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Differential Responses of *Azolla microphylla* Kaulf and *Azolla filiculoides* Lam. to Lead Nitrate

Muthukumaras, wamy Eyini[1], Natesan Anjana Devi[1], Chinnathambi Pothiraj[1], Muthukrishnan Jayakumar[2], and Bong-Seop Kil[3]*

[1]Research Centre in Botany, Thiagarajar College (Autonomous), Madurai 625 009, India
[2]Research Department of Botany, VHNSN College, Virudhunagar 626 001, India
[3]Division of Life Science, Wonkwang University, Iksan 570-749, Korea

The aquatic fern *Azolla* is used as a green manure for rice production systems in warm temperate and tropical regions throughout the world. We used lead nitrate in nutrient media to assess the tolerance/response of two *Azolla microphylla* and *Azolla filiculoides* to heavy metals. While both species showed negative responses in growth with an increase in lead concentrations, They were distinctly different in their tolerance for higher concentration of the heavy metal. This was apparent in their growth and biochemical characteristics. *A. microphylla* was more tolerant of the higher concentrations of lead nitrate (25 and 50 ppm), whereas *A. filiculoides* had a higher rate of lead uptake.

Keywords: aquatic fern, *Azolla filiculoides, Azolla microphylla*, green manure, heavy metals

Different species of the aquatic fern (*Azolla*) have been successfully used as biofertilizer for lowland rice (Setty et al., 1987; Wibur Ventura et al., 1987; Singh et al., 1988). In addition to their agricultural role, they may be able to treat wastewater by absorbing and incorporating the dissolved materials and heavy metals (Wolverton and McDonald, 1979, 1981; Jain et al., 1992; Song, 1994). Sutton and Portier (1989) sampled different stages of development in *Azolla caroliniana*, and counted the fronds produced after ten days of growth. In this system, they found that low concentrations of *Azolla* extracts stimulated *Lemna* growth, but higher concentrations reduced its growth.

To assess the differences in response to lead, we have investigated the effects of culture solutions, containing different concentrations of lead nitrate, on the survivability, growth, and rate of lead uptake by *Azolla microphylla* and *Azolla filiculoides*.

MATERIALS AND METHODS

Plant Material and Growth Conditions

A. microphylla and *A. filiculoides* cultures were procured from the Tamil Nadu Agricultural University, Coimbatore, (India). The cultures were grown in 250-mL conical flasks, using a nitrogen free, liquid culture [IRRI(-NO_3)] medium as described by Watanabe et al. (1977).

Stock solutions were prepared by dissolving reagent grade lead nitrate in distilled water. The solution was then added to individual conical flasks, each containing 100 mL of the culture medium. This provided overall lead nitrate concentrations ranging between 10 and 1000 ppm. Fresh *Azolla* cultures (500 mg) were inoculated in each conical flask.

The conical flasks were kept in partial sunlight at room temperature (29 ± 2°C). Five replicate samples were maintained for each concentration of lead nitrate. The control set of conical flasks comprised *Azolla* samples growing in culture media free of lead nitrate.

The threshold level of toxicity was arbitrarily determined by identifying the concentration of lead nitrate in the particular culture medium in which *Azolla* inoculum was still alive after 15 days. Three concentrations, including the one showing the threshold level of toxicity (50 ppm), were used for further study.

Measurement

We used *Azolla* fronds from 15-day-old samples that were recovered from the culture media containing 10, 25, and 50 ppm of lead nitrate. Factors for study included doubling time, relative growth rate (Subudhi and Watanabe, 1981), heterocyst frequency (Kannaiyan and Kumar, 1993), total nitrogen content (Markham, 1942), proline content (Bates et al., 1973),

*Corresponding author; fax +82-653-857-8837
e-mail bskil@wonnms.wonkwang.ac.kr

catalase activity (Kumar and Khan, 1982) and the rate of lead uptake (De, 1987).

RESULTS

For 10 ppm lead nitrate, doubling time for *A. microphylla* was greater than for *A. filiculoides*. At higher concentrations, however, the latter species grew more slowly, with doubling rates of 1.7 and 8.7 days at 25 and 50 ppm, respectively (Fig. 1).

Similarly, the relative growth rate of *A. microphylla* was greater only at higher concentrations of lead nitrate (Fig. 2). Heterocyst frequency of its symbiont was nearly 8% more than that of *A. filiculoides* at 25 ppm.

Total nitrogen increased for both species when treated with 10 ppm of lead nitrate. At higher concentrations, total nitrogen content decreased rapidly in *A. filiculoides*, but only gradually in *A. microphylla* (Fig. 3).

Proline content increased three-fold in *A. microphylla* grown in the presence of 50 ppm lead nitrate. In contrast, this concentration caused a loss of proline in *A. filiculoides*, to only 74% of the initial content (Fig. 4).

Catalase activity decreased gradually in *A. microphylla* in response to 25-ppm and 50 ppm lead concentrations. *A. filiculoides*, however, showed a dramatic decrease in catalase activity at all tested concentrations of lead nitrate (Fig. 5). Lead uptake by *A. micro-*

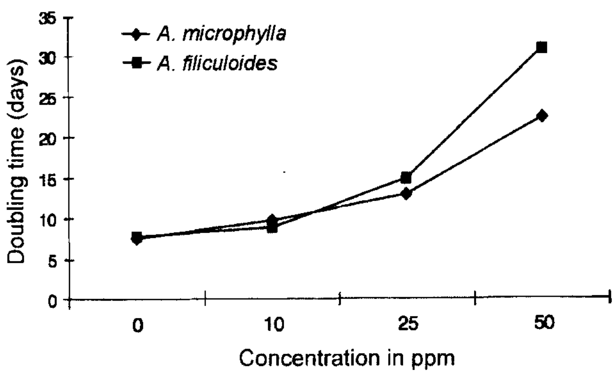

Figure 1. Effect of lead nitrate on the doubling times of *A. microphylla* and *A. filiculoides*.

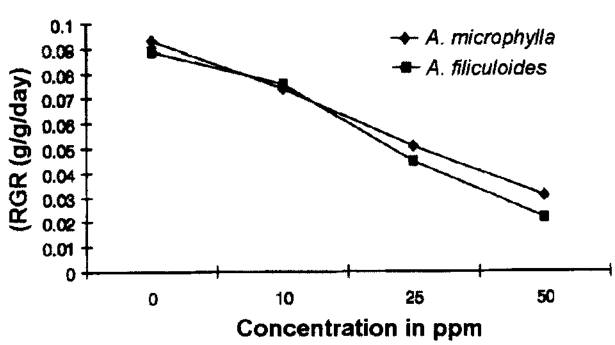

Figure 2. Effect of lead nitrate on the relative growth rates of *A. microphylla* and *A. filiculoides*.

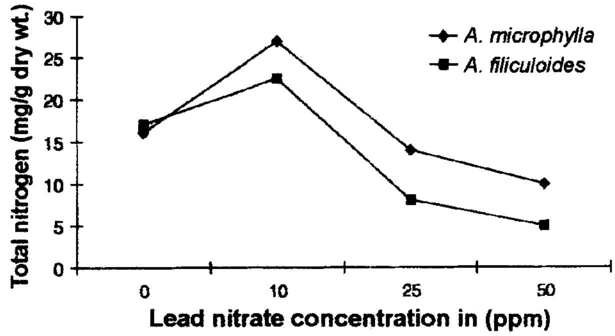

Figure 3. Effect of different levels of lead nitrate on total nitrogen contents in *A. microphylla* and *A. filiculoides*.

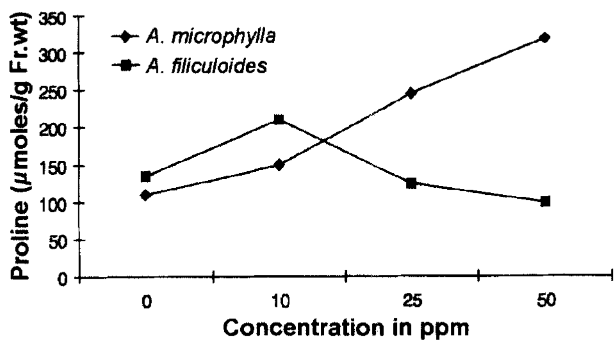

Figure 4. Effect of lead nitrate on proline contents of *A. microphylla* and *A. filiculoides*.

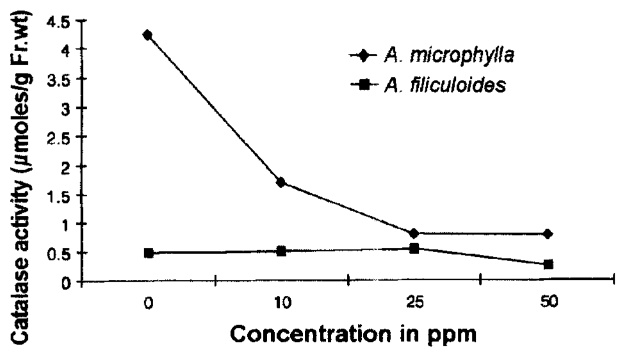

Figure 5. Effect of lead nitrate on catalase activities in *A. microphylla* and *A. filiculoides*.

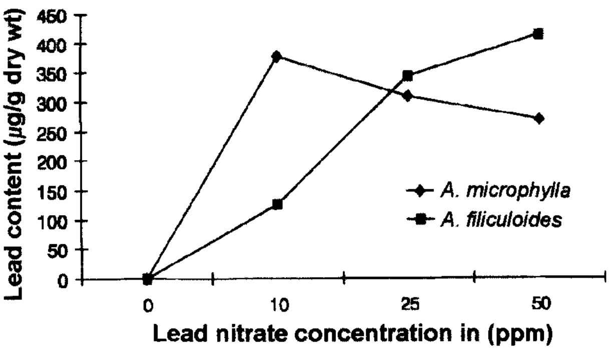

Figure 6. Uptake of lead by *A. microphylla* and *A. filiculoides* in the presence of different concentrations of lead nitrate.

phylla tended to decrease at 25 and 50 ppm concentrations, compared with the rate at 10 ppm. *A. filiculoides* accumulated 2.8 and 3.4 fold more lead at 25 and 50 ppm, respectively, compared with its uptake at 10 ppm (Fig. 6).

DISCUSSION

The differences between species in doubling times, relative growth rates, and symbiont heterocyst frequency indicates that the growth potential of *Azolla* is influenced by environmental variables (Kannaiyan and Somporn, 1987). This includes the presence of heavy metals. For example, shoots of *Ruta graveolens* that were treated for seven days with a mixture of Ni, Cu, Co, Cr, Cd, and Pb, exhibited both qualitative and quantitative differences in phenolic content (Zobel et al., 1999).

Because the symbiont can provide the nitrogen requirements of *Azolla* (Peters and Calvert, 1983; Song et al., 1994), the presence of lead in the culture medium affects the growth of *Azolla* through its inhibitory influence on the symbiont *Anabaena azollae* and its heterocyst frequency.

Proline accumulation is an indicator of stress tolerance (Stewart and Lee, 1974). The three-fold increase in accumulation by *A. microphylla*, at 50 ppm lead nitrate, showed that this species clearly is more tolerant of lead than is *A. filiculoides*. This fact can be correlated with the higher growth rate in *A. microphylla*.

The rates of decrease in catalase activity differed between species, indicating variable sensitivity to lead concentration and growth inhibition (Das et al., 1978; Park and Chung, 1994). The susceptibility of *A. filiculoides* was shown by the drastic decrease in catalase activity. This resulted in more accumulated hydrogen peroxide and a greater inhibition of growth at higher concentrations of lead, compared with *A. microphylla*. Thus, lead tolerance and rates of uptake seem to be mutually exclusive processes in the two species of *Azolla*.

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