Available online at www.sciencedirect.com
ScienceDirect
Environmental and Experimental Botany 58 (2006) 206–215
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Arsenic hyperaccumulation by aquatic macrophytes in the Taupo Volcanic Zone, New Zealand

Brett Robinson [a,d,*], Nick Kim [b], Monica Marchetti [d], Christophe Moni [d], Lina Schroeter [d], Carlo van den Dijssel [d], Georgie Milne [c], Brent Clothier [d]

[a] *Institut für Terrestrische Ökologie, Universitätstrasse 16, ETH Zentrum CHN F24, CH-8092 Zürich, Switzerland*
[b] *Environment Waikato, P.O. Box 4010, Hamilton, New Zealand*
[c] *Institute of Natural Resources, Massey University, Palmerston North, New Zealand*
[d] *Hort Research, Private Bag 11 030, Palmerston North, New Zealand*

Received 26 April 2005; received in revised form 27 July 2005; accepted 30 August 2005

Abstract

Geothermal activity in the Taupo Volcanic Zone (TVZ), New Zealand, has resulted in elevated (0.01–0.1 mg L^{-1}) levels of arsenic (As) in many of the region's soils, lakes and rivers. Some aquatic plants in the TVZ are known to accumulate inordinate amounts of As. We sampled 28 species of aquatic macrophytes and 11 terrestrial species from the TVZ along with ambient waters, sediments and soils. Selected aquatic species were grown in controlled conditions to examine As-uptake, tolerance, translocation and depuration. The geometric mean As concentration in 184 aquatic macrophytes was 125 mg kg^{-1} dry weight, while the geometric mean As concentration of the 36 terrestrial samples was <0.5 mg kg^{-1} in the above-ground portions. Some aquatic species had >1000 mg kg^{-1} As, the threshold for As hyperaccumulation. The average As concentrations in the aquatic sediments and waters were 38 mg kg^{-1} and 0.021 mg L^{-1}. We found a strong and positive correlation between Fe and As, along with a geometric mean Fe concentration of 3798 mg kg^{-1}. Aquatic macrophytes accumulated less As under controlled conditions compared to plants collected in situ. Arsenic was not translocated from the submerged to emergent portions of watercress. High-As specimens rapidly released As when placed in water containing <0.01 mg L^{-1} As. Our results are consistent with the hypothesis that As accumulation by aquatic plants occurs via physicochemical adsorption to the plant's surface, facilitated by co-deposition of other adsorptive species such as hydrated Fe oxides. Accumulation appears independent of any specific As-uptake mechanism. The aquatic plants *Rorippa nasturtium-aquaticum* (L.) and *Mentha* spp. are foraged as vegetables in the TVZ. In these species, we found respective As concentrations of up to 138 and 88 mg kg^{-1} on a fresh weight basis. Consumption aquatic plants taken from waters with geothermal inflows may present a human health risk.

Keywords: Arsenic; Hyperaccumulation; Aquatic macrophytes; Human health; Bioindication; Phytoremediation

1. Introduction

The Taupo Volcanic Zone (TVZ) covers an area of 600,000 ha in the central North Island of New Zealand. It extends from the Bay of Plenty in the north, southwest to Mount Ruapehu in a long narrow belt. The area features four active volcanoes that include Mt. Tarawera, Mt. Tongariro, Mt. Ngauruhoe and Mt. Ruapehu. The area has widespread hydrothermal activity, and an abundance of hot springs, geysers and mud pools. The TVZ contains over 32 natural lakes, including Lake Taupo, the largest lake in New Zealand, and Lake Rotorua. New Zealand's longest river, the Waikato, flows out of Lake Taupo, through several geothermal fields and northwestward to the Tasman Sea. Seven hydroelectric power dams have been built along this river to exploit the 366 m fall to the sea (Liddle, 1982).

* Corresponding author. Tel.: +41 44 633 6073; fax: +41 44 633 1123.
E-mail address: Brett.Robinson@env.ethz.ch (B. Robinson).

0098-8472/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2005.08.004

Geothermal activity in the TVZ has resulted in elevated arsenic (As) concentrations in some lakes and rivers (Aggett and Aspell, 1978). The As loading in surface waters is exacerbated by the commercial exploitation of geothermal bores for electricity (Axtmann, 1975). The condensate has elevated As concentrations. Historically, As has also been introduced into the aquatic environment from runoff of As-based pesticides (Hill, 1975), aquatic pesticides used to control lake weed (Tanner and Clayton, 1990) and leaching from timber treatment sites that used copper-chromium-arsenic (CCA) biocides.

The predominant form of As in the environment is arsenate [As(V)] since arsenite [As(III)] is oxidised by atmospheric oxygen (Pepper et al., 1987). For most of the year, over 90% of the As in the Waikato River, which flows through the TVZ, is present as As(V) (Aggett and Aspell, 1978), but in the summer months the levels of As(III) increase, probably because of microbial reduction (Freeman, 1985).

Some aquatic macrophytes growing in the TVZ and Waikato River contain high concentrations of As (Reay, 1972; Liddle, 1982). Robinson et al. (1995a) reported >1000 mg kg^{-1} (ppm) As (dry weight) in samples of *Egeria densa* and *Ceratophyllum demersum* growing in the Waikato River system.

A survey of watercress (*Rorippa nasturtium-aquaticum*) growing in the region revealed concentrations as high as 1766 mg kg^{-1} As (dry weight) in the shoots (Robinson et al., 2003). On a fresh weight basis, the average leaf As concentration was 29 mg kg^{-1}. This is of concern to human health, because foraged watercress is consumed as a vegetable. Plant accumulation of As may also facilitate its entry into the food chain.

The effects of sub-acute As poisoning include an increased incidence of cancer, particularly of the bladder, liver and lungs (Morales et al., 2000; Storelli and Marcotrigiano, 2001). Inorganic As is considerably more toxic than organic As (FSANZ, 2002). There is currently some debate over appropriate tolerable daily intakes (TDIs) for inorganic As, as older numbers come up for review. Regulatory TDIs now range from 0.3 to 2.1 μg kg^{-1} of body weight per day (USEPA, 1993; ATSDR, 2000; Baars et al., 2001). However, progressively more conservative values are being adopted.

Liddle (1982) investigated the uptake of As by *C. demersum*. He found that plants grown in As solutions with <0.1 mg L^{-1} As (III or V) reached a maximum concentration in 1 or 2 days, while plants in >0.5 mg L^{-1} As required up to a week. Arsenic concentration in plants increased with increasing concentration of As in the growing medium. At a similar As concentration in the growing medium, plants grown under laboratory conditions contained far less As than plants collected under natural conditions in the Waikato River.

Arsenic uptake by plants is associated with the phosphate ion ($H_2PO_4^-$). Arsenic(V) is taken up as an $H_2PO_4^-$ analogue (Khattak et al., 1991; Meharg and Macnair, 1991; Pickering et al., 2000). Aquatic plants have been shown to accumulate trace elements by absorption followed by passive or active transport across membranes (Forstner and Wittmann, 1981; Smies, 1983).

Ma et al. (2001) reported As concentrations in the Brake fern, *Pteris vittata*, of up to 22,000 mg kg^{-1} (2.2%) on a dry weight basis. This is the first terrestrial species reported to take-up As to concentrations greater than 1000 mg kg^{-1}, the threshold for a plant to be considered a 'hyperaccumulator' (Brooks et al., 1977). Hyperaccumulator plants could be used for the phytoremediation of As-contaminated sites (Ma et al., 2001). This technology relies on plants that translocate inordinate amounts of one or more metal(loid)s, including As, into their above-ground biomass. Arsenic phytoremediation would involve the repeated cropping until the soil's As concentration has reached an acceptable level. After each cropping, the plant biomass would be removed from the area and may be burned to reduce its volume, whereupon it could be stored in an appropriate area, such as a contained landfill that does not pose a risk to the environment. The possibility of using hyperaccumulator plants to extract As from aquatic environments has also been suggested (Brooks and Robinson, 1998).

The aim of this study was to investigate the nature and extent of As-uptake by aquatic and terrestrial plant species that occur in a high-As environment. In particular, we sought to measure As-uptake by aquatic species in both controlled conditions and in the field with a view to determining the potential implications for human health, phytoremediation and the fate of As.

2. Materials and methods

Aquatic plants were sampled from 11 sites along the Waikato River, and from four other waterways with geothermal inflows (Fig. 1). Whole specimens were taken and washed in river water to remove sediment. Further washing in distilled water was carried out upon returning to the laboratory. Water and sediment samples were collected from all sites and stored in sealed containers.

Leaves were collected from selected terrestrial plant species growing near geothermal springs at five locations, along with accompanying soil samples from four of the five locations (Fig. 1).

2.1. Watercress and native ferns grown in soil

The greenhouse experiment was performed at HortResearch, Palmerston North, New Zealand (40.2°S, 175.4°E). Five species of native ferns (*Asplenium bulbiferum*, *Blechnum discolor*, *Histiopteris incisa*, *Pneumatopteris penningera* and *Polystichum vestitum*) as well as watercress (*R. nasturtium-aquaticum*) were grown in 15 L buckets containing soils spiked with sodium arsenate (Analytical Reagent [AR] grade) to give final concentrations of 0, 55 and 105 mg kg^{-1}. There were five replicates for each treatment.

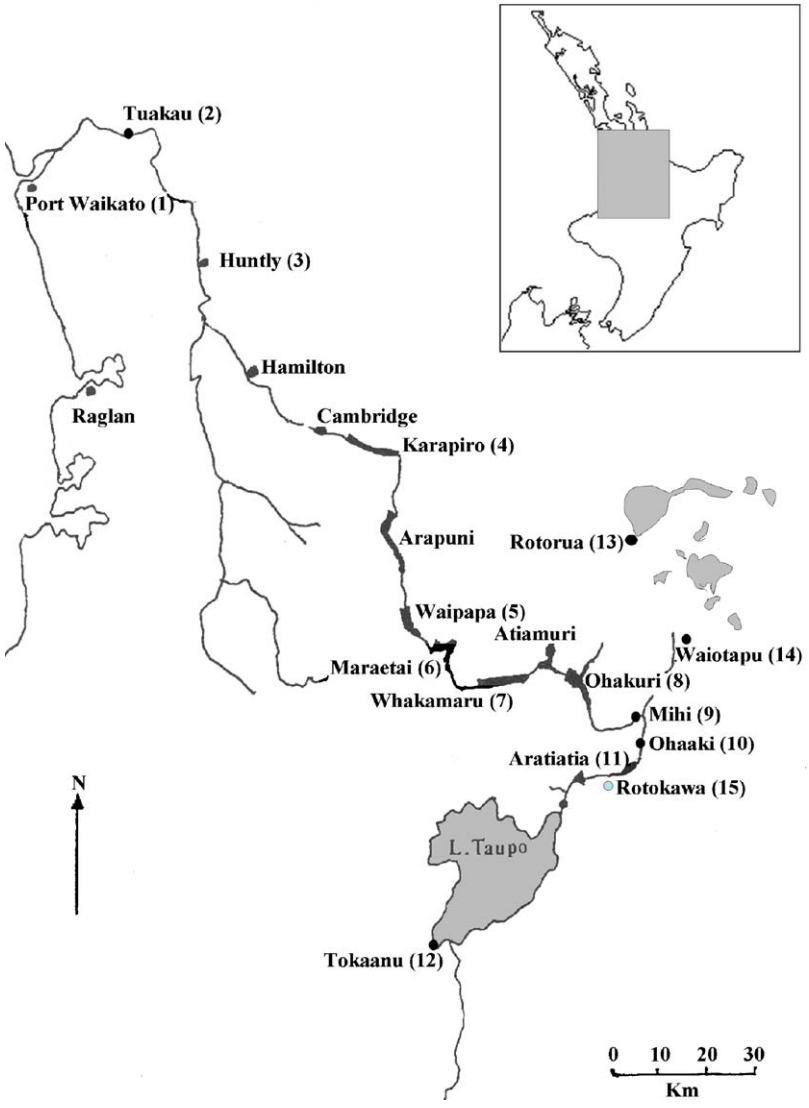

Fig. 1. Sampling locations from the Waikato River and Taupo Volcanic Zone in the North Island of New Zealand. The samples collected from the numbered locations are shown in Tables 1–3.

The soil used was Manawatu fine sandy loam (*Dystric Fluventic Eutropchrept*) (Vogeler et al., 2001), with a pH of 4.4, total organic carbon 6.3% and an exchange capacity of 13.4 cmol(+) kg^{-1}. Soils were prepared by adding varying amounts of a 1% As solution to 15 kg of soil. This was mixed using a concrete mixer. Soils were fertilised with Nitrosol™ liquid fertiliser (N:P:K. of 8:3:6 Rural Research Limited, P.O. BOX 12773 Penrose, Auckland, New Zealand) at a rate recommended by the manufacturer. Planting occurred at the end of October 2002, some 6 weeks after preparation of the pots. This delay was to allow time for the added As to reach an equilibrium with the soil. Plants were watered according to evaporative demand. Pots were arranged in a randomised block design within a greenhouse. Plant samples were taken for analysis in mid-January, 2003, 10 weeks after planting.

2.2. The uptake of As from spiked river water by aquatic plants

The As-uptake and tolerance of watercress (*R. nasturtium-aquaticum*), *Myriophyllum propinquum* and *C. demersum* were determined in simulated aquatic conditions by growing free-floating plants in 2 L jars filled (to 1.5 L) with As-spiked river water. Each jar was covered with clear plastic containing two small holes: one for a bubbler tube and the other to let the air escape. In addition, cuttings of watercress (*R. nasturtium-aquaticum*) were grown hydroponically in 250 mL Erlenmeyer flasks to elucidate its capacity to tolerate, translocate and store As. Here, the plant was suspended through a polystyrene bung, so that half of the stem and nascent roots were suspended in solution, while the aerial stems and leaves remained isolated from any As solution.

For the growth experiment, we collected watercress plants from waterways around the Manawatu Region, near the city of Palmerston North, New Zealand. This area has no known As contamination. Plants with a fresh weight of approximately 18 g were accurately weighed, and then one plant was placed into each jar or Erlenmeyer flask. The jars and flasks were filled with river water (Turitea Stream, Palmerston North) and As added (as 1000 mg As L^{-1} AR Na_2HAsO_4) to give approximate final solutions of 0.1, 0.3, 1 and 3 mg L^{-1} As. Jars and Erlenmeyer flasks containing no added As (controls) were also prepared. Additional treatments of 10 and 30 mg L^{-1} were prepared for watercress in the Erlenmeyer flasks. There were three replicates for each species at each concentration. The experiment was set up in May, 2003 and lasted for 6 weeks. After 3 weeks, the solutions in the Erlenmeyer flasks were replaced. Aliquots of 4 mL of solution were taken from each container at the start of the experiment and then at 48 h intervals, to determine when the solutions had reached a quasi-equilibrium concentration. We assumed this point to have been reached when there was only a negligible change in the solution As concentration, presumably caused by plant-growth. All plants in all treatments had reached this point after 6 weeks. The plants in jars were then removed, washed thoroughly in distilled water and the fresh and dry weights determined. The dried plant material was then analysed for As.

The aerial portions of watercress grown in flasks were excised, washed in distilled water, the stems and leaves separated and the fresh and dry weights determined. The submerged portions were washed in distilled water, the roots and stems separated and the fresh and dry weights determined.

The volume of the ambient solution in the jars was measured at the end of the experiment. On average, 110 mL (7%) of solution had evaporated from each jar. Here, we report the final solution concentrations. Evapotranspiration from the Erlenmeyer flasks was not determined. The reported concentrations therefore represent initial values.

2.3. Arsenic depuration by aquatic plants

An experiment was set up to determine the retention of As by aquatic plants when placed in an ambient solution containing negligible amounts of As. Live specimens of watercress (*R. nasturtium-aquaticum*) and *M. propinquum* were collected from Tokaanu hot springs at the Southern end of Lake Taupo. The ambient water concentration was 0.18 mg L^{-1} As. Upon returning to the laboratory, 18 g portions of fresh material were placed in jars containing 1.5 L of Turitea River water with an As concentration of less than 0.01 mg L^{-1}. There were five replicates of *R. nasturtium-aquaticum* and three of *M. propinquum*. Water samples (5 mL aliquots) were taken regularly for 25 days. At the end of the experiment, the plants and waters were treated the same as those in the As-uptake experiment described above. The initial As concentrations of the plants tested were calculated at the end of the experiment by adding the amount of As remaining in the plant to that contained in the ambient water and dividing the sum by the final dry biomass of the plant. This calculation assumes that there was negligible growth during the experiment. The calculated initial As concentrations were compared to 18 other specimens of *R. nasturtium-aquaticum* and 10 other specimens of *M. propinquum* that were collected from the same site.

2.4. Sample preparation and As determination

Plant material and soils were placed in a drying cabinet at 80 °C until a constant weight was reached. Plant material was ground using a mortar and pestle. Soil samples were sieved to <1 mm size using a nylon sieve and stored in sealed plastic bags.

Between 0.15 and 0.2 g of ground plant material, or 0.5 g of sieved soil or sediment sample, were accurately weighed into 50 mL Erlenmeyer flasks. Ten mL of concentrated nitric acid (69%) were added to each flask and the mixtures heated and evaporated on a heating block until a final volume of about 3 mL was reached. The samples were then diluted to 10 mL using distilled water and stored in polythene containers.

All samples of plant material, soils and waters were analysed for As using graphite-furnace, atomic-absorption spectroscopy. Due to the high concentrations of As measured in some plant samples, plant standards were unavailable. Thus, for quality assurance, 15 dried and ground aquatic plant samples from the TVZ were sent to a commercial laboratory (Hill Laboratories, Hamilton, New Zealand) for the determination of As and other elements by inductively coupled plasma mass spectroscopy (ICPMS). The species analysed (with the site no. shown in Fig. 1) were: *Agrostis sp*. (12), *Ceratophyllum demersum* (4,4,6,7,8), *E. densa* (10), *Elodea canadensis* (12), *Juncus sp*. (1), *Lagarosiphon major* (11), *Mentha spicata* (12), *M. propinquum* (12,12), *Polygonum salicifolium* (12) and *R. nasturtium-aquaticum* (12). Our results were in good agreement ($R^2 = 0.978$) with those of the commercial laboratory.

2.5. Statistical treatment of data

MINITAB (Minitab Inc., Pennsylvania State University, University Park, Pennsylvania) was used for ANOVA (at the 5% level) and correlation analyses. Data that were log-normally distributed were log-transformed for statistical analyses. For log-normal data, we report geometric means and standard deviation ranges rather than means and standard deviations. With log-normal data, the geometric mean is a better measure of central tendency than the arithmetic mean. Use of geometric means reduces distortions caused by a few anomalous values and produces a value of better centrality.

3. Results

3.1. Arsenic accumulation in terrestrial and aquatic plants from the TVZ and Waikato River

The average As concentrations in the waters, sediments and soils, as well as aquatic and terrestrial plants collected from the TVZ, are presented in Table 1. The geometric mean As concentration in the water samples was over twice that of the New Zealand Drinking Water Standard (0.01 mg L^{-1}). The pH of the water samples ranged between 5.9 and 6.4, with a geometric mean of 6.3 (pH data are not shown in the tables or figures).

There was a clear demarcation between terrestrial plants, which accumulated negligible concentrations of As, and aquatic species that had As concentrations manifold higher than the ambient waters from where they were taken.

A summary of As concentrations in 28 aquatic species and 11 terrestrial species from the Waikato River and TVZ is given in Tables 2 and 3, respectively. The data clearly display the difference in As accumulation between aquatic and terrestrial plants. All aquatic plants accumulated measurable As concentrations with nine species (30%) exceeding 1000 mg kg^{-1} on a dry weight (d.w.) basis, the threshold for hyperaccumulation (Brooks et al., 1977). By contrast, 83% of the terrestrial plants (Table 3) were below detection limits for As (<0.5 mg kg^{-1}). All the aquatic plants accumulated As to concentrations greater than 5 mg kg^{-1}. None of the terrestrial plants had As concentrations surpassing 11 mg kg^{-1}.

The *bioaccumulation coefficient* (B.C.) is defined here as the plant/water concentration quotient. The B.C. indicates the efficacy of plant As-accumulation. The geometric means of the bioaccumulation coefficients for the aquatic plants tested in this experiment are shown in Table 2. The results presented here show only the plant/water bioaccumulation coefficient. Some of the aquatic plants tested were rooted to sediments that had an As concentration that was many times the level of the water. An ANOVA test revealed that there was no significant difference ($p = 0.25$) in the As concentrations of rooted and free-floating plants.

Table 1
The geometric means and standard deviation ranges of the As concentrations waters (mg L^{-1}), as well as soils, sediments, aquatic and terrestrial plants (mg kg^{-1} dry weight) from the Taupo Volcanic Zone

Sample type	No. of samples	Geomean [As] and S.D. range
Soils	4	50 (33–79)
Sediments	23	38 (16–93)
Terrestrial plants	36	<0.5 (30 samples)–11
Waters	23	0.021 (0.005–0.078)
Aquatic plants	184	125 (12–1222)

The sampling locations are shown in Fig. 1.

3.2. Arsenic accumulation in ferns and watercress from As-spiked soil

The ferns and watercress grew well in all the treatments, and there were no signs of As toxicity. None of the fern species or the watercress had foliar As concentrations greater than 0.5 mg kg^{-1}, the detection limit for As, even at the highest soil concentration of 105 mg kg^{-1}. There were no significant differences in the soil As concentration before and after the experiment, indicating that leaching losses were minimal.

3.3. Arsenic tolerance and accumulation by aquatic plants under controlled conditions

None of the free-floating plants in jars showed necrosis or growth distortion in any of the treatments. All three species increased in biomass (Fig. 2A). However, there was a significant negative correlation ($r = -0.87$, $p < 0.01$) between biomass production by *C. demersum* and the ambient As concentration. There was also a significant decrease ($p = 0.02$) in the growth of *M. propinquum*, between 0.17 and 0.29 mg L^{-1} in the ambient solution. The growth of watercress (*R. nasturtium-aquaticum*) was not significantly affected.

Fig. 2B shows the final As concentrations in the plants after 6 weeks of growth. In the control treatment, the As concentrations of the control plants and that of the ambient water were below the detection limits of 0.5 mg kg^{-1} and 0.01 mg L^{-1}, respectively. This indicates that there were negligible amounts of As in the plants at the beginning of the experiment. When As was added to the ambient solution, there were significant differences ($p < 0.01$) between the extent of accumulation for each species: *C. demersum* ≫ *M. propinquum* > *R. nasturtium-aquaticum*. At the highest As levels in the ambient solution, 3.9 mg L^{-1}, the plant As concentrations were similar to those sampled from the TVZ and Waikato River. However, under these conditions, the bioaccumulation coefficients (Fig. 2C) for all three species were 1–2 orders of magnitude lower than the plants collected in situ.

Fig. 3A shows the As accumulation in various portions of watercress grown hydroponically in Erlenmeyer flasks. On average, the submerged roots and stems had an As concentration an order of magnitude higher than the aerial stems and leaves. The growth of watercress (Fig. 3B) was unaffected at and below 1.03 mg L^{-1}. This threshold is well above water As concentrations found in the aquatic environment of the TVZ or Waikato River. Plants grown above this concentration showed signs of stress, in particular through the disintegration of the submerged portions. At the highest concentration of 25.8 mg L^{-1} the fresh weight decreased during the experiment, indicating that the plant was dying.

3.4. Depuration of As by aquatic plants

When placed in uncontaminated river water at <0.01 mg L^{-1} As, As-rich watercress (*R. nasturtium-*

Table 2
Arsenic accumulation in the submerged portions of aquatic species collected from the Taupo Volcanic Zone

Species	Location	No. of samples	% dry matter	Plant Geomean [As] and S.D. range	Water Geomean [As]	Geomean B.C.[a]
+Hot water algae	12	3	3	3019 (1058–8617)	0.32	9345
+Cold water algae	9, 13	10	8.5	36.7 (7.3–184.9)	0.03	1452
Alisma plantago aquatica (L.)	9	2	10.2	40.3 (25.8–63.1)	0.02	1853
Callitriche stagnalis (Scop)	6, 8, 9	3	6.4	4215 (3402–5223)	0.09	49307
Callitriche petriei (R. Manson)	9, 12	2	11.0	422 (343–520)	0.03	15190
+*Ceratophyllum demersum* (L.)	1, 3–6, 8–12	16	6.9	284 (119–769)	0.03	10606
Convolvulus arvensis (L.)	13	1	10.9	17.0	0.03	580
Cyperus eragrostis (Lam.)	1, 2	5	17.8	47.5 (21–105)	0.05	1049
Cyperus ustulatus(J. & K. Presl)	9	1	28.7	20.5	0.04	584.9
+*Egeria densa* (Planch.)	2, 3, 5, 7–11	8	6.1	568 (189–1701)	0.02	24847
+*Elodea canadensis* (Michx.)	12	6	6.2	68 (2.5–1837)	0.01	4987
Glyceria maxima (Hartman)	3, 6, 8, 9	5	17.6	13.9 (3.7–52.5)	0.04	352
Juncus spp.	1, 2, 6, 8	14	24.5	9.8 (2.4–39.4)	0.08	116
+*Lagarosiphon major* (Ridley)	7, 9–13	11	7.8	127 (7.7–2107)	0.02	8502
+*Lemma minor* (L.)	10, 12, 13	9	2.9	15 (0.6–360)	0.01	1793
Lotus corniculatus (L.)	7	2	10.7	637 (344–1179)	–	–
Lycopus europaeus (L.)	2	2	15.9	102 (91–114)	0.03	3343
Mentha piperata/var. citrata (Ehrh.)	8, 10, 12	4	9.1	167 (14.8–1884)	0.04	4361
Mentha spicata (L.)	12	3	9.9	181 (88.7–370.4)	0.13	1361
Myosotis laxa (Lehm.)	10	1	4.7	64.2	0.01	4758
+*Myriophyllum propinquum* (A.Cunn)	12	10	4.7	2101 (873–5056)	0.08	27860
Nymphaea alba (L.)	6, 8, 9	6	8.0	16.5 (7.5–36.4)	0.03	483
Polygonum hydropiper (L.)	2, 9, 12	8	8.1	114 (19.3–672)	0.05	2423
Polygonum salicifolium (Brouss.)	9, 10, 13	4	7.4	44 (10.0–197.8)	0.01	3126
Potamogeton orchreatus (Raoul.)	8	5	7.3	144.6 (26.5–808)	0.01	16188
Rorippa nasturtium-aquaticum (L.)	8, 10, 12	33	4.6	369 (58.7–2325)	0.04	9174
Rumex crispus (L.)	10	1	11.5	3.9	0.01	294
Rumex obtusipholius (L.)	9	1	24.1	97.3	0.04	2274
Typha orientalis (C. Presl)	12	3	9.5	174 (113–267)	0.09	2042
Veronica aquatica (L.)	8–10, 12	6	3.8	10.0 (3.4–29.0)	0.02	459

The numbers in the "Location" column refer to sites shown in Fig. 1. "% dry weight" indicates the percentage dry matter in the fresh weight plant. Plant concentrations are in mg kg^{-1} dry weight, water concentrations are in mg L^{-1}. The "hot water" and "cold water" algae represent an assemblage of prokaryotic and eukaryotic photosynthetic-organisms that included both single celled and filamentous species. Plus (+) denotes species that lack extensive root systems.

[a] Bioaccumulation coefficient, defined here as the plant/water concentration quotient.

aquaticum) and *M. propinquum* released 62(±25)% and 21(±5)% of their total As into the ambient water. The calculated geometric means and standard deviation ranges of the initial concentrations were 2364 (1964–2485) mg kg^{-1} As for *R. nasturtium-aquaticum* and 1976 (1353–2886) mg kg^{-1} As for *M. propinquum*. The measured concentrations from other specimens from the same site were 1390 (666–2904) mg kg^{-1} As for *R. nasturtium-aquaticum* and 2102 (874–5056) mg kg^{-1} As for *M. propinquum*. The final ambient solution As concentrations for *R. nasturtium-aquaticum* and *M. propinquum* were 0.42 (0.25–0.69) and 0.24 (0.16–0.37) mg L^{-1}. During this

Table 3
Arsenic concentrations (mg kg^{-1} dry weight) in the leaves of terrestrial species collected from the Taupo Volcanic Zone

Name	Location	No. of Samples	Minimum (no. of samples below detection limit)	Maximum
Asplenium polyodon (G. Forst.)	14	1		<0.5
Blechnum capense (L.)	12, 13	5	<0.5 (3)	1.2
Cyathodes juniperina (JR. & G. Forst.)	12	1		<0.5
Dicranopteris linearis (Burm.)	11	1		<0.5
Histiopteris incsia (Thunb.)	11, 13–15	6	<0.5 (5)	1.4
Kunzea ericoides (A.Rich.)	11–14	5		<0.5
Leptospermum scoparium (JR. & G. Forst.)	12–15	6	<0.5 (5)	11
Lycopodium cernuum (L.)	11	1		<0.5
Paesia scaberula (A.Rich.)	13, 15	3	<0.5 (2)	0.6
Pittosporum tenuifolium (Gaertn.)	12	1		<0.5
Pteridium esculentum (Forst. F.)	12, 15	4		<0.5

The numbers in the "Location" column refer to sites shown in Fig. 1. The soil concentrations for sites 11, 12, 14 and 15 were 80.2, 33.2, 36.4 and 67.9, respectively. No soil was sampled from site 13.

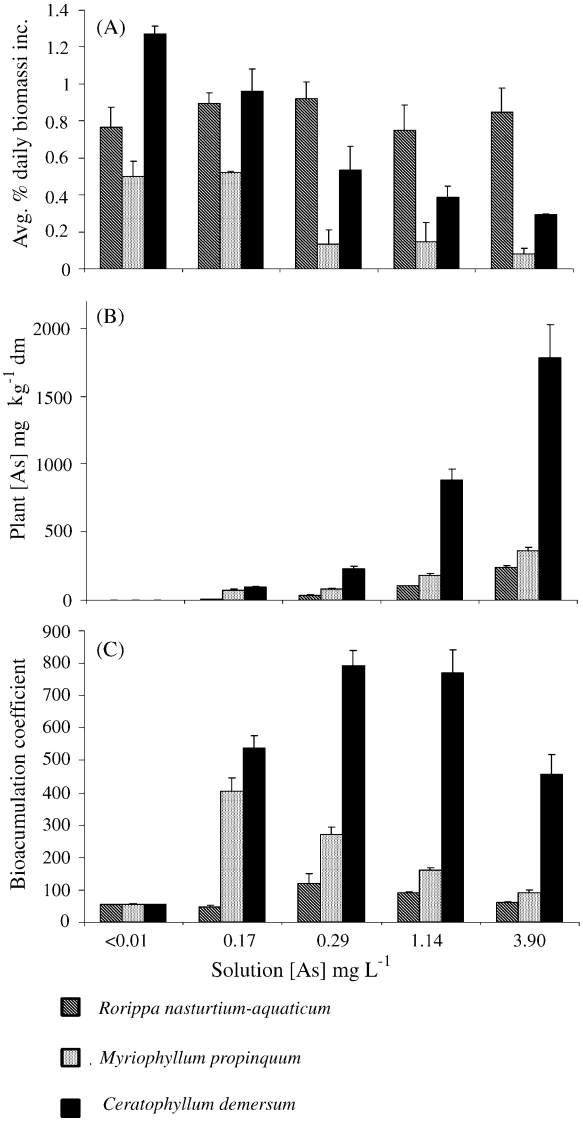

Fig. 2. (A–C) The growth (average % daily increase over 42 days), As-uptake (mg kg^{-1} dry weight) and bioaccumulation coefficients (plant/water concentration quotient) of submerged aquatic plants under controlled conditions. The bars represent the standard error of the mean.

time, the plants showed no signs of necrosis and had visible growth. The change in biomass was not recorded.

Watercress did not reach equilibrium even after 25 days, although over half of the final concentration in the ambient solution was reached after 5 days. There was no consistent trend for *M. propinquum*. However, like watercress, over half of the final concentration in the ambient solution was achieved after 5 days.

3.5. Accumulation of other elements by aquatic plants

The results of the multi-element analysis are given in Table 4. In addition to As, the plants also had elevated levels of other chalcophile elements, notably Fe, Sb, Hg, Tl and Se. The Fe concentrations in the aquatic plants were signifi-

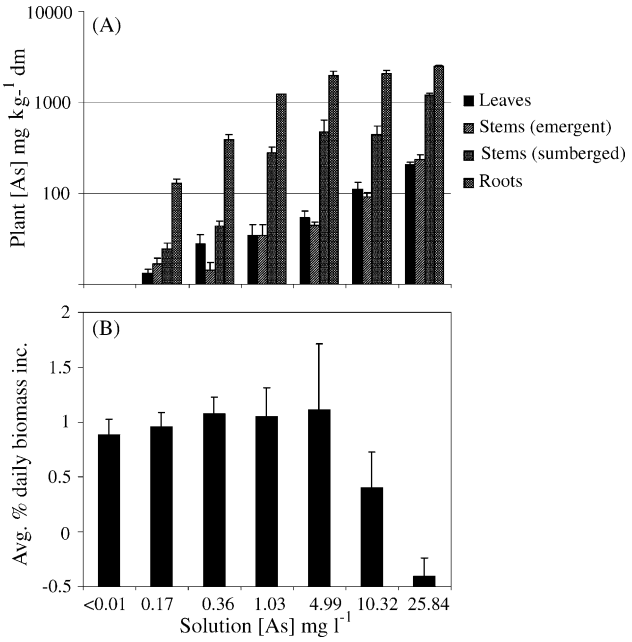

Fig. 3. (A and B) The As accumulation and growth (average % daily increase over 42 days) of watercress grown hydroponically. Bars represent the standard error of the mean.

Table 4
The geometric means, standard deviation ranges and maximum values of elemental concentrations (mg kg^{-1} dry weight) in 15 selected aquatic plants from the Taupo Volcanic Zone, New Zealand

	Geometric mean	Standard deviation range	Maximum value
Al	526	278–996	1960
As	413	91–1870	3190
B	46	23–90	147
Ba	60	32–113	189
Be	0.07	0.03–0.16	0.50
Ca	7700	4700–12400	16100
Cd	0.12	0.05–0.25	0.36
Co	1.6	0.6–4.0	8.5
Cr	3.6	1.3–10	18
Cs	16	7.6–34	55
Cu	6.0	3.4–11	15
F	7.7	5.9–10	12
Fe	3798	1157–12467	34900
Hg	0.29	0.06–1.3	2.8
K	27600	15200–50000	56100
La	0.48	0.25–0.92	1.8
Li	18	9.6–33	56
Mg	3300	1800–5900	6500
Mn	950	332–2717	4850
Mo	0.71	0.37–1.4	3.3
Na	7500	4600–12100	24200
Ni	1.6	1.0–2.6	3.8
Pb	0.67	0.36–1.3	1.6
Rb	92	31–267	312
Sb	2.4	0.2–23	121
Se	0.36	0.12–1.0	1.5
Sr	81	48–136	181
Tl	0.69	0.26–1.9	10
U	0.11	0.05–0.23	0.46
V	10.7	2.2–52	132
Zn	117	55–247	568

cantly and positively correlated with all the chalcophilic elements, including As ($r = 0.68$, $p < 0.01$). The concentrations in Table 4 are the means of 10 aquatic species. Therefore, the results do not show any species-specific patterns that may have been present.

4. Discussion

4.1. Contrasting As-uptake between aquatic and terrestrial vegetation

All the aquatic plants in the TVZ and Waikato River had As concentrations 100–50,000 times that of the ambient water. Most specimens of *M. propinquum* were over the 1000 mg kg^{-1} threshold. There have been no previous reports of As hyperaccumulation by this species.

Previous studies of aquatic plants from the TVZ (Liddle, 1982; Robinson et al., 1995a, 2003; Brooks and Robinson, 1998) focused on four species: *C. demersum*, *E. densa*, *Lagarosiphon major* and *R. nasturtium-aquaticum*. These previous studies reported average As concentrations in *C. demersum*, *E. densa*, *L. major* and *R. nasturtium*, of 400, 500, 300 and 400 mg kg^{-1}, respectively. Although sampled at different locations, our results were in good agreement.

Meanwhile, As was not taken up to any extent by terrestrial vegetation. Outridge and Noller (1991) reported a difference in trace-element accumulation between aquatic and terrestrial species. In terrestrial systems, solubilisation of As in the rhizosphere is necessary to allow root uptake. An efficient As transport and storage system would be needed for leaf accumulation to occur. Neither of these need be the case when the plant grows in an aqueous medium. Here the metalloid is already present in a bioavailable form and can be absorbed or adsorbed by the leaves.

When comparing the results of our experiment with those of Ma et al. (2001), who showed *P. vittata* could hyperaccumulate As from a soil containing less As than the highest treatment in this trial, it is clear that our ferns have no specialised As-uptake mechanism.

4.2. Mechanisms of As-uptake by aquatic plants

For the aquatic plants used in our experiments, As was accumulated to a greater extent in situ than under controlled conditions. This is similar to the findings of Liddle (1982) for *C. demersum*. These differences may be due to the chemical composition of the water, plus interactions with sediments. In the Waikato River, contamination of plant material with sediment would, on a dry weight basis, reduce the measured As concentration, as the mean As concentration is an order of magnitude lower in the sediment than in *C. demersum* (Table 1).

An alternative hypothesis is that suspended oxides of Fe may bind to the surface of aquatic plants and adsorb As and other dissolved elements. In this case, As accumulation would thus occur via physicochemical adsorption rather than biological uptake. The Fe concentrations in the aquatic plants in this study were well in excess of those required for metabolic processes. Salisbury and Ross (1992) report average Fe concentrations in maize and cherry leaves at 120 and 58 mg kg^{-1}, respectively. Our geometric mean was 3798 mg kg^{-1} and the maximum value was 34,900 mg kg^{-1} (Table 4), namely 3.5%.

The significant and positive correlation between As and Fe concentrations is also consistent with As being incorporated into Fe oxides attached to the surface of the plant. This phenomenon was observed by Blute et al. (2004) on the roots of *Typha latifolia* (cattail) growing in contaminated wetland sediments. Chen et al. (2005) demonstrated that iron plaques on the surface of rice roots not only bound As, but also promoted its uptake by the root.

Further supporting evidence that As is surface-bound comes from the observation that As was rapidly desorbed in the depuration experiment. If As were internalised into cellular structures, then it would be expected that natural depuration would be limited. Finally, the hypothesis is supported by the observation that the total dry weight As concentrations were highest in plants with the highest surface areas (and therefore highest surface area-to-weight ratios), notably *M. propinquum*.

When watercress was grown in soil or hydroponically, the lack of As accumulation in the aerial portions at solution concentrations similar to those found in the Waikato River and TVZ indicated that this species has no efficient As-translocation mechanisms. At higher solution concentrations, some As accumulation in the stems and leaves occurred; however, the growth was negatively affected and the plant showed signs of necrosis. Again, this evidence is consistent with the hypothesis that there is no specific uptake or tolerance mechanism for As accumulation in watercress.

4.3. Arsenic in the food chain and potential human exposure

Plant As accumulation may facilitate the entry of this toxic element into the food chain and provide an exposure pathway to humans. The effect on humans of consuming As-rich watercress is unclear. Some local people report no ill effects, despite having consumed watercress from As-rich areas for many years. Any As toxicity from consuming watercress will depend on the amount and frequency eaten, how the watercress is prepared, what it is consumed with and the chemical form of As in the plant. In this respect, the finding that most As is likely to be adsorbed to the plant surfaces would imply that most of the As in watercress is likely to be the more toxic inorganic form. Transformation to plant organoarsenicals generally presupposes biochemical mediation.

For the purposes of this work, the most conservative international figure of 0.3 μg kg^{-1} of body weight per day will be applied (DEFRA, 2002). Further, due to the likelihood that As

in watercress is mostly present in a surface-adsorbed form, an assumption will be made that all of the As in the watercress is inorganic. Use of a conservative approach is standard for the initial risk-assessment of ingested dietary contaminants. The TDI may be taken as a threshold level below which adverse effects are unlikely.

Therefore, a 70-kg person should consume no more than 0.15 mg of As per week. Assuming the person consumes watercress from Lake Ohakure, which contains an average of 9 mg kg^{-1} As, and that this is the only source of As in the person's diet, then they would need to consume just 16 g of fresh watercress per week to exceed the TDI. It is probable, therefore, that routine ingestion of watercress would cause a person's total inorganic As dose to move beyond the threshold where adverse effects are unlikely, and into an area where they become more probable.

The potential As-contribution from watercress is also high in relation to background levels of As in the New Zealand diet. On the basis of New Zealand total diet survey data for 1997/1998 (Vannoort et al., 2000), making a standard assumption that 10% of As in food is inorganic, the weekly ingestion of 7 g of watercress containing 9 mg kg^{-1} As would be sufficient to double dietary intakes of inorganic As.

Values of up to 138 mg kg^{-1} As on a fresh weight basis have been found in this study, indicating that, in some cases, the TDI for As may be exceeded many-fold if the plant is consumed. However, it would require an epidemiological study to indicate any cancer risk associated with the consumption of As-rich aquatic plants. This is because it would be difficult to separate cancer caused by As from other cancer-causing activities such as smoking and excessive drinking.

The presence of other toxic heavy metals such as Sb, Hg and Tl may exacerbate As toxicity.

Humans may also be exposed to As indirectly. Much of the Waikato River is surrounded by farmland, and stock occasionally have access to aquatic weeds. Fishing is popular in the region. However, a previous study (Robinson et al., 1995b) showed that As levels in fish were unlikely to pose a risk to human health.

In a lake or river system, the amount of plant-bound As at any one time may be a significant portion of the total amount of As in the river. If, for example, low river flows or pesticides kill the plants, then there may be a large pulse of As released into the water.

4.4. Aquatic macrophytes for the bioindication or phytoremediation of As contaminated water

The bioaccumulation coefficients for the aquatic plants in this study are higher than any previously reported for terrestrial species. They may therefore be useful for the phytoremediation of As from contaminated drinking water. The possibility of using plants to remove As has been discussed by Brooks and Robinson (1998). More generally, the use of aquatic plants to remove pollutants from contaminated water has been described by Wolverton (1975) and Wolverton and McDonald (1975a,b).

Alternatively, aquatic plants may be used to bioindicate the level of water-contamination. The high-As bioaccumulation coefficients would make As detection far simpler than direct measurement of the water. Robinson et al. (1995a) measured As concentrations in *C. demersum* and the ambient water from 25 locations along the Waikato River. There was a highly significant ($r = 0.60$, $p < 0.01$) and positive correlation between the plant As concentration and that of the ambient water. This indicates that the As concentration of the plants could be used to predict the As concentration of the river water. Moreover, the plants (average 412 mg As kg^{-1}) contained around 10 times the As concentration of the sediments (average 46 mg As kg^{-1}) that, in turn, had over 1000 times the As concentration of the ambient water (average 0.042 mg As L^{-1}).

Other factors, such as the pH, water temperature and nutrient availability will doubtless affect As accumulation. Further work needs to be carried out on these effects to determine how well these bioindicators would perform under various scenarios.

5. Conclusions

We found a clear distinction in As concentrations between aquatic and terrestrial plants. Arsenic accumulation is widespread among aquatic plants, and we found concentrations of 100–1000 mg kg^{-1} in several previously untested species. Arsenic accumulation in other aquatic species will undoubtedly be found as further surveying is conducted.

Arsenic accumulation by aquatic macrophytes may make them valuable tools for the bioindication and phytoremediation of As in fresh waters. Additionally the ecological impacts of As accumulation are likely to be profound. Clearly, no aquatic plants should be consumed from waterways with geothermal inflows, or with other sources of As contamination, either by humans or livestock.

Our experiments provide indirect evidence that As is adsorbed onto the surface of the plant via physicochemical reactions, probably as co-precipitation with Fe. This hypothesis could be confirmed by microanalyses of the tissues to determine whether As is present inside the cells or simply bound to the outer cell walls. In this latter case, it could be argued that the plants are not true hyperaccumulators, as they may lack specialised As-uptake and storage mechanisms. Nevertheless, this does not change their potential usefulness as bioindicators, or make them any less toxic if consumed.

Acknowledgements

We are most grateful to Prof. Mary Beth Kirkham who provided a constructive review of the manuscript. We thank Environment Waikato for financial support for this study.

References

Aggett, J., Aspell, A.C., 1978. Arsenic from geothermal sources. New Zealand Research and Development Committee Report #35. University of Auckland, Auckland, New Zealand.

ATSDR (Agency for Toxic Substances and Disease Registry), 2000. Toxicological Profile for Arsenic. U.S. Department of Health and Human Services, Atlanta, GA, USA, p. 244.

Axtmann, R.C., 1975. Environmental impact of a geothermal power plant. Science 187, 795–803.

Baars, A.J., Theelen, P.J.C.M., Janssen, J.M., van Apeldorn, M.E., Meijrink, M.C.M., Verdam, L., Zeilmaker, M.J., 2001. Re-evaluation of human-toxicological maximum permissible risk levels. RIVM Report 711701 025. National Institute of Public Health and the Environment. Bilthoven, The Netherlands.

Blute, N.K., Brabander, D.J., Hemond, H.F., Sutton, S.R., Newville, M.G., Rivers, M.L., 2004. Arsenic sequestration by ferric iron plaque on cattail roots. Environ. Sci. Technol. 38 (22), 6074–6077.

Brooks, R.R., Lee, J., Reeves, R.D., Jaffré, T., 1977. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J. Geochem. Explor. 7, 49–77.

Brooks, R.R., Robinson, B.H., 1998. Aquatic phytoremediation by accumulator plants. In: Brooks, R.R. (Ed.), Plants that Hyperaccumulate Heavy Metals: Their Role in Archaeology, Microbiology, Mineral Exploration, Phytomining and Phytoremediation. CAB International, Wallingford, pp. 203–226.

Chen, Z., Zhu, Y.G., Liu, W.J., Merhag, A.A., 2005. Direct evidence showing the effect of root surface iron plaque on arsenite and arsenate uptake into rice (*Oryza sativa*) roots. New Phytol. 165 (1), 91–97.

DEFRA & EA (Department for Environment, Food and Rural Affairs and the Environment Agency), 2002. Soil guideline values for arsenic contamination. Report SGV 1, Environment Agency. Bristol, UK.

Food Standards Australia New Zealand (FSANZ), 2002. http://www.foodstandards.gov.au (FSANZ New Zealand, P.O. Box 10599, The Terrace, Wellington).

Forstner, U., Wittmann, G.T.W., 1981. Metal Pollution in the Aquatic Environment. Springer-Verlag, New York.

Freeman, M.C., 1985. The reduction of arsenate to arsenite by an *Anabaena* bacteria assemblage isolated from the Waikato River. N. Z. J. Mar. Fresh. 19, 277–282.

Hill, C.F., 1975. Impounded Lakes of the Waikato River. In: Jolly, V.H., Brown, J.M.A. (Eds.), New Zealand Lakes. Auckland University Press, Auckland.

Khattak, A.K., Page, A.L., Parker, D.R., Bakhtar, D., 1991. Accumulation and interactions of arsenic, selenium, molybdenum and phosphorus in alfalfa. J. Environ. Qual. 20, 165–168.

Liddle, J.R., 1982. Arsenic and other elements of geothermal origin in the Taupo Volcanic Zone. PhD Thesis. Massey University, Palmerston North, New Zealand.

Ma, L.Q., Komar, K.M., Tu, C., Zhang, W., Cai, Y., Kennelly, E.D., 2001. A fern that hyperaccumulates arsenic. Nature 409, 579.

Meharg, A.A., Macnair, M.R., 1991. Uptake, accumulation and translocation of arsenate in arsenate-tolerant and non-tolerant *Holcus lanatus* L. New Phytol. 117, 225–231.

Morales, K.H., Ryan, L., Tsung, L.K., Meei, M.W., Chien, J.C., 2000. Risk of internal cancers from arsenic in drinking water. Environ. Health Persp. 108 (7), 655–661.

Outridge, P.M., Noller, B.N., 1991. Accumulation of toxic trace elements by freshwater vascular plants. Rev. Environ. Contam. T. 121, 163.

Pepper, I., Galanti, N., Sans, J., Lopez-saez, J.F., 1987. Reversible inhibition of root growth and cell proliferation by pentavalent arsenic in *Allium cepa*. Environ. Exp. Bot. 28 (1), 9–18.

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 Physiol. 122, 1171–1177.

Reay, P.F., 1972. The accumulation of arsenic from arsenic–rich natural waters by aquatic plants. J. Appl. Ecol. 9, 557–565.

Robinson, B.H., Brooks, R.R., Outred, H.A., Kirkman, J.H., 1995a. The distribution and fate of arsenic in the Waikato river system, North Island, New Zealand. Chem. Spec. Bioavailab. 7 (3), 89–96.

Robinson, B.H., Brooks, R.R., Outred, H.A., Kirkman, J.H., 1995b. Mercury and arsenic in trout from the Taupo Volcanic Zone and Waikato River, North Island, New Zealand. Chem. Spec. Bioavailab. 7 (1), 27–32.

Robinson, B.H., Duwig, C., Bolan, N.S., Kannathasan, M., Saravanan, A., 2003. Uptake of arsenic by New Zealand watercress (*Lepidium sativum*). Sci. Total Environ. 301 (1), 67–73.

Salisbury, F.B., Ross, C.W., 1992. Plant Physiology, fourth ed. Wadsworth, Belmont, CA.

Smies, J., 1983. Biological aspects of trace element speciation in the aquatic environment. In: Leppard, G.G. (Ed.), Trace Element Speciation in Surface Waters and its Ecological Implications. Plenum Press, New York, pp. 177–193.

Storelli, M.M., Marcotrigiano, G.O., 2001. Total, organic, and inorganic arsenic in some commercial species of crustaceans from the Mediterranean Sea (Italy). J. Food Protect. 64 (11), 1858–1862.

Tanner, C.C., Clayton, J.S., 1990. Persistence of arsenic 24 years after sodium arsenite herbicide application to Lake Rotoroa, Hamilton, New Zealand. N. Z. J. Mar. Fresh. 24, 173–179.

USEPA, 1993. Integrated Risk Information System (IRIS). Arsenic. Oral reference dose assessment last reviewed January 1993. Carcinogenicity assessment last revised 1998. http://www.epa.gov/iris/index.html (accessed January 2005).

Vannoort, R., Cressey, P., Silvers, K., 2000. 1997/98 New Zealand Total Diet Survey Part 2: Elements, Selected Contaminants & Nutrients. HP3353. Ministry of Health, Wellington, New Zealand.

Vogeler, I., Green, S.R., Clothier, B.E., Kirkham, M.B., Robinson, B.H., 2001. Contaminant transport in the root zone. In: Iskandar, I.K., Kirkham, M.B. (Eds.), Trace Elements in Soil. Bioavailability, Flux, and Transfer. Lewis Publishers, Boca Raton, FL, pp. 175–197.

Wolverton, B.C., 1975. Water hyacinths for removal of cadmium and nickel from polluted waters. National Aeronautics and Space Administration (NASA). Technical Memorandum TM-X-72721. Washington, DC.

Wolverton, B.C., McDonald, R.C., 1975a. Water hyacinths and alligator weeds for removal of lead and mercury from polluted waters. National Aeronautics and Space Administration (NASA). Technical Memorandum TM-X-72723. Washington, DC.

Wolverton, B.C., McDonald, R.C., 1975b. Water hyacinths and alligator weeds for removal of silver, cobalt and strontium from polluted waters. National Aeronautics and Space Administration (NASA). Technical Memorandum TM-X-72727. Washington, DC.