the As remediation efficiency of As in diverse barnyard grass and rice varieties (and species).

The objective of this study is to find the most suitable biotype of barnyard grass and rice for the phytoremediation with respect to As-contaminated soils. To this end, a hydroponic test was carried out to obtain basic information regarding the plants' ability to tolerate and/or uptake As from a solution containing arsenic. Because the hydroponic approach is based on a soil-free medium, it is possible to exclude a number of uncertainties associated with soil-related factors (such as adsorption of As on soil, effect of soil microbes, and other relevant factors) that can affect the remediation capacity of plants.

Materials and methods

Chemicals used

A standard inorganic As solution (inductively coupled plasma (ICP) grade, 1000 mg L^{-1}) was used for the quantification of As in the samples by inductively coupled plasma optical emission spectrophotometer coupled with a hydride generator (HG-ICP-OES). The working standards of As at various concentrations were prepared by the appropriate dilution from the stock solution with purified and deionized water (Elix 3, Nihon Millipore, Tokyo, Japan). Arsenic (V) was added into the solutions as disodium hydrogen arsenate heptahydrate [$Na_2HAsO_4.7H_2O$ (special grade)]. Concentrated nitric acid and 30 % H_2O_2 (analytical grade) were used for the extraction of As from soil and plant samples. For the analysis by HG-ICP-OES, 1 % sodium borohydrate solution (prepared by dissolving an appropriate amount of extra pure $NaBHO_4$ in 0.5 % sodium hydroxide solution), 40 % KI, and 1:1 trace metal grade HCl were used. All of the chemicals were obtained from Wako Pure Chemical Industries Ltd, Japan. Deionized water was used in all standard and sample preparations. All glass and plastic ware were cleaned prior to use by overnight soaking in 1 mol L^{-1} nitric acid.

Plant materials

Four barnyard grass and two rice varieties were used for the present experiment. Among the four barnyard grass varieties, two were Japanese barnyard grass, namely, Himetainubie (*Echinochloa crus-galli* L. Beauv. var. *formosensis* Ohwi) and Inubie (*Echinochloa crus-galli* L. Beauv. var. *crus-galli*), and the other two were Bangladeshi barnyard grass varieties, namely, Choto shama (*Echinochloa crus-galli* L.) and Boro shama (*Echinochloa colonum* L.). The two rice species consisted of Japonica rice, namely, Nipponbare (*Oryza sativa* L. cv. Nipponbare) and Indica rice BR-29 (*Oryza sativa* L.). The barnyard grass and rice seeds were soaked in deionized water, kept at 30 °C for 1 day, and germinated in a plastic tray at the same temperature in an incubator. Then the germinated seeds were cultured in a modified Kasugai nutrient solution (Yoshiba 1990) in a growth chamber (at 25 °C for 14 h light periods and 20 °C for 10 h dark period with an average light intensity of 300 μmol m^{-2} s^{-1}) until three leaf stages prior to use in the experiment. The pH of the solution was maintained at 5.5–6.2. Six seedlings of barnyard grass or rice were transplanted in the pots of 4/5 strength of the aforementioned Kasugai nutrient media. The media were pretreated with As at four concentration levels (0, 1, 5, 10 mg As L^{-1}) with sodium arsenate ($Na_2HAsO_4.7H_20$). Each pot was filled with 250-mL nutrient solution with the appropriate amount of As. The seedlings were allowed to grow in the As-containing solutions for 15 days in the growth chamber. Periodical agitation and pH adjustment were done at 3-day intervals to maintain proper aeration and to keep the pH at a constant level (5.5–6.2). The experiment was carried out at completely randomized design (CRD) with three replications.

Determination of As in plants

After harvesting, the seedlings were taken and washed several times using tap water. Such cleaning procedures were repeated subsequently using deionized water to remove the adjacent solution from root. The seedlings were separated into root, and shoot and fresh weights were taken. Then the samples were dried at 80 °C for 48 h, weighed, ground, and prepared for extraction. These samples were digested using the procedure developed for As analysis by Cai et al. (2000). Briefly, 0.1 g of plant sample was taken in an Erlenmeyer flask. Then 10 mL of concentrated HNO_3 was added to it and heated at 130 °C for 1 h covered with a watch glass. After cooling, 1 mL of H_2O_2 was added to it and heated again at 130 °C until a small amount of sample (approx. 1 mL) remained at the bottom of the flask. The flask was then allowed to cool and the content was diluted with deionized water up to 50 mL. All the samples were filtered through a quantitative paper filter (5C, Advantech, Tokyo, Japan) followed by a 0.20-μm polytetrafluoroethylene disposable syringe filter (DISMIC-25HP from Toyo Roshi Kaisha Ltd., Tokyo, Japan). Analysis of As in the plant samples and in the remaining solution was carried out individually by ICP-OES (Optima 7300 V ICP-OES Spectrometers) coupled with a hydride generator. The detection limit of As is 0.2 ppb using hydride generator. The relative standard deviation (RSD) in the analysis was set at 2 % prior to analysis. The recovery was kept within 95–105 % using standard (Japan Calibration Service System, JCSS) at 10 sample intervals to get consistency of measurement.

All the data were analyzed using Microsoft® Excel software. Student's *t* test was used to evaluate the statistical significance test. The biomass of the plants was analyzed by one way ANOVA using Tukey's test. The results were expressed as a mean of three replicates with ±standard error (SE). Bioaccumulation factor (BF) values were obtained from the ratio of As concentration between shoot and solution.

$$\mathrm{BF} = \text{Concentration of As shoot}/\text{Concentration of As the solution.}$$

Results and discussion

Effect of As on plant growth

To select a candidate plant for phytoremediation, it is important to maintain good growth in the As-contaminated medium. Figure 1a shows the difference in biomass production among the biotypes in each treatment, whereas Fig. 1b presents the effect of As

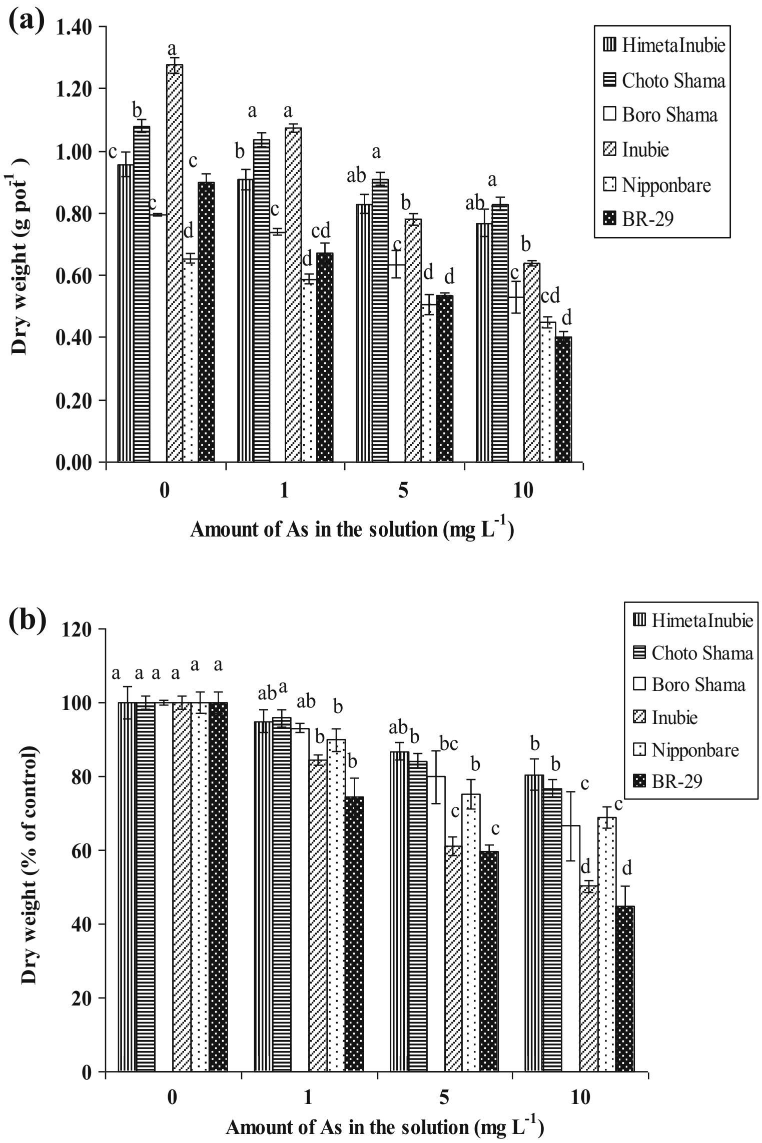

Fig. 1 Growth of different barnyard grass and rice varieties in As-treated solution. **a** The dry biomass per pot (biomasses were compared among the plant varieties in each treatment) and **b** the dry biomass as percent of control (biomasses were compared among the treatments in each variety). *Bars* indicate the ±standard error of the means (n=3). The *columns with the same letter* are not significantly different at P<0.05 as determined by Tukey's test

treatments on plant biomass (in each rice and barnyard grass) compared to the control (mg As L^{-1}). The growth of the plants was affected slightly by the application of As in the solution at the levels of 1–10 mg As L^{-1}. A gradual and slight growth reduction was found, regardless of the biotypes (of plants) at increasing doses of As in the nutrient solution (Fig. 1b). The reduction in growth might occur due to interference with the metabolic processes of plants due to phytotoxicity. The trend of growth reduction was also more or less similar. In the case of Choto Shama, such reduction in plant growth was seen to occur at a minimal level. Moreover, Choto shama produced the highest biomass at all As-treated solutions (1–10 mg As L^{-1}) among the six biotypes tested here (Fig. 1a), which suggests a better suitability for phytoremediation in terms of biomass production.

Upon exposure to excess As (either in soil or in solution culture), plants tended to suffer from a reduction in root growth and a decrease in shoot growth (Cox et al. 1996; Abedin and Meharg 2002). The effect of As on dry matter production of rice was assessed by Marin et al. (1992). They found that when rice seedlings were grown in a nutrient solution containing As (V) at 0.05 to 0.8 mg As L^{-1}, the total dry matter production was unaffected. In our experiment, the total dry biomass of Choto shama, Boro Shama, and Himetainubie was not greatly distinguished at 1 mg As L^{-1} (compared to control). However, a slight reduction in dry biomass compared to control was found in the case of BR-29, Nipponbare, and Inubie at 1 mg As L^{-1} treatment (at control total dry biomasses were as follows: Inubie=1.3 g, Choto Shama=1.06 g, Himetainubie=0.96 g, BR-29=0.9 g, Boro shama=0.80 g, and Nipponbare=0.66 g/pot. In contrast, the values were as follows: Inubie=1.08 g, Choto shama=1.04 g, Himetainubie=0.90 g, Boro Shama=0.74 g, BR-29=0.67 g, and Nipponbare=0.59 g/pot after treatment at 1 mg As L^{-1}). At control and at 1 mg As L^{-1}, Inubie produced the highest biomass. However, Choto shama and Himetainubie produced a good biomass in all the As-treated solutions without much reduction despite increases in the As level. This observation thus indicates the improved tolerance capacity of arsenic by those species. Among the barnyard grass varieties tested, Boro shama produced the lowest biomass, while it was BR-29 among the rice species compared to their respective biomass at control. This may be explained by their relative susceptibility to the interference of As on plants growth as described above.

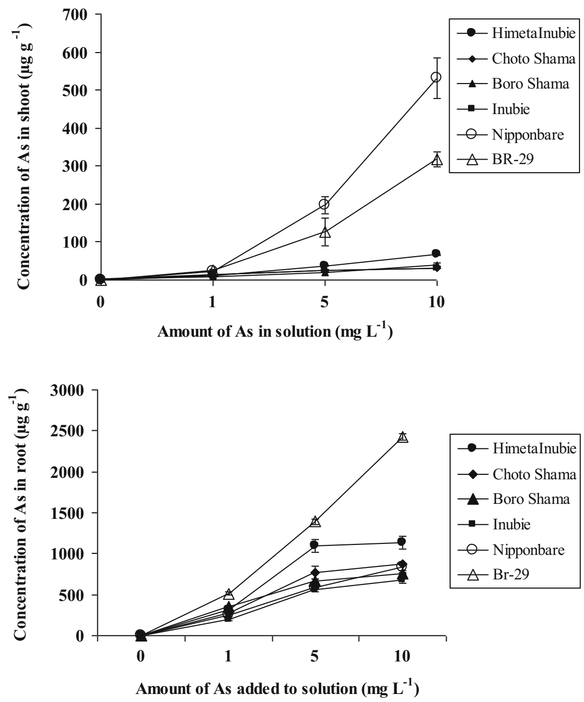

Fig. 2 Concentration of As in the shoot and root component between different barnyard grasses and rice varieties after growing in As-containing solution. *Bars* indicate the ±standard error of the means (n=3)

Concentration of As in the plants

Absorption of a sufficient amount of As by the plants is a key factor for effective phytoremediation. The greater the amount of As absorbed by the plants, the greater the removal of As from the contaminated media. Absorption of As by plants is influenced by many factors including plant species (Walsh and Keeney 1975). A higher concentration of As was found in root than in shoot in all the varieties and species of barnyard grass and rice plants. The concentrations of As were gradually increased with the increase of As in the solution both in shoot and root of the plants (Fig. 2). At the highest As level in the solution (10 mg L^{-1}), the highest concentration of As was found in the root of BR-29 (2430 μg As g^{-1}). Likewise, the highest concentration of As was found in the shoot of Nipponbare (531 μg As g^{-1}). BR-29 showed a sharp increase in absorption of As in both root and shoot after 1 mg L^{-1} treatment, suggesting the complete breakdown of barriers between solution-root and root-shoot (a protective mechanism of plant to prevent toxic metal accumulation) at high concentrations. The shoot/root ratio of As is of particular importance to assess the translocation of metals in the aboveground part of the plants which is prerequisite to phytoremediation for phytoextraction (a subset of phytoremediation). According to Marin et al. (1992), the shoot/root ratio of As concentration was not distinguished between the two cultivars of rice. However, in this study, the shoot/root ratio of As differed noticeably by variety and species of barnyard grass and rice. In the root, concentrations of As were found in the order of BR29>Himetainubie>Choto shama>Nipponbare≥Boro shama≥Inubie. In the shoot, the relative ordering was seen as follows: Nipponbare>BR29>Himetainubie>Choto shama≥Boro shama≥Inubie. In all treatments, the shoot As concentration was higher in rice than in barnyard grass (Fig. 2), indicating that the translocation of As from root to shoot was higher in rice than that of barnyard grass. As we found that the concentration of As in root and shoot of the plants differed significantly among the plants and among the biotypes, it may be reasonable to infer that the capacity of barnyard grass and rice to absorb As from a media and translocation of As in to the aboveground part does not only depend on the type of plants but also on their biotypes.

Table 1 Bioaccumulation factor (BF) values of different barnyard grass and rice varieties after growing in As-treated solutions

Plants	Bioaccumulation factor (BF) values		
	1 mg L^{-1} As	5 mg L^{-1} As	10 mg L^{-1} As
Himetainubie	11.33±0.70	7.63±0.40	6.70±30.3
Inubie	12.67±2.30	4.94±0.44	3.18±0.06
Boro shama	7.58±0.63	3.87±0.11	4.05±0.43
Choto shama	13.71±0.57	5.02±0.10	3.08±0.05
Nipponbare	23.46±0.24	39.43±2.53	53.12±5.23
BR-29	25.72±3.71	25.40±7.37	31.78±1.98

BF values were obtained from the shoot/solution ratio of As concentration. Values are presented as a mean of three replicates ±standard error

Bioaccumulation factors

The BF of a plant for a given metal is the ratio of the compound accumulated in the aboveground plant parts in relation to the amount of metal in the growth medium (Yoon et al. 2006). It is an important factor when one considers the potential of phytoextraction for a given species. The plants which have poor BF value could be suitable for phytostabilization of metals to suppress their availability to the plants in the soil. However, the more the BF value of a plant, the more the plant is suitable for phytoremediation through phytoextraction. Many researchers also consider the bioconcentration factor between the concentration of heavy metals in the root (or in total plant biomass) relative to their concentration in the growth media, for phytoremediation (Malik et al. 2010; Ali et al. 2012). In this study, we considered only the As accumulation in the aboveground plant parts. Table 1 shows the BF values of As in different barnyard grasses and rice varieties (and species). Although the highest concentration of As was found from BR-29, the highest BF value was recorded in case of Nipponbare in all treatments. There was no significant difference in the BF values between Himetainubie, Choto shama, Inubie, and Boro shama. It was reported that the bioaccumulation factors decreased with the increase in soil heavy metal concentrations (Efroymson et al. 2001; Zhao et al. 2003). In our study, the BF values of barnyard grass varieties decreased slightly with the increase of As in the solution. This decrease may be due to limited root-to-shoot transport, when internal As concentration is high, as reported by Pence et al. (2000) in the case of Zn accumulation by *Thlaspi caerulescens*. However, in both the species of rice, BF values increased with the increase in the As concentration in the media. This may reflect the effect of breakdown of a defense mechanism such as root-shoot barrier at high metal concentrations as described above. The BF values of barnyard grass were within the range of 6.7–11.3, while those of rice were 23.5–53.1. At the As treatment of 1 mg L^{-1}, the BF values were more than 10 in all the barnyard grass and rice varieties tested. According to Baker (1981), the BF value of >1 implies that the plant is an "accumulator," while <1 is an "excluder." Therefore, all of the plants tested here should belong to As accumulators in a soil-free medium. Moreover, Himetainubie and Nipponabare are the best candidates for the phytoremediation in terms of BF values.

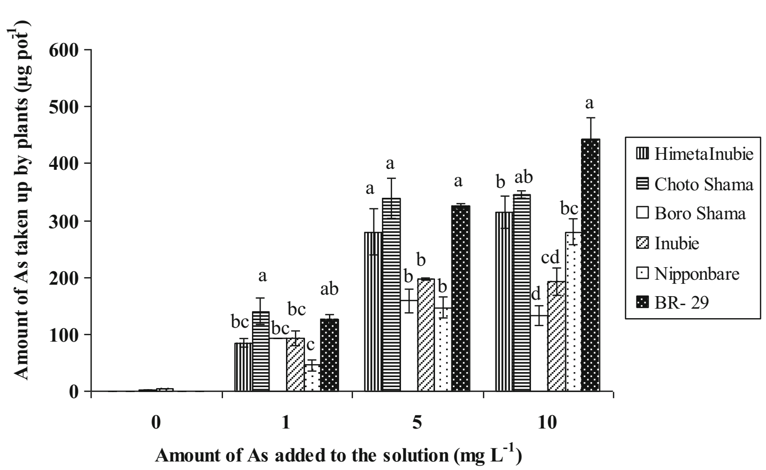

Fig. 3 Amount of As in different barnyard grasses and rice varieties after growing in As-containing solution. The bars indicate the ±standard error of the means (n=3). The *columns with the same letter* are not significantly different at P<0.05 as determined by Tukey's test

Uptake of As by the barnyard grasses and rice varieties

According to the shoot/root ratio of As, rice varieties generally showed better translocation than the barnyard grasses. Nonetheless, Choto shama took up the highest amount of As per pot with the treatments of As at 1 and 5 mg L^{-1} (Fig. 3). This observation may suggest its potential for biomass production at those As-treated solutions. At 10 mg L^{-1} treatment of As, the highest uptake was observed from BR-29 followed by Choto shama and Himetainubie. However, except for the 10 mg L^{-1} As level, there was no significant difference in As uptake between Choto shama and BR-29. With the increase of As concentration in solution, the rate of its uptake increased sharply in both rice varieties compared to the barnyard grasses; it thus suggests the enhanced As absorption of the former at the higher doses of arsenic relative to the latter. The trends of As uptake in all the plants were distinguished from each other in all the treatments due to differences in their tolerance of As and biomass production capacity.

In this experiment, high levels of As (up to 10 mg L^{-1}) were used to test the plant's capacity to tolerate As, although such a concentration level cannot be found commonly in the surface soil of Bangladesh (Meharg and Rahman 2003). Nevertheless, in certain areas with elevated As levels (e.g., in areas irrigated with contaminated groundwater), the soil As level can reach up to 57 mg kg^{-1} (Alam and Sattar 2000) or up to 83 mg kg^{-1} in survey from different locations (Ullah 1998). Taking into account the actual soil As level, the least level of As treatment in this experiment (1 mg L^{-1}) should be realistic to assess the remediation capacity of plants under the actual field conditions of As-affected areas. At this treatment level, the relative order of As uptake was seen as Choto shama>BR-29>Himetainubie≥Boro shama≥Inubie>Nipponbare. Himetainubie, Choto shama, and BR-29 were able to maintain a steady uptake rate even at 10 mg L^{-1} As (in the solution), indicating their enhanced potential to remove As. BR-29 is a widely cultivated rice variety in Bangladesh. The ability of BR-29 rice to uptake and translocate As poses a threat to cultivate this variety in the As-contaminated areas as a food crop. However, it can be a suitable option for phytoremediation of arsenic-contaminated soil as it has great potential to remediate arsenic.

Conclusion

This research investigated the phytoremediation potential of some barnyard grasses and rice varieties (and species) against arsenic. Among the varieties and species tested, Choto shama, BR-29, and Himetainubie were found to have greater potential for arsenic removal. Nipponbare can also be suitable because it can translocate the highest amount of As in the plant shoot. These four varieties of barnyard grass and rice could be suitable for phytoremediation of different levels of As-contaminated areas, especially in lowland soils. The BF values are higher in both of the rice than those of barnyard grass varieties. However, in the field condition, barnyard grass will be more tolerant of the environmental stress to yield more biomass than rice. If both plants are employed together for phytoremediation under the actual field conditions, it can facilitate an effective remediation. Further research is desirable to explore the efficacy of the barnyard grasses and rice varieties under different contaminated conditions of arsenic.

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