Uptake of arsenic by New Zealand watercress (*Lepidium sativum*)

Brett Robinson[a,*], Céline Duwig[b], Nanthi Bolan[c], M. Kannathasan[d], A. Saravanan[d]

[a]*Hort Research, Private Bag 11030, Palmerston North, New Zealand 14850*
[b]*Laboratoire d'étude des Transferts en Hydrologie et Environnement, LTHE-UMR (CNRS, INPG, IRD, UJF), BP 53 X, 38041 Grenoble, France*
[c]*Institute of Natural Resources, Massey University, Palmerston North, New Zealand*
[d]*Tamil Nadu Agricultural University, Coimbatore, India*

Abstract

Watercress (*Lepidium sativum*) is consumed as a vegetable, especially by the indigenous community in New Zealand. An investigation was carried out on the accumulation of arsenic by watercress, following earlier reports of inordinate arsenic concentrations in some aquatic macrophytes collected from the Waikato River, North Island, New Zealand. The Waikato River and some other aquatic systems in Taupo Volcanic Zone, New Zealand have elevated arsenic concentrations due to geothermal activity. Watercress, river water and sediment samples were collected from 27 sites along the Waikato river and analysed for arsenic. Greenhouse trials with watercress grown in beakers containing added arsenic were conducted to confirm the ability of this species to accumulate arsenic. At a number of sites, the concentration of arsenic in both the water and the watercress samples exceeded the World Health Organisation (WHO) limit for drinking water (0.01 mg l^{-1}) and foodstuffs (2 mg kg^{-1} on a fresh weight basis). The average leaf and stem arsenic concentrations were, respectively, 29.0 and 15.9 mg kg^{-1} on a fresh weight basis. Plants grown in solutions of >0.4 mg l^{-1} arsenic concentration had fresh weight arsenic concentrations above the WHO limit. Despite these higher concentrations, arsenic levels in plants grown under greenhouse conditions were approximately fivefold lower than in plants growing in the Waikato River, possibly because under natural conditions, the watercress is rooted in sediment containing on average approximately 35 mg kg^{-1} arsenic. It is recommended that watercress from the Waikato River, or other areas with elevated water arsenic concentrations, should not be consumed.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Watercress; Waikato River; Arsenic; Phytoremediation

1. Introduction

It is widely known that some aquatic macrophytes growing in the Taupo Volcanic Zone and Waikato River contain high concentrations of arsenic (Reay, 1972; Liddle, 1982). Robinson et al. (1995) reported >1000 mg kg^{-1} (ppm) arsenic (dry weight) in samples of *Egeria densa* and *Ceratophyllum demersum* growing in the Waikato River system. This arsenic is derived from geothermal activity to some extent exacerbated by commercial exploitation of geothermal power.

*Corresponding author. Tel: +64-217-49867; fax: +64-635-46731.
E-mail address: brobinson@hortresearch.co.nz (B. Robinson).

Liddle (1982) conducted experiments involving the uptake of arsenic by *Ceratophyllum demersum.* He found that plants grown in arsenic solutions with <0.1 mg l^{-1} arsenic (III or V) reached equilibrium in 1 or 2 days, while plants in >0 5 mg l^{-1} of this element, needed up to a week to reach equilibrium. Arsenic concentration in plants increased with increasing concentration of arsenic in the growing medium. At a similar arsenic concentration in the growing medium, plants grown under laboratory conditions contained far less arsenic than plants collected under natural conditions in the Waikato River. A plant grown in 0.5 mg l^{-1} of arsenic reached an equilibrium concentration of 67 mg kg^{-1} arsenic (dry weight). It was noted that the majority of the arsenic in the plant was stored in the leaves.

Arsenic uptake by plants is associated with the phosphate uptake mechanism, where presumably arsenate is taken up as a phosphate analogue (Khattak et al., 1991; Meharg and Macnair, 1991; Pickering et al., 2000). To date, there is only one report (Ma et al., 2001) of a terrestrial plant, *Pteris vittata,* that hyperaccumulates arsenic (up to 2.2% on a dry matter basis). It was suggested that this fern could be used for the phytoremediation of arsenic-contaminated sites. The possibility of using hyperaccumulator plants to extract arsenic from aquatic environments was discussed by Brooks and Robinson (1998).

In the course of our work on the uptake of arsenic by aquatic plants and other organisms in the Waikato River system (Robinson et al., 1995), we observed an inordinate accumulation of arsenic in watercress (*Lepidium sativum*). Four samples taken from the river at a site near a geothermal power station (Ohaaki near Broadlands) averaged 412 mg kg^{-1} (dry weight). This prompted further studies on this plant as it is used as a food source and its metal concentration could have serious implications for public health, as this amount is well in excess of 2 mg kg^{-1} fresh weight, the WHO limit for arsenic in foodstuffs (Brandsetter et al., 2001).

The predominant form of arsenic in the environment is As(V) since As(III) is oxidised by atmospheric oxygen (Pepper et al., 1987). For most of the year, over 90% of the arsenic in the Waikato

River is present as As(V) (Aggett and Aspell, 1978), but in the summer months the levels of As(III) increase, probably because of bacterial action (Freeman, 1985). In this experiment, As(V) was used because it approximates the majority of arsenic in the river.

The aim of this experiment was to determine if *L. sativum* accumulated arsenic, and if so what was the maximum level of arsenic in the surrounding water before arsenic levels in *L. sativum* exceeded 2 mg kg^{-1}, the WHO limit for arsenic in foodstuffs.

2. Materials and methods

2.1. Sample collection from the Waikato River

L. sativum samples were taken at several sites (Fig. 1) along the Waikato River in late May, 2000. As a control, samples were taken from the Tiritea stream near Massey University, an area with no known arsenic contamination. Water and sediment samples were also collected from the Waikato River and control sites at the same time as the *L. sativum*. Plant material was washed thoroughly in the river and placed in plastic bags until return to the laboratory where further washing in distilled water was carried out.

2.2. Greenhouse experiments

The control watercress samples from Tiritea stream, which accumulated an insignificant amount of arsenic, were used in the greenhouse experiments. Excess water was removed from the fresh material, and approximately 12-g portions of plant weighed out and rinsed in distilled water. They were then floated in 1-l beakers containing 0.8 l of arsenic solution. Each beaker contained an aerator to ensure adequate oxygen. The experiments were conducted in greenhouse facilities at Massey University, Palmerston North, New Zealand (latitude 40.2° S, longitude 175.4° E). Temperatures ranged from 13 to 23 °C.

The arsenic solutions were prepared by taking a measured amount of 1000 mg l^{-1} of sodium arsenate solution and making the volume up to 0.8 l with distilled water. Plants were grown in solu-
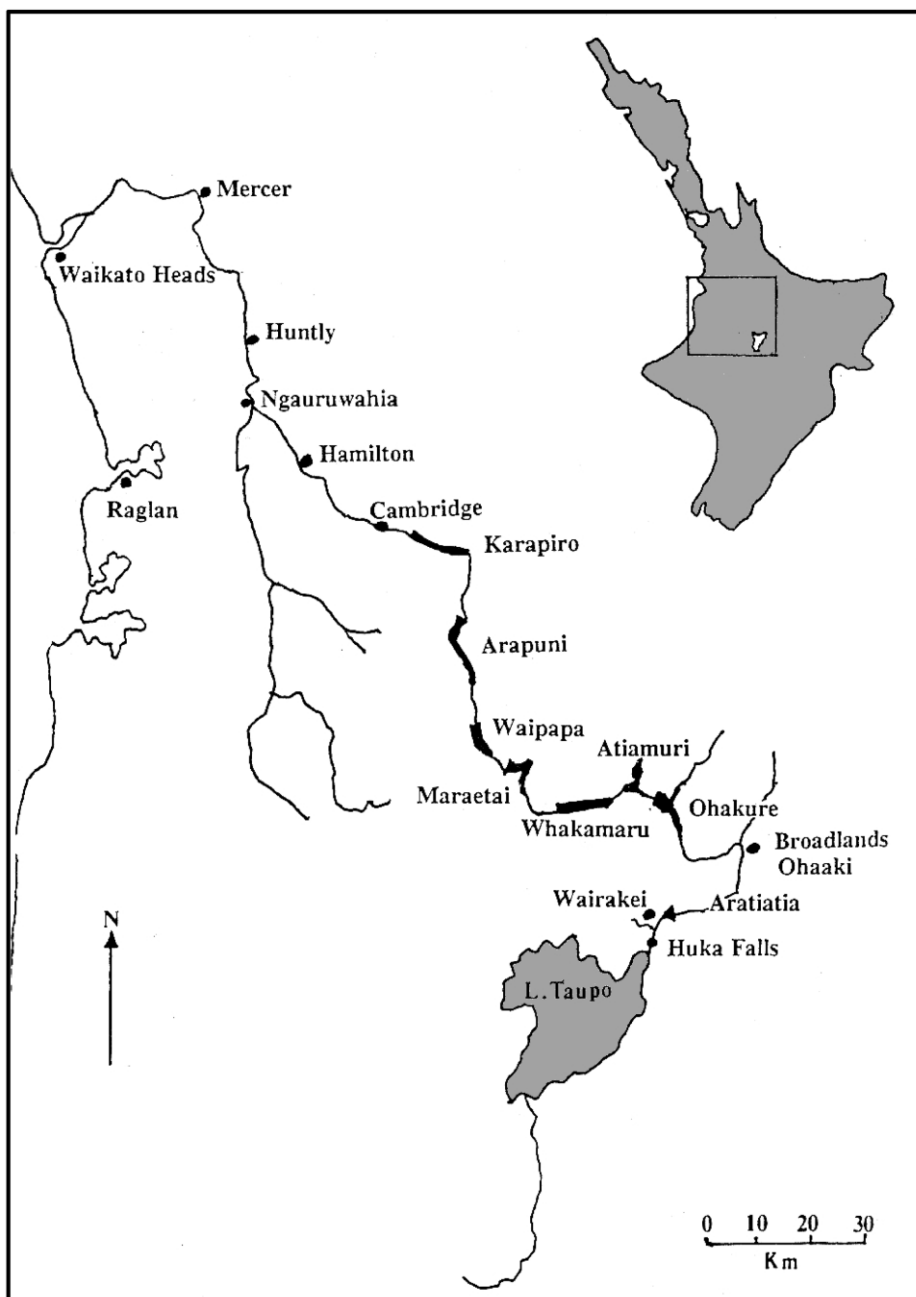
Fig. 1. Sampling sites along the Waikato River, North Island, New Zealand.

tions containing various levels (<0.01–0.8 mg l^{-1}) of arsenic. There were three replicates each of six treatments and the experiment lasted 8 days.

The control consisted of a beaker containing 0.8 l of 0.8 mg l^{-1} of arsenic but no plant. Aliquots of 4 ml of solution were taken from each beaker

initially at the start of the experiment and then at 48-h intervals, to determine when the plants had reached an equilibrium concentration. We assumed this point to have been reached when there was only a negligible change in solution concentration caused by plant growth.

2.3. Sample preparation and As determination

Leaves and stems were separated, weighed and placed in a drying cabinet at 80 °C until a constant weight was reached. Approximately 0.2 g of ground material from each sample was accurately weighed into 50-ml Erlenmeyer flasks. Concentrated nitric acid (10 ml) was added to each flask and the mixtures heated on a heating block until a final volume of approximately 3 ml was reached. The samples were then diluted to 10 ml using distilled water and stored in polythene containers.

Sediment samples were dried at 80 °C and sieved to <1-mm size using a nylon sieve. Approximately 0.2-g quantities of sediment were accurately weighed into 50-ml Erlenmeyer flasks. Concentrated nitric acid (10 ml) was then added and the mixtures boiled until a final volume of 3 ml was reached. A further 10 ml of concentrated hydrochloric acid was then added and the mixtures again evaporated to 3 ml. After filtration, the solutions were diluted to 10 ml with distilled water.

All samples of plant material, sediment and waters were analysed for arsenic using hydride-generation atomic absorption spectroscopy. Sodium borohydride was used as a reducing agent in a direct introduction method similar to that described in Godden and Thomerson (1980). The limit of detection was approximately 4 ng for loadings of up to 5 ml of sample (i.e. 0.8 $\mu g \ l^{-1}$ [ppb] for waters and 4 $\mu g \ kg^{-1}$ for sediments and plant material). Repeated measurements indicated that the reproducibility was approximately 10% at concentrations of arsenic close to the limit of detection. Reproducibility improved at higher arsenic concentrations. Reagent blanks were below detection limits, i.e. <0.8 $\mu g \ l^{-1}$ in the solution. The fresh weight arsenic concentration was calculated using the percentage water that the plants contained (an average of 92.7% with a standard deviation of 0.4%).

Table 1
Arsenic concentrations for waters (mg l^{-1}), sediments (mg kg^{-1}) and plant samples (mg kg^{-1}) taken from along the Waikato River, North Island, New Zealand

	Average	Standard error	Range
Water ($n=27$)	0.041	0.006	0.001–0.1532
Sediments ($n=5$)	40.5	5.3	14.0–85.0
Leaves ($n=27$)			
Fresh weight	28.97	6.06	0.1–103.0
Dry weight	499.6	90.2	12.0–1766.0
Stems ($n=27$)			
Fresh weight	15.9	3.78	1.9–76.0
Dry weight	280.6	54.7	43.0–1226.0

3. Results and discussion

3.1. Arsenic in watercress, water and sediments from the Waikato River

Table 1 summarises the arsenic concentrations in *L. sativum*, sediments and waters from the Waikato River sampling sites. Nearly all samples taken were above the WHO limit for arsenic in foodstuffs (2 mg kg^{-1} fresh weight) and the leaves, on average, were 14 times this value. Arsenic concentrations in the leaves were nearly twice that of the stems, consistent with the distribution of metal in most plants (Brooks, 1998). Although the arsenic concentrations in the *L. sativum* samples are highly variable, this is the usual pattern for water plants in the Waikato River. For example, Liddle (1982) reported arsenic values ranging from 265 to 1121 mg kg^{-1} for *Ceratophyllum demersum* growing in nearby Lake Ohakure. Fig. 2 shows the significant positive correlation ($r=0.77$, $P<0.001$) between the arsenic concentrations in the watercress and that from the ambient water. It is clear from Fig. 2 that even if the ambient water concentration is less than the WHO limit for drinking water (0.01 mg l^{-1}) the arsenic concentration in watercress may be above the WHO limit for foodstuffs (2 mg kg^{-1}).

All but four of the water samples taken had arsenic concentrations in excess of the WHO limit for arsenic in drinking water (0.01 mg l^{-1}). This concentration may be greater in the summer
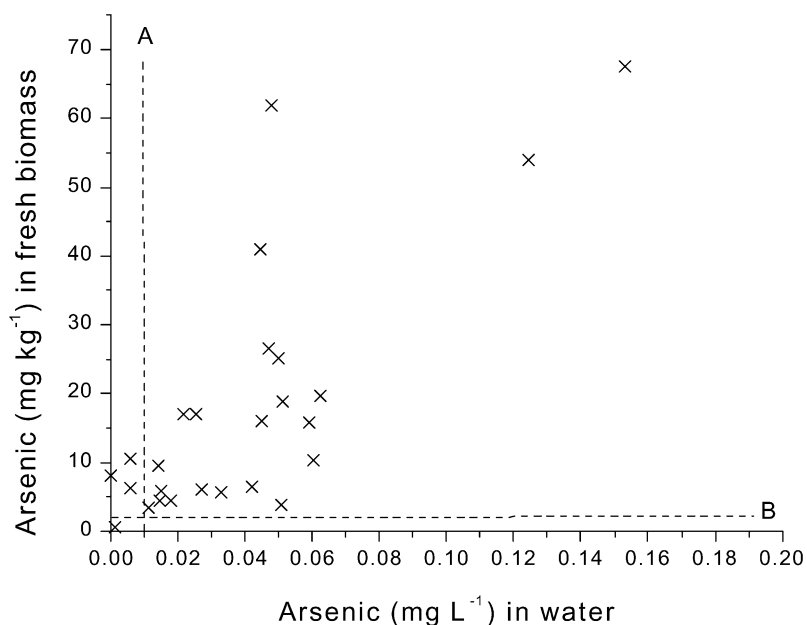
Fig. 2. The arsenic concentration (mg kg^{-1} fresh weight) in the shoots of *L. sativum* vs. water arsenic concentration (mg l^{-1}) in samples collected along the Waikato river. Lines A and B represent WHO limits for arsenic in drinking water and foodstuff.

months when the river flow is lower. The arsenic concentration in the sediments (Table 1) was on average 988 times higher than the ambient water concentration.

3.2. Arsenic accumulation by watercress in greenhouse experiments

At the completion of the greenhouse trials, the arsenic concentrations in all the plants had reached equilibrium, indicated by negligible decrease in the arsenic concentrations of the surrounding solutions. The arsenic levels in the shoots (leaves + stems) of the plants at the end of the greenhouse trials ranged from 0.02 (control) to 106 mg kg^{-1} dry weight and 0.0018–7.79 mg kg^{-1} fresh weight (Fig. 3). The arsenic concentrations in the plants were strongly positively correlated ($r = 0.96$, $P < 0.001$) with the arsenic concentrations of the surrounding water. There was a slight increase in the arsenic concentration in the control beaker, this probably being due to evaporation.

All the plants in the arsenic solutions accumulated this element, the plant/water arsenic concen-

tration ratio increasing exponentially from 3:1 to 10:1 as the arsenic concentration in the surrounding water increased from 0.05 to 0.8 mg l^{-1}. This type of increase is usually associated with active exclusion: at low concentrations the plant is able to exclude the element, and this barrier breaking down at higher concentrations. Active exclusion is not, however, consistent with the theory that arsenic accumulation occurs via the phosphate uptake pathway. If phosphate is taken up preferentially over arsenate, then the higher plant/water concentration ratio at high solution arsenic concentrations may be explained by an increase in arsenic competition for the phosphate uptake sites in the rootzone.

At equilibrium, the arsenic concentrations were approximately fivefold less than in plants taken from the Waikato River, even though both were in arsenic solutions of similar concentrations. This is similar to the findings of Liddle (1982) for *Ceratophyllum demersum*. A possible reason for the difference is that the watercress in this experiment was free floating, whereas in the river it is rooted to the sediment layer. Sediments from the Waikato
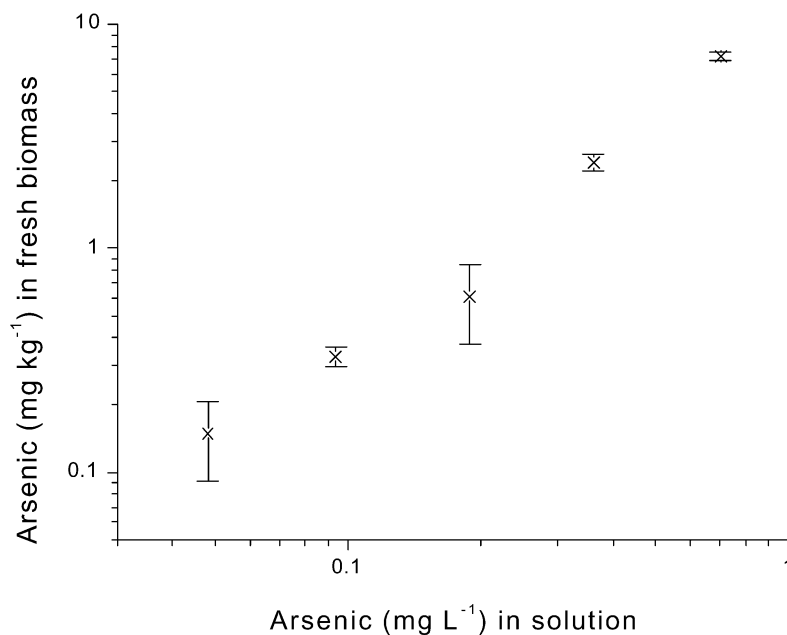
Fig. 3. Arsenic concentration $(mg\ kg^{-1}\ fresh\ weight)$ in the shoots of *L. sativum* vs. the arsenic concentration $(mg\ l^{-1})$ in the ambient solution when the plant concentration had reached equilibrium.

River were found to contain on average of 40.5 mg kg^{-1} arsenic (Table 1). If this is a major source of arsenic to the plant, then it would be expected that the plants growing in a high arsenic sediment would have a higher arsenic concentration. Further work could be conducted to explore this possibility. Despite the lower arsenic concentrations in the watercress of this experiment, plants grown in arsenic solutions of 0.4 mg l^{-1} or greater, were above 2 mg kg^{-1} on a fresh weight basis (the WHO limit for arsenic in foodstuffs).

The ability of *L. sativum* to take up large amounts of arsenic from substrates containing relatively low concentration of this element indicate the plant may have potential for phytoremediation by extracting arsenic from contaminated soils or water. In our greenhouse experiments, the arsenic taken up by the watercress reduced the arsenic concentration in the solutions by 7.3% on average. Before any conclusions can be drawn in this area, however, an investigation is needed of the plant's biomass production and arsenic uptake in terrestrial as well as aquatic environments.

4. Conclusions

Watercress should not be taken from zones where there may be elevated arsenic concentrations in aquatic systems. This is particularly relevant in geothermal areas where As-rich geothermal fluids increase the arsenic burden on aquatic systems. The development of geothermal power stations and geothermal heating systems that discharge geothermal fluids into waterways may render previously safe locations unsuitable for watercress collection.

Acknowledgments

References

Aggett J, Aspell AC. Arsenic from Geothermal Sources. New Zealand Research and Development Committee Report, #35 1978.

Brandsetter A, Lombi E, Wenzel WW, Adriano DC. Arsenic contaminated soils: I. Risk assessment. Adriano DC, editor. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risks of Metals, 2nd ed. New York: Springer 2001:715–737.

Brooks RR. Phytochemistry of hyperaccumulators. In: Brooks RR, editor. Plants that Hyperaccumulate Heavy Metals: their Role in Archaeology, Microbiology, Mineral Exploration, Phytomining and Phytoremediation. Wallingford: CAB International, 1998. p. 15–53.

Brooks RR, Robinson BH. Aquatic phytoremediation by accumulator plants. In: Brooks RR, editor. Plants that Hyperaccumulate Heavy Metals: their Role in Archaeology, Microbiology, Mineral Exploration, Phytomining and Phytoremediation. Wallingford: CAB International, 1998. p. 203–226.

Freeman MC. The reduction of arsenate to arsenite by an *Anabaena* bacteria assemblage isolated from the Waikato River. N Z J Mar Freshwater Res 1985;19:277–282.

Godden RG, Thomerson DR. Generation of covalent hydrides in atomic-absorption spectroscopy. Analyst 1980;105:1137–1156.

Khattak AK, Page AL, Parker DR, Bakhtar D. Accumulation and interactions of arsenic, selenium, molybdenum and phosphorus in Alfalfa. J Environ Qual 1991;20:165–168.

Liddle JR. Arsenic and other elements of geothermal origin in the Taupo Volcanic Zone. Ph.D Thesis, Massey University, Palmerston North, New Zealand 1982.

Ma LQ, Komar KM, Tu C, Zhang W, Cai Y, Kennelly ED. A fern that hyperaccumulates arsenic. Nature 2001;409:579.

Meharg AA, Macnair MR. Uptake, accumulation and translocation of arsenate in arsenate-tolerant and non-tolerant *Holcus lanatus* L. New Phytol 1991;117:225–231.

Pepper I, Galanti N, Sans J, Lopez-Saez JF. Reversible inhibition of root growth and cell proliferation by pentavalent arsenic in *Allium cepa*. Environ Exp Botany 1987;28:9–18.

Pickering IJ, Prince RC, George MJ, Smith RD, George GN, Salt DE. Reduction and coordination of arsenic in Indian Mustard. Plant Physiol 2000;122:1171–1177.

Reay PF. The accumulation of arsenic from arsenic-rich natural waters by aquatic plants. J Appl Ecol 1972;9:557–565.

Robinson BH, Brooks RR, Outred HA, Kirkman JH. The distribution and fate of arsenic in the Waikato River system, North Island, New Zealand. Chem Speciation Bioavailability 1995;7(3):89–96.