B.K. SARANGI, T. CHAKRABARTI
National Environmental Engineering Research Institute,
Maharashtra, India,
E.mail: bk_sarangi@neeri.res.in
E.mail: twmneeri@sancharnet.in

CHARACTERIZATION OF AN ECOTYPE OF BRAKE-FERN, *PTERIS VITTATA,* FOR ARSENIC TOLERANCE AND ACCUMULATION IN PLANT BIOMASS

An ecotype of brake fern (Pteris vittata) was assessed for arsenic tolerance and accumulation in its biomass under in vivo and in vitro condition; using soil, and agar-gelled Murashige and Skoog (MS) medium supplemented with different concentrations of arsenic. The plants were raised in soil amended with 100–1000 mg arsenic kg^{-1} soil, and MS medium was supplemented with 10–300 mg arsenic L^{-1} medium using $Na_2HAsO_4 \times 7H_2O$. The spores and haploid gametophytic-prothalli were raised in vitro on MS medium supplemented with arsenic. The field plants showed normal growth and biomass formation in arsenic amended soil, and accumulated 1908– 4700 mg arsenic kg^{-1} dry aerial biomass after 10 weeks of growth. Arsenic toxicity was observed above >200 mg arsenic kg^{-1} soil. The concentrations of arsenic accumulated in the plant biomass were statistically significant ($p < 0.05$). Normal plants were developed from spores and gametophyte prothalli on the MS media supplemented with 50–200 mg arsenic L^{-1} medium. The in vitro raised plants were tolerant to 300 mg arsenic kg^{-1} of soil and accumulated up to 3232 mg arsenic kg^{-1} dry aerial biomass that showed better growth performance, biomass generation and arsenic accumulation in comparison to the field plants.

Introduction. Arsenic is bioactive and potentially toxic. Long-term exposure to low concentrations of arsenic in drinking water can lead to skin, bladder, lung and prostrate cancer, cardiovascular diseases, diabetes, anemia and reproductive, developmental, immunological and neurological effects [1–4]. Mining and processing of ores of other elements such as Au, Ag, Cu and Sn in particular had led to extensive arsenic pollution of mining regions throughout the world [5–7]. The use of arsenic-based pesticides as lawn herbicide and insecticides for rice, orchards and cotton had led to considerable contamination of domestic and agricultural land [6, 8]. Arsenic contamination in soil is one of the major sources of arsenic in drinking water [3, 7, 9]. It has been reported that increased arsenic level in soil leads to build up of arsenic in plants and crops such as cereals, vegetables and fruits [10]. Arsenic contamination in soil and water has spread to an alarming dimension in some eastern states and coastal parts of India, and other parts in the world [11]. The remediation of arsenic-contaminated soil and removal of arsenic from contaminated water is an important current issue [2]. A number of plants, known as hyperaccumulators [12], have been identified accumulating large quantity of contaminants and a variety of metals in its aboveground biomass potential for phytoremediation of pollutants [13]. Phytoremediation is an emerging technology that utilizes the ability of plants to accumulate metals from soil and groundwater [3, 14–20, 37].

Tolerance to arsenic toxicity is reported in a large number of plant species, such as *Agrostis tenuis* [21], *Holcus lanatus* [22, 23], *Deschampsia cespitosa* and *Agrostis capillaris* [24], *Silene vulgaris* [25, 26], *Bidens cynapiifolia* [4], *Calluna vulgaris* [27], *Cytisus striatus* [28], Indian mustard [29] and other plant species [30–32]. Majority of this arsenic tolerant plants were identified from abandoned mine site where the concentration of arsenic in the soil was extremely high. On the other hand, plant species like *Deschampsia cespitosa* [24] and *Silene vulgaris* [26] identified from uncontaminated soils also exhibited resistance to arsenic. Under normal conditions, arsenic concentration in terrestrial plants is less than 10 mg arsenic Kg^{1} dry biomass [33]. At higher concentrations, arsenic interferes with plant metabolic processes, inhibits growth and leads to death. Biomass production and crop yield of a variety of species are significantly reduced at elevated arsenic concentrations [34]. The yields of barley (*Hordeum vul-*

gare) and rye grass (*Lolium perenne*) were significantly decreased with application of 50 mg arsenic Kg^{-1} of soil [10]. The studies on uptake, accumulation and translocation of arsenic in both arsenic-tolerant and non-tolerant plants have indicated wide difference in arsenic tolerance among plant species [21, 23, 36–41].

The brake fern, *Pteris vittata* (also known as Chinese brake / ladder brake) is reported as an arsenic hyperaccumulator plant that could accumulate high amount of arsenic in its biomass [36] and tolerate up to 1500 mg arsenic kg^{-1} soil in arsenic amended and contaminated soil. This plant was identified around California from the South-East of the USA, and reported to be available in similar mild climatic areas of the world. Investigations of Tu and Ma [39] showed that 50 mg arsenic Kg^{-1} soil was best for growth and arsenic accumulation in this fern species, and as high as 2.2 % arsenic was accumulated in the above ground plant biomass and about 26 % arsenic was removed from the soil. After 8–20 weeks of transplantation, the arsenic bioconcentration factor (BF) reached 1000 to 1450. The arsenic translocation factor (TF) in the leaves was 1.2 and 42 at the 2nd week and the 8th week respectively, after of transplantation. Though, Zhang et al. [40] reported that after 20 weeks of growth in moderately contaminated soil, arsenic translocation and accumulation in the young parts and old parts of brake fern was 4893 mg Kg^{-1} and 7575 mg Kg^{1} respectively. There are other fern species, which are also reported to be efficient in accumulating arsenic in their above ground biomass. Visoottiviseth et al. [41] assessed the potential of 36 native plant species of Thailand from mine tailings, where arsenic concentration in the soil was up to 16 g Kg^{-1}, and reported that plant species with the highest leaf arsenic concentrations did not occur with the highest frequency in the contaminated sites. They reported two species of ferns (*Pityrogramma calomelanos and Pteris vittata*), a herb (*Mimosa pudica*) and a shrub (*Melastoma malabrathricum*) potential for phytoremediation of arsenic. Francesconi et al. [42] reported that the silver fern (*Pityrogramma calomelanos*) could accumulate 2760 to 8350 mg arsenic Kg^{-1} plant biomass in old fronds, and 5130 to 5610 mg arsenic Kg^{-1} in young fronds.

The identification of brake fern to be an efficient hyperaccumulator of arsenic has opened tremendous potentiality for phytoremediation of arsenic contaminated soil [38, 39]. However, Meharg [32] and Gumaelius et al. [43] reported that some members of the genus *Pteris*; like *Pteris straminea* and *P. tremula* also do not hyperaccumulate arsenic, while another species of *Pteris cretica* was found to be hyperaccumulator of arsenic. Gumaelius et al. [43] also investigated arsenic accumulation in the gametophyte and sporophytes of *P. vittata* and compared with non-accumulating fern *Ceratopteris richardii*. The present work was carried out to characterize arsenic tolerance and accumulation in an ecotype of *Pteris vittata* collected from the Indian subcontinent. Arsenic tolerance of spores, prothallic-gametophyte and sporophytes were assessed in arsenic supplemented Murashige and Skoog [44] growth medium under in vitro conditions, and the sporophytes were assessed in arsenic supplemented soil under glass house conditions.

Materials and methods. ***Plant propagation under glass-house conditions***. The ecotype of *Pteris vittata* plant was collected from the Kerala state of India, and used in this study (Fig. 1). The parent plants were maintained in the glass house in soil pots. The potting material was a mixture of garden soil, sand and farmyard manure (FYM) in a ratio of soil : sand : FYM – 2 : 1 : 1. Young sporophytes were developed on soil from spores of stock plants, and used in the experiments. Young plants with 2–3 fronds were transplanted to single pot with 0.5 kg of soil mixture and allowed to grow for at least one month prior to experimental use. One to four months old plants of equal height and with equal number of fronds were used in the experiments.

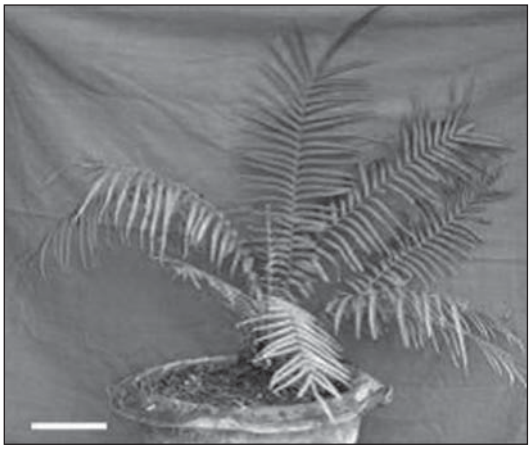

Fig. 1. Sporophyte of *Pteris vittata* ecotype. Bar represents 11.5 cm

Preparation of arsenic amended soil for plant growth. The soil mixture was amended with different concentrations of arsenic for arsenic treatment experiments by adding $Na_2HAsO_4 \cdot 7H_2O$ to the soil. The soil mixture was mixed with a basal dose of fertilizer [N : P : K – 180 : 60 : 120 mg kg^{-1} soil], and 500 mL Hoagland nutrient solution [45] Kg^{-1} soil mixture to supplement optimum level of macro and micro nutrients and trace minerals. Two kg of air-dried soil mixture was amended with arsenic to get the desired concentrations of arsenic in the soil. Calculated amounts of $Na_2HAsO_4 \cdot 7H_2O$ in aqueous solution were added to the soil mixture to obtain 50, 100, 200, 500 and 1000 mg total arsenic Kg^{-1} soil. The arsenic amended soil was thoroughly mixed and kept moist for one week before planting saplings and used for treated experiments. In a similar manner, soil pots were prepared without arsenic for raising plants without arsenic, and used for control experiments.

One to 4 months aged *Pteris vittata* saplings were used for the experiments. One healthy fern plant was transplanted in one packet, amended with 50/100/200/500/1000 mg arsenic kg^{-1} soil and the control was maintained on arsenic free soil. Five replicates were maintained in each concentration of arsenic amended soil. The plants were watered when the topsoil looked dry, and leaching of water from the soil packets was prevented. Experimental plants in control and treated soil were grown inside glass house under similar conditions at an average temperature of 30 °C (day) and 24 °C (night), under a day and night photoperiod of 14 hours light :10 hours dark period.

The morphological changes of the plants, appearance of new fronds, coloration of fronds, and overall growth behavior, were recorded at an interval of one week, and when noticeable changes were observed. Live and dead aerial parts of the plants were collected at different time intervals for arsenic analysis.

Preparation of arsenic supplemented culture media for in vitro experimentations under aseptic condition. Agar gelled Murashige and Skoog (MS) plant tissue culture medium amended with different concentrations of arsenic was utilized to asses arsenic tolerance of the ecotype under in vitro conditions. Filter-sterilized aqueous $Na_2HAsO_4 \cdot 7H_2O$ solution was added to molten MS media at <50 °C; to final concentration of 10, 20, 50, 100 and 200 mg arsenic L^{-1} culture medium, inside laminar airflow cabinet following standard aseptic procedure. These media were used in the in vitro experiments after gelling.

Culture of spore, prothallus, gametophytes and prothallic-sporophytes. Spores were collected from the sori of mature fronds from the field grown sporophytes, decontaminated and cultured on arsenic supplemented culture media under in vitro conditions. A piece of leaflet having mature sporangia was wrapped in a packet prepared from filter paper, and incubated under warm and dry conditions for 3 days. The filter packet with the spores was dipped in 70 % ethanol for 1 minute followed by treatment with 0.1 % aqueous mercuric chloride solution for 3, 5, 7 or 9 minutes and washed 4 times with sterile distilled water. Subsequently, the filter packet was soaked in sterile water for 7 minutes, and the spores were spread on the surface of the culture medium. In other case, 1cm leaf cuttings were directly treated with ethanol and mercuric chloride as described above; the sporangia were ruptured in 0.5ml sterile water and the entire spore suspension was plated on the agar-gelled medium. All aseptic works were carried out under the laminar airflow cabinet. Spores were plated on agar-gelled MS [44] and Knudson culture medium [46] without growth hormones. The in vitro cultures were maintained at 25 ± 2 °C, 1500-lux illumination, 60 % relative humidity and 16 hours light: 8 hours dark photoperiod.

The prothallus, gametophyte and prothallic-sporophyte that developed from spores, were subcultured on control (without arsenic) and treated MS medium (supplemented with different concentrations of arsenic). The cultures were incubated under specified illumination, photoperiod, temperature and humidity mentioned previously. The spores were cultured in vitro on full, half and quarter strength MS, and Knudson hormone free medium supplemented with 20–100 mg of arsenic L^{-1} of culture medium. The gametophyte prothalli were cultured in arsenic amended culture medium supplemented with 100 to 300 mg of arsenic L^{-1} of medium, and subcultured to fresh arsenic supplemented medium at an interval of one month. The gametophytes or sporophytes were subcultured to fresh medium with similar or higher concentrations of arsenic, and the responses of the tissue or organ on the culture medium were monitored from time to time.

Growth of in vitro raised sporophytes in arsenic amended medium and arsenic amended soil. The gametophyte prothalli formed 2–4 cm height sporophyte plants under in vitro, henceforth called as tissue culture derived plants. These tissue culture plants were subcultured to fresh arsenic amended growth medium (100, 200 and 300 mg arsenic L^{-1}) at an interval of one month and maintained for 6–8 months on the same medium. These sporophytes were subsequently transplanted to soil amended with the same or higher concentrations of arsenic and allowed to grow inside glass house conditions.

Arsenic analysis in plant tissue. The live biomass (green) and dead biomass (dry and brown) were collected from field plants after 2, 4, 6, and 10 weeks of growth in control and arsenic amended soil. Likewise live and dead fronds were collected from the tissue cultured plants, generated in vitro from spores using arsenic amended culture medium and subsequently transplanted to arsenic amended soil, when the sporophytes produced sufficient biomass in the arsenic amended soil. These biomass were thoroughly washed in tap water, rinsed three times with deionized water, oven dried at 60 °C for 72 hours and the dry weight was determined, and used for estimation of arsenic in the biomass. Known amount of biomass was digested with analytical grade 6.5ml concentrated HNO_3 and 2.5 ml concentrated HCl using the Microwave Sample Preparation System (Ethos 900, Milestone Micro-wave Lab. System U.S.) at 300 watt–15 minutes program. The sample solution was filtered through Whatman filter paper No. 42 and volume of the filtrate was adjusted to 100 ml with double distilled water. The concentration of arsenic in the filtrates was determined by the Inductively Coupled Plasma spectrometer (ICP-AES, JOBIN YVON, France) using arsenic standard (MERCK, ICP standard, product no 1.70303.0100) at concentrations of 0, 1, 5 and 10 mg arsenic L^{-1} and blank.

Statistical method. The results of effect of soil arsenic (50 to 1000 mg arsenic kg^{-1} of soil) on plant growth at different durations (2–10 weeks), and arsenic accumulation in the plant biomass under control and treated conditions were statistically analyzed. The significance of the differences in values of arsenic, estimated in plant biomass, was determined by Pearson Bivariate Correlation Coefficient and Students *t*-test, using statistical software Statistica version 5.1 (Texas, USA).

Results and discussion. ***Affect of arsenic on growth and morphology of the sporophytes.*** Field grown fern sporophytes (4 months old, with 4–6 numbers of fronds and about 10 cm height) were transplanted on control and arsenic amended soil, with 50, 100, 200, 500 and 1000 mg arsenic kg^{-1} of soil. After two weeks, new frond primordia appeared in control and treated plants (with 50–100 mg arsenic) whereas, the fronds turned brown in soil amended with 200 mg arsenic. The fronds of sporophytes planted in soil >200 mg arsenic kg^{-1} of soil dried by two weeks. The symptoms of arsenic toxicity were first visible in older and larger fronds, the leaflets in the apical part of fronds started browning from its tip, and it further extended to other leaflets and rachis of the affected frond. The toxic effect began with browning of the leaflets followed by drying, and fronds turned black. The sporophytes transplanted in soil amended with $\geqslant$500 mg of arsenic kg^{-1}soil dried and turned black by 5th week. The results of growth performance indicated a dose dependent cumulative effect of arsenic toxicity in the plants in comparison to the control plants (Table 1). Growth and increase in size of the sporophytes was not affected up to 100 mg arsenic kg^{-1} soil, and growth response was similar to the sporophytes raised in control soil (Fig. 2). New frond primordia appeared after the second week of transplantation in control as well as treated soil and the fronds remained partly brown up to two weeks, but afterwards they enlarged and turned green like the normal fronds. The height of the control and treated plants was increased 6–8 cm by the 10th week and the rate of increase in height was maximum at the 4th week. Whereas, in case of the plants treated with >200 mg arsenic kg^{-1} of soil the increase in plant height was marginal, maximum up to 1cm within the same duration (Fig. 2). It is presumed that the partial browning of the sporophytes, in control and treated with 100 mg arsenic kg^{-1} soil, up to the 2nd week could be due to the transplantation shock to plants in the soil during acclimatization.

Our findings are comparable with the findings of Tu and Ma [39], who studied the effect of soil arsenic on biomass production, arsenic uptake and accumulation in *Pteris vittata* identified in California. In their study, young plants were grown on arsenic amended soil (950–1500 mg arsenic Kg^{-1} soil) in green house, and the highest plant

Table 1

Morphological changes of *Pteris vittata* plants grown in control (soil without arsenic) and treated (soil amended with different concentrations of arsenic) soil

Culture duration (in weks)	Control	Treated (Arsenic in soil mg kg^{-1})				
		50	100	200	500	1000
1	No visible change					
2	A few leaflets of larger fronds turned brown	A few leaflets of larger fronds turned brown	A few leaflets of large fronds turned brown	Tips and parts of large, and lower fronds turned brown	Peripheral parts of large fronds turned brown	Many fronds turned brown
3	New frond primordia appeared	New frond primordia appeared	New frond primordia appeared	As above	Venation of the fronds were visibly brown	Brown fronds dried
4	New primordia developed to fronds	New primordia developed to fronds	New primordia developed to fronds	Browning of fronds increased	As above, new frond primordia initiated	More fronds turned brown
5	More new primordia appeared	More new primordia appeared	As above, brown fronds dried	One new primordia initiated	Brown fronds died	Browning of fronds increased
6	No visible changes	Initiation of more primordia	More new frond primordia initiated and developed	Majority of fronds (75 %) started browning	More fronds turned brown	More fronds turned completely brown
7	More primordia appeared and developed to fronds	Primordia developed to fronds	No visible changes	Browning increased	All fronds turned brown	Brown fronds died. All fronds started browning
8	No visible chan ges, plants were green	No visible changes, plants were green	Upper part of fronds (10 %) browned	Browning of old fronds increased	Plant turned brown and dried	Plant turned brown and dried
9	More new primordia developed	More new primordia developed	No change. Plants were green	Old fronds dried. New fronds were green	–	–
10	Plant height increased by 6–8 cm	Plant height increased by 6–8 cm	Plant height increased by 6–8 cm	Plants were alive as above	–	–

growth and biomass accumulation (3.9 g plant $^{-1}$) were reported with 50 mg arsenic kg^{-1}soil. They reported arsenic toxicity three days after transplanting, the fronds turned dark brown followed by necrosis in the leaf tips and margins, and plants died after one week. They reported 64 to 107 % increase in the fern biomass than control up to 100 mg arsenic kg^{-1} of soil, and it did not increase at 200 mg arsenic, whereas at 500 mg arsenic the above ground biomass was reduced by 64 %. The reduction in biomass is a common phenomenon of arsenic phytotoxicity [35]. Tu et al. [38] also reported slow growth of plants for 6–8 weeks after transplantation in arsenic soil, though subsequently, the biomass increased rapidly and nearly quadrupled every 4–week.

Arsenic accumulation in aerial biomass of field plants. The alive and dead fronds of plants, raised under different concentrations of soil arsenic were colleted after the 2nd, 4th, 6th and 10th weeks from the date of plantation from four different sets of plants, and analyzed to estimate the quantity of arsenic accumulated in the biomass. The results are presented in Tables 2 through 5.

The trends of arsenic accumulation in the plant biomass under different concentrations of soil

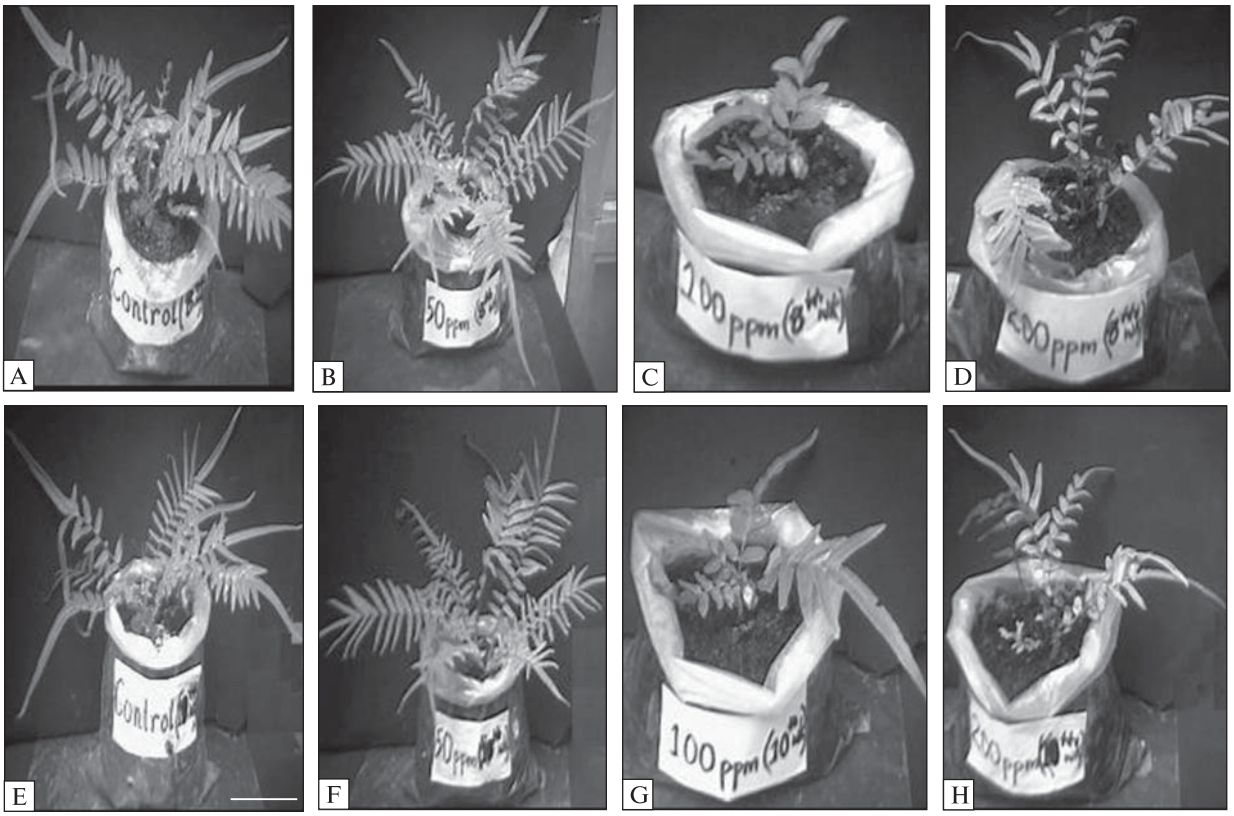

Fig. 2. Growth performance of field grown sporophytes of *P. vittata* in control and As spiked soil after 8 weeks (A – control, B – 50 mg As, C – 100 mg As, D – 200 mg As Kg^{-1} soil) and 10 weeks (E – control, F – 50 mg As, G – 100 mg As, H – 200 mg As Kg^{-1}soil). Bar represents 10 cm

arsenic after 2,4,6 and 10 weeks of growth were analyzed (Fig. 3). The arsenic accumulation in the plant biomass indicates that accumulation was more at higher levels of soil arsenic, and when duration of plant growth was more. Thus, arsenic accumulation in this ecotype of brake fern varied in accordance to the level of arsenic in the soil and duration of plant growth. The amount of arsenic accumulated in plant biomass (mg of arsenic kg^{-1} dry weight) grown in the soil amended with 100 and 200 mg arsenic kg^{-1} soil was comparable to that with 500 and 1000 mg arsenic kg^{-1} soil (Fig. 3). Whereas, in case of the plants grown with similar concentration of soil arsenic, arsenic accumulation in plant biomass was quantitatively more after ten weeks than the plants grown for less duration (Fig. 4).

The analyses indicate that, the rate of arsenic transportation to the shoot in arsenic amended soil was less in all concentrations of arsenic up to 4 weeks of plant growth, which, however, increased sharply between 6 and 10 weeks (Fig. 5). This ecotype of

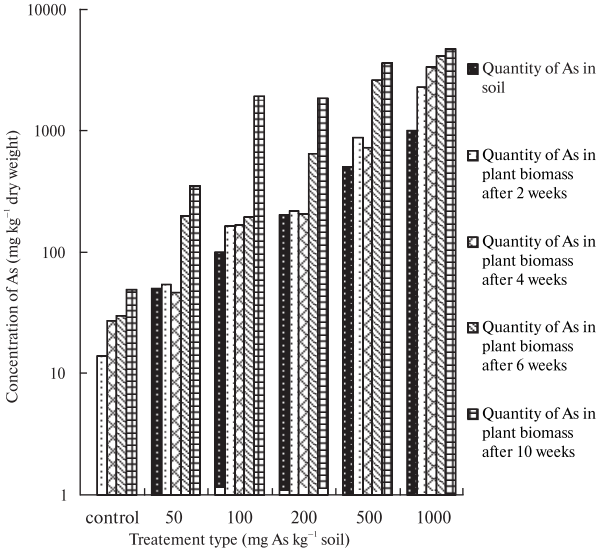

Fig. 3. Correlation of quantity of arsenic accumulated in biomass (dry weight) of the *Pteris vittata* ecotype with respect to different levels of soil arsenic; 50, 100, 200, 500 and 1000 mg arsenic kg^{-1} of soil after 2, 4, 6 and 10 weeks of growth

Table 2

Plant height, biomass and quantity of arsenic accumulated in plant biomass of the *Pteris vittata* ecotype after 2 weeks of arsenic treatment

Concentration of arsenic in soil (mg kg^{-1})	Height of plant (cm, ±SD)	Fresh weight of plant (g, ±SD)	Quantity of arsenic in plant biomass after 2 weeks (mg kg^{-1})
Control	19.5 ± 2.5	4.47 ± 1.2	14
50	14.5 ± 2.0	4.86 ± 1.0	54
100	22 ± 2.1	3.64 ± 0.5	164
200	15 ± 1.5	4.54 ± 0.8	216.46
500	26.5 ± 3.5	2.25 ± 0.9	874
1000	17.5 ± 1.1	4.46 ± 1.5	2274

Table 3

Plant height, biomass and quantity of arsenic accumulated in plant biomass of the *Pteris vittata* ecotype after 4 weeks of arsenic treatment

Concentration of arsenic in soil (mg kg^{-1})	Height of plant (cm, ±SD)	Fresh weight of plant (g, ±SD)	Quantity of arsenic in plant biomass after 4 weeks (mg kg^{-1})
Control	17 ± 0.5	5.728 ± 0.5	27
50	21 ± 1.5	14.94 ± 4.5	46.54
100	19,5 ± 2.5	8.94 ± 2.1	165.08
200	15 ± 1.9	6.26 ± 0.5	206.25
500	12 ± 0.5	7.84 ± 2.5	720
1000	14.5 ± 1.3	3.9 ± 0.5	3308

Table 4

Plant height, biomass and quantity of arsenic accumulated in plant biomass of the *Pteris vittata* ecotype after 6 weeks of arsenic treatment

Concentration of arsenic in soil (mg kg^{-1})	Height of plant (cm, ±SD)	Fresh weight of plant (g, ±SD)	Quantity of arsenic in plant biomass after 6 weeks (mg kg^{-1})
Control	21 ± 1.5	12.05 ± 0.5	30.00
50	9 ± 0.5	1.09 ± 0.5	198.29
100	24 ± 1.5	15.79 ± 3.5	191.35
200	12.5 ± 0.5	2.39 ± 0.9	637.89
500	11.5 ± 2.5	1.46 ± 0.7	2573.67
1000	12 ± 3.5	1.23 ± 0.9	4106.41

brake fern accumulated 1908 mg arsenic kg^{-1} of dry biomass when grown in arsenic amended soil for a period of 10 weeks with lower concentrations of arsenic (50–100 mg arsenic kg^{-1} soil). Whereas, at higher concentrations of arsenic (500–1000 mg arsenic kg^{-1} soil), in the same growth period, more quantity of arsenic (4700 mg arsenic kg^{-1} of dry plant biomass) was accumulated in the plant biomass. Moreover, under high concentrations of soil arsenic (1000 mg arsenic kg^{-1} soil), very high quantity of arsenic was accumulated in the plant biomass as early as after 2 weeks (Fig. 4). However, under 1000 mg As the increase in plant biomass was negligible and aerial parts of the plants gradually turned black and subsequently the plants died. The variances in arsenic concentrations in the plant biomass at different soil arsenic concentrations (control vs1000 mg, 50 vs1000 mg, 100 vs1000 mg, 200 vs100 mg, 200 vs500 mg and 500 vs1000 mg arsenic kg^{-1} soil), and at different duration of growth (2nd vs10th, 4th vs 10th and 6th vs10th week) were statistically analyzed. The differences were significant at 95 % confidence level ($^{*}p < 0.05$).

Arsenic accumulated in dead and live fronds of this fern ecotype was estimated to determine the rate of arsenic accumulation in old and young aerial parts. The concentrations of arsenic in the aerial biomass after 2 weeks and 4 weeks of growth in arsenic amended soil are presented in Table 6. The results show that the rate of arsenic accumulation was almost similar between live and dead fronds after 2 and 4 weeks of growth at all concentrations of soil arsenic, indicating that the rate of arsenic translocation to the aerial parts was almost similar in all fronds. The comparative rate of arsenic accumulation after the 2nd and the 4th weeks of growth with relation to all concentrations of soil arsenic was analyzed (Fig. 6). It was found that larger amount of arsenic was deposited in dead biomass of plants than that of live biomass in case of plants grown up to 4 weeks. This could be because the plants grown for 4 weeks in arsenic amended soil were exposed to arsenic for more duration, which led to accumulation of more amount of arsenic in plant biomass in comparison to the plants grown in the arsenic amended soil for 2 weeks.

The study indicates that this ecotype of brake fern is efficient in extracting arsenic form the soil up to 100 mg arsenic kg^{-1} soil, and can tolerate up to 200 mg arsenic kg^{-1} soil. This genotype of the brake fern accumulated 1908 mg arsenic kg^{-1} dry biomass in lower concentrations of soil arsenic (100 mg arsenic kg^{-1} soil) after 10 weeks of growth.

Under high soil arsenic (1000 mg kg^{-1} soil), the plant biomass accumulated as high as 4700 mg arsenic kg^{-1} dry weight, in the same 10 weeks growth period. Further, under high concentration of soil arsenic (1000 mg kg^{-1} soil) after 2 weeks of growth, this genotype also accumulated high amount of arsenic (2300 to 2428 mg kg^{-1} dry weight) in the plant biomass.

Our findings on arsenic accumulation by this ecotype of *Pteris vittata* are comparable to the *Pteris vittata* ecotype reported by Tu and Ma [39]. The brake fern was reported to be a hyperaccumulator of arsenic by Ma et al. [36]. This species was identified at California, which accumulated up to 15,861 mg arsenic kg^{-1} plant biomass in the aerial shoots after 2 to 6 weeks and arsenic toxicity was severe ⩾500 mg arsenic Kg^{-1} in soil. However, in the same species of brake fern (ladder brake) Tu and Ma [39] reported that arsenic accumulation was maximum in the young plants under 50 mg arsenic Kg^{-1} soil, based on the results after 12 to 18 weeks of growth of the plants with 50–1500 mg arsenic Kg^{-1} soil in green house conditions. Under low concentrations of soil arsenic, the younger fronds accumulated high concentration of arsenic, whereas, at high arsenic concentration in soil accumulation was more in the older fronds, presumably, due to older fronds receiving arsenic for a longer time in comparison to the younger fronds. Further study on *Pteris vittata* indicated that arsenic accumulation in the fronds increased with duration of growth [38]. The mature and young fronds had 6610 and 5570 mg arsenic Kg^{-1} biomass respectively and arsenic concentrations were highest in old fronds (13 800 mg Kg^{-1} in dry biomass). In the same ecotype of *Pteris vittata* Zhang et al. [40] reported that arsenic accumulation was 4893 mg Kg^{-1} and 7575 mg Kg^{-1} plant biomass in young and old parts of the plants respectively, after 20 weeks of growth. Concentration of arsenic was lowest in the roots of plants, substantially high in fronds, and old fronds had highest concentrations of arsenic. Arsenic accumulation by this ecotype of *Pteris vittata* from the Indian subcontinent is less in comparison to the *Pteris vittata* ecotype identified at California [36], but our results with this ecotype are similar to the findings reported for *Pteris vittata* by other workers [38–40].

Ma et al. [36] estimated that the bioaccumulation factor (BF) for arsenic by brake fern was as high as 193 as an indicator of efficient arsenic accu-

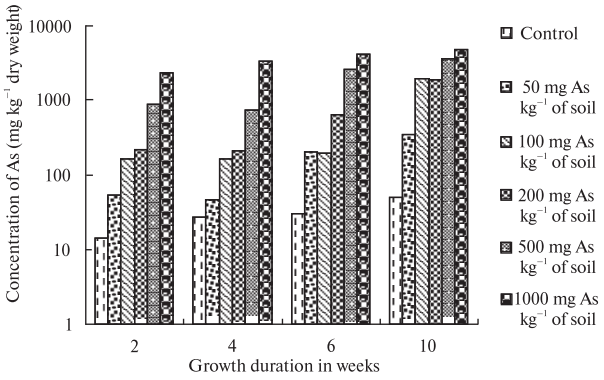

Fig. 4. Comparison of quantity of arsenic accumulated in plant biomass (dry weight) of the *Pteris vittata* ecotype after 2 weeks, 4 weeks, 6 weeks and 10 weeks of growth at different levels of soil arsenic (50, 100, 200, 500 and 1000 mg arsenic kg^{-1} soil)

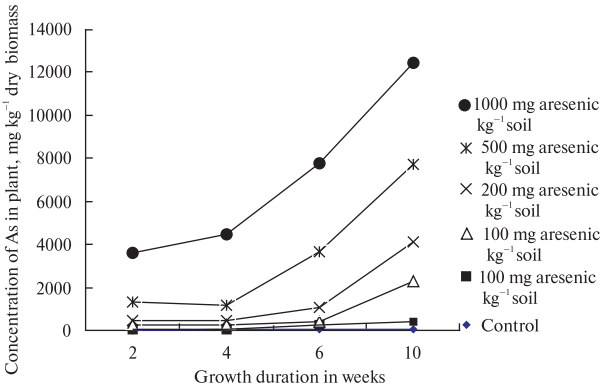

Fig. 5. Rate of arsenic accumulation in the ecotype of *Pteris vittata* after 2, 4, 6 and 10 weeks at different concentrations of soil arsenic (50–1000 mg arsenic kg^{-1} soil)

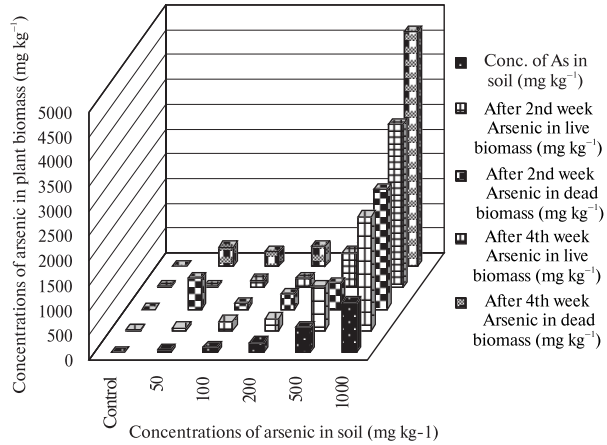

Fig. 6. Quantity of arsenic accumulated in live and dead biomass of *Pteris vittata* ecotype treated with different concentrations of arsenic amended in soil, after two and four weeks

mulation in plant biomass from soil. While, Tu et al. [38] reported that the BF for arsenic accumulation in the fronds of *Pteris vittata* based on water-soluble arsenic was increased to >1000 in the fronds after 8 week of transplanting. In this ecotype of brake fern studied by us, the difference in arsenic accumulation between live and dead fronds was marginal after two weeks of growth in arsenic amended soil (100 and 200 mg arsenic kg^{-1} soil). However, after 4 weeks of growth in arsenic amended soil, the difference in arsenic accumulation was significant between dead and live fronds (Table 6). Tu and Ma [39] have also reported that *Pteris vittata* had highest BF value for arsenic with 50 mg arsenic Kg^{-1} soil. In this ecotype of brake fern, the bio-concentration factor of arsenic in the plant biomass was up to 19 at 100 mg arsenic Kg^{-1} soil after 10th week (Table 5). The BF for arsenic varied with respect to different concentrations of soil arsenic and duration of growth.

Table 5

Plant height, biomass and quantity of arsenic accumulated in plant biomass of *Pteris vittata* ecotype after 10 weeks of arsenic treatment

Concentration of arsenic in soil (mg kg^{-1})	Height of plant (cm)	Fresh weight of plant (g)	Quantity of arsenic in plant biomass after 6 weeks (mg kg^{-1})
Control	17 ± 3.5	5.72 ± 0.5	48.89
50	21 ± 1.5	14.94 ± 1	347.54
100	19,5 ± 0.5	8.945 ± 1.5	1908.53
200	15 ± 1.5	6.26 ± 0.3	1827
500	12 ± 3.5	7.84 ± 2.5	3593
1000	14,5 ± 3.0	3.9 ± 0.7	4700

Table 6

Quantity of arsenic accumulated in the live and dead biomass of control and arsenic treated *Pteris vittata* ecotype after 2 weeks and 4 weeks, mg kg^{-1}

Concentration of arsenic in soil	After the 2nd week		After the 4th week	
	Arsenic in live biomass	Arsenic in dead biomass	Arsenic in live biomass	Arsenic in dead biomass
Control	14	Dead fronds absent	27	Dead fronds absent
50	54	620	39	386
100	190	122	158	296
200	242	309	199	404
500	900	516	713	477
1000	2300	2428	3301	4736

Growth performance of the sporophytes developed from spores on arsenic supplemented MS medium in vitro and arsenic amended soil. Spores of the *Pteris vittata* plant were aseptically cultured on full, half and quarter strength Murashige and Skoog and Knudson hormone free medium supplemented with various concentrations of arsenic to assess tolerance to arsenic toxicity. The spores germinated gametophyte prothalli in all media, but development of sporophytes from the gametophytes was better supported in full strength MS medium (Fig. 7, B–D). The gametophyte prothalli were cultured in arsenic amended MS medium with different concentrations of arsenic to assess arsenic tolerance at organ level. After 2–3 months, the gametophytic-prothalli generated fern sporophytes on control MS medium, as well as arsenic treated medium supplemented with 20–100 mg arsenic L^{-1} of medium (Fig. 8, C–F). The tender sporophytes were morphologically normal, and did not show visible symptoms of arsenic toxicity. These prothallic gametophytes and sporophytes were further cultured on higher concentrations of arsenic (Fig. 9 and 10), up to 300 mg arsenic L^{-1} of medium.

These tissue culture derived sporophytes were separated from the gametophytic-prothalli after 2–4 cm height and transplanted on fresh growth medium amended with 100, 200 and 300 mg arsenic L^{-1} of medium (Fig. 10). They were subcultured to the same medium at an interval of one month and maintained under in vitro conditions for 6–8 months. These plants developed intricate root systems, height and the above ground biomass of the plants increased to more than double after ten weeks. Under in vitro conditions with 200 mg arsenic L^{-1} of growth medium, sporophytes were similar to control plants. The sporophytes treated with 300 mg arsenic L^{-1} MS medium had less biomass in comparison to the plants treated with 200 mg arsenic L^{-1} of medium (Fig. 10); however, the plants were green and alive after repeated subculture to ar senic supplemented medium with 300 mg arsenic L^{-1} of medium. Observations of growth response of these sporophytes in arsenic amended growth medium up to 8 months are presented in Table 7. Subsequently, these in vitro raised sporophytes

were transplanted on arsenic amended soil and raised under glass house.

Performances of in vitro raised Pteris vittata sporophytes after transplantation to arsenic amended soil. The *Pteris vittata* sporophytes; 8–10 cm in height, with 10–20 fronds and six to eight months old, which were developed in vitro on arsenic (150, 200 and 300 mg L^{-1}) amended MS media, were transplanted on arsenic amended soil (150, 200, 300 and 500 mg kg^{-1} soil) and grown in glass house. The sporophytes selected with150 and 200 mg arsenic L^{-1} MS media were transplanted to soil amended with the same and higher (300 and 500 mg arsenic kg^{-1} soil) concentrations of arsenic. It was observed that after transplantation to arsenic amended soil these sporophytes established in less time, in comparison to the sporophytes developed ex vitro in soil. A few fronds started browning in control (soil not amended with arsenic) as well as in treated plants (soil amended with different concentrations of arsenic), probably due to the transplantation shock. However, both in control and treated plants new frond primordia emerged quickly by the 4th week, and enlarged to normal sized fronds. The length of new fronds increased to ⩾10 cm after 6 weeks, the leaflets were long, and all plants were green and healthy. In case of the plants treated with 300 mg arsenic kg^{-1} of soil, the new fronds looked brown up to the 4th week, but, subsequently the fronds in control and treated plants looked alike. The growth rate of sporophyte plants in 100 and 200 mg arsenic kg^{-1} soil was normal (Fig. 11), whereas the growth rate was slow in 300 mg arsenic kg^{-1} soil. All fronds of control plants and treated with 100, 200 and 300 mg arsenic kg^{-1} of soil were enlarged in length to ⩾20 cm by the 9th week and the numbers of fronds were ⩾20. In case of the plants treated with 300 mg arsenic kg^{-1} soil, the lower 2–3 fronds of the plant developed black patches on the peripheral parts of leaflets and adjoining the venation of the frond, but growth performances of the treated plants were similar to control plants. Whereas, the height of the sporophytes and size of the fronds did not increase in soil treated with 500 mg arsenic kg^{-1} of soil, the fronds turned black and the sporophytes degenerated.

The in vitro generated *Pteris vittata* sporophytes grew better with 200 and 300 mg arsenic kg^{-1} soil after transplantation to arsenic amended soil

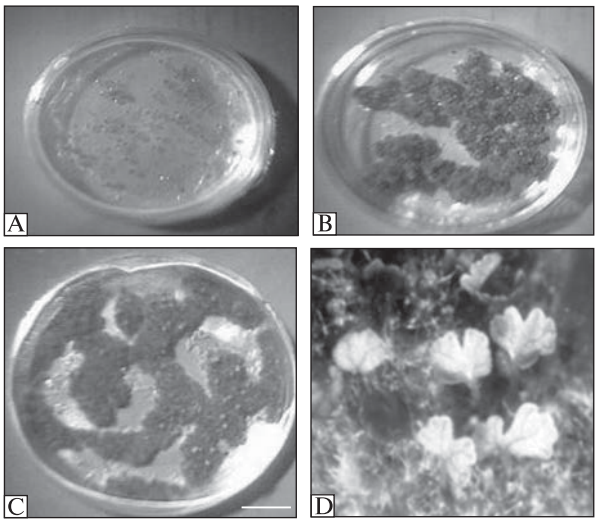

Fig. 7. Spore culture: development of gametophytes (A – Knudson medium, B – MS medium), and sporophytes (C, D – microscopic view of nascent sporophytes) on arsenic fee agar-gelled media after 4–6 weeks. Bar represents 13 mm (A, B, C),1.0 mm (D)

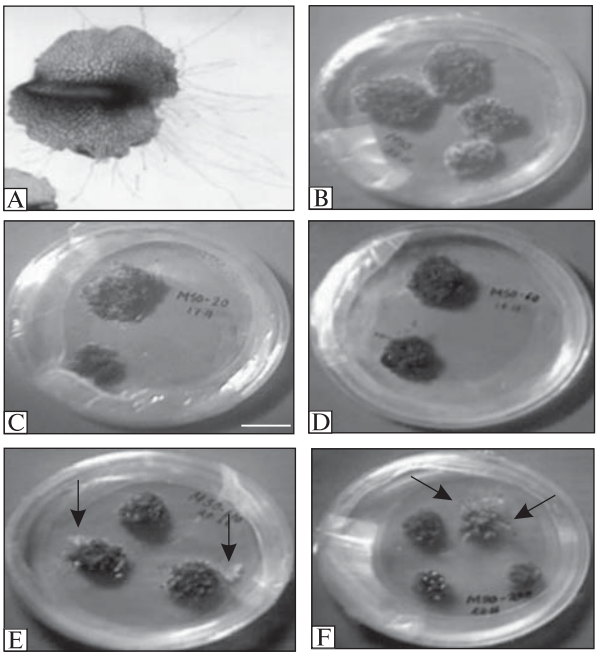

Fig. 8. Growth responses of gametophyte prothalli of *Pteris vittata* in MS agar-gelled media: without arsenic (A – single gametophyte prothalli microscopic view; B – clump of gametophyte), and supplemented with different concentrations of arsenic (C – 20 mg, D – 60 mg, E – 100 mg and F – 200 mg As L^{-1}) after 6–9 weeks. Shows development of sporophytes. Bar represents 0.25 mm (A), 13 mm (B–F)

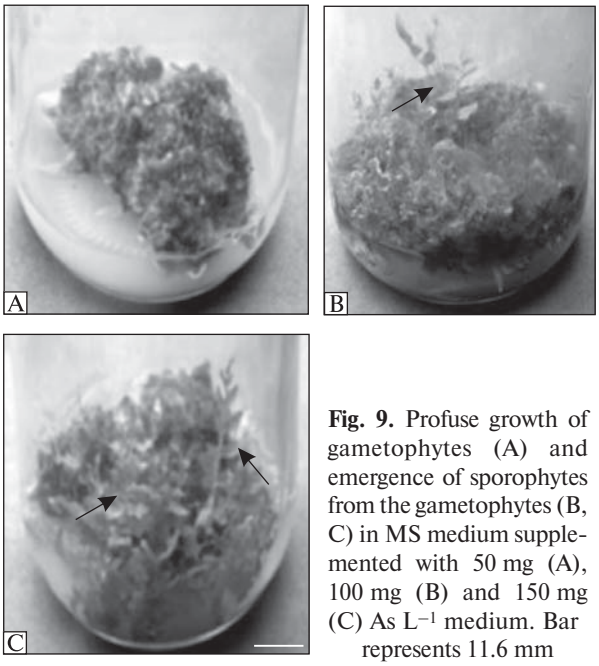

Fig. 9. Profuse growth of gametophytes (A) and emergence of sporophytes from the gametophytes (B, C) in MS medium supplemented with 50 mg (A), 100 mg (B) and 150 mg (C) As L^{-1} medium. Bar represents 11.6 mm

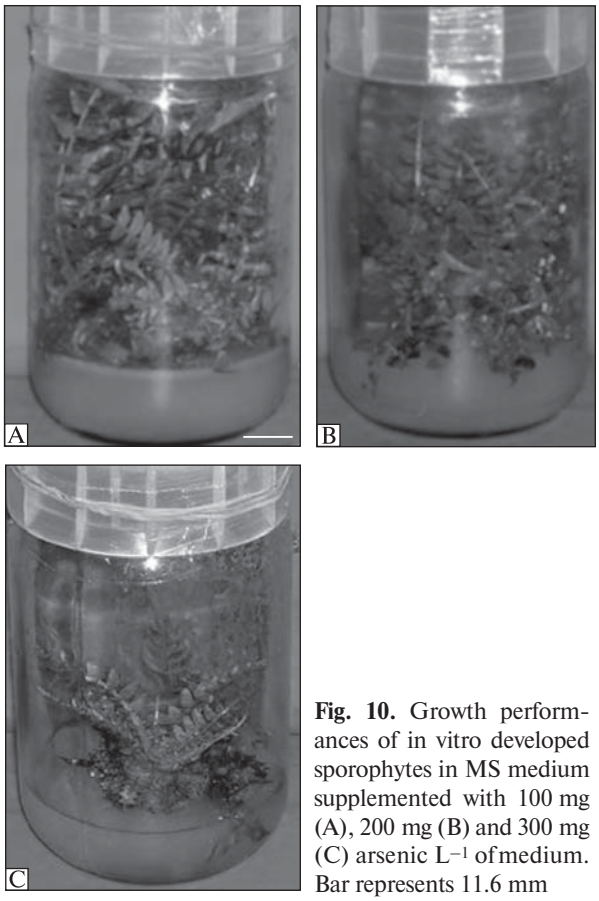

Fig. 10. Growth performances of in vitro developed sporophytes in MS medium supplemented with 100 mg (A), 200 mg (B) and 300 mg (C) arsenic L^{-1} of medium. Bar represents 11.6 mm

(Table 8), as compared to the sporophytes raised in soil and treated with similar concentrations of arsenic amended soil (Table 1). It was also observed that the size of plants, number of fronds and generation of biomass were more in case of the in vitro raised plants (Fig. 11) in comparison to plants generated in ex vitro condition and transplanted in arsenic amended soil (Fig. 2). This could be because the in vitro raised plants were treated with arsenic from the very early stage of growth, and the plants were adapted to tolerate higher concentrations of arsenic when transplanted to arsenic amended soil under field conditions. Presumably, as a result of arsenic induced enhanced expression of genetic traits and physiological adaptations of the in vitro raised plants due to prolonged arsenic treatment from early stage of growth and development in vitro. Further biochemical characterization of in vitro raised and field raised arsenic treated plants and investigations with genetic markers specific for arsenic tolerance would justify for this difference in their nature.

Arsenic accumulation in the biomass of in vitro raised sporophytes after 12 months growth on arsenic amended soil. The in vitro developed plants grew luxuriantly in 100, 200 and 300 mg arsenic kg^{-1} of soil, and generated normal biomass by twelve months (Table 8 and Fig. 11). The average numbers of live and dead fronds in twelve months old plants didnrt show significant difference between control and arsenic treated plants. The concentrations of arsenic accumulated in the live and dead fronds of these plants were estimated using Inductively Coupled Plasma (ICP) Spectrophotometer as described earlier under the Materials and Methods. The amounts of arsenic, in live and dead fronds of plants (mg of arsenic kg^{-1} of dry weight, Table 8) indicated greater accumulation of arsenic in the live fronds in comparison to the dead fronds in all concentrations of arsenic (100, 200, 300 and 500 mg arsenic kg^{-1} of soil). It would seem that arsenic was actively transported to the live fronds that were in a physiologically active state of growth in comparison to the dead fronds. The maximum concentration of arsenic in live frond of plants was 3232 mg kg^{-1} dry weight, which were grown under 300 mg arsenic kg^{-1} of soil for duration of 12 months. The plants grown under 500 mg arsenic kg^{-1} of soil had a very few fronds, but some of them were still green. The live and dead fronds

of these plants treated with 500 mg arsenic had 3732 mg and 3237 mg arsenic kg^{-1} dry biomass respectively. The observations indicated that the estimated concentration of arsenic in the live fronds of this ecotype of *Pteris vittata* induced toxic effect and could be the threshold concentrations within the accumulated plant biomass.

The *Pteris vittata* plants collected by Ma et al. [36] accumulated 11.8–64 mg arsenic kg^{-1} above ground biomass in uncontaminated soil (arsenic level: 0.47–7.56 mg arsenic kg^{-1} soil), and up to 1400–7500 mg arsenic kg^{-1} biomass in arsenic contaminated soil (arsenic level: 18.8–1603 mg arsenic kg^{-1} soil). Our investigations with this Indian ecotype of brake-fern also indicate similar observations within the study period of 10 weeks. The growth characteristics and biomass accretion of that plant in arsenic contaminated soil indicated that biomass increase took place after a slow growth for 6–8 weeks [38], arsenic accumulation in the fronds increased with

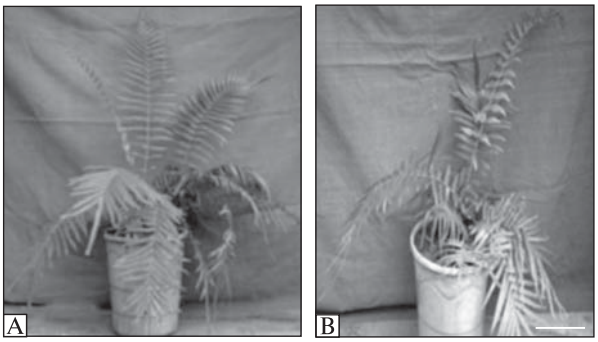

Fig. 11. Growth performance of in vitro developed sporophytes of *P. vittata* in As spiked treated soil (A–100 mg K^{-1}, B–200 mg Kg^{-1}) after 12 months. Bar represents 10.2 cm

growth of the plant that was a function of time, and highest concentrations of arsenic was in old fronds. Gumaelius et al. [43] reported that gametophytes of *P. vittata* grow normally in the medium containing 20 mM arsenate, and accumulate >2.5 % of their dry weight as arsenic in a manner similar to that the

Table 7

In vitro growth response of the *Pteris vittata* sporophytes in arsenic amended MS media supplemented with 50, 100 and 200 mg arsenic L^{-1} medium

Type of Culture	Growth response, weeks				
	2	**5**	**7**	**10**	**32**
MS medium Control	Ht – 2, Fnd – 7, 2–3, Lft – 1, greenish brown, Rt – Short	Ht – 4, Fnd – 10, 4–5, Lft – 1–2, green, Rt – 2–3	Ht – 5, Fnd – 10, 4–5, Lft – 2, green, Rt – Several, brownish	Ht – 8, Fnd – 12–14, 6–7, Lft – 3–5, green, Rt – Numerous	Ht – ⩾15, Fnd – 30, 12, Lft – 2–4, green, Rt – Profuse, intricate, brown to black, + –50 times
MSAs150	Ht – 1.5, Fnd – 4, 1–2, Lft – Small, brownish, Rt – Not seen	Ht – 1.5, Fnd – 5, 2, Lft – Small, brownish, Rt – Slender	Ht – 2, Fnd – 6, 2, Lft – Small, Rt – Few	Ht – 2, Fnd – 8, 3, Lft – Small, Rt – Scarce	Ht – ⩾15, Fnd – 28, 10, Lft – 2–4 cm, green, Rt – Profuse, intricate, brown to black, + –50 times
MSAs200	Ht – 3, Fnd – 5, 2–3, Lft – Small, green, Rt – Short	Ht – 4.5, Fnd – 7–8, 4, Lft – Enlarged, green, Rt – Several, elongated, brown	Ht – 4.5, Fnd – 7–8, 6.5, Lft – Large and green, Rt – Numerous, intricate	Ht – 7.3, Fnd – 10, 7, Lft – Large and green, Rt – Elongated, blackish	Ht – ⩾15, Fnd – 30, 10, Lft – 2–4 green, Rt – Profuse, intricate, brown to black, + –50 times
MSAs300	Ht – 3 cm, Fnd – 4, 3 cm, Lft – Small, green, Rt – Not seen	Ht – 3 cm, Fnd – 4, 3 cm, Lft – Small, green, Rt – Diminutive	Ht – 3 cm, Fnd – 4, 3 cm, Lft – Small, green, Rt – Meager	Ht – 3 cm, Fnd – 5, 4 cm, Lft – Small, green, Rt – Scarce	Ht – 8 cm, Fnd – 8, 5 cm, Lft – 1–2, green, Rt – Scarce, + –15 times

Note. As – mg arsenic L^{-1} MS medium; Ht – height of plants in cm; ⩾ – to the size of culture container; Fnd – no of fronds and length in cm; Lft – size of leaflets in cm, and color; Rt – number of roots, size and color; + – increase in plant size from initial size.

Table 8

Morphological features and quantity of arsenic accumulated in the aerial biomass of in vitro developed *Pteris vittata* sporophytes after growth in arsenic amended soil for 12 months

Concentration of arsenic mg kg^{-1} soil	Height of plant (cm)	Average length of fronds (cm)	Average number of fronds		Concentration of arsenic in plant biomass (mg kg^{-1} of dry weight)	
			Live	Dead	Live	Dead
Control (without arsenic)	34 ± 5	32 ± 7	26 ± 3	7-dry 3-partly dry	24.5	17
As150	34 ± 5	30 ± 2	25 ± 2	7-dry 3-partly dry	1751	951.5
As200	32 ± 3	32 ± 5	28 ± 5	8-dry 4-partly dry	2046.5	1326.5
As300	32 ± 5	30 ± 5	28 ± 5	8-dry 4-partly dry	3232	2132
As500	8 ± 5	9 ± 5	2	8-dry	3731.5	3236.5

Note. As – mg arsenic kg^{-1} of soil.

sporophytes. Whereas, the gametophytes of the related non-accumulating fern *Ceratopteris richardii* die at even low (0.1 mM) arsenic concentrations, and the gametophytes of the related arsenic accumulator *Pityrogramma calomelanos* tolerate and accumulate arsenic to intermediate levels compared to *P. vittata* and *C. richardii*. Their study also revealed natural variability in arsenic tolerance in the gametophyte populations from 40 different *P. vittata* sporophyte plants collected at different sites in Florida. The spores and prothallic-gametophyte of this ecotype of *Pteris vittata* grow in MS culture medium in vitro with 20–100 mg/L and up to 300 mg/L arsenic respectively. Germination of spores, growth performance of prothallic-gametophytes as well as development of saprophytes from the gametophytes was normal under arsenic supplemented growth medium in vitro.

Conclusion. Arsenic tolerance in plants may result from arsenic exclusion through avoidance or restriction of arsenic uptake and transport to the shoots [31, 47] or accumulation of higher concentration of arsenic within plant tissue in comparison to the surroundings [15,18, 31]. The molecular analysis of arsenic metabolism in terrestrial plant species have shown that arsenate-induced hytochelatins (PC) accumulation and PC-based arsenic sequestration are responsible for both normal and enhanced arsenate tolerance [30, 48–50].

The results of arsenic tolerance and accumulation by this ecotype of *Pteris vittata* used in this study show that arsenic accumulation in the plant biomass was statistically significant ($^{*}p < 0.05$) at all concentrations of soil arsenic (50, 100, 200, 500 and 1000 mg kg^{-1} soil), and at different durations (2^{nd}–10^{th} week, 4^{th}–10^{th} week and 6^{th}–10^{th} week). The in vitro raised *Pteris vittata* plants were tolerant to higher concentrations of arsenic (300 mg arsenic kg^{-1} of soil) in comparison to the plants raised in ex vitro conditions (field grown plants) using $NaH_2AsO_4 \cdot 7H_2O$ as the source of arsenic, which is more toxic to plants in comparison to its potassium salt. The growth performances of field grown plants were similar to control plants at 100 mg arsenic Kg^{-1} soil. But, the field grown plants generated poor biomass under ⩾200 mg arsenic kg^{-1} soil and the fronds turned brown between 3–6 weeks (Table 1). However, at this concentration of soil arsenic, the field plants accumulated 216 and 1827 mg arsenic kg^{-1} dry biomass of fronds after 2 and 10 weeks of treatment respectively (Table 2 and 5). On the contrary, the in vitro raised plants showed normal growth performance and biomass accumulation in 100, 200 and 300 mg arsenic kg^{-1} soil, which was better than the field plants (Table 7 and 8). Under high concentrations of soil arsenic (300 mg arsenic kg^{-1} soil), the in vitro selected plants accumulated 3232 mg arsenic and 2132 mg arsenic kg^{-1} live and dead frond biomass respectively (Table 8). Arsenic accumulation was more in live as well as dead fronds in case of in vitro raised plants (Table 8) in

comparison to the field raised plants (Table 6). The in vitro raised *Pteris vittata* plants were more tolerant to arsenic stress and accumulated more arsenic in the biomass. The mechanism of arsenic hyper accumulation in the plant biomass of *Pteris vittata* could be through compartmentalization or by molecular chaperons such as phytochelatins, which is being investigated.

This ecotype of brake-fern identified is hardy, perennial and survives under high temperature at 40–45 °C (data not presented), and adapt to new environment easily. It could be useful for phytoextraction of arsenic from contaminated soil. Further studies are carried out to assess genetic variations between the ecotypes of *Pteris vittata* plants for arsenic uptake and metabolism, and find out the reason for better survival and tolerance of in vitro plants in comparison to the field plants under higher concentrations of arsenic. This could help in selection of brake fern species and clones showing better arsenic hyper accumulation and suited for remediation of arsenic contaminated soils.

The authors gratefully acknowledge the assistances provided by trainee students for bench works, the instrumentation division of NEERI for arsenic estimation through ICP and Mr. A. Kulkarni, E. B. Division for statistical analysis. The authors are thankful to Director NEERI for providing facility for the work.

Б.К. Саранги, Т. Чакрабарти

ИЗУЧЕНИЕ ЭКОТИПА ПАПОРОТНИКА *PTERIS VITTATA* НА УСТОЙЧИВОСТЬ К МЫШЬЯКУ И НАКОПЛЕНИЕ РАСТИТЕЛЬНОЙ БИОМАССЫ

Экотип птериса ленточного (*Pteris vittata*) был исследован на устойчивость к мышьяку и его накопление в биомассе в условиях *in vivo* и *in vitro* с использованием почвы и агаризованной среды Мурасиге-Скуга (MS), содержащих мышьяк в разных концентрациях. Растения выращивали в почве, содержащей 100–1000 мг мышьяка на 1 кг почвы, или в среде Мурасиге-Скуга, в которую добавляли 10–300 мг/л $Na_2HAsO_4 \times \times 7H_2O$. Споры и гаплоидные гаметофитные проростки росли *in vitro* на среде MS с мышьяком. Растения, которые росли в почве, содержащей мышьяк, характеризовались нормальным ростом и накоплением биомассы и через 10 недель выращивания накапливали 1908–4700 мг мышьяка на 1 кг сухой надземной биомассы. Токсичность мышьяка проявлялась при его концентрации в почве свыше 200 мг/кг. Концентрации мышьяка, которые накапливались в растительной биомассе, были статистически значимыми ($p < 0.5$). Из спор и гаметофитных проростков, которые выращивали на среде MS с 50–200 мг/л мышьяка, развивались нормальные растения. Полученные *in vitro* растения были устойчивы к мышьяку в концентрации 300 мг/кг почвы и накапливали мышьяк до 3232 мг/кг сухой надземной биомассы, что означает улучшенные ростовые характеристики, формирование биомассы и накопление мышьяка по сравнению с растениями, выращенными в поле.

Б.К. Сарангі, Т. Чакрабарті

ВИВЧЕННЯ ЕКОТИПУ ПАПОРОТІ *PTERIS VITTATA* НА СТІЙКІСТЬ ДО МИШ'ЯКУ ТА НАКОПИЧЕННЯ РОСЛИННОЇ БІОМАСИ

Екотип птериса стрічкового (*Pteris vittata*) був досліджений на стійкість до миш'яку та його накопичення в біомасі в умовах *in vivo* і *in vitro* з використанням грунту та агаризованого середовища Мурасіге-Скуга (MS), що містять миш'як в різних концентраціях. Рослини вирощували на грунті, що містить 100–1000 мг миш'яку на 1 кг грунту, чи в грунті Мурасіге-Скуга, в котрий додавали 10–300 мг/л $Na_2HAsO_4 \cdot 7H_2O$. Спори та гаплоїдні гематофітні паростки росли *in vitro* на середовищі MS з миш'яком. Рослини, які ростуть на грунті, що містить миш'як, характеризувались нормальним ростом і накопиченням біомаси та через 10 тижнів вирощування накопичували 1908–4700 миш'яку на 1 кг сухої надземної біомаси. Токсичність миш'яку проявлялась при його концентрації в грунті більше 200 мг/кг. Концентрації миш'яку, котрі накопичувалися в рослинній біомасі, були статистично значимими ($p < 0.5$). Зі спор та гаметофітних паростків, котрі вирощували на грунті MS з 50–200 мг/л миш'яку, розвивались нормальні рослини. Отримані *in vitro* рослини були стійкими до миш'яку в концентрації 300 мг/кг грунту та накопичували миш'як до 3232 мг/кг сухої надземної біомаси, що означає покращання ростових характеристик, формування біомаси та накопичення миш'яку в порівнянні з рослинами, вирощеними на полі.

REFERENCES

1. *Tamaki S., Frankenberger W.T.* 1992. Environmental biochemistry of Arsenic. Reviews of Environmental Contamination and Toxicology 124:79–110.
2. *U.S.* EPA, 2000a. Environmental Protection Agency, National Primary Drinking Water Regulations; Arsenic and Clarifications to Compliance and New Source Contaminants Monitoring; Proposed Rule. Federal Register, Part II, 40 CFR Parts 141 and 142, Vol. 65 (121): 38888–38983.

3. *U.S.* EPA, 2002. Proven Alternatives For Above Ground Treatment Of Arsenic In Ground Water. EPA–542-S-02–002, June, 2002.
4. *Bech J., Poschenrieder C., Llugany M., Barcelo J., Tume P., Tobias F.J., Barranzuela J.L., Vasques E.R.* 1997. Arsenic and heavy metal contamination of soil and vegetation around a copper mine in northern Peru. Science of Total Environment 203:83–91.
5. *Nriagu J.O.* 1994. Arsenic in the Environment Part I: Cycling and Characterization, John Wiley and Sons, Canada.
6. *Hazardous Waste Consultant*, 2002. Technologies for treating arsenic in groundwater. Hazardous Waste Consultant 20: 114 –119.
7. *Welch A.H., Westjohn D.B., Helsel D.R., Wanty R.B.* 2000. Arsenic in ground water of the United States: occurrence and geochemistry. Ground Water 38: 589–604.
8. *Meharg A.A., Hartley-Whitaker J.* 2002. Arsenic uptake and metabolism in arsenic resistant and non-resistant plant species. New Phytologist 154:29–43.
9. *National* Research Council, 1999. National Research Council. Arsenic in Drinking Water. National Academy Press: Washington, DC.
10. *Jiang Q.Q., Singh B.R.* 1994. Effect of different forms and sources of arsenic on crop yield and arsenic concentration. Water Air Soil Pollution 74:321–343.
11. *Christen K.* 2001. The arsenic threat worsens. Environmental Science and Technology 35: 286A-291A.
12. *Brooks R.R.* 1998. Plants that Hyper Accumulate Heavy Metals. Their role in Phytoremediation, Microbiology, Archaeology, Mineral Exploration and Phytomining. CAB International.
13. *Raskin I., Kumar N.P.B.A., Dushenkov S., Salt D.E.* 1994. Bioconcentration of heavy metals by plants. Current Opinion in Biotechnology 5:285–290.
14. *Baker A.J.M.* 1987. Metal tolerance. New Phytologist 106:93–111.
15. *Baker A.J.M., Mcgrath S.P, Reeves R.D, Smith J.A.C.* 2000. Metal hyper accumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal polluted soils. In: Terry, N., Banuelos, G. (Eds.), Phytoremediation of Contaminated Soil and Water. Lewis, Boca Raton, pp.85–107.
16. *U.S. EPA*, 2000b. US EPA, Office of Research and Development. Introduction to Phytoremediation. EPA/600/R-99/107. 2000.
17. *Raskin I, Ensley B.D.* 2000. Phytoremediation of Toxic Metals, John Wiley and Sons, Inc., New York
18. *Dahmani-Muller H., Van Oort F., Gelie B., Balabane M.* 2000. Strategies of heavy metal uptake by three plant species growing near a metal smelter. Environmental Pollution, 109: 231–238.
19. *Reeves R.D., Schwarz C., Morel J.L., Edmondson J.* 2001. Distribution and metal-accumulating behavior of Thlaspi caerulescens and associated metallophytes in France. International J Phytoremediation 3:145–172.
20. *Licht L.A, McCutcheon S.C., Wolfe N.L., Carreira L.H.* 1995. Phytoremediation of organic and nutrient contaminants. Environmental Science and Technology 29:318–323.
21. *Porter E.K., Peterson P.J.* 1975. Arsenic accumulation by plants on mine waste (United Kingdom). The Science of The Total Environment 4: 365–371.
22. *Macnair M.R, Cumbes Q.* 1987. Evidence that arsenic tolerance in *Holcus lanatus* L. is caused by an altered phosphate uptake system. New Phytologist 107:387–394.
23. *Meharg A.A., Macnair M.R.* 1991b. Uptake, accumulation and translocation of arsenate in arsenate-tolerant and non-tolerant *Holcus lanatus* L. New Phytologist 117: 225–231.
24. *Meharg A.A., Macnair M.R.* 1991a. The mechanisms of arsenate tolerance in *Deschampsia cespitosa* (L.) Beauv. and *Agrostis capillaris* L. New Phytologist 119: 291–297.
25. *Paliouris G., Hutchinson T.C.* 1991. Arsenic, cobalt and nickel tolerances in two populations of *Silene vulgaris* (Moench) Garcke from Ontario, Canada. New Phytologist 117: 449–459.
26. *Sneller F.E.C., Van Heerwaarden L.M., Kraaijeveld-Smit F.J.L., Ten Bookum W.M., Koevoets P.L.M., Schat H., Verkleij J.A.C.* 1999. Toxicity of arsenate in *Silene vulgaris*, accumulation and degradation of arsenate-induced phytochelatins. New Phytologist 144: 223–232.
27. *Sharples J.M., Meharg A.A., Chambers S.M., Cairney J.W.G.* 2000. Symbiotic solution to arsenic contamination. Nature 404: 951–952.
28. *Bleeker P.M., Schat H., Vooijs R., Verkleij J.A.C., Ernst W.H.O.* 2003. Mechanisms of arsenate tolerance in *Cytisus striatus*. New Phytologist 157: 33–38.
29. *Pickering I.J., Prince R.C., George M.J., Smith R.D. George G.N., Salt D.E.* 2000. Reduction and coordination of arsenic in Indian mustard. Plant Physiology 122:1171–1177.
30. *Schmöger M.E.V., Oven M., Grill E.* 2000. Detoxification of arsenic by phytochelatins in plants. Plant Physiology 122:793–801.
31. *Koch I., Wang L., Ollson C., Cullen W.R., Reimer K.J.* 2000. The predominance of inorganic arsenic species in plants from Yellowknife, Northwest Territories, Canada. Environ Science and Technology 34: 22–26.
32. *Meharg A.A.* 2003. Variation in arsenic accumulation-hyperaccumulation in ferns and their allies. New Phytologist 157: 25–31.
33. *Matschullat J.* 2000. Arsenic in the geosphere – a review. Science of Total Environment 249: 297–312.
34. *Carbonell-Barrachina A.A., Burlo F., Burgos-Hernanadez A., Lopez E., Mataix J.* 1997. The influence of arsenic concentration on arsenic accumulation in tomato and bean plants. Scientia Horticulturae. 71:167–176.

35. *Kabata-Pendias A., Pendias H.* 1991. Arsenic. In: Trace Elements In Soils And Plants. CRC Press, Boca Raton, Florida, pp 203–209.
36. *Ma L.Q., Komar K.M. Tu C., Zhang W., Cai Y., Kennelly E.D.* 2001. A fern that hyperaccumulate arsenic. Nature 409:579.
37. *Baker A.J.M., McGrath S.P., Sidoli C.M.D., Reeves R.D.* 1994. The possibility of in-situ heavy metal decontamination of polluted soils using crops of metal accumulating plants. Resource Conservation Recycling 11: 41–49.
38. *Tu C., Ma L.Q., Bondada B.* 2002. Arsenic accumulation in the hyper accumulator Chinese Brake and its utilization potential for phytoremediation. Journal of Environmental Quality 31:1671–1675.
39. *Tu C., Ma L.Q.* 2002. Effects of arsenic concentrations and forms on arsenic uptake by the hyper accumulator Ladder Brake. Journal of Environmental Quality 31:641–647.
40. *Zhang W., Cai Y., Tu C., Ma L.Q.* 2002. Arsenic speciation and distribution in an arsenic hyperaccumulating plant. The Science of Total Environment 300:167–177.
41. *Visoottiviseth P., Francesconi K., Sridokchan W.* 2002. The potential of Thai indigenous plant species for the phytoremediation of arsenic contaminated land. Environmental Pollution 118: 453–461.
42. *Francesconi K., Visoottiviseth P., Sridokchan W., Goessler W.* 2002. Arsenic species in an arsenic hyperaccumulating fern, *Pityrogramma calomelanos*: a potential phytoremediator of arsenic-contaminated soils. Science of Total Envronment 284:27–35.
43. *Gumaelius L., Lahner B., Salt E.D., Banks J.A.* 2004. Arsenic Hyperaccumulation in Gametophytes of *Pteris vittata*. A new model system for analysis of arsenic hyperaccumulation. Plant Physiology 136: 3198–3208.
44. *Murashige T., Skoog F.* 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15:473–497.
45. *Hoagland D.R., Arnon D.I.* 1938. The water culture method for growing plants without soil. *California* Agriculture Experimental Station Circular. 347:461
46. *Knudson L.* 1946. A nutrient solution for the germination of orchid seed. Bulletin of American Orchid Society 15: 214–217.
47. *Dushenko W.T., Bright D.A., Reimer K.J.* 1995. Arsenic bioaccumulation and toxicity in aquatic macrophytes to gold-mine effluent: relationship with environmental partitioning, metal uptake and nutrients. Aquatic Botany 50:141–158.
48. *Salt D.E., Smith R.D., Raskin I.* 1998. Phytoremediation. Annual Review of Plant Physiology and Plant Molecular Biology 49: 643–668.
49. *Zenk M.H.* 1996. Heavy metal detoxification in higher plants – a review. Gene 179: 21–30.
50. *Hartley-Whitaker J., Ainsworth G., Vooijs R., Ten Bookum W.M., Schat H., Meharg A.A.* 2001. Phytochelatins are involved in differential arsenate tolerance in *Holcus lanatus L.* Plant Physiology 126: 299–306.

Received 23.08.07