Acid rock drainage (ARD) from gold tailings dams on the Witwatersrand basin impacts on tree seed fate, viability, inorganic content and seedling morphology

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ABSTRACT

Trees of the genus *Acacia* are common on and around gold mine tailings dumps (`slimes' dams) in southern Africa, suggesting inherent tolerances to the prevailing environmental conditions and hence potential for use in rehabilitation. *Acacia karroo, Acacia hebeclada* ssp. *hebeclada* and *Acacia hereroensis* are the dominant indigenous acacia's growing on and around gold slimes dams in the Witwatersrand Basin of South Africa. We compared seed fate, seed inorganic composition, germination and seedling morphology of acacia trees growing on saline and ARD-polluted soils over perched seepage to that of trees growing in unpolluted substrata (i.e. beyond the influence of ARD). Acacia trees growing on ARD-polluted substrata suffered less seed predation than neighbouring trees on un-polluted substrata, but produced less viable seed, and seed of lower mass. Even when viable, seeds from *A. karroo* and *A. hebeclada* growing in slimes-polluted soils exhibited lower germinability than seeds from unpolluted sources. Seed embryo's derived from slimes-impacted trees contained lower levels of N and P than trees on unpolluted soils, but accumulated K and Mo despite the low availability of these elements in acid slimes. Seeds from polluted sources also contained higher concentrations than those from unpolluted sources of elements known to be more available in acid slimes, specifically S, Mn, Fe, Co, Ni, Cu and Zn. The seed testa generally contained more Cl and Ca than the embryo, and appreciable amounts of S, K, Mn, Fe, Ni, Zn, Rb, Sr and Mo. However the total concentrations of all inorganics in seeds were below the levels normally considered toxic. Eleven seedling abnormalities were identified during germination: five associated with radicle development and five associated with cotyledon development. Twin seedlings were also observed. No plumule abnormalities were observed.

The frequency of seed abnormalities was higher for trees growing on ARD-polluted substrata than for trees on unpolluted substrata. The frequency of radicle abnormalities was in the order of *A. karroo* > *A. hereroensis* > *A. hebeclada*, whereas that of cotyledon abnormalities was highest in *A. hereroensis*. Although most ARD-associated abnormalities were lethal, a few were apparently advantageous and associated with faster seedling growth: twin radicle, branched radicle and tricotyledon seedlings emerged more rapidly than normal seedlings, and had higher relative growth rates.

In addition, despite their lower mass, normal seedlings derived from parents growing in ARD-polluted substrata exhibited significantly higher survival rates when planted in acidic gold slimes dam material than those derived from parents in unpolluted soils. ARD-pollution of soils around gold slimes dams therefore appears to be exerting strong selective pressures for tolerant local land-races of vegetation. In some cases trees are growing on or adjacent to gold mine tailings of up to a century in age. Cuttings and seed for use in the rehabilitation of gold and uranium mine tailings and ARD-impacted environments in the goldfields should therefore be derived from these local land-races.

1. INTRODUCTION

Gold mine tailings dumps in the Witwatersrand Basin have been in existence for approximately 100 years. The `footprint’ of gold mining activities far exceeds the boundary of tailings deposits, with salinity and acid rock drainage (ARD) impacting heavily on the surrounding soils and water in the goldfields (Naiker *et al.*, 2003; Weiersbye, 2002a; Winde, 2001; Witkowski & Weiersbye, 1998a). The establishment of large belts of tolerant vegetation (trees) on and around slimes dams is being tested as a cost-effective measure for the containment of water-borne pollution on the goldfields (Weiersbye, 2002b; 2002c). Mitigation of pollution footprints and management of the component vegetation requires in depth characterization of the extent and sources of pollution, and of the resilience of the exposed ecosystems to pollution impacts. Plants that colonize gold mine tailings and persist on the surrounding saline and ARD-polluted substrata have been subjected to intense selection pressures and thus have rehabilitation potential. The surviving land-races are exhibiting a remarkable combination of tolerances, i.e. to salinity, acidity, nutrient deficiencies, ion imbalances, and excessive availability of some metals and radionuclides, as well as to seasonal drought and frost, alternating with waterlogged anaerobic conditions.

The genus *Acacia* comprises some 1200 species in tropical and sub-tropical regions and is well represented in South Africa, Australia and South America (Thomson *et al.*, 1994). Many acacia species exhibit considerable tolerance of stressful edaphic conditions such as acid or alkaline soils, salinity, and arid to waterlogged conditions. The genus is therefore considered one of the most promising general purpose tree crops for arid, infertile or degraded areas and includes species that provide erosion control and soil stabilization, soil nutrient enrichment and shelter, timber, fuelwood, charcoal, fibres, fodder, bee forage, gums, tannins, dyes and medicinal compounds (Felkner, 1981; Fagg & Stuart, 1994). The Fabaceae is one of the dominant plant families on soils polluted by ARD and saline seepage from gold and uranium slimes dams in South Africa, and 14 species of *Acacia* are common on these substrata in the Witwatersrand Basin (Weiersbye *et al.*, 2002a). Despite the poor growing conditions, these acacia’s exhibit relatively good regeneration potential on slimes-dams, saline and ARD-polluted soils, maintaining levels of seed production, viability and germination that are higher than that of most other species growing in the same environments (Witkowski & Weiersbye, 1998b).

However, despite being the dominant woody genera represented on gold slimes dams, a survey of seed germination responses across 227 woody species growing on gold slimes polluted soils found that seedling developmental abnormalities were commonest in the genus *Acacia* (Witkowski & Weiersbye, 1998b). When grown in saline, acidic and/or metaliferous soils, seedling abnormalities during growth may be attributable to ion imbalances and the uptake of toxic amounts of trace elements by the developing seedling. However, pre-germination abnormalities would be associated with the conditions of seed ontogeny and therefore parent growing conditions. The elemental composition of seeds is genetically determined but relative amounts vary among species and genotypes, and depending on growing conditions (Bewley & Black, 1994). Exclusion of toxic amounts of salts or metals from developing seeds during parent growth in metaliferous substrata, as well as preferential allocation to seeds of elements in limited supply, has been proposed as important survival and competitive strategies for seedlings in these environments (Tyler & Zohlen, 1998).

In addition to the production of viable seed, seed germination strongly limits the establishment of plants under saline conditions and even salt-tolerant plants are most sensitive to salinity at the germination and seedling stage (Catalan *et al.*, 1994). Salinity can affect seed germination either by creating an osmotic potential that prevents imbibition or through toxic effects of Na and Cl ions on seed tissues (Bewley & Black, 1982). Similarly, acid-tolerant species are most sensitive to low pH in the early stages of growth, possibly as a consequence of high levels of soil Al and interference with P uptake. Although it is well established that seed germination percentage and germination rate is decreased by germination in external solutions of increasing salt (Catalan *et al.*, 1994; Lovato *et al.*, 1994; Rehman *et al.*, 1996; 2000), acid and metal concentrations, there is little information on the effects on imbibition and germination of the internal, i.e. seed, solution concentrations of Na, Cl and metals as a consequence of seed production by parents growing in saline or acidic or metaliferous environments.

We describe the impacts of tree growth in saline and ARD-polluted substrata on the fate, germination and inorganic contents of seeds, in comparison to trees of the same provenance growing on unpolluted soils.

2. METHODS

2.1 DESCRIPTION OF THE STUDY SITE AND SUBSTRATA

The study was carried out between 1998 and 2002 on deep-level gold mines near Klerksdorp, Carletonville and Welkom in the Upper Witwatersrand Basin within a region of 15 000 km^2. Slimes dams in the Klerksdorp study area are situated on doleritic and sandy soils, with regions of chert, in the A2 vegetation subdivision (O'Connor & Bredenkamp 1997) of the grassland biome at 1300 to 1350 m. The slimes dams in the Carletonville study area are situated on quartzite and dolomitic soils in the C6+7 subdivisions at 1600 to 1650 m, whereas those in the Welkom study area are on clayey to sandy soils within the A2 and B3 subdivisions at 1300 to 1400 m. The vegetation type in the Carletonville study area comprised bankenveld, xeric grasslands (klipveld) and *Acacia karroo* savanna (Acocks 1988). In the Klerksdorp study area the vegetation comprised dry *Cymbopogon-Themeda* veld, klipveld and *Acacia karroo* savanna (Bredenkamp & Brown 1995a, 1995b). In the Welkom study area the dams are surrounded by mesic to hydromorphic grasslands on clays, with some semi-karroid vegetation and frequent endorheic saline pans supporting halophytic vegetation.

Gold slimes dams consist of crushed rock slurry from which most gold has been extracted using the all-sliming process (Bosch, 1987). The ore is derived from gold and uranium-bearing conglomerates associated with the sediments of the Witwatersrand Basin at depths of up to 3 500 meters below surface. Pyrite is the dominant sulphide in the ore (up to 3%), with an additional 2% of other sulphides (Annhaesser, 1987). The slimes particles are cohesionless, silicaceous and of the size range associated with clays and silts but lacking a reactive clay content. The pH of freshly deposited slimes is alkaline, but the high pyrite content results in rapid acidification on exposure to air and water. The surface slimes deposits are consequently aerobic, with an acidic pH to a depth of approximately 100-300 mm. With depth the slimes becomes more saturated, anaerobic and alkaline. Plants rooting in slimes dams, slimes-inundated soils and in ARD are therefore exposed to strong pH, moisture, air and redox potential gradients, acidic, saline and damp conditions, with low availability of Mg, N, P and K and high availability of some transition elements and radionuclides (Weiersbye, 2002a; Weiersbye *et al*., 1999; 2002a; Witkowski & Weiersbye, 1998a).

2.2 SEED FATE AND VIABILITY

Seventy-five individual acacia trees (*A. karroo: N*=20*, A. hereroensis: N*=25 and *A. hebeclada*: *N*=30) were selected on ARD-polluted soils around 25 slimes dams (4 in Carletonville, 6 in Klerksdorp and 15 in Welkom), and another 35 trees were selected on unpolluted soils in the same regions (*A. karroo, N*=10, *A. hereroensis, N*=10 and *A. hebeclada, N*=15). *A. karroo* was sampled from all three regions, whereas *A. hereroensis* did not occur in the Welkom study sites, and *A. hebeclada* did not occur in the Carletonville study sites. Trees `On' ARD-polluted soils were growing in ARD-inundated soils within 100 metres of slimes dams. Trees `Off' ARD-polluted soils were growing between two and 10 km away from slimes dams (the absence of ARD or chloride contamination confirmed by the quality of local borehole water). Trees growing beside roads or in towns were avoided. Within each species, pods were collected from similarly sized large trees. Seed fate and viability were assessed in seed batches collected in 1998 and 1999 for a total of 11 912 seeds (1 640 pods) from all 110 trees. The number of seeds per pod was recorded, and seeds categorized as either `predated', `aborted' or `intact'. Intact seeds were then dissected and assessed for viability using the tetrazolium chloride vitality test (Moore, 1985), modified for acacia seeds (Mbalo & Witkowski, 1997).

2.3 SEED MASS, IMBIBITION AND GERMINATION

Seed mass, imbibition and germination was assessed in 1998 and 1999 for the same seed batches. For seed mass and percentage imbibition, twenty-five seeds per tree were air dried for 1 month before weighing, and then allowed to imbibe in water for 48 hours before reweighing in order to determine percentage moisture uptake. Subsequent to weighing, seeds were dissected and assessed for viability. Seeds of *A. hebeclada* and *A. karroo* possess physical dormancy and require mechanical disruption of the testa to facilitate imbibition, whereas seeds of *A. hereroensis* lack significant physical dormancy (Weiersbye & Witkowski, 2002). For assessing imbibition and germination rates, 100 intact seeds from each tree were scarified with the point of a soldering iron and 100 were left unscarified. Seeds were incubated in sterile water under controlled environment conditions at a temperature of 30°C with a photoperiod of 14 hours light and 10 hours dark. Light was provided at an intensity of 702±63 µmol/m/s by Fluora 77 Plant Growth Lighting Tubes and incandescent lamps (Osram) and dishes were randomly repositioned in the incubator once every 24 hours. Total % germination of viable seeds was recorded (ungerminated seeds were subsequently assessed for viability), as well as the lag period to germination, the peak value for germination (i.e. the day on which most seeds germinated) and the t_{50} germination (i.e. the number of days taken for 50% of seeds to germinate). Germination was taken as the day on which the emergent radicle measured at least 1 mm.

2.4 INORGANIC COMPOSITION OF VIABLE SEEDS

Seed inorganic contents were assessed for 5 individual viable seeds from each of 6 individual trees (3 trees ‘On’ and 3 trees ‘Off’ polluted substrata) in 1999 and 2000 in the Klerksdorp region only (i.e. the only region where all three species co-occurred on the same substrata). In order to determine which seeds were viable, seeds were cracked in half, and one half subjected to tetrazolium chloride vitality testing (Moore, 1985). The other half of the viable seed and the seed testa were lyophilized and then digested in nitric acid and hydrogen peroxide, the dissolution made up to volume using ultra-pure water and analyzed for elemental concentrations using ICP-OES against appropriate multi-element and plant tissue standards (Pritchard, MacKay & Points, 1996).

2.5 SEEDLING ABNORMALITIES

For the assessment of seedling abnormalities rates, seed batches were collected annually from 1998 to 2001, from a subset of five trees growing ‘On’ and 5 trees ‘Off’ ARD-polluted substrata in the Klerksdorp region (including the same trees as used for seed inorganic analysis). One hundred intact seeds per tree/annum were air-dried for one month and weighed, and 1000 intact seeds/tree/annum (including the weighed seeds) were assessed for germination percentage and appearance of the seedlings in the first 14 days of growth (under the same conditions each year in an incubator at 30°C).

2.6 SEEDLING SURVIVAL

The `normal’-appearing seedlings derived from parent trees growing ‘On’ versus ‘Off’ ARD-polluted substrata during 2001 were planted into 20-litre bags of acidic slimes supplemented with 2% organic matter and compatible rhizobia and arbuscular mycorrhizal fungi (as described by Weiersbye *et al.* 1998; 2002b). The number of seedlings comprised 250 per site for *A. hebeclada*, 1400 per site for *A. karroo* and 1500 per site for *A. hereroensis.* Seedling survival under outdoor conditions was monitored for 1 year.

3. RESULTS

3.1 SEED FATE AND VIABILITY

The number of intact seeds per pod, and the percentages of intact seeds tended to be higher for trees growing on rather than off ARD, whereas levels of seed abortion were similar for both substrata (Table 1). However, the higher percentage of intact seeds on ARD appeared to be a consequence of reduced seed predation. The difference in seed predation between substrata probably confounded the seed abortion data (seed abortion occurs as a consequence of predation attempts as well as ion imbalances during development). Assessment of seed abortion as a consequence of growth on polluted soils would therefore require that pods were protected against predation. Seed intactness was however a poor indicator of viability and seed viability was uniformly reduced in all three species growing on as opposed to off ARD-polluted soils.

3.2 SEED MASS AND IMBIBITION OF UNTREATED SEEDS

Mean viable seed mass tended to be greater for *A. hereroensis* and *A. hebeclada* trees growing off polluted soils than for those grown on polluted soils, whereas viable seed masses for both substrata were similar for *A. karroo* (Table 2). Seed mass was of the order nonviable<semi-viable<viable seeds for all three species. The percentage water uptake of seeds over 48 hours was of the order viable<semi-viable<nonviable. Percentage water uptake by viable seeds over 48 hours differed between species (seeds of *A. hebeclada* were

impermeable). Within each species there were no differences between substrata for short-term seed water uptake (over 48 hours; Table 2), but subsequent imbibition rates were higher for seeds from polluted soils (Table 3).

3.3 SEED GERMINATION INDICES

Seeds of *A. hebeclada* and *A. karroo* possess physical dormancy and therefore require mechanical disruption (i.e. scarification) of the seed testa in order to facilitate imbibition. Seeds of *A. hereroensis* have limited physical dormancy and will imbibe and germinate without pre-treatment. 100% of viable seeds from all three species growing off polluted soils could be germinated, as could all viable seeds of *A. hereroensis* from trees on polluted soils (Table 3). For *A. hebeclada* and *A. karroo,* there tended to be a higher percentage of seeds germinating for trees off polluted soils than on polluted soils, whereas equal percentages germinated from both substrata for *A. hereroensis*. This suggested that slimes-induced mechanical or chemical changes to the seed testa may reduce seed germinability for species with physical dormancy, even when these seeds are viable and scarified. Seeds from polluted soils imbibed more rapidly than seeds from unpolluted soils: un-scarified seeds from *A. hebeclada* and *A. karroo* growing off polluted soils were slower to imbibe than seeds from trees growing on polluted soils. When seeds were scarified there was however little difference in the mean days to imbibition and peak imbibition, suggesting the differences were related to pollution-induced changes in the physical characteristics of the testa that impose physical dormancy on these two species. There was little difference in germination percentage or progress between substrata for *A. hereroensis* seeds. However, unscarified seeds of *A. hebeclada* and *A. karroo* from trees growing on unpolluted soils germinated more rapidly than seeds from trees on polluted soils, but there were fewer differences in germination between substrata once seeds were scarified.

3.4 INORGANIC COMPOSITION OF VIABLE SEEDS

Seed embryo’s derived from trees growing in ARD contained lower levels of N (data not shown) and P than trees not exposed to ARD, but accumulated K and Mo despite the low availability of these elements in acidic conditions, as well as elements known to be more available in ARD-polluted soils, especially S, Mn, Fe, Co, Ni, Cu, Zn, Rb and Sr (Figs 1 & 2). The seed testa contained more Cl and Ca than the embryo (with higher Ca concentrations from polluted sources), and appreciable amounts of S, K, Mn, Fe, Ni, Zn, Rb, Sr and Mo (Figs 1 & 2).

3.5 SEEDLING ABNORMALITIES

Eleven gross seedling abnormalities were identified during seed germination. Five were associated with radicle development: no radicle, club radicle, twin radicle, branched radicle and dwarf radicle; and five were associated with cotyledon development: tricotyledon, fused cotyledons, minus cotyledons, mismatched cotyledons and heart-shaped cotyledon. Radicle and cotyledon abnormalities were only exhibited by the same germinant in the case of dwarf radicle and radicleless. Twin seedlings (with two radicles, two plumules and three to four cotyledons fused at the base) were also observed. No plumule abnormalities were observed. The percentage of seed abnormalities was higher for trees growing on ARD-polluted soils than for trees growing in unpolluted soils (Fig. 3). The commonest radicle abnormalities were radicleless and club radicle (both lethal), whereas the commonest cotyledon abnormalities were tricotyledon and fused cotyledons. Overall, the percentage of radicle abnormalities was in the order of *A. karroo* > *A. hereroensis* > *A. hebeclada*, whereas that of cotyledon abnormalities was in the order of *A. hereroensis* > *A. karroo* > *A. hebeclada*. No abnormalities were observed in *A. hebeclada* seeds derived from unpolluted soils.

3.6 SEEDLING SURVIVAL

Despite their lower seed mass, apparently normal seedlings derived from trees growing in ARD-polluted soils exhibited higher survival percentages over a one-year period when planted in acidic slimes material than those derived from trees in the unpolluted soils (Fig. 4). Most ARD-associated abnormalities were lethal, but a few apparently contributed to faster seedling growth: twin radicle, branched radicle and tricotyledon seedlings emerged more rapidly than normal seedlings, and had higher relative growth rates (data not shown).

4. DISCUSSION

Seeds of leguminous trees have high nutritive contents (Liptay & Arevalo, 1998) and are usually highly attractive to seed predators such as insect larvae (Ernst *et al.*, 1990). However, seed predation by insect larvae was lower for acacia trees on ARD polluted soils than for those on unpolluted soils. This could be associated with many factors. Seed predation is known to be highly variable between species, sites and between years (Ernst *et* al., 1990). For example, local microclimate changes with the proximity of the slimes dams, tree densities are lower on polluted soils (and may contribute to lower predator population densities), and trees growing on polluted soils near slimes dams may also be less attractive to herbivores due to a higher dust load, altered metabolism or reduced palatability. All of the former have been identified as factors influencing pollination, disease and predation in general of plants growing in low fertility soils, metaliferous soils and polluted soils (Boyd & Martens, 1998; Bryant *et al.*, 1989; Ruohomaki *et al.*, 1996). Conversely, there is also evidence for increased herbivory of trees growing in metaliferous polluted environments as pollution impact appears to inhibit the induction of plant resistance mechanisms that regulate herbivore populations dynamics (Zvereva & Koslov, 2000 and references therein). Lowered palatability of seeds from polluted sources in comparison to unpolluted sources could be a consequence of the lower N, P and Mg contents of embryos, elevated embryonic and seed testa contents of S and ‘heavy’ metals observed in this study, pollution-induced changes in testa permeability (that affect oviposition), or elevated levels of distasteful secondary compounds in pods and foliage in response to growth in polluted soils.

Seed mineral accumulation during ontogeny is strongly influenced by parental growing conditions (Liptay & Arevalo, 1998). Whereas nutrients that are non-limiting will be accumulated by seeds, there is evidence from plants grown in silicate versus calcareous soils that elements that are less available are also accumulated preferentially in seeds (at the cost of foliar concentrations) in order to serve as a starting source for seedling growth (Tyler & Zohlen, 1998). The results of this study support the preferential allocation of nutrients in limited supply to the embryo. In legumes, the cotyledons are the principal storage organs for the seed, whereas the seed coat may, in addition to providing a hard protective barrier, possess ion exchange properties. The results of this study suggest that the success of acacia seedlings in establishing in saline and ARD-polluted substrata, and in gold slimes material, may be in part due to their ability to accumulate Ca, K and Mo in the seed, and for the seed testa to act as a sink for excess Na, Ca, Cl, Mn and Fe. Ca and K are essential for membrane function but are displaced by Na, whereas Mo is of low availability in acidic soils but is essential for biological nitrogen fixation (Bewley & Black, 1994). Higher seed Ca, K and Mo contents would therefore be expected to facilitate seedling survival in saline and acidic environments.

Seeds of many acacia species are able to germinate in saline environments and the seed coat has been observed to act as a buffer between the embryo and the external NaCl solution by accumulating Cl and Na ions (Rehman *et al.*, 1998; 2000). Ca and K leakage from plant tissues is associated with germination and growth under saline conditions, causing Ca and K deficiencies and plants must therefore maintain relatively high concentrations of Ca and K in order to grow in saline environments (Rengel, 1992). The differences between acacia species in seed allocation of nutrients, especially Cl, Ka and Ca, between the embryo and the testa that we found is in agreement with that found for a range of acacia species of varying salt tolerances (Rehman *et al.,* 1996; 1998).

In addition to the maintenance of higher tissue Ca and K, and low Cl and Na uptake, faster germination by salt-tolerant species is also a strategy that may allow the seedling to escape salt injury (Rogers *et al.*, 1995). Salt has been reported to affect the germination rate more than the germination percentage (Lovato *et al.*, 1994; Rehman *et al.*, 2000). Seed germination is initiated by imbibition, the process of which in acacia's is dependent on increased permeability of the testa. The three acacia species examined differed in testa permeability, in their percentage imbibition and in the rate of water uptake. These differences in imbibition may have accounted for the inter-species differences in germination rate. Within each species, viable seeds were characterized by slower (i.e. more controlled) imbibition than non-viable seeds, and the more rapid imbibition of unscarified viable seeds from polluted sources suggests that development in slimes increases testa permeability to water. More rapid imbibition would be expected to result in more rapid germination. However, imbibed unscarified seeds derived from polluted sources were slower to germinate than those from unpolluted sources. These differences in imbibition and germination rates between polluted and unpolluted sources were marginal for *A. hereroensis* (which lacks significant physical dormancy), but marked for the two species with physical dormancy (*A. hebeclada* and *A. karroo*). Since differences in imbibition and germination between polluted and unpolluted sources for the latter two species were then nullified by scarification of the testa, this indicates that some effects of pollution on seed germinability are exerted by changes in testa composition (resulting in increased permeability to water, but reduced permeability to radicle emergence). A higher proportion of the radicles in seeds derived from polluted sources emerged laterally from the seed testa, suggesting that higher concentrations of inorganics were contributing to mechanical strength in the seed testa (e.g. Si, Ca) or that higher concentrations of potentially reactive metals in the testa and embryo (e,g. Al, Mn and Fe) were inhibiting radicle development even when seeds were viable and fully imbibed.

With the exception of predation, growth of acacia trees in ARD from gold and uranium slimes dams impacts negatively on all aspects of regeneration by seed. Indices of seed viability used in this study were vital seed staining (for potential viability; Moore, 1985), seed germinability (Steiner *et al.*, 1999) and normal appearance of seedlings. The viability of seeds derived from polluted sources was reduced in terms of all three indices in comparison to seeds from unpolluted sources. High levels of developmental abnormalities in seeds derived from ARD polluted substrata were exhibited during germination. Salt toxicity in seeds results in abnormal development and in grain seedlings is usually reflected by a decrease in K concentrations (Petruzzelli *et al.*, 1992). However, we found that higher, and not lower, K concentrations were associated with seeds from ARD polluted sources. The abnormalities found in the seeds from polluted sources may therefore be associated with ion imbalances, and higher concentrations of some Class B transition metals (`heavy' metals). Excess amounts of unsequestered Mn, Fe, Cu and Zn ions are known to cause oxidative stress, membrane and chromosomal damage in plants (Cuypers *et al.*, 1999). All the seeds examined excluded U, although the concentrations of this radionuclide can be elevated in plants growing around slimes dams (Weiersbye *et al.*, 1999; Weiersbye & Cukrowska, 2002). Although the total concentrations of most metals in seeds from ARD-polluted substrata were higher than those from unpolluted sources, the levels of each metal were not of the range normally considered toxic when considered in isolation (Marschner, 1998). The presence of radicle and cotyledon abnormalities, but no discernable plumule abnormalities, suggests that seed tissue partitioning of potentially reactive transition metals may contribute to the observed pattern of abnormalities. A parallel study using nuclear microscopy to map the distribution of elements within viable acacia seeds derived from the same sources demonstrated that concentrations of Fe, Mn, Ni, Cu and Zn were indeed highest in the tissues exhibiting abnormalities: in the proradicle, and on the dorsal surface of the cotyledon base adjoining the embryo (I.M. Weiersbye & W.J. Przybylowicz, unpublished).

Most of the abnormalities observed in seedlings from ARD-polluted substrata were lethal or impaired seedling growth and survival. A few ARD-associated abnormalities were apparently advantageous, contributing to faster seedling growth: twin radicle, branched radicle and tricotyledon seedlings emerged more rapidly than normal seedlings, and had higher relative growth rates. In addition, despite their lower seed mass, normal seedlings derived from parents growing in ARD-polluted soils exhibited higher survival rates when planted in acidic slimes material than those derived from parents in unpolluted soils.

5. CONCLUSIONS

This study demonstrates that despite indigenous *Acacia* species forming a dominant (and presumably more tolerant) component of vegetation on gold mines, growth in ARD polluted substrata still impairs tree regeneration. Seed production, mass, viability, germinability and seedling performance (in the case of lethal and sub-lethal abnormalities) are adversely affected by parent tree growth in saline ARD.

However, the survival in acid gold slimes of ‘normal’ appearing seedlings derived from parents in ARD-polluted soils is better than that of seedlings derived from parents in unpolluted soils. Gold slimes dams and ARD-pollution of soils are therefore exerting strong selective pressures for the development of tolerant land-races of vegetation. In some cases trees are growing on or adjacent to gold mine tailings of up to a century in age. Seed for use in the rehabilitation of gold and uranium mine tailings and ARD-impacted environments in the goldfields should therefore be derived as far as possible from the local land-races.

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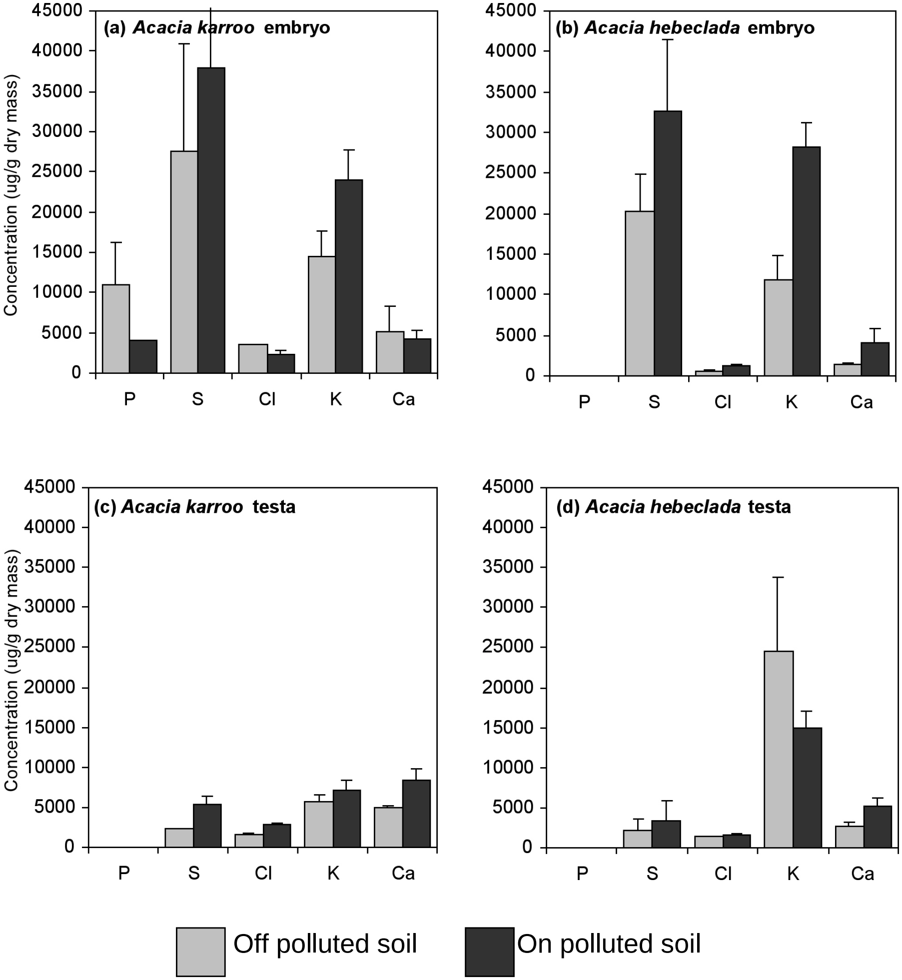

Figure 1. Concentration of major inorganics in the embryo and testa of Acacia karroo and Acacia hebeclada seeds derived from trees growing `On' versus `Off' ARD-polluted substrata. Data are means ± standard errors.

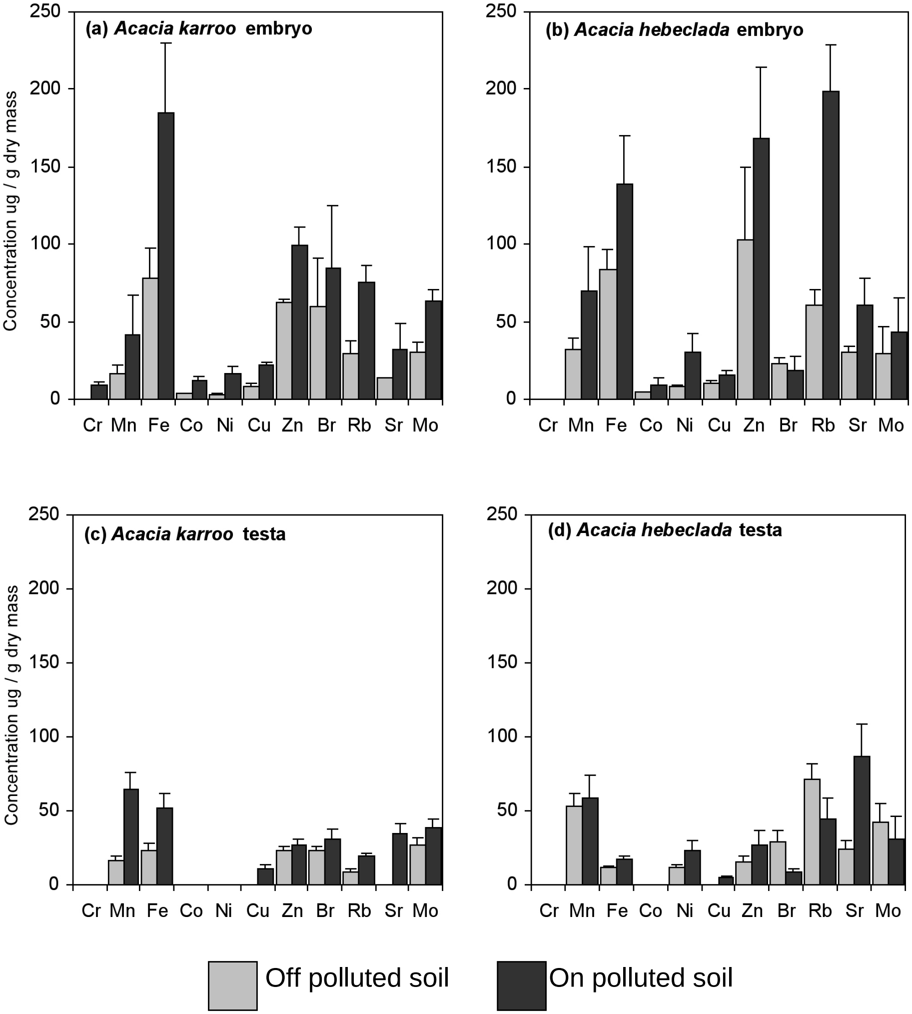

Figure 2. Concentration of minor inorganics in the embryo and testa of Acacia karroo and Acacia hebeclada seeds derived from trees growing `On' versus `Off' ARD-polluted substrata. Data are means ± standard errors.

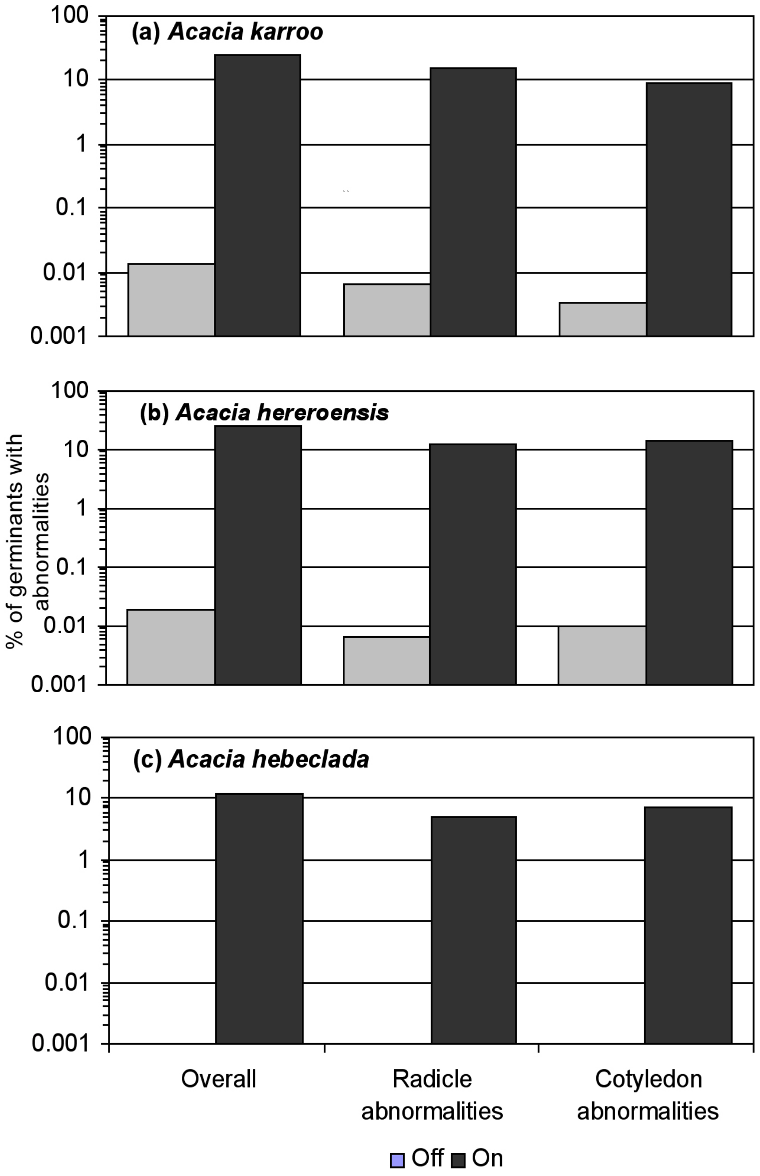

Figure 3. The percentage of abnormalities in seedlings derived from trees growing `Off' versus `On' ARD-polluted substrata. Data are means ± standard errors.

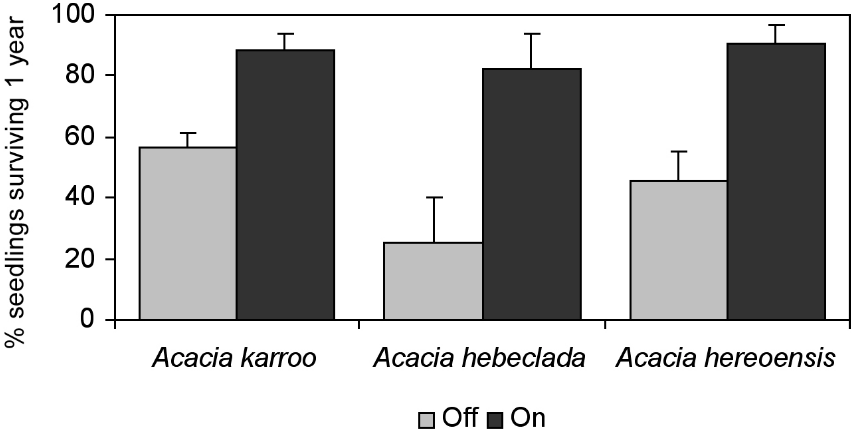

Figure 4. The percentage survival in acidic slimes of seedlings derived from trees growing `Off' versus `On' ARD-polluted' substrata.

*Table 1. The numbers of trees, pods and seeds assessed and the fate and viability of acacia seeds derived from `Off' versus `On' ARD-polluted soils. Data are means ± standard errors and *indicates significant difference between seeds (t-test, P<0.05), with * placed adjacent to the greater of the two values.*

Species	Seed Origin	No. of trees	No. pods sampled	No. seeds sampled	No. of seeds/pod	No. intact seeds/pod	% Intact	% Aborted	% Predated	% Viable	% Viable (of intact)
Acacia hebeclada:	Off	10	160	1013	6.45±0.40	1.97±0.39	29.2±4.5	**37.5±5.2***	**41.7±4.5***	**20.4±5.2**	**70.0±5.7**
	On	20	265	2337	**8.77±0.42***	**3.54±0.44***	**38.6±4.5**	19.7±1.9	33.2±2.2	17.4±6.3	65.3±2.6
Acacia hereroensis:	Off	10	200	1182	5.91±0.14	1.49±0.22	25.0±3.4	46.1±1.9	**28.9±3.6***	**29.2±1.1***	**87.2±3.2**
	On	25	400	2171	5.47±0.12	**1.83±0.17**	**33.4±3.1**	45.2±3.0	21.4±1.6	20.0±2.0	80.0±8.0
Acacia karroo:	Off	15	270	2312	8.67±0.24	6.25±0.23	77.4±2.6	9.3±1.1	**12.8±1.7***	**74.9±4.0***	**89.3±4.8***
	On	30	345	2897	8.19±0.29	**7.27±0.24***	**83.9±1.7**	9.8±1.8	6.8±1.5	58.3±5.0	75.3±6.4

Table 2. Seed dry mass and percentage water uptake (as % of seed dry mass after water supply for 48 hours) for viable, semi-viable and non-viable seeds derived from Off' versus 'On' ARD -polluted soils.
Data are means ± standard errors.

	Viability category	Dry mass (mg)	Percentage water uptake (% of seed dry mass)
***Acacia hebeclada*:**			
On	Non-Viable	103.5±23	59±26
	Semi-viable	117.5±15	30±14
	Viable	118.3±6.8	0.0±0.0
Off	Non-viable	123.5±32	27±7
	Semi-viable	152±25.0	24±24
	Viable	181.5±29	0.0±0.0
***Acacia hereroensis*:**			
On	Non-Viable	48.4±7	166±72
	Semi-viable	61.0±3	139±22
	Viable	70.6±5	19±5
Off	Non-viable	64.5±6	144±15
	Semi-viable	86.5±8	35.0±7
	Viable	89.0±14	17±4
***Acacia karroo*:**			
On	Non-Viable	38.3±4	104±23
	Semi-viable	41.92±9	20±7
	Viable	48.33±7	22±14
Off	Non-viable	39.3±5	171±40
	Semi-viable	49.7±14	28±24
	Viable	48.0±10	10±10

Table 3. Summary of seed treatment (no treatment or scarified) effects on the progress of seed imbibition and germination in acacia seeds derived from `Off' versus `On' ARD-polluted soils.

Data are means ± standard errors.

			OF VIABLE SEEDS IMBIBITION:			GERMINATION:				
Origin	Treatment	% Viability (of intact seeds)	% imbibed	Mean days to imbibition	Peak imbibition (days)	% Germinated	Mean days to germination	Peak Germination (days)	Germination Lag (days)	t_{50} (days)
Acacia hebeclada:										
Off	None	70.0±5.7	76.7±23.3	11.9±0.7	6.5±1.5	76.7±23.3	17.4±0.6	16.3±0.8	9.0±6.0	17.0±2.0
On	None	65.3±2.6	64.7±17.9	5.1±2.4	4.3±1.5	31.4±16.0	21.4±4.4	17.0±9.8	8.0±2.4	23.5±3.7
Off	Scarified	70.0±5.7	100.0±0.0	1.0±0.0	1.0	100.0±0.0	11.0±0.0	11.0	11.0	11.0
On	Scarified	65.3±2.6	81.0±19.0	2.1±0.0	1.5±0.4	66.7±33.3	11.6±0.3	14.0±0.0	5.0±2.4	10.5±2.9
Acacia hereroensis:										
Off	None	87.2±3.2	100.0±0.0	3.2±0.7	3.0±0.0	100.0±0.0	4.3±0.4	4.0±0.0	3.5±0.5	4.0±0.0
On	None	80.0±2.6.	100.0±0.0	3.0±0.3	2.6±0.2	100.0±0.0	4.6±0.4	3.9±0.4	3.2±0.2	4.2±0.2
Acacia karroo:										
Off	None	75.0±16.7	27.3±22.7	7.7±0.3	6.5±1.5	27.3±22.7	19.6±4.1	13.5±1.5	10.0±2.0	17.0±5.0
On	None	49.9±10.6	35.7±4.0	5.0±1.7	5.0±2.3	25.5±6.2	27.4±0.6	28.0	25.0	28.0
Off	Scarified	89.3±4.8	100.0±0.0	2.1±0.0	1.0	100.0	8.0±1.4	7.5	4.0	4.0
On	Scarified	75.3±6.4	94.4±5.6	2.0±0.0	2.0±0.0	94.4±5.6	9.8±2.0	10.9±2.8	8.8±3.1	12.6±3.0