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Performance of *Eucalyptus* species on capped arsenic-rich gold mine tailings in the Victorian Goldfields, Australia

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Abstract

An experimental tailings research facility was constructed to allow long-term trials to be conducted in order to investigate the performance and variation in growth and arsenic foliar content in 16 taxa of eucalypts comprising five Eucalyptus spp. with various provenances. A 30 cm deep cover of slurried oxide waste residue was poured on to consolidated arsenic-rich sulphidic gold tailings and was capped with a 10 cm layer of local topsoil. The Eucalyptus were planted in September 2008. The study reported here focusses on the growth responses of candidate Eucalyptus species and provenances in relation to substrate variables (cover depth and arsenic mobility). Three provenances of Eucalyptus cladocalyx grew the fastest and, on average, produced the largest stem volumes. The local provenance of E. goniocalyx was the poorest. Among the other species, Corymbia maculata with provenances from New South Wales and Western Australia (WA) ranked second, E. camaldulensis with provenances from Victoria, WA and South Australia ranked third, and E. tricarpa ranked after these. Owing to its ability to grow under arsenic-rich conditions, more detailed testing of E. cladocalyx involving long-term monitoring of growth, biomass partitioning and foliar arsenic content is required to improve the selection of suitable species and provenances for use in arsenical mineral waste rehabilitation.

1 Introduction

Gold mining operations generate large volumes of fine residues (tailings), with tailings storage facilities often being the largest area on mine sites requiring rehabilitation. These mineral wastes are often physically and chemically unstable. One of the major environmental issues present at Stawell Gold Mine, the largest active mine in the Victorian Goldfields, Australia, is the elevated level of arsenic (As) in tailings (> 2,000 mg kg^{-1}). The aim of the present study was to develop cost-effective revegetation protocols for sulphide-bearing gold mine tailings that result in land of high economic and/or environmental value, which provide an economic asset to the former mining community once the mine ceases to operate.

Traditional covers required at least a 300 mm thick compacted clay layer over the tailings to seal off the toxic wastes, plus a greater thickness of protective neutral cover material. This is expensive and has not always been successful in tailings surface stabilisation. Moreover, shallow covers are generally not conducive to vegetative rehabilitation. The covers represent partially 'open' geochemical systems with the purpose of limiting acid and metal release to environmentally acceptable levels, rather than the unrealistic goal of infinite encapsulation. However, the weathering process will continuously form oxidation products such as secondary minerals, which may be detrimental to plant growth. Formation of a permanent and self−sustaining vegetation cover over tailings will minimise surface erosion and reduce the transfer of oxygen and water to the sulphidic tailings.

There is a need to mitigate high levels of As contamination in terrestrial ecosystems in order to reduce the threat to humans and the environment. In recent years, there has been an interest in plants to alleviate the contamination of As in soil. Phytoextraction of As employs plants that are able to accumulate and tolerate

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high levels of As with minimal to no physiological stress. An ideal candidate for some phytoextraction projects is the fern Pteris vittata, a hyperaccumulator of As. The fern can accumulate more than 2,000 µg As g^{-1} d.w. in its aboveground biomass (Ma et al., 2001; Chen et al., 2002). There is, however, considerable variation in uptake capacity among Pteris taxa, and only a limited number of other candidates species for As hyperaccumulation have subsequently been identified (Meharg, 2003; Wang et al., 2006). For cases where phytoextraction is not an option, phytostabilisation is an alternative approach to limit the mobility of As in contaminated environments. It employs As-excluder plants with minimal uptake and translocation of As, and a potential to progressively detoxify As over time.

To date, all known As excluders are woody species, but a greater understanding is required of the mechanism by which these species survive and perform under As-rich conditions in the long term. Since the recognition of suitable woody species for As phytostabilisation, many studies have been directed towards northern hemisphere species (Medejón et al., 2004; Chang et al., 2005; French et al., 2006; Vázquez et al., 2006; Tlustoš et al., 2007) but very few studies have investigated southern hemisphere species (Hartley, 1977; Craw et al., 2007; King et al., 2008). Furthermore, these few studies have mainly focussed on colonisation, As accumulation and tree height as indicators of tree performance, lacking other more informative indices of growth performance such as stem diameter.

King et al. (2008), for example, examined four *Eucalyptus* species (*E. cladocalyx, E. melliodora, E. polybractea* and *E. viridis*) and demonstrated that *E. cladocalyx* may have potential for As phytostabilisation. All four species accumulated low concentrations of foliar As (ranging from 0.29 to 5.14 µg As g^{-1}), and the authors identified important variations within species both in foliar As concentrations and tree height, suggesting that an opportunity existed for further improvement by cloning or selective breeding. *Eucalyptus* is a diverse genus of trees native predominately to Australia, which includes over 700 species now distributed worldwide due to their fast growth rates and adaptability to different environmental conditions in appropriate climates. However, other than the work by King et al. (2008), there has been no study examining the performance of eucalypts in As phytostabilisation. The need to identify other *Eucalyptus* spp. and provenances with superior As exclusion and fast growth rates, as well as good long-term survival, prompted the establishment of this extended *Eucalyptus* spp. provenance trial.

The aim of the work was to examine variation in growth and foliar As content in five *Eucalyptus* spp., including *E. cladocalyx*, and to provide a more comprehensive basis for further comparison and provenance selection.

2 Materials and methods

A field trial was conducted on a Tailings Experimental Research Facility (TERF) dam located at Stawell Gold Mine (37°03’59 latitude, 142°48’15 longitude and altitude 203 m) in Western Victoria, Australia. Stawell Gold Mine is approximately 240 km northwest of Melbourne and is the largest and oldest operating gold mine in Victoria. The TERF commenced in September 2001 and consists of a small dam filled with fresh sulphidic tailings poured from spigots positioned around its periphery. It was completed in May 2002. Perforated PVC pipes were laid at the base of the TERF before filling to allow collection of drainage water and to speed up the drying time of the tailings. Experimental plots were created in September 2002 when the surface of the tailings had sufficiently consolidated to allow the use of earth-moving machinery. A trial cover was implemented in November 2006 on the sulphidic tailings that by then had been exposed to weathering and hence had oxidised for four years.

The final TERF has ~3 m high walls, forming two experimental cells each with a dimension of 75 × 155 m. One of the two experimental cells used for experimentation in this study was 40 × 50 m; the other cell had been used previously in a field trial conducted by King et al. (2008) who described the prevailing geochemical conditions of the tailings substrate. Briefly, these are rich in sulphides mainly as pyrite and As mainly as arsenopyrite, with great acid-neutralising potential and enriched with salitre gypsum. The total As concentration in tailings ranges from 1,000 to 2,000 mg kg^{-1}. In contrast to previous work (King et al., 2008), in this study the experimental cell was capped with two different layers. The top layer was 5 cm thick and

consisted of a slightly acidic sandy loam (pH 5.5) with low electrical conductivity (EC < 0.5 mS cm^{-1}). A base layer consisted of 30 cm of oxide waste from heavily leached waste rock that was milled to produce a fine material. The top layer was constructed with material stockpiled from the top 10 cm of the original land surface when it was removed by previous mining operations from open cuts and tailings storage facility floors. The base layer had an alkaline pH (8.2), low electrical conductivity EC < 0.5 mS cm^{-1} and no net acid-generation potential and was demonstrated in a small-scale glasshouse trial to be chemically benign for plants.

Stawell Gold Mine has a temperate climate. Mean annual rainfall at Stawell TERF over the period 2007 to 2011 was 562 mm, mean max/min temperature was 19.7/8.4°C and the mean daily solar irradiance was 21 MJ (m^{-2}) (Bureau of Meteorology, Australia). The vegetation surrounding the Stawell TERF is a mix of five major ecological classes, including Box Ironbark Forest; Heathy Woodland; Grassy Dry Forest; Yellow Gum Woodland; and Valley Grassy Forest, for which about 200 species of vascular plants have been recorded; 75% of the total are indigenous species, and the remaining 25% are introduced species (Comino et al., 2004).

2.1 Plant material

Seedlings of five species of *eucalypts* – *E. cladocalyx* (sugar gum), *E. camaldulensis* (river red gum), *Corymbia maculata* (spotted gum), *E. tricarpa* (red ironbark), and *E. goniocalyx* (long-leaved box) – were transplanted into the trail plots in late spring (September) 2007. Species were chosen according to several criteria:

- *E. Cladocalyx*, with six provenances, was previously reported to grow in As-rich contaminated land (King et al., 2008) and to show a high growth rate in acidic and moderately saline conditions and in drought conditions.
- *E. Camaldulensis*, with four provenances, has been found to be tolerant to both drought-salinity and waterlogged conditions.
- *C. maculate*, with two provenances, has already been widely used in many mining restoration operations in Western Australia (WA) and Queensland, and it produces good timber and can be useful for ecological restoration of mine tailings as it attracts honeyeaters (birds that feed on nectar).
- *E. Tricarpa*, with three provenances, is endemic to the Victorian Goldfields, where soils are naturally enriched in As (20 µg As g^{-1} d.w.) and so may possibly be more tolerant to toxicity of this metalloid in tailings.
- *E. goniocalyx*, like the previous species, is endemic and so may also have tolerance to As. It also has a potential benefit as a cash crop due to its content of high-value cineole (*eucalyptus* oil).

Provenances of the different species were selected from latitudes ranging from 32 to 37° south. In total, therefore, 16 different provenances were established in the Stawell TERF for the field trial. The number of parent trees representing each provenance ranged from 1 to 28. Prior to germination, *Eucalyptus* provenance seeds of similar size were selected and surface-sterilised in sodium hypoclorite 1% (v/v) for 15 min. They were then sown into Premium Vermiculite™ (Exfoliators, Australia) in seed trays. Germination occurred within 5–8 days under glasshouse conditions. After 14 d and prior to the field trial, seedlings were carefully transferred into potting mix (1 L volume plastic bag) for acclimatisation to soil conditions for about 7 d. Seedlings of similar appearance and size (height of 25 cm) were then hardened by gradually exposing them to full sunlight prior to planting out in the TERF trial.

2.2 Field trial

A Latin Rectangle was used to provide randomisation and planning of the field trial layout (Healy, 1951). The rectangle arrangement was a same provenance neighbouring-restricted design in rows and columns,

where the number of rows was not the same as the number of columns. Restrictions were not allocated for provenance location in column or row due to an assumption that substrate distribution was consistent, and fertility trend lines within the experimental plot were assumed to be insignificant. Based on the Rothamsted classic long-term comparative experiments with field crops (Dyke, 1988), provenance plots were separated by other provenances whether from the same or a different species, to minimise interference by the same provenance; headlands and sidelands (1 m wide) were included within the layout to minimise any interference by other species and collection of plant and soil materials. A watering system was established for the first six months to ensure success in establishment and survival during the dry summer season, which consisted in an aboveground hose dripping system for each individual.

The survival and performance of plants were measured on an annual basis during the spring season (September to November) and over a four-year period from 2008 to 2011. Every surviving tree transplanted into the field trial plot after the first season (Sept 2008) was noted and measured for stem height (SH) and based-diameter (SD), as well as diameter at breast height as an estimator of biomass production and tree performance. Relative growth rate (RGR) using dry biomass (Hall et al 1993) was not calculated, but estimates of relative stem height and diameter growth were made using SH and SD data. The equation used for RGR of (SH/SD) was $d\log_e (SH/SD) / dt$, with a non-linear model fitted with the assumption of variance in the amounts of SH and SD added each unit of time over a period of four years (Paine et al., 2011). The diameter of the seedlings that were less than 1.3 m was assessed at 0.1 m above the tree base. Tree height was measured using a graduated metric pole. Foliar material was collected over the same period using a method adapted from King et al. (2008). Tissue was washed in a 2% v/v solution of QuantumClean® detergent pH 6.25 (Rowe Scientific, Melbourne) to remove traces of As from any surface deposits, then it was submerged in As-free phosphate solution (20 mM, pH 6.25) to further remove As from the leaf surfaces and stomata pores. The foliar tissue was finally rinsed in deionised water for a minute to remove any excess phosphate solution. After washing, all plant tissues were oven-dried at 70°C for 4 d before acid digestion.

2.3 Arsenic availability

Tailings cores were collected from the field trial to investigate the effects of roots on the availability of As by determining any differences in As availability between planted and nil (plant-free) plots. After four seasons of growth, in September 2011, soil cores were extracted from 18 points of planted plots and nil plots, beginning with 1 core at the centre of each nil plot (total 8 plots) and 2 cores at the centre of planted plots (total 10 plots), collecting from close to the rhizosphere zone. Cores from planted plots were taken randomly in the field from each *Eucalyptus* spp. using an auger. The parent tailings material was struck at a depth of 35 cm, and a sample was taken at a depth of ≈ 50 cm for all cores. Core samples of nil plots were homogenised and thus divided into 10 samples; the same procedure was also applied to cores from the planted plots. A total of 20 samples (10 nil plots and 10 planted plots) each of about 200 g of fresh tailing material were then stored in plastic containers under dark and ice conditions (5°C) prior to sample analysis.

Two extractants commonly used for the determination of As in solution were used to estimate the bioavailable fraction of As in collected cores samples, NH_4NO_3 and $(NH_4)_2SO_4$ (Anawar et al., 2008). The method of As extraction used by Wenzel et al. (2001) was employed. Air-dried tailings were sieved to < 425 µm and subsamples of c. 1 g were extracted at pH 7 with a soil solution ratio of 1:25 w/v using a rotary shaker at 150 rpm for about 1 h at room temperature. Supernatants were recovered by centrifugation, then filtered using 0.45 µm PTFE membranes (2–10 ml filtration volume). Before analysis samples were stored in dark and cold conditions 4°C.

Eucalypt roots from cores from the planted plots were also collected at the time of core collection and washed in 2% v/v solution of QuantumClean® detergent pH 6.25 (Rowe Scientific, Melbourne), then soaked in As-free phosphate solution (20 mM, pH 6.25) to further desorb As from root surfaces and intercellular spaces. Root tissues were finally rinsed in deionised water to remove excess phosphate solution. After washing, root tissues were oven-dried at 70°C for 4 d before analysis.

2.4 Arsenic analysis

Oven-dry samples were mechanically ground (< 1 mm), and subsamples of 100 mg d.w. were used for acid digestion. An adapted open-vial wet digestion method of Krachler et al. (2001) was used. The powdered foliar materials were predigested in 4 ml vials containing 2 ml of concentrated HNO_3 (68–70%) for 24 h, and digested at 125°C max. for 3 h. Hydrogen peroxide (0.02 µL, 30%) was added to each open vial at 2.5 h of digestion. Samples that were completely clear and homogeneous were carefully transferred into 2 ml volumetric containers and made to the mark with water. All samples including the tailings extractions were analyzed by HG-AFS (Hydride Generator Atomic Fluorescent Spectroscopy). For high concentrations of As in samples, in particular for tailings samples, a 100× dilution using a mix solution containing 30% HCl, 2.5% KI and 0.5% ascorbic acid was required in order to maintain stability during detection. The minimum detection limit for As in plant and soli material was 0.0001 µg g^{-1} As d.w. Arsenic-spiked samples and reference plant material samples gave recoveries of 98±3%.

2.5 Statistics

Survival data were analysed on the basis of the presence or absence of living trees, independent of size or form. All statistical tests were performed using either IBM SPSS statistics (v. 14) or Sigmaplot Software (v.12). Variables including plant performance and foliar tissue As concentrations were transformed using their square-root values to satisfy normality assumptions. A General Linear *r* Model approach and the mean slope of relative stem volume growth rate were used to assess differences in stem volume increase with time. Due to mortality and therefore unequal sample size, one-way ANOVA was used to assess differences in stem volume and foliar As content within species. Two-way ANOVA was used to assess the differences in As bioavailability in the rhizosphere and tailings materials for the factor of extractants [(NH_4NO_3) or ($(NH_4)_2SO_4$]. Association between plant performance and As concentration was assessed using Pearson correlation analysis.

3 Results

3.1 Survival

After four years (2011), mean survival for *E. camaldulensis* was 96%, the highest survival proportion for the trial. The second highest survival was 0.92 for *E. tricarpa*, followed by 0.8 for *C. maculata*, 0.56 for *E. cladocalyx*, and 0.55 for *E. goniocalyx* (Figure 1). It is noteworthy that almost all of the mortality occurred during the first year; there was a minor amount of additional mortality in four of the *E. cladocalyx* provenances in the second year. Within *E. camaldulensis*, the top two provenances – Lake-Albacutya from Victoria (VIC) and salt-tolerant from WA – had little or no mortality, followed by the growth-enhanced variety from WA and *C. maculata* provenance Barclays-Deniliquin from New South Wales (NSW), which also showed little mortality. The poorest performing provenance was *E. cladocalyx* provenance Wirrabara from South Australia (SA), with a survival of just 0.41 (Figure 1).

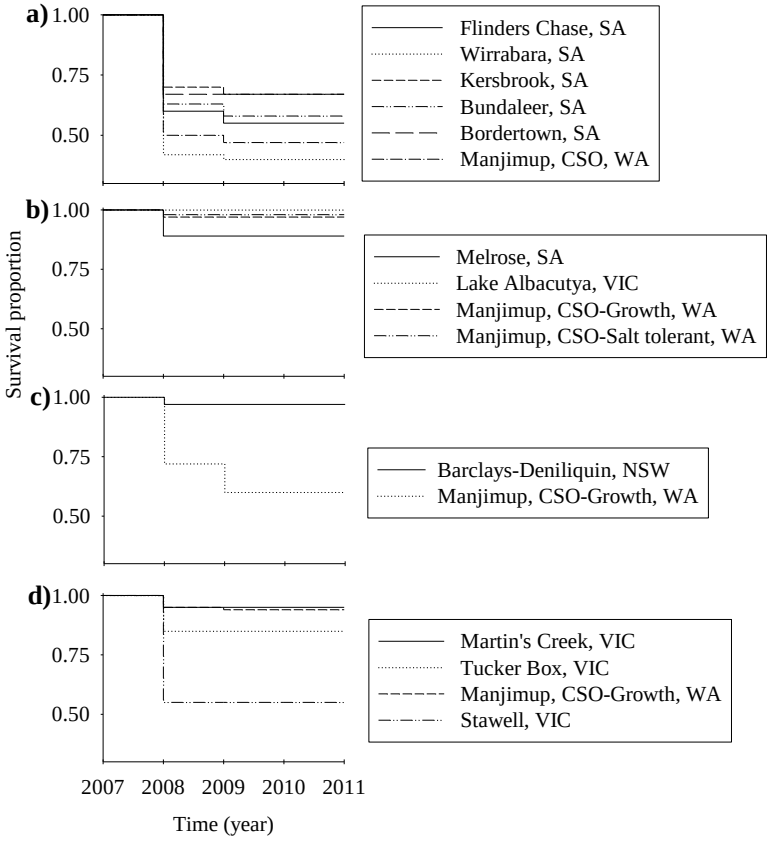

Figure 1 Survival of *Eucalyptus* spp. provenances at the TERF fieldsite. Saplings of *Eucalyptus* spp. established were *E. cladocalyx* provenances; (a) Flinders (I-a), Wirrabara (I-b), Kersbrook (I-c), Bundaleer (I-d), Bordertown (I-e) and Manjimup (I-f) *E. camaldulensis* provenances; (b) Melrose (II-a), Lake Albacutya (II-b), Manjimup growth (II-c) and Manjimup salt-tolerant (II-d) *C. maculata* provenances; (c) Barclays-Deniliquin (III-a) and Manjimup growth (III-b); *E. tricarpa* provenances; (d) Martin's Creek (IV-a), Tucker Box (IV-b) and Manjimup growth (IV-c) and *E. goniocalyx* provenance; and (d) Stawell (V-a)

3.2 Stem volume

Stem volume was calculated using stem height and stem base diameter. Averaging across provenances, the stem volume for *E. cladocalyx* was 15±1.5 (×10^3) cm^3, followed by *C. maculata* at 9.3±2.3 (×10^3) cm^3, *E. camaldulensis* at 7.5±1.5 (×10^3) cm^3 and *E. tricarpa* at 7.4±1.1 (×10^3) cm^3. *E. goniocalyx* with one provenance had the lowset stem volume at 6.7±1.4 (×10^3) cm^3. Stem volume growth rate for each provenance from 2007 to 2011 was analysed using a linear model to describe the average stem volume growth rate (Figure 2). This model showed a remarkably good fit, with all provenances having a significant fit ($P < 0.05$) and most of the provenances an $r^2 > 0.9$ (data not shown). A one-way ANOVA was performed using the mean slope values, and significant differences were detected between provenances ($F = 10.23$, $P < 0\ 001$). A Tukey's multiple comparison test was then undertaken, which showed amongst other things that the three fastest-growing provenances belonged to *E. cladocalyx* from SA (Wirrabara, Bordertown and Bundaleer), with the top provenance (Wirrabara) having a stem growth rate that was significantly higher than all but the Bordertown and Bundaleer provenances. The fourth fastest-growing was an *E. camaldulensis* provenance (Manjimup-Salt tolerant) from WA, and the two provenances with the lowest mean stem growth rate were *E. camaldulensis* Manjimup and *E. tricarpa* Manjimup, both from WA.

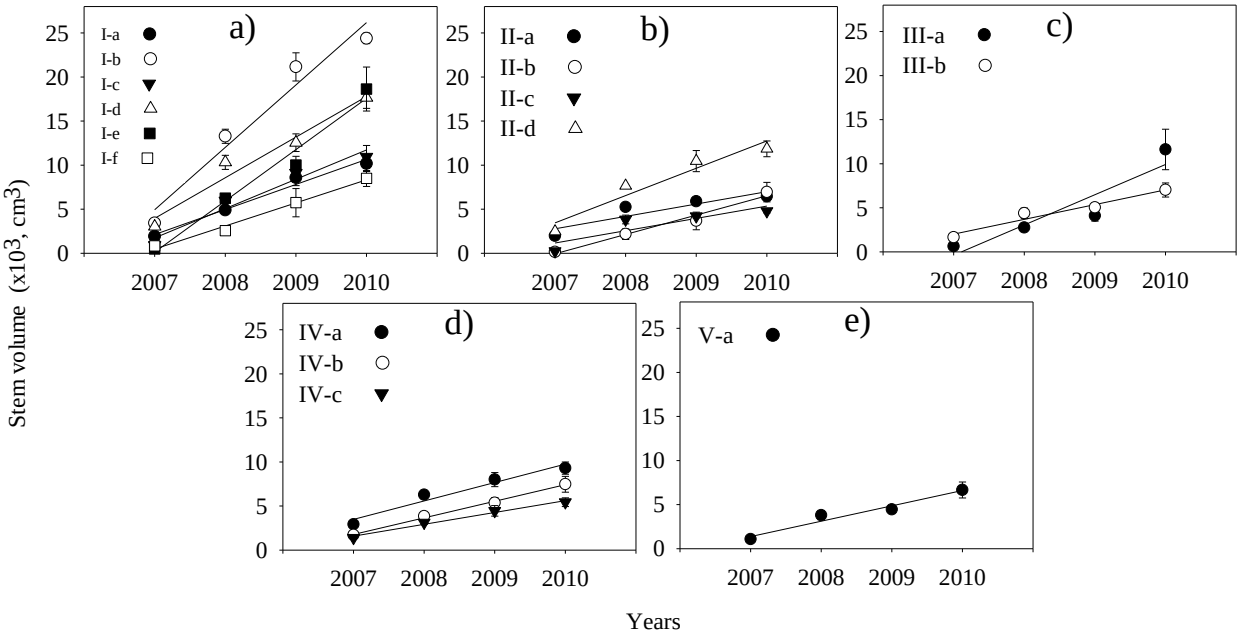

Figure 2 Relationship between stem volume and time for the *Eucalyptus* spp. provenances: *E. cladocalyx* provenances; (a) *E. camaldulensis* provenances; (b) C. maculata provenances; (c) *E. tricarpa* provenances; (d) and *E. goniocalyx* provenance Stawell (e). Figure 1 shows the name code for *Eucalyptus* spp. provenances where, for example, I = species and a provenance. Data points are means ±se. A linear regression was applied for each provenance from 2007 to 2011. One-way ANOVA was performed between provenances using the slope values to compare the stem volume growth rate, and significant differences were detected (F = 10.02, P < 0.001)

3.3 Arsenic accumulation

At age four years in 2011, the average foliar arsenic content for *E. goniocalyx* was 5.6±0.3 µg As g^{-1} d.w., the highest of all species (Figure 3). *Eucalyptus cladocalyx* was lower at 3.8±0.2 µg As g^{-1} d.w., followed by *C. maculata* at 3.4±0.6 µg As g^{-1} d.w., *E. camaldulensis* at 3.1±0.5 µg As g^{-1} d.w., and *E. tricarpa* at 2.5±0.3 µg As g^{-1} d.w., which was half the concentration for *E. goniocalyx* (Figure 3). One-way ANOVA was performed for all provenances comparing foliar arsenic content in 2011, and significant differences were detected between provenances (F = 24.65, P< 0.001). A Tukey's multiple comparison test was then undertaken, which showed that *E. goniocalyx* was significantly higher in foliar As than all other provenances.

The As content of root samples (data not shown) averaged 60±8.7 µg As g^{-1} (n = 12), approximately an order of magnitude higher than foliar concentrations. These samples were collected from the following provenances: *E. cladocalyx* Wirrabara, Kersbrook, and Bundaleer from SA; *E. camaldulensis* Melrose SA, Lake-Albacutya VIC, and Manjimup, CSO-salt tolerant, WA; *C. maculata* Barclays-Deniliquin, NSW, and Manjimup, CSO-Growth, WA; *E. tricarpa* Martin's Creek, Tucker Box from VIC, and Manjimup, CSO-Growth, WA; and *E. goniocalyx* Stawell VIC.

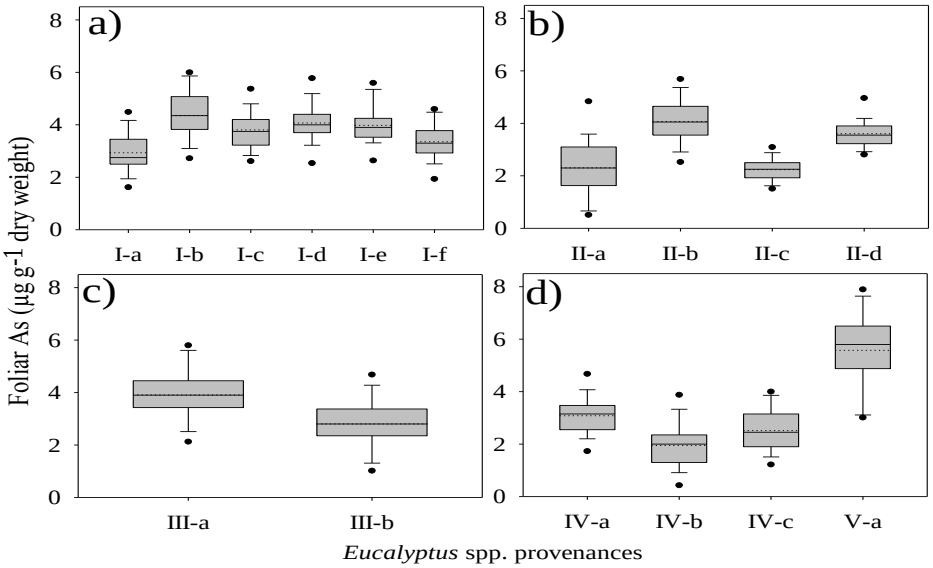

Figure 3 Foliar arsenic content of *Eucalyptus* spp. provenances, plants were measured in 2011: *E. cladocalyx* provenances; (a) *E. camaldulensis* provenances; (b) *C. maculata* provenances; (c) *E. Tricarpa*; and (d) *E. goniocalyx* provenances. Figure 1 shows the name code for *Eucalyptus* spp. provenances where, for example, I = species and a provenance. Each box represent more than 20 analysed plants (leaves) for total arsenic from a given provenance (delimited by 25th and 75th percentiles with errors bars denoting 5th and 95th percentiles) and includes the mean (dotted line), median (solid line) and outliers (black dotes). One-way ANOVA was performed for all provenances using the mean value of foliar arsenic content, and significant differences were detected (F = 24.65, P < 0.001)

3.4 Relationship between growth and arsenic content

The relationship between stem growth rate and foliar arsenic accumulation for provenances was investigated (Figure 4). A regression was performed including all provenances using the mean foliar arsenic concentrations in 2011 and the mean stem volume growth rate. A weak positive relationship was detected (r^2 = 0.2048; F = 5.51, P < 0.05; Figure 4). A regression at the level of individuals between the foliar arsenic concentrations and the stem volume growth rates showed a weaker but more significant positive relationship (r^2 = 0.0430; F = 13.93, P < 0.001). There was a particularly good correlation for the six *E. cladocalyx* provenances, which is important given that this species accounted for the largest number of provenances in the trial (r^2 = 0.71; F = 9.87, P < 0.05). This relationship could relate to the As tolerance mechanism such that fast-growing provenances are more tolerant than slow-growing provenances to As. It is noteworthy, however, that *E. goniocalyx* was clearly delimited from the main cluster of provenances, showing the highest content of foliar As and a growth rate that was amongst the poorest. The relationship between foliar As content and stem volume growth was also examined at the level of individuals within provenances. Surprisingly, no significant correlations were detected (P > 0.05), except for *E. tricarpa* Martin's Creek from Victoria, which showed a significant weak negative relationship (r^2 = 0.28, P < 0.05).

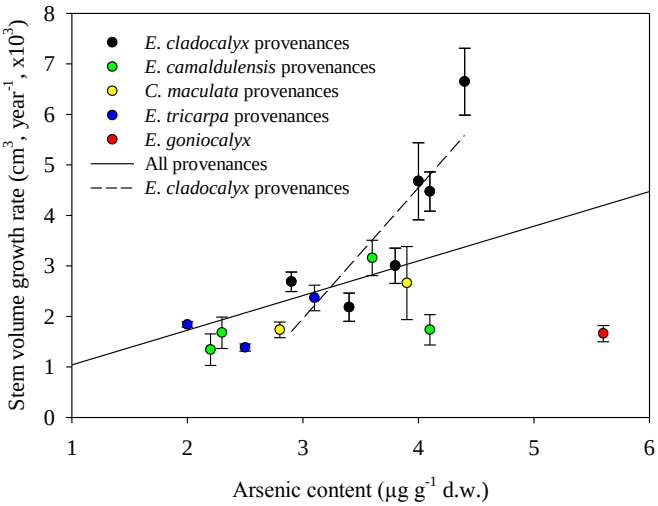

Figure 4 **Relationship between stem volume growth rate and foliar arsenic content of *Eucalyptus* spp. provenances. Regressions were performed using the mean foliar arsenic content in 2011 and the mean stem volume growth rate for each provenance. Across all provenances a weak positive relationship (solid line; r^2 = 0.20; F = 5.51, P < 0.05) was found. A moderate positive relationship was found for the *E. cladocalyx* provenances (dotted line; r^2 = 0.71; F = 9.87, P < 0.05)**

3.5 Bioavailability of arsenic

Significant differences existed in As bioavailability between rhizosphere and tailings substrates (Figure 5). The mean extractable fraction of arsenic from rhizosphere using $(NH_4)_2SO_4$ was 0.80±0.04 µg As g^{-1}, whereas from the tailings it was 0.52±0.02 µg As g^{-1}. In contrast, using NH_4NO_3 as an extractant, the mean extractable fraction of As in the rhizosphere was 0.43±0.02 µg As g^{-1} and in tailings it was 0.30 ± 0.01 µg As g^{-1}. Two-way ANOVA was performed using the factors of extraction type (NH_4NO_3 or $(NH_4)_2SO_4$) and substrates type (from rhizosphere or tailings), and a significant difference was detected in arsenic bioavailability in substrate types (F = 72.12; P< 0.001) and in extraction methods (F = 21.61; P < 0.001). Importantly, a significant interaction (F = 0.44; P = 0.51) was not found. The analysis shows that $(NH_4)_2SO_4$ extracted twice as much arsenic from both the rhizosphere and tailings substrates compared with NH_4NO_3 and that arsenic is about 1.5× more available in the presence of roots compared with tailings alone, regardless of the method to determine As bioavailability.

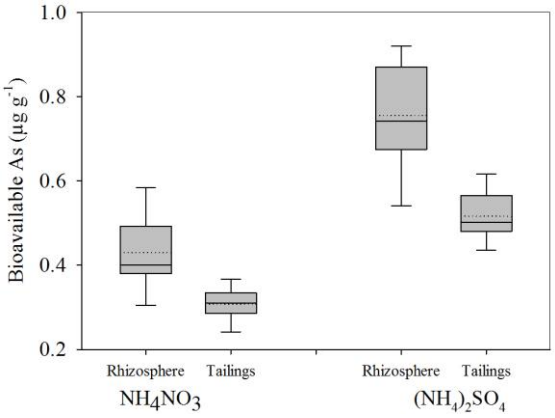

Figure 5 **Bioavailability of arsenic in rhizosphere and substrate samples from the fieldsite. Rhizosphere and tailings were compared using two extraction methods: NH_4NO_3 and $(NH_4)_2SO_4$. For comparison, values shown were squared root back transformed to match normality and variance assumptions. Each box represents 13 samples from a given substrate type (delimited by 25th percentiles with error bars denoting 5th and 95th percentiles) and includes the mean (dotted line) and median (solid line)**

4 Discussion

The survival proportions of the eucalypts species varied significantly on the capped sulphidic tailings. Among the best-performing species were *E. camaldulensis*, with the top two provenances, and *E. tricarpa* and *C. maculata*. The majority of *E. cladoclayx* provenances and the *E. goniocalyx* single provenance had the lowest survival proportions. There are many factors that could contribute to the differences in survival proportion. Certainly the environment and provenance-related effects (genetic differences) could affect the survival proportions. With regard to environmental effects, it is noteworthy that assessment of capping layers in the TERF dam in September 2009 showed an uneven spread of the top layer, which resulted in a thinner cap in some areas, in particular in the southern corner of the experimental trial. Growing on a shallower growth substrate would have limited plant growth. Poorly capped tailings are known to prevent plant establishment and retard plant growth (Robinson et al., 2006). Other environmental factors such as watering regimes were uniform for all individuals, so it is likely that factors such as early root development, frost sensitivity and disturbance during the planting procedure accounted for mortality. This is supported by the fact that almost all of the mortality across all of the provenances occurred within the first year after planting. Once established, all of the surviving plants were able to grow relatively well.

Appraisal of performance using the stem volume growth rate showed that *E. cladocalyx* provenance Wirrabara from SA produced the fastest-growing trees in this trial. A previous study using various amendments showed that *E. cladocalyx* F. Muell stem height was the tallest (at 364±133 cm tall) of the three other tested *Eucalyptus* spp. (E. *viridis* R.T. Baker, E. *polybractea* R.T. Baker and E. *melliodora* Blakely) after just over five years since establishment (King et al., 2008). In that study, the origin of the seed lot for *E. cladocalyx* F. Muell was not mentioned. In this study, *E. cladocalyx* provenance Bordertown from SA was another strong performer, ranking second in stem volume growth rate. Another strong performer was *E. cladocalyx* provenance Bundaleer from SA, ranking third in growth rate. These three provenances were delimited as the top performing group by the post-hoc (Tukey's) analysis, although there was more or less continuous variation in performance between the top and bottom groups (data not showed). Interestingly, the best performing trees did not come from local Victorian provenances; five of the top six provenances are from SA. One factor influencing performance may have been root development, with the SA provenances possibly being more deep-rooted than their Victorian counterparts. Deep rooting may be an advantage on tailings, given that they are very wet at depth and tend to dry out in the upper layers. Based on stem volume growth rate, the above-mentioned three *E. cladocalyx* provenances are the best option for growth under those environmental and edaphic conditions on the Stawell Gold Mine tailings field trial.

E. camaldulensis provenance Manjimup, CSO-Growth from WA was the poorest performer in stem volume growth rate. It is noteworthy that significant insect damage was observed in *E. camaldulensis* and to a greater degree in *E. goniocalyx*, with the majority of the damage done at the top part of the tree (data not shown). In contrast, King et al. (2008) reported that at five years of age, *E. viridis* (the smallest species in their trial at 134±55 cm) only incurred moderate insect damage. In this study, the poorest-growing *E. goniocalyx* provenance Stawell, VIC, after four years of age, had a relatively high incidence of insect damage (data not shown) yet was 280±31 cm in height – almost three times that of *E. viridis* previously reported over a similar period of time. These results show the potential importance of the selection of *Eucalyptus* provenances, and surprisingly that the local *E. goniocalyx*, which is potentially the best adapted species to the local conditions, was amongst the poorest-growing trees.

At four years of age, all established provenances showed little arsenic uptake into mature fully expanded leaves. The greatest accumulation of As in the trial was observed in *E. goniocalyx* with 5.6±0.3 µg As g^{-1} d.w., which was significantly higher than the rest of the provenances (Figure 3). The second highest group in terms of foliar As content (as defined by the Tukey's *post hoc* analysis) ranged from 4.4 to 3.6 µg As g^{-1} d.w. and included *E. cladocalyx* provenance Wirrabara from SA, the fastest-growing species, along with Bundaleer from SA, *E. camaldulensis* Lake-Albacutya from VIC, *E. cladocaly* Bordertown, SA, *C. maculata* Barclays-Deniliquin, NSW, *E. cladocalyx* Kersbrook, SA and *E. camaldulensis* Manjimup, CSO-Salt tolerant, WA. It is noteworthy that these do not form a distinct group, given the continuous nature of the data between the second highest and the lowest provenances (in terms of foliar As concentration). These results

are in agreement with previous findings in studies from Australasia and the northern hemisphere. For instance, in New Zealand, Craw et al. (2007) found that in woody species, growing in high arsenic mine waste, such as *L. scoparium, U. europaeus, F. excorticate* and *G. littoralis*, accumulated < 10 µg As g^{-1} in the foliar tissue. Similarly in Australia, King et al. (2008) reported low foliar content of As in four *eucalypt* species (*E. cladocalyx, E. melliodora, E. polybractea* and *E. viridis*), ranging from 0.29 to 5.14 ug As g^{-1}. Moreover, in the northern hemisphere, studies have reported low concentration in foliar tissue (< 10 µg As g^{-1}) in *Populus* spp. and *Salix* spp. Chang et al., 2005; French et al., 2006; Madejón et al., 2004).

Among all species, the *E. cladocalyx* provenances were largely responsible for the positive relationship (r^2 = 0.71; F = 9.87, P < 0.05) detected between foliar As concentration and stem volume growth rate. The relationship could reflect the possibility that fast-growing provenances are more tolerant to As compared with slow-growing provenances. Alternately, higher growth rate may have been correlated to greater utilisation of water and other resources from the capped tailings material and to subsequent increased uptake of As from tailings. It is noteworthy that *E. goniocalyx* was relatively separate from the main group of provenances and that it showed the highest content of foliar As, but its growth rate was amongst the poorest. It is also noteworthy that when the relationship between foliar As concentration and stem volume growth rate was examined for all individuals within the provenances in 2011 (as distinct from the provenance means), the relationship between the variables was not significant (P > 0.05), except for *E. tricarpa* Martin's Creek from Victoria, which showed a significant weak negative relationship (r^2 = 0.28, P< 0.05). Similar findings were observed by King et al. (2008), who detected no relationship between foliar As concentration and individual stem height after five years. However, in that study no relationship between As foliar content and growth rate (stem volume or any other mass measure) was investigated. In this study, although As was one of among many environmental stressors, it is likely that the accumulation of As triggered changes in the plant metabolism, including detoxification responses resulting in variation of growth and biomass production.

Significant variation was found for the mean values of arsenic foliar concentration between provenances (F = 24.65, P< 0.001) accumulating little As aboveground (a minimum of 2.0±0.18 to a maximum of 5.6±0.31 µg As g^{-1}) compared with the total content of As in tailings (> 2000 µg As g^{-1}). These results are broadly in line with King et al (2008), who found significant variation in foliar As between species. In their study, two possible reasons for such variation were proposed: 1) the availability of As varied due to the heterogeneity of the tailings, and 2) the capacity of plants to take up available As varied due to genetic variability. At the time of the King et al. (2008) study, the mechanisms of tolerance to As in *Eucalyptus* spp. were poorly understood. The mechanisms of tolerance to As in roots of *Eucalyptus* spp. have been previous investigated (Sanchez-Palacios, unpublished), and variation was found between *E. camaldulensis* and *E. cladocalyx* under the conditions of solution culture. In particular, it was shown that in *E. Camaldulensis*, root uptake of inorganic As in the form of AsIII involved the formation of complexes with phytochelatins (to form of AsIII-PC3), which accounted for > 95% of the total As. These complexes are stored preferentially in two root tissues, the endodermis and exodermis, whereas in *E. cladoclayx* 95% of AsIII-PC3 was stored preferentially in the endodermis. These results suggest that the variation in foliar As content observed in this trial could be attributed to genetic variability at the root level (e.g., differences in sequestration capacity) as well as to the complex and heterogeneous soil matrix. Nevertheless, the relatively high degree of variability in foliar As observed in the solution culture experiments indicates that the genetic factors may be the most important ones. The way plant roots responded indicates there is a strong inherent control of As uptake, but in the tailings matrix there are many and likely variable conditions controlling the bioavailability of As that have to be taken into account.

In this study, the results showed that after establishment, *Eucalyptus* spp. were able to tolerate As-rich conditions and grow relatively well. These results are in line with previous finding by King et al. (2008) involving *Eucalyptus* spp. under similar tailings conditions, and other studies elsewhere involving woody plants such as poplars and willows (French et al., 2006; Madejón et al., 2004; Tlustoš et al., 2007). Whilst a higher As content of foliar tissue may increase the risk of As toxicity in aboveground parts, the good performance of *Eucalyptus* spp. shows that they can still be suitable for phytostabilisation purposes.

Perhaps the most concerning aspect of especially the higher values of foliar As recorded here is the potential impact on herbivore performance and As transfer into the food chain. There is some evidence that As can be transferable from leaf tissue into herbivores. For instance, preliminary analysis of an insect (*Paropsis* sp.) exposed to *E. cladocalyx* leaves with As concentration of about 8 µg As g^{-1} d.w. over an eight day period under controlled conditions showed an accumulation of As threefold higher than that in the leaves (Bates, unpublished results). Arsenic as a food chain contaminant particularly via grain consumption is of great concern (Zhao et al., 2010). However, transfer of As via terrestrial plants to the primary levels of the food chain is relatively poorly studied.

After four years of the trial, the significant differences in the bioavailability of As between the rhizosphere and tailings substrates suggest that the presence of tree roots increased the bioavailability fraction of As at a depth of 50 cm (Figure 5). Similarly, King et al. (2008) reported great variation in the As fraction extracted from soil at various times throughout the trial and at various depths. However, King et al. (2008) suggested that variation in the extracted fractions was not attributable to the presence of trees, given the remarkable consistency of values in pH across the trial site and between various depths, except for the upper layer (about 30 cm depth), where pH was significantly more acidic. This was attributed to the presence of grasses. In this study, no grasses were present amongst the trees, and soil cores were collected from near the roots (the rhizosphere) and at a depth of about 50 cm. Moreover, the significant differences in As bioavailability between the rhizosphere and tailings were confirmed using two different extraction methods.

5 Conclusions

Species and provenances differed significantly in their growth and foliar arsenic content. *E. cladocalyx* grew the fastest, producing the largest stem volume over the study period but one of the lowest survival rates of *eucalypts* in this trial. The most productive provenances were all from SA (Wirrabara, Bordertown and Bundaleer). Of these, Wirrabara was the most likely to produce the best-performing plants, which also showed considerable uptake of As into aboveground parts. *E. goniocalyx* notably had the highest levels of foliar As yet was amongst the poorest performers with respect to growth and survival, although it is noteworthy that a weak positive relationship between foliar As concentration and growth rate was detected. Owing to its ability to grow well under As-rich conditions, more detailed testing of *E. cladocalyx*, especially of the top provenances in this trial, is needed. It would be useful to examine growth and survival performance in the longer term to see if there are significant changes in foliar As content. The most desirable phytostabilisation system involves trees with very low foliar As levels, to minimise the risk of transfer into the food chain. As noted previously, it is worth considering selective breeding of desirable genotypes since there is some evidence from the results here that foliar As level is genetically influenced.

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