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Effect of arsenic contaminated irrigation water on *Lens culinaris* L. and toxicity assessment using *lux* marked biosensor

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Abstract

Contamination of irrigation water represents a major constraint to Bangladesh agriculture, resulting in elevated levels in the terrestrial systems. *Lux* bacterial biosensor technology has previously been used to measure the toxicity of metals in various environmental matrices. While arbuscular mycorrhizal fungi have their most significant effect on phosphorus uptake, but showed alleviated metal toxicity to the host plant. The study examined the effects of arsenic and inoculation with an arbuscular mycorrhizal fungus, *Glomus mosseae*, on lentil (*Lens culinaris* L. cv. *Titore*). Plants were grown with and without arbuscular mycorrhizal inoculum for 9 weeks in a sand and terra-green mixture (50:50, *V*/*V*) and watered with five levels of arsenic (0, 1, 2, 5, 10 mg As/L arsenate). The results showed that arsenic addition above 1 mg/L significantly reduced percentage of mycorrhizal root infection. On further analysis a close relationship was established with the vegetative and reproductive properties of lentil (*L. culinaris*) plants compared to the percentage bioluminescence of the soil leachate. However, arbuscular mycorrhizal fungal inoculation reduced arsenic concentration in roots and shoots. Higher concentrations of arsenic (5, 10 mg As/L arsenate) reduced the mycorrhizal efficiency to increase phosphorus content and nitrogen fixation. Therefore, this study showed that increased concentration of arsenic in irrigation water had direct implications to the lentil (*L. culinaris*) plants overall performance. Moreover the use of bioassay demonstrated that mycorrhiza and clay particle reduced arsenic bioavailability in soil.

Key words: bioassay; contaminated water; lentil; trace metals; vegetative response
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Introduction

Arsenic contamination of irrigation water represents a major constraint in Bangladesh agriculture, resulting in elevated levels in the soils. In some areas of Bangladesh, arsenic contamination of groundwater reached up to 2 mg/L (British Geological Survey, 2000; Tondel et al., 1999), while the national limit of arsenic in drinking water is 0.05 mg/L. The people of Bangladesh not only drink the arsenic contaminated groundwater, but also irrigate their crops with this water, as groundwater is the main source of irrigation. Groundwater is extensively used for irrigation of crops resulting in elevated levels of arsenic in soils, rice arsenic uptake (Duxbury et al., 2003; Meharg and Rahman, 2003), vegetables (Alam et al., 2003) and other agricultural products (Abedin et al., 2002).

Due to the extensive use of irrigation water for agricultural purposes in Bangladesh the need to understand the speciation behaviour of arsenic is important. For example a wide range of redox conditions may prevail in wetland soils, and this influences arsenic speciation (Brannon and Patrick, 1987). So far, four arsenic species (arsenite, arsenate, monomethyl arsenic acid (MMAA) and dimethyl arsinous acid (DMAA)) have been found in the water/plant/soil system (Sohrin et al., 1997). In general, the ratios of As(III) and As(V), as well as total arsenic concentrations, are controlled by pH, redox conditions of groundwater, adsorption/desorption process of metal hydroxides and the source of arsenic (Abdullah et al., 1995; Nickson et al., 1998). Organic sources of arsenic are considered less toxic to plants than inorganic sources (Sheppard, 1992). Arsenic chemical form was reported to be more important than the arsenic level in determining phytotoxicity in *Spartina alterniflora* and *Spartina patens* (Carbonell-Barrachina et al., 1998), turnip (Carbonell-Barrachina et al., 1999) and rice (Marin et al., 1992). The speciation of arsenic in the environment is of critical importance because organic and inorganic compounds differ largely in their toxicity (Leonard, 1991). For example As(III) is reported to be 25–60 times more toxic than As(V), and several hundred times more toxic than organic arsenicals (Morrison et al., 1989).

Lentil (*Lens culinaris* L.), which is the main pulse

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crop in Bangladesh is grown in the dry season, therefore irrigation is needed for successful cultivation. In the case of other annual grain legumes, lentil can fix atmospheric nitrogen via symbiotic rhizobia in root nodules and hence has potential in maintaining soil fertility (Crook et al., 1999). Background concentrations of arsenic in Bangladesh soils are 4–8 mg As/kg. However, in areas where irrigation is carried out with arsenic contaminated groundwater, soil arsenic concentrations can reach up to 83 mg/kg (Ullah, 1988; Meharg and Rahman, 2003). Arsenic levels in surface soils (depth 0–15 cm) ranged from 0–31.8 mg/kg, with higher concentrations in the 15–30 cm soil, reaching 56 mg/kg (Alam and Sattar, 2000). In both cases, soil arsenic levels were correlated with local well water concentrations, suggesting that the soils had become contaminated through irrigation with arsenic-contaminated water. Apart from the effect of irrigation water humans may be exposed to arsenic from a variety of environmental sources, but drinking water and dietary sources form the major exposure routes for most people (Tao and Bogler, 1999).

When considering the toxicity of arsenic to plants, the role of mycorrhizal associations with plants must also be considered. One of the principal role of mycorrhizal fungi is phosphorus uptake (Smith and Read, 1997). This could potentially be a problem on arsenic contaminated substrates because P and As uptake in plants are similar. However, there is also growing evidence that mycorrhizal fungi may alleviate metal or metalloid (e.g., arsenic) toxicity to the host plant by acting as a barrier to uptake (Leyval et al., 1997).

To evaluate the bioavailable fraction of metals/metalloids in environmental matrices bioassay techniques were necessary. Therefore, a tool-kit of techniques was developed, including whole cell biosensor to complement chemical assays. A luminescence based bacterial biosensor (*Escherichia coli* HB101 pUCD607) (via a multi-copy plasmid) was used for toxicity assessment. Although chemical analysis of environmental samples can produce a measure of the total concentration of the chemical present in the sample, it will not indicate what is bioavailable in the sample (Steinberg et al., 1995) and hence what the likely toxic impact will be. Bioavailability is important for many organic compounds, particularly when they are associated with humic and other natural complexing agents (Shaw et al., 2000). There is an increasing trend towards the use of biological techniques for monitoring the hazards associated with environmental pollution by industry (Mwinyihija et al., 2005a) and regulators alike. The detection of chromium toxicity using biological techniques involving microorganism (Cervantes et al., 2001; Mwinyihija et al., 2005b) and plants (Prasard et al., 1991) has previously been applied for environmental diagnostics. The toxicity of chlorinated phenols has also been assessed by bioluminescence-based ecotoxicity tests (Lagido et al., 2001).

A novel biological technique involving microorganism like *lux* (reporter genes encoding marine bacterial luminescence (i.e., light production)) bacterial biosensor technology has been used to measure the toxicity of metals in a number of matrices ranging from aqueous solutions of single compounds to industrial effluents (Mwinyihija et al., 2005b; Sinclair, 1999). The light emission intensity is proportional to the concentration of the toxic analyte over a certain concentration range, allowing one to perform a quantitative analysis. Paton et al. (1995) used the luminescence response of a chromosomally *lux*-marked bacterium, *P. fluorescens*, to assess the toxicity of metal salts. Chaudri et al. (1999) used the luminescence response of *lux*-marked bacteria to assess the toxicity of zinc in pore water in a long-term sewage sludge field. Gälli et al. (1994) used naturally luminescent marine bacteria (Microtox) to test the toxicity of soil from a site contaminated with various pesticides, dyes and other chemicals.

The advantages associated with the use of genetically modified bacterial biosensors over other forms of ecotoxicity testing are that they are rapid, sensitive, easy to culture and maintain, flexible in terms of selecting for environmental relevance, and reliable tools which integrate the many factors contributing to environmental toxicity (Wild et al., 1993). *Lux* bacterial biosensor assays of toxicity can be linked to sample manipulation to assess the scope and the nature of possible remediation strategies (Mwinyihija et al., 2005b).

This study simulated the arsenic level in Bangladesh irrigation water using potted experiment in the green house at the University of Aberdeen, UK. This approach incorporated the use of sand and terra-green mixture to study properly the effect of Arbuscular Mycorrhizal fungus and arsenic on the root system of the lentil plant. This is a novel technique used for the first time incorporating the use of biosensor to evaluate the toxicity of irrigation water on lentil (*Lens culinaris* L.) crop. The aim of this study was to assess the toxicity (using *lux*-marked biosensor *Escherichia coli* HB101 pUCD607) of arsenic contaminated irrigation water on *L. culinaris* growth and nutrient uptake. This was activated by analysing the collected soil leachate and comparing the level of uptake by lentils.

1 Materials and methods

1.1 Preparation of growth medium and leachate

The experimental pots were filled with 1.2 kg sand and terra-green (50:50, *V*/*V*). The substrate was sieved (2 mm) and then autoclaved (121°C) for 1 hr on two consecutive occasions. Leachate was obtained by using 10 g of air dried growth medium soil. The growth medium soil was then placed into 100 mL conical flask and mixed with 25 mL of deionised water and agitated for 3 hr. After shaking, the material was passed through the two types (125 mm diameter and 0.22 μm diameter) of filter papers sequentially.

1.2 Growing plants

The plants were grown in the greenhouse under controlled environmental conditions with a 16 hr/8 hr day/night regime and a day/night temperature of 20/15°C. Bulk

inoculum of *Glomus mosseae* (UK115) was obtained from INVAM (International culture collection for vesicular arbuscular mycorrhizal fungi) and used directly for the experiment. Infected roots and substrate containing fungal mycelium and spores, were used for mycorrhizal inoculum. The inoculum (50 g/pot) was then spread in a thin layer and 100 g sterilised substrate was spread over the inoculum. The INVAM inoculum UK115 was grown on Sorghum plants (*Sorghum vulgare*). Non mycorrhizal pots were treated with the same amount (50 g/pot) of sterilised sand/terra green. Seeds of lentil (*Lens culinaris* L. cv. *Titore)* were surface sterilised with 10% H_2O_2 for 10 min and germinated for 48 hr on moist filter paper in a Petri dish. Three germinated seeds were transplanted into each pot. *R. leguminosarum* was applied directly onto the pre-germinated seeds at planting time with medium viscosity carboxymethyl cellulose (CMC) as a liquid inoculum (0.5 mL/seedling) containing 3.3 $\times$ 10^8 cells/mL. To each pot 200 mL nutrient solution was applied as modified Hoagland's solution (1/10 N of a full-strength solution and without P, NH_4NO_3 1 mmol/L, $CaCl_2{\cdot}2H_2O$ 10 mmol/L, KCl 7 mmol/L, $MgSO_4{\cdot}7H_2O$ 4 mmol/L, FeNaEDTA 0.4 mmol/L, H_3BO_3 0.4 mmol/L, $ZnSO_4{\cdot}7H_2O$ 0.4 mmol/L, $CuSO_4{\cdot}5H_2O$ 0.4 mmol/L, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.4 mmol/L, $MnCl_2{\cdot}4H_2O$ 0.4 mmol/L) at the start of the experiment and thereafter at 100 mL/week. Pots were watered every alternate day with arsenate contaminated water as a solution of $Na_2HAsO_4{\cdot}7H_2O$ in deionised water at concentrations of 0 (control treatment), 1.0, 2.0, 5.0 and 10.0 mg As/L. To each pot 3.1 L of irrigation water was used.

1.3 Measurements

Nine weeks after sowing, shoots and roots were harvested separately. Roots were collected by sieving the pot contents through a 1 mm mesh and were washed with tap water to remove adhering substrate. Roots were stored in 50% ethanol for measuring percent mycorrhizal infection, root length and chemical analysis. Plant biomass was determined after drying shoot, root and pod material at 70°C for 48 hr. Total root length was determined with Win-Rhizo 5.0A software (Regent Instruments, Quebec, Canada) at 200 dpi resolution.

1.4 Chemical analysis

Soil and plant samples were digested with HNO_3-H_2O_2 (Tang and Miller, 1991) to determine total arsenic. Concentrated HNO_3 (2.5 mL) was added to about 200 mg of dry sample weighed into a 75 mL digestion tube and allowed to stand overnight. Then, the same amount of H_2O_2 was added and the digestion tubes were placed on a heating block and the temperature gradually raised to 120°C. The samples were allowed to digest for 3 hr, after which the volume was reduced to 3–4 mL. The digests were cooled and made up to 10 mL with deionised water in graduated tubes. Arsenic concentrations were determined using a hydride generation atomic absorption spectrometer (FI-HG-AAS, Perkin Elmer Analyst 300 fitted with a flow injection analysis system, FIAS 100 (Perkin Elmer, USA). Total N and P of shoot and root were determined by colorimetry following sulphuric acid-hydrogen peroxide digestion (Allen, 1989). Total N was measured by a continuous flow analyser (model-Technicon Autoanalyser II, USA) and total P concentrations were measured by a flow injection analyser (model-Tecator FIAster 5010 analyser, Haganas, Sweden).

1.5 Determination of nitrogenase activity and N_2 fixation

Nitrogenase activity was measured by an indirect method, the Acetylene Reduction Assay. Entire root systems of three lentil plants were enclosed in 150 mL bottles capped with a suba-seal, and to 10% acetylene in the dark at 20°C for 12 hr. Ethylene was determined using a Perkin-Elmer 8420 gas chromatograph (GC) (Perkin-Elmer Co., Beaconsfield, UK) equipped with H_2-FID detector and a porpak-T (80-100 mesh) column. One milliliter of gas from the sealed flasks was taken to the vacutainas (3.35 mL) and the ethylene gas measured as μL/L basis. Then total amount of C_2H_4 gas in the flask was measured at the unit of nL and converted into nmol.

1.6 Mycorrhizal infection

Entire root systems were cut into pieces of 1 cm. From these, 10% were randomly selected for mycorrhizal detection. Roots were cleared in 10% KOH for 10 min at 90°C in a water bath, rinsed in water, and then soaked in 0.1 mol/L hydrochloric acid for at least 2 hr. After soaking, the roots were stained in an acidic glycerol solution containing 0.05% Trypan Blue for 30 min at 90°C in a water bath. The roots were de-stained overnight in the mixture of lactic acid:glycerol:water (14:1:1, *V/V/V*) (Kormanik and McGraw, 1982) and mounted on glass slides. Mycorrhizal infection was determined on 30–50 root pieces using a compound microscope (×400) with an eye-piece crosshair which was moved to randomly 100 selected positions per sample to determine percentage of root length infected (McGonigle et al., 1990).

1.7 Bioassay

1.7.1 Preparation of *lux*-marked *Escherichia coli* HB101 (pUCD607)

Escherichia coli HB101 (pUCD607) had been previously *lux*-marked (and derived from *E. coli* K12 strain) using plasmid pUCD607 (Rattray et al., 1990) (with a *lux* CDABE cassette from *V. fischeri*, Shaw and Kado, 1986) using calcium chloride transformation. The plasmid is under the control of a constitutive promoter of a tetracycline resistance gene (Close et al., 1984). The plasmid (pUCD607) also contains resistance genes for ampicillin, kanamycin and spectinomycin/streptomycin. *E. coli* HB101 (pUCD607) light production is constitutive, with the *lux* cassette downstream of a strong constitutive promoter.

1.7.2 Freeze-drying of *lux* bacterial biosensors *E. coli* HB101 (pUCD607)

Cells of *E. coli HB101* were grown on Luria Bertani

Glucose (LBG) agar plates containing 50 μg/L of kanamycin for 2 days in a incubator at 25°C. An overnight culture of bacteria was inoculated with 10 mL of LBG medium containing 50 μg/L of kanamycin with a single colony from the agar plate. The cultures were grown overnight on an orbital shaker incubator at 25°C, 200 r/min. Aliquots (500 μL) of these bacterial cultures were used to inoculate 2 conical flasks each containing 500 mL of LBG medium amended with 50 μg/L of kanamycin. These flask cultures were then incubated on an orbital shaker at 25°C, 200 r/min until an OD_{550} of 0.6, with relative luminescence units of 1×10^6, was reached. The cultures were harvested by centrifugation in a MSE Coolspin 2 at 2000 ×*g*, 4°C for 40 min. The supernatant was discarded and the cells were resuspended in 130 mL of sterile Mist desiccans (100 mL of horse serum, 30 mL of LBG medium and 10 g of glucose). Aliquots (1 mL) of resuspended cultures were pipetted into 5 mL sterile freeze-drying vials with rubber stoppers. The vials were placed in liquid nitrogen for at least 90 min and then transferred to a Modulyo Edwards freeze drier (General Scientific Instrument Services Inc., USA) for 16–24 hr at –80°C under vacuum. Freeze dried cultures were stored at –20°C. Optical density was measured using a Cecil instruments CE373 linear readout grating spectrophotometer (Cambridge, England) at 550 nm. Light output was measured in relative light units (RLU) using a BioOrbit 1251 luminometer (Labtech International, Uckfield, UK).

1.7.3 Toxicity testing using *Escherichia coli* HB101 pUCD607

Determination of toxicity was based on the bioluminescence response of the lux-modified biosensor, *E. coli* HB101 pUCD607 (Amin-Hanjani et al., 1993). The biosensor was stored at –20°C and resuscitated from freeze dried prior to bioassay. Freeze dried cultures of *Escherichia coli* HB101 pUCD607 were resuscitated in 10 mL of sterile 0.1 mol/L KCl (contained in a Universal). One mL of KCl was added and the culture resuspended by mixing (drawing up and down 5 times into a PI000 Gilson PIPETMAN pipettes, USA). The resuspended culture was transferred back to the universal and the culture placed in a shaking (200 r/min) incubator (25°C) for 1 hr.

1.7.4 Sample addition and luminometry measurements

One hundred microlitre of the resuscitated biosensor suspension was added to the samples at 15 sec intervals, accurately timed for measurement in the Bio Orbit 1253 luminometer (Labtech International, Uckfield, UK). Each sample was exposed to the sensor for exactly the same time. Samples were incubated for 15 min before light output measurements were carried out at 15 sec intervals. This ensured the same exposure time to the potentially toxic elements for cells in each of the cuvettes. The output from the luminometer resulting from each assay carried out was recorded in RLU (equating to mV/(10 sec·mL)). The light output was then converted to percentage maximum bioluminescence (*B*, %). This was calculated against a blank of double deionised water adjusted to pH 5.5, the optimum pH for bioluminescence.

$$B = I_S / I_C \times 100\%$$

where, I_S is RLU's emitted by the cells exposed to the sample and I_C is RLU's emitted by the cells exposed to the control.

The percentage maximum bioluminescence was determined for the three sample replicates. A mean of this determination was then calculated. The assay performance was monitored by reference to the response of the control, the reproducibility of the response to the three replicates and the response to a standard of trichlorophenol. Effect of exposure time on toxicity to a range of standard solutions of Zn and Cu were prepared by dilution with double deionised water at pH 5.5.

1.8 Experimental design and statistical analysis

The experiment was a completely randomised block design with five replications and a factorial combination of *Glomus mosseae* (inoculated and uninoculated) and five doses of arsenic contaminated irrigation water. All data were analysed using a general linear model of analysis of variance (ANOVA) to determine the effect of factors and interactions between factors using Minitab 13. Before ANOVA shoot As and root As concentrations were transformed by Box-Cox transformation. Regression analyses were carried out using excels. Root length was expressed as root tolerance index (RTI) and shoot height was expressed in terms of relative shoot height (RSH), both were calculated by dividing length of the arsenic treatments with the length of the zero exposure treatment. Plant element (N, P, As) content was determined by multiplication of weight and the respective concentration.

2 Results

2.1 Effect on growth of lentil

Shoot dry weight decreased with increasing concentration of arsenate in irrigation water (Fig. 1a). The effect of arsenic on root dry weight, flower number, pod dry weight, root tolerance index and relative shoot height per pot followed a similar pattern to shoot dry weight (Fig. 1b–f).

Mycorrhizal inoculation increased shoot dry weight ($p = 0.077$), root dry weight ($p = 0.092$), and flower number ($p = 0.092$), pod dry weight ($p < 0.001$) and relative shoot height ($p = 0.091$) (Fig. 1). There was no significant interaction between arsenic addition and mycorrhizal inoculation except for pod dry weight.

2.2 Effects on nitrogen fixation and N, P and As content

Nitrogen fixation, N and P content in lentil decreased significantly ($p < 0.001$) with increasing arsenic concentration in irrigation water (Fig. 2). The level of arsenic content increased significantly ($p < 0.001$) with increasing arsenic concentration of irrigation water. Nitrogen fixation, N and P content increased significantly ($p < 0.001$) with mycorrhizal inoculation (Fig. 2). Arsenic interaction with mycorrhiza significantly decreased N fixation and total P

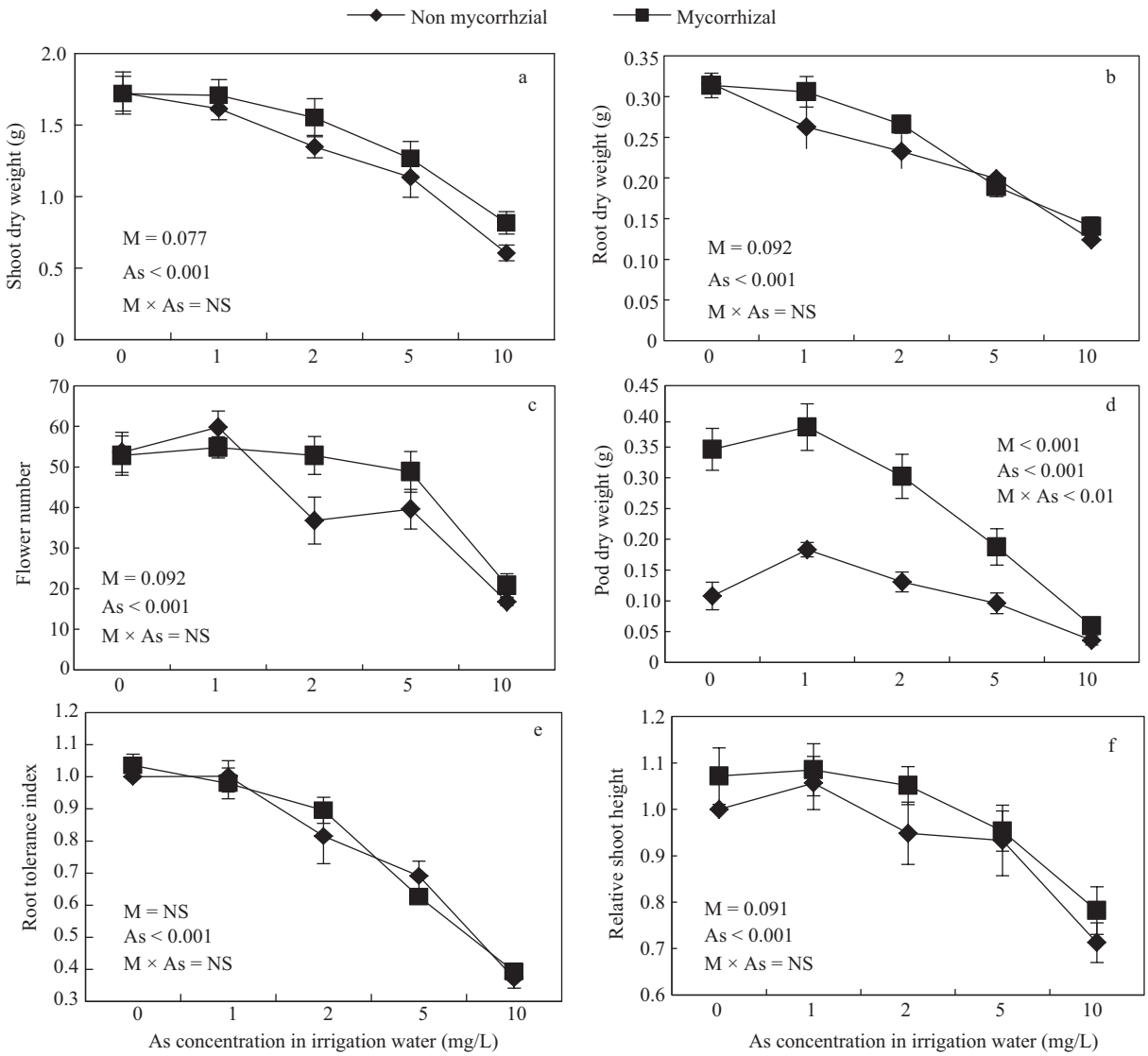

Fig. 1 Mycorrhizal effect on shoot dry weight (a), root dry weight (b), flower number (c), pod dry weight (d), root tolerance index (e), and relative shoot height (f) of lentil at five levels of arsenic contaminated irrigation water. The error bars are ± standard errors of five replicates. M: mycorrhiza; As: arsenic; NS: non significant.

content, that is higher concentrations of arsenic (5, 10 mg As/L arsenate) when compared to lower concentrations (1, 2 mg As/L arsenate) reduced the mycorrhizal efficiency to increase P content and N fixation (Fig. 2a, c).

2.3 Effect on mycorrhizal infection

Arsenic addition above 1 mg As/L significantly reduced percent root length infection ($p < 0.001$). At the highest level of arsenic addition, 6% of the root length was attributed to mycorrhizal (Fig. 3).

2.4 Effect on bioluminescence

The level of arsenic content in plant increased with increasing concentration in irrigation water (Fig. 2d) and bioluminescence decreased with increasing concentration of arsenic in irrigation water (Fig. 4). Prior to bioassay the soil leachate was filtered using two types of filter papers (125 mm diameter (to allow colloidal particles to pass through and 0.22 μm diameter (to filter colloidal particulate matter out of solution)) but when the two filter papers were compared a significant ($p < 0.001$) difference was observed (Fig. 4). Table 1 shows the correlation of the vegetative and reproductive responses of lentil plants (*Lens culinaris* L.) to that of percentage bioluminescence of the soil leachate. A close relationship was established with the highest correlation observed on root length ($r = 0.86$) and the lowest noted on pod dry weight ($r = 0.63$).

When the leachate was filtered using 125 mm diameter filter paper the mycorrhiza inoculated sample indicated a 50% bioluminescence ($y = -6.4779x + 103.07$, $R^2 = 0.9837$) at arsenic concentration of 8.19 mg/L. However,

Table 1 Correlation and *p* values for vegetative and reproductive properties of lentil (*Lens culinaris* L.) plants compared to the percentage bioluminescence of the soil leachate

Plant responses	Correlation	*p* value
Plant height	0.82	< 0.01
Shoot dry weight	0.85	< 0.001
Root dry weight	0.83	< 0.001
Root length	0.86	< 0.001
Pod dry weight	0.63	< 0.001
Flower number	0.76	< 0.001

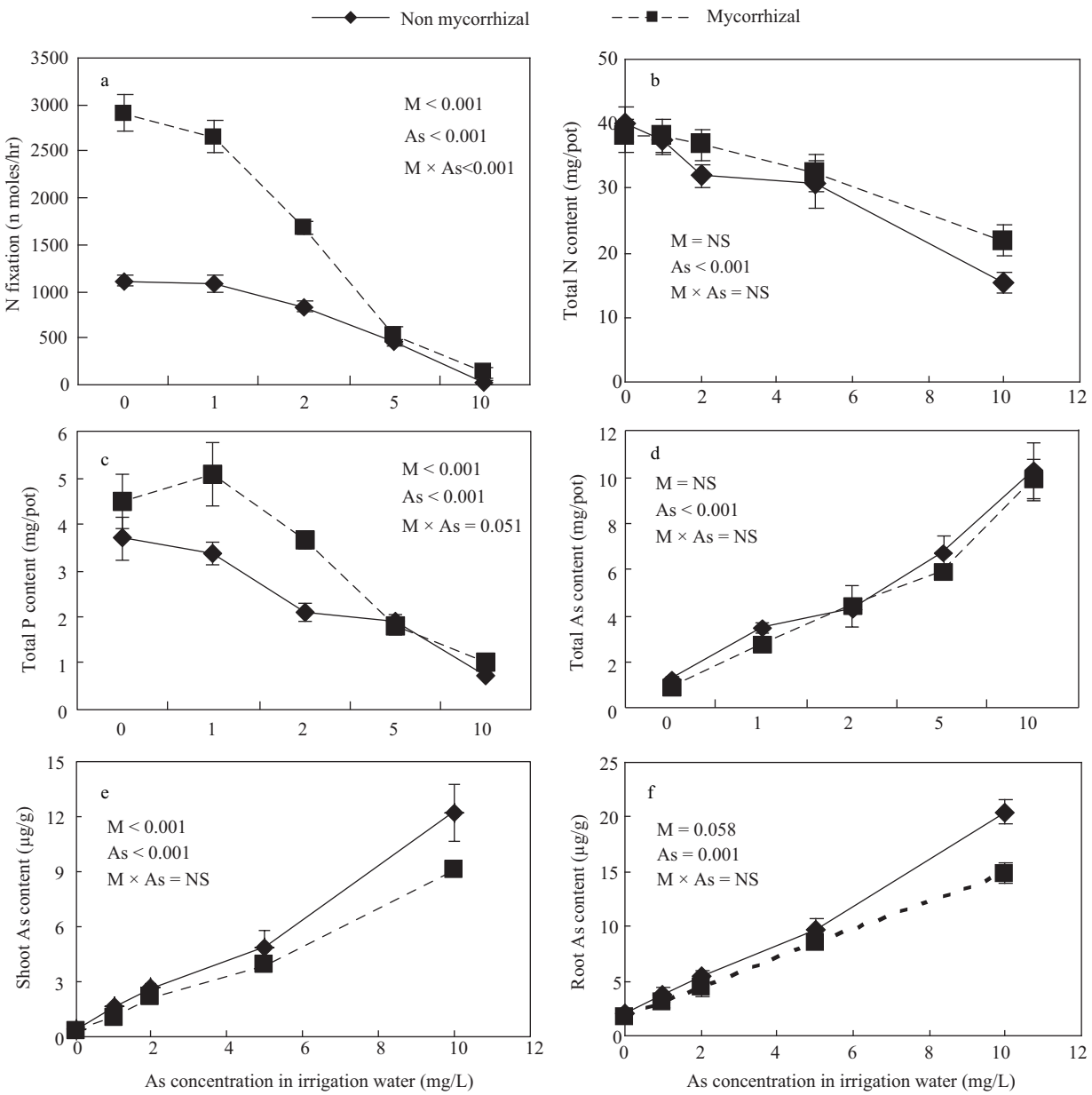

Fig. 2 Mycorrhizal effect on the nitrogen fixation (a), N (b), P (c) and As (d, e, f) content of lentil at five levels of arsenate contaminated irrigation water. The error bars are ±standard errors of five replicates. M: mycorrhiza, As: arsenic and NS: non significant.

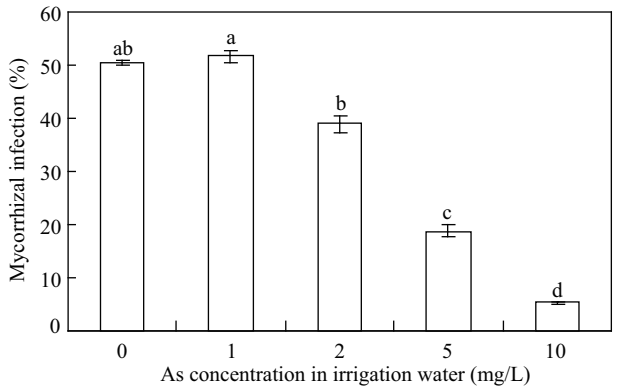

Fig. 3 Arsenic effect on mycorrhizal infection. The error bars are ±standard errors of five replicates. Different letters indicate significant difference between treatments.

for non-mycorrhizal sample with the same filter paper, it was observed that the concentration of arsenic was 6.92 mg/L at 50% bioluminescence ($y = -7.446x + 101.57$, $R^2 = 0.9866$). However, when non-mycorrhizal samples were measured (using 0.22 µm filter paper) at 50% bioluminescence ($y = -8.2036x + 97.805$, $R^2 = 0.9891$) the treatment showed arsenic concentration of 5.83 mg/L. While mycorrhizal inoculated samples showed 6.34 mg/L ($y = -7.5588x + 97.934$, $R^2 = 0.9994$) at 50% bioluminescence. Comparatively, when a 0.22 µm was used instead of 125 mm filter paper a lower arsenic concentration was obtained indicating that the result of bioluminescence was affected with the particulate fraction of the soil leachate (Fig. 4). In addition, the results also demonstrated the effect of mycorrhizal inoculation in reducing toxicity.

3 Discussion

Soil ecosystems have been extensively contaminated with toxic heavy metals due to various human activities e.g.

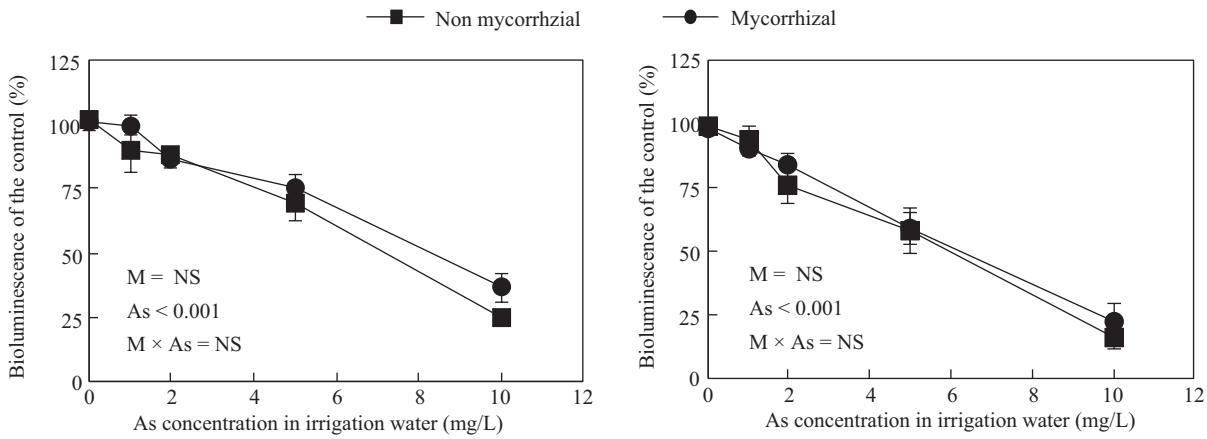

Fig. 4 Effect of arsenic contaminated irrigation water in bioluminescence percentage with 125 mm filter paper (a) and with 0. 22 μm filter paper (b). M: mycorrhiza, As: arsenic and NS: non significant.

contaminated irrigation water, disposal pits and landfills, sometimes leading to mobilisation of these metals up the food chain, thereby threatening human health (Naidu et al., 1997). To evaluate the effect of arsenic contaminated irrigation water on leguminous (*Lens culinaris* L.) plant growth, nutrient uptake and subsequently assess toxicity, several factors were investigated. The study evaluated factors such as growth response of lentils; effect on nitrogen fixation, N, P and As uptake; effect on mycorrhizal infection and effect on bioluminescence (to evaluate the bioavailable fraction of arsenic). Determination of toxicity during the study was based on the bioluminescence response of the *lux*-modified biosensor, *E. coli* HB101 pUCD607 (Amin-Hanjani et al., 1993). The biosensor's environmental relevancy and wide pH range (3–10) (Palmer et al., 1998) provided reliable results, which indicated an increase of toxicity on increased arsenic concentration.

The experimental data in this study showed that an increase on arsenic concentration in irrigation water resulted in high arsenic content in vegetative responses (e.g., root and shoot) and soil leachates. Fundamentally arsenic is not an essential nutrient for plants and appears not to be involved in specific metabolic functions when supplied at low concentrations (Lepp, 1981; Marin et al., 1993). It was observed during the current study that at higher concentrations, arsenic could interfere with the metabolic process and can hamper normal growth and development of plants through arsenic induced phytotoxicity this was in concurrence with studies by Marin et al. (1993). Plants can develop toxicity symptoms (Carbonell-Barrachina et al., 1995; Marin et al., 1992); reduction in root growth (Carbonell-Barrachina et al., 1998; Tang and Miller, 1991); wilting (Cox et al., 1996) and necrosis of leaf blades (Odanaka et al., 1987); reduction in leaf area and photosynthesis (Marin et al., 1993); decrease in shoot growth (Carbonell-Barrachina et al., 1998; Cox et al., 1996; Tang and Miller, 1991); lower fruit and grain yield (Carbonell-Barrachina et al., 1995; Tsutsumi, 1980); and sometimes plant death (Marin et al., 1993).

Similarly in comparison to lentil, decreased dry matter production was recorded when arsenate was applied to garden pea (Päivöke and Simiola, 2001), apricot (Creger and Peryea, 1994) and rice (Tang and Miller, 1991). Also Carbonell-Barachina et al. (1995) observed a drastic decrease in growth parameters (maximum decrease of 76.8% in leaf fresh weight) and in the fruit yield (maximum reduction of 79.6%) of tomato when exposed to different concentrations of arsenite (0–10 mg As/L).

Reduced root length growth in response to arsenic exposure was demonstrated in this study and has been reported by a number of investigators (Hartley-Whitaker et al., 2001; Kapustka et al., 1995; Meharg and Macnair, 1992; Sneller et al., 1999). A significant reduction in root length with increasing concentration of arsenic was attributed to the fact that plant roots represent the first point of contact with the arsenic species in the nutrient media.

In addition increased shoot dry weight, root dry weight, flower number, pod dry weight and relative shoot height was demonstrated with mycorrhizal inoculation. The role of AM fungi in the tripartite symbiosis (legume+rhizobia+AM fungi) could be significant in soils with low available P content (such as the growth medium used in this study) as nitrogen fixation is impaired by an inadequate P supply (Graham and Rosas, 1979). Other related research findings suggest that growth and yield increases of legumes inoculated with AM fungi and rhizobia are generally associated with enhanced nitrogen fixation and/or P uptake (Pacovsky et al., 1986).

Lens culinaris was found during this study to be sensitive to arsenic and that higher concentrations of arsenic in irrigation water produced arsenic contents of this crop higher than the recommended values for forage (Abedin et al., 2002). Moreover, the experimental data showed that arsenic decreased N and P content in lentil. Airas et al. (2004) suggested that arsenate altered the mineral balance at various levels, hydrolysis of reserve phytate and mobilization of nutrients from the cotyledons, as well as uptake and transport of mineral elements. The elements that were most affected were phosphorus, nitrogen, sodium, magnesium, manganese, zinc, and sulphur. However, in this investigation, the observed disturbances in growth

and the decreased N and P uptake of *Lens culinaris* under arsenate stress could not have been caused by one mechanism alone, but factors regulating the selectivity of ion uptake and transport.

To further understand the mode of plant tolerance to sources of toxicity in the soil matrices the effect of mycorrhizas were studied. Bioluminescence data on mycorrhiza showed that their presence reduced toxicity in some of the experimental plant vegetative responses and soil leachate. No significant changes were observed for arsenic content in lentil, however, less arsenic concentration in shoot and root were demonstrated (Fig. 2c, d). This observation was probably due to mycorrhizas/plant symbioses, which essentially is of great importance in promoting nutrient and heavy metal uptake (Smith and Read, 1997). Previous studies related to AM fungi on contaminated sites showed that the presence of AM fungi could facilitate plant re-establishment (Khan et al., 1999). Growth stimulation, better mineral nutrition and lower heavy metal uptake are among the benefits of mycorrhizal plants growing in soils with very high concentrations of metals (Leyval et al., 1991; Schüepp et al., 1987; Weissenhorn and Leyval, 1995). However, some researchers have found that mycorrhizal plants may have increased heavy metal uptake. For example, Weissenhorn and Leyval (1995) reported a higher uptake of metals by mycorrhizal plants. Dueck et al. (1986), studying the effects of AM fungi on the growth and Zn uptake of two grass species growing in soil amended with Zn sulphate, observed that mycorrhizal colonisation of the roots alleviated the negative effects of excess Zn on plant development, but had no effects on Zn concentrations in the shoots.

During this study it was also observed that there was a reduced nitrogen fixation with increasing concentration of arsenic in contaminated irrigation water. Heavy metal toxicity to symbiotic nitrogen fixation has received significant attention in related studies elswhere. For instance a complete absence of symbiotic nitrogen fixation by white clover (*Trifolium repens* L.) was identified at only moderate metal concentration in sewage sludge amended soil from the Woburn plots (McGrath et al., 1988). Nitrogen fixation measurements using ^{15}N-isotope dilution revealed no nitrogen fixation in the sludge-amended soil while nitrogen fixation in the control soil was above 60%.

The results therefore, indicated a positive effect of mycorrhizal inoculation and infection on lentil growth, nitrogen fixation and P nutrition when arsenate contaminated irrigation water was applied to the lentil crop. However, arsenic showed negative effect on plant growth and nitrogen fixation. Importantly, at higher concentration of arsenic, mycorrhizal activity was decreased, this reduction equally caused a decrease of nitrogen fixation and P uptake (Fig. 2a, c).

There have been variable findings in terms of mycorrhizal infection and metal toxicity to plants. For example, in mine spoils heavily polluted with Zn and Cd, Gildon and Tinker (1983) found 35% colonized roots of naturally growing clover. Diaz and Honrubia (1993), studying *Medicago sativa*, found less AM fungal spores in Zn and Pb contaminated soil than the soil not altered by mining activity. On a Zn and Cd contaminated site close to a zinc refinery, *Agrostis capillaris* was extensively colonised by AM fungi, while a low percentage of colonisation was observed beside an old copper mine (Griffioen and Ernst et al., 1990). In this investigation, there was (Fig. 3) a significant reduction in mycorrhizal infection due to arsenic.

To be able to evaluate the toxicity effect of arsenic in irrigation water on plant uptake and determine its bioavailability status, *lux*-marked biosensor (used to provide the bioavailable fraction of the contaminant) was successfully applied to complement on the chemical analysis (which provided the total concentration of the contaminant). The effect of arsenic on increasing order of sensitivity showed that root length > root dry weight > shoot dry weight > plant height > pod dry weight > flower pod number. This was closely related to Kapustka et al. (1995) who proposed that the sensitivity of vegetative response follows the order: root length > root mass > shoot length > total mass (root+shoot) > shoot mass > germination. The vegetative responses (root length (r = 0.86), root dry weight (r = 0.83), shoot dry weight (r = 0.85), plant height (r = 0.82), pod dry weight (r = 0.63) and flower number (r = 0.76) had a positive correlation to the bioluminescence. This observation demonstrated that an increase in arsenic toxicity (through irrigation water) resulted to a decrease of all the measured vegetative and reproductive responses of lentil plant.

The bioassay results further indicated that the effect of filtration was probably attributed to physically held (e.g. arsenic complexes) but not chemically bond arsenic compounds (e.g., arsenite are more soluble in aqueous media) in the soil matrices. This observation is related to the behaviour of clay which are negatively charged silicate minerals and surfaces of Fe-, Al- and Mn-oxides or hydroxides which have net negative charges, therefore, they preferably adsorb negatively charged ions, not oxyanions of arsenic. However the sorption of arsenic oxyanions from soil leachate occurs on clay and other colloidal surfaces (Sadiq, 1997). Sopper (1992) further reported that binding of metals to organic materials and clay minerals, precipitation, complexation and ionic interactions significantly reduces their inhibitory influences on microbial activity, so that toxicity is substantially less in a soil system compared to pure culture systems. Clay soils are also considered to play a major role in the immobilisation of heavy metals by sesquioxides, which are involved, in two main steps. Firstly, fast surface adsorption which is depended on cation exchange capacity (CEC) and specific adsorption that is also related to metal ion hydrolysis followed by a slow process consisting of the substitution of a matrix ion, the occlusion by re-crystallization and diffusion into the sesquioxides pores (Brümmer et al., 1986). Some investigators found a significant correlation between clay content and arsenic sorption in surface soils (Nightingale, 1987). Therefore, arsenic contaminated irrigation water might have a greater impact on plant uptake of arsenic in clay soil rather than in sandy soil.

4 Conclusions

The main criterion of the study was based on assessing toxicity (using *lux*-marked biosensor *Escherichia coli* HB101 pUCD607) of arsenic contaminated irrigation water on leguminous plant (*Lens culinaris* L.) growth and nutrient uptake, which was successfully achieved. Significant effect of arsenic on plant growth and productivity was observed indicating that increased concentration of arsenic in irrigation water had direct implications to the plants' overall performance. The effect of arsenic on increasing order of sensitivity showed that root length > root dry weight > shoot dry weight > plant height > pod dry weight > flower pod number. Furthermore when the soil leachate toxicity was measured a positive correlation was observed between the vegetative and reproductive responses and percentage bioluminescence indicating that arsenic in irrigation water was bioavailable. When the effect of mycorrhiza was evaluated it was found to increase nitrogen fixation, N and P uptake. The presence of mycorrhiza also showed a reduction in shoot and root arsenic uptake as well as toxicity based on filtration (to evaluate the clay arsenic adsorption capacity) of soil leachate using 125 diameter and 0.22 μm filter paper. These results therefore, inferred that the higher the concentration of arsenic in the irrigation water the higher the toxicity observed (indicating higher bioavailable fraction of arsenic) in lentil plant and the soil leachate solution. However, the observable limitation in this study was the use of artificial soil, which was not a true representative of the field conditions existing in Bangladesh. Moreover the substrate used particularly lacked the presence of iron oxides (prevalent in Bangladesh soil) considered critical to phosphate and arsenate sorption in field soils. Finally, in future studies there is need to use arsenic specific sensors where *lux* reporter genes are fused to arsenic resistance promoters to provide a much more reliable status quo on arsenic bioavailability fraction in Bangladesh agricultural field soil samples.

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