IMPACTS OF ACID MINE DRAINAGE ON THE REGENERATION POTENTIAL OF HIGHVELD PHREATOPHYTES

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Abstract

Phreatophytes are deep-rooted plants, reliant to some extent on groundwater. Saline and acid mine drainage (AMD, or acid neutral drainage, AND) from gold, uranium and coal mining has contaminated shallow groundwater in many areas of the Witwatersrand Basin, and is predicted to increase with the decanting of mine water from old underground workings. Knowing how phreatophytes respond to AMD is essential for rehabilitation and long-term land management post-mining. For long term persistence, phreatophytic plants must tolerate this contamination and still be capable of regeneration. Previous studies (1998 – 2002) of >200 species common on gold mine tailings storage facilities (TSFs, or mine dumps), and AMD-polluted soils of the highveld showed these environments negatively affected all aspects of reproductive biology, including seed production, nutrient-content, metal-content, viability and germinability. Herbaceous species, grasses and weedy species were most severely affected, whereas woody plants appeared most tolerant.

Therefore, the aim of this study was to determine how growth in AMD-contaminated groundwater impacts on the regeneration of common phreatophytic woody species that hold promise for use in rehabilitation programmes. AMD-tolerant woody species are being tested to help minimize seepage of AMD from TSFs into underlying aquifers, and for the rehabilitation of polluted soils (Weiersbye *et al*., 2002). Design and management of this engineered vegetation requires an understanding of potential tolerance to future contamination scenarios. In addition, the end land-use most commonly designated for mined lands in gold mine Environmental Management Progress Reports (EMPRs) is `grazing', which implies rangelands populated by livestock. However, impacts on phreatophyte regeneration could result in changed vegetation structure, and hence negatively affect key rangeland ecosystem services.

Seeds were collected in 1998 and 2005 from deep-rooted trees, shrubs and sub-shrubs growing in (a) unaffected rangelands ('Off" AMD) and (b) AMD-contaminated rangelands ('On' AMD) around gold and uranium mine TSFs. For most species examined, growth on AMD with >1000 mg/l total dissolved solids (TDS) was associated with lower seed production compared with 'Off' AMD plants, whereas seed ontogeny on AMD resulted in lower percentage viable seed and percentage germination, and lower viable seed mass. Seed shelf life was also impaired as lipid oxidation and percentage senescence in storage was higher for AMD-grown seed. Not all impacts of AMD were negative, the percentage of intact seeds was higher for many AMD-grown plants due to lower pre-dispersal seed predation. However, even when seeds were germinable, germination was slower for most species, resulting in an extended period of vulnerability for germinating seeds from 'On' AMD. With the exceptions of *Tamarix* spp, *Acacia hebeclada* ssp. *hebeclada, Acacia erioloba* and *Ziziphus mucronata,* seed ontogeny in sodium chloride-rich AMD was more deleterious to seed germination and establishment than calcium sulphate-rich AMD. For calcium-sulphate rich AMD, although viable seed mass declined with decreasing water quality for all species, only two species exhibited significant declines in seed viability in response to AMD of >1000<5000 mg/l TDS. At TDS of >7000 mg/l, seed viability for five species was still between 25-50% that of seeds from relatively uncontaminated groundwater (<500 mg/l TDS), whereas seed viability was negligible for all species at TDS exceeding 20 000 mg/l. However, it is important to note that all study sites had naturally acidic or saline soils, and had further been exposed to AMD for decades. It is therefore to be expected that the local flora is dominated by tolerant species and individuals.

This study demonstrated that the impact of mining discharges on phreatophytes can be remote from point sources, and exert strong selection pressures for tolerance. Selection for tolerant land-races results in genetic erosion of populations. To maintain biodiversity, rehabilitation planting in affected areas, such as riparian zones, must consider both the requirement for tolerant land-races, and the need to maintain genetic diversity through inclusion of seed from the wider population. The findings also have implications for community nurseries that we are fostering to provide tolerant indigenous planting material to the mining industry. These nurseries need reliable germination protocols, high seedling recruitment and provision of AMD-tolerant seed and clonal material. Knowledge of which species should be collected, from what environments, and the expected germination and storage rates will contribute to commercial viability.

1. Introduction

Tailings storage facilities (TSFs, commonly termed mine dumps) and `footprints' (areas where tailings have been removed, leaving contaminated soil) cover 400 to 500km^2 in the Witwatersrand basin gold fields, comprising ± 6 billion tonnes of gold and uranium tailings (Chevrel *et al.,* 2003), and containing ± 30 million tonnes of sulphur (Witkowski & Weiersbye 1998) and 430 000 tons of low-grade uranium (Winde, 2001). Tailings volume increases by ±105 million tonnes per annum, or 200 000 tonnes of tailings per tonne of gold (Chamber of Mines of South Africa, 2004). Although the TSFs are localized, the zone of influence is large due to wind- and water-borne emissions, in particular acid mine drainage (AMD). A substantial literature is dedicated to the deleterious impacts of AMD on freshwater ecosystem functioning worldwide, and contamination of groundwater by AMD and heavy metals is a well-recognized phenomenon for sulphide ore mines. The impacts of gold and coal mines on water resources in South Africa have long been recognised (Rudd, 1973), and priority areas of research by the Water Research Commission for over 15 years. On lime-rich substrata in the receiving environment, such as dolomites, AMD may be neutralized and is then known as acid neutral drainage (AND). However, although concentrations of some metals in solution then decline, AND still has elevated concentrations of calcium sulphate and occasionally chlorides. We have used the term AMD throughout this report as our focus is contamination of mining origin, regardless of subsequent chemical interactions in the receiving environments. Of particular concern in South Africa are the impacts of AMD on soils, groundwater and watercourses (Funke, 1990; Pulles, 1992; Hodgson *et al.*, 2001; Rosner *et al*., 2001; Winde *et al.,* 2004; Coetzee *et al.*, 2004; Sutton *et al*., 2006), and the potential for bioaccumulation of potentially hazardous contaminants in flora, fauna and humans (Weiersbye *et al.*, 1999; Naiker *et al*., 2003; Haywood, 2004; McIntyre, 2005; Tutu *et al*., 2005; Steenkamp *et al*., 2005).

The AMD-tolerant flora of gold mine tailings and contaminated soils on the Witwatersrand Basin has been described (Weiersbye *et al*., 2006). The majority of gold and coal mines in South Africa occur on highveld grasslands, with some on savannas. A low proportion of these areas are conserved, and the grassland biome is classified as critically endangered due to habitat loss, degree of fragmentation and development threats (WWF, 2002). The principle development threats are mining and cropping. Highveld grassland and savanna is highly diverse, with high levels of endemism, although almost 60% of the grassland biome has already been irreversibly transformed with consequent loss of biodiversity. Most species (±83%) in grasslands are actually non-grasses (Reyers & Tosh, 2003), with a high proportion in both biomes being facultative or obligate phreatophytes. Although less abundant than grasses and herbs, phreatophytes such as acacia's make significant contributions to nutrient cycling and provide browse, habitat for wildlife and livestock, and livelihood goods (Seymour & Milton, 2003; Shackleton *et al*., 2007).

Gold and coal mining on the Witwatersrand Basin has impacted on some localities for over a century, with pollutant emissions subjecting flora to intense selection pressures. Previous studies showed that growth in gold tailings and AMD-polluted soils generally negatively affected reproductive biology in the 227 species assessed (Witkowski & Weiersbye, 1999) The surviving ecotypes are exhibiting a remarkable combination of tolerances to salinity, acidity, nutrient imbalances and trace metal toxicities, as well as to the extremes of the highveld climate (Weiersbye & Witkowski, 1998; Weiersbye *et al*., 2006). Although genetic erosion of populations of long-lived species through selection for AMD-tolerance could take many years to become apparent, reduced genetic diversity and selection for genetically-distinct races has recently been confirmed for some perennial herbaceous legumes on AMD-polluted soils (Angus, 2005).

This paper summarizes how AMD impacts on the regeneration biology of the vegetation class that we identified as the most tolerant component in previous studies, i.e. phreatophyte sub-shrubs, shrubs and trees. Although the impacts of AMD on regeneration of woody species is less than the significant deleterious effects on herbaceous species, impacts on the regeneration of long-lived and keystone woody species could result in long-term changes in vegetation structure, and hence affect ecosystem functioning. For example, the Fabaceae is one of the dominant plant families on soils polluted by AMD in South Africa, and contains species that provide significant ecosystem services in the grassland and savanna biomes (reviewed in Seymour & Milton, 2003). Indigenous acacia's maintain high percentages of seed viability and germination on AMD-polluted soils. However, despite being a dominant woody genus on highveld gold mines, a survey of germination in tree seed derived from AMD-polluted soils found that developmental abnormalities were commonest in the genus *Acacia*, and occurred at significant levels (Weiersbye & Witkowski, 2003). Most of the abnormalities observed were typical of salt toxicity or ion imbalances during seed ontogeny (Bewley & Black, 1994), and most were lethal or impaired seedling growth and survival.

Declines in woody biomass have occurred on highveld gold mines, and along the riparian zone of the Vaal River in the last century due to changes in land-use. Further declines in woody biomass due to excessive AMD are of concern, as this biomass actually represents one of the mechanisms by which AMD could be mitigated (Versfeld *et al*., 1998). Woody vegetation can be an efficient sink for nutrients and heavy metals (Pulford & Watson, 2003), and the ratio of woody to grassy biomass is a key determinant for the susceptibility of groundwater and streams to nitrate and sulphate contamination - both major pollutants associated with mining and agriculture. Losses of woody biomass around mining pollution point sources, and along riparian zones, could therefore predispose water resources to contamination. Woody species have the inherent capacity to modify resource allocation under stress conditions (Mooney *et al*., 1991), but it is not known to what extent this mechanism could buffer affected plants against AMD-stress, and permit continued reproduction and seedling recruitment. Understanding how phreatophyte species respond to AMD is therefore important for both rehabilitation and rangeland management in the Witwatersrand Basin region.

2. Methods

2.1 Description of the study site and substrata

The study was carried out on gold mines between Johannesburg and Welkom in the Upper Witwatersrand Basin within a region of 25 000 km^2. The North West Province study areas 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 Gauteng study areas are on quartzite and dolomitic soils in the C6+7 subdivisions at 1600 to 1650 m, whereas those in the Free State are on Ecca shales and clayey to sandy soils within the A2 and B3 subdivisions at 1300 to 1400 m. The vegetation and veld types (Acocks, 1988) were bankenveld, xeric grasslands (klipveld) and *Acacia karroo* savanna in Gauteng, dry *Cymbopogon-Themeda* veld, klipveld, *Acacia karroo* and *A. erioloba* savannas, *Rhus lancea* open woodland and wet grassland in North West Province, while the Free State sites comprised mesic to hydromorphic grasslands on clays, large areas of semi-karroid vegetation with endorheic saline pans supporting halophytic vegetation, and occasional thickets. The quality of mine water across the study regions varies, and is broadly classified in different regions in terms of calcium sulphate- and/or sodium chloride- enrichment (Funke, 1990; Pulles, 1992; Hodgson *et al.*, 2001). The detailed geochemical environment of the study sites, climate and the flora has already been described (Witkowski & Weiersbye, 1998; 1999; Weiersbye & Witkowski, 1998; Weiersbye *et al*., 2006).

2.2 Plant and seed selection and harvesting

Seeds were harvested from plants growing on groundwater for which the AMD contamination status was known. Groundwater with a total dissolved solids (TDS) concentration of <500 mg/l was assumed to be uncontaminated or least contaminated by AMD, whereas groundwater with a TDS concentration of >1000 mg/l, dominated by sulphates, was assumed to be contaminated. Growth on contaminated groundwater was confirmed by foliar chemical analysis as described (Weiersbye *et al*., 2007). Phreatophyte foliar chemistry is a reliable indicator of groundwater contamination in semi-arid regions (Erdman & Christensen, 2000). The quality of groundwater cannot be precisely determined for the rooting zone of each plant, and so was broadly categorized. No plants were selected in the category >500<1000 mg/l TDS. Although plants were sampled from AMD with TDS >15 000<40 000 mg/l these data are not presented as replication was low. All the `On AMD' and `Off AMD' plants were situated on land zoned as mining and rangelands. Plants growing beside roads, in croplands, near metallurgical plants, on known nitrate contamination from graveyards, rock dumps, feedlots and agricultural facilities, and in towns, were avoided in order to minimize the influence of run-on and other emissions chemistry. Depth to groundwater differed between `On AMD' and `Off' AMD plants (being shallower in most of the former) and this may have influenced the findings.

2.3 Seed fate, mass and viability

Seed fate and viability were assessed for seed batches collected in 1998 and 2005 as described by Weiersbye & Witkowski (2002). Number of seeds per fruit for between 100 and 1000 fruits per individual was recorded, and seeds categorized as either `predated', `aborted' or `intact'. Sub-samples of 25 to 50 air-dry (ambient) intact seeds were weighed before volume and density determination. Seed mass and density are useful indices of seed quality within species, as they vary with predation and abortion. 100 - 1000 intact seeds per plant (including those weighed) were then assessed for viability using the tetrazolium chloride vitality test (Moore, 1985). Any "intact" seeds found to have endogenous predation were added to the `predated' category.

2.4 Seed imbibition and germination

Seed imbibition and germination (radicle emerged >1 mm) were assessed in the 1998 and 2005 seeds. 100 - 1000 intact seeds per plant were treated to break dormancy immediately before incubation under controlled environment conditions (Weiersbye & Witkowski, 2002). Total percentage imbibition of viable seeds was recorded by mass difference, in addition to mean time (days) taken for imbibition, and peak value for imbibition (date which most species imbibed). Subsequently, total percentage germination of viable seeds was recorded, as well as lag period to germination, peak value for germination (day on which most seeds germinated) and t_{50} germination (days for 50% of seeds to germinate). Ungerminated seeds were assessed for viability.

3. Results

3.1 Seed fate

For hard-coated seeds (most legume species), percentage intact seed was higher for plants growing `On' rather than `Off' AMD (*Acacia erioloba, A. hebeclada* ssp. *hebeclada, A. hereroensis, A. robusta* ssp. *robusta* & *Mundulea sericea*, Table 1). Exceptions were *A. karroo and Elephantorrhiza elephantina.* Higher percentage intact seed in legumes appeared to be a consequence of reduced seed predation 'On' AMD. Opposite trends were found for some softer seeded species (*Grewia flava, Gymnopsoria buxifolia*, *Rhus pyroides* var. *pyroides,* & to a lesser extent *R. lancea* & *Euclea crispa* ssp. *crispa*) and there were no apparent trends in seed fate for the two species with armoured fruit (*Ziziphus* ssp.). Seed predation may have been underestimated as seed abortion can occur as a consequence of early predation attempts. Two species did exhibit higher abortion on AMD, but reliable assessment of abortion due to AMD would require protection of fruits against predation.

3.2 Seed mass and viability

Seed intactness was a poor indicator of percentage viability and germinability under AMD conditions, both of which were significantly reduced in most species assessed (Table 1). Dry mass per seed was greater for viable relative to non-viable seeds, although seed density was the best indicator of viability for all species examined (data not shown, Witkowski & Weiersbye, 1999). In species for which seed was compared between calcium sulphate-rich and sodium chloride-rich AMD, the latter resulted in much lower seed mass, percentage viability, percentage germination and seedling survival. Exceptions were *A. erioloba, A. hebeclada* ssp. *hebeclada, Tamarix usneoides, T. ramosissima* and *Ziziphus mucronata.* Data for seed from two species on sodium chloride-rich AMD is shown in Figure. 1.

For most species growing on calcium-sulphate and metal-sulphate rich AMD, water quality poorer than the >3000<5000 mg/l TDS category was associated with declines in percentage viability and mean mass of intact seed (Figure. 2). There was little variation between 1998 and 2005, although a number of trees on the poorest water quality (i.e. >20 000 mg/l TDS) had died by 2005.

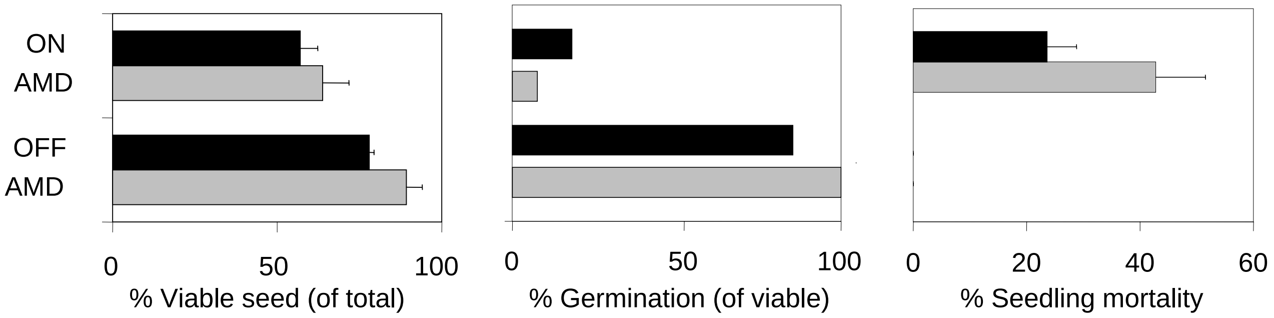


Figure 1. Influence of seed ontogeny (mean ± SE) in sodium chloride-rich AMD for *Acacia karroo* ▒ and *Rhus lancea* ■ : (a) viability, (b) germination, (c) seedling mortality.

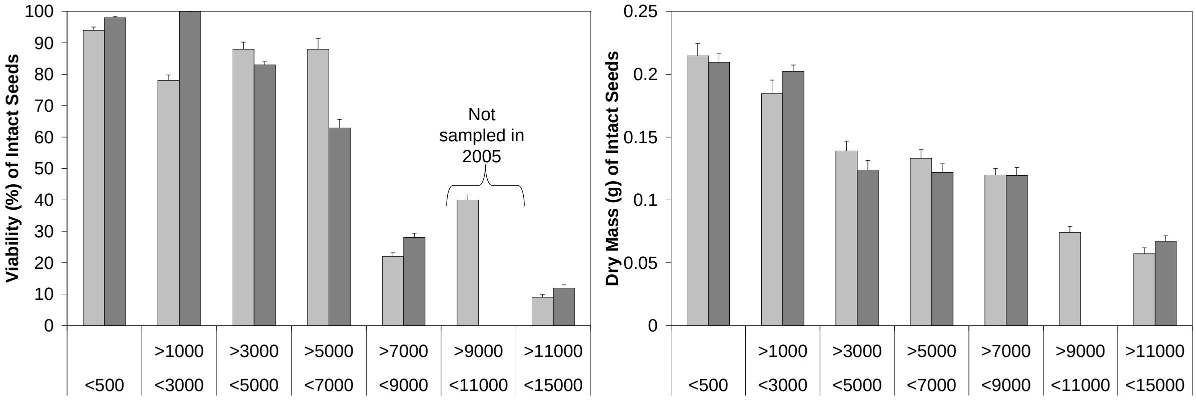
3.3 Seed imbibition and germination dynamics

Imbibition rates of AMD-grown seed varied from slower (most legumes, & *G. buxifolia*), to faster (*R. lancea*) to no different (*D. lycioides* ssp *lyciodes* and *Z. mucronata*), to those 'Off' AMD (Table 2). However, despite the range of seed types, germination in most species followed a similar pattern as a consequence of seed ontogeny in AMD. With the exception of *A. hereroensis,* AMD-grown seeds tended to germinate more slowly. All species except *E. elephantina* and *A. erioloba* also exhibited reduced germination of viable seed in the germination progress tests, which was largely consistent with the % viability and germination tests (Table 1).

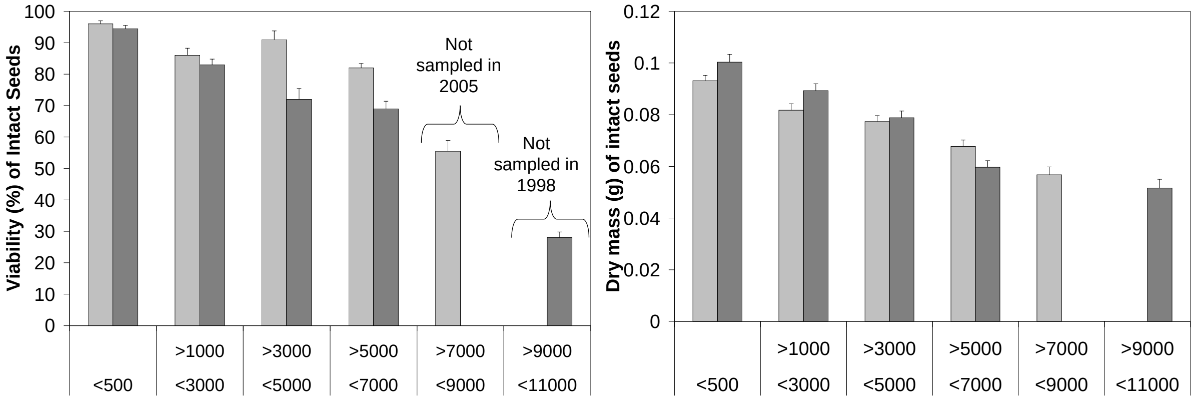
Table 1. Fate, percentage viability and percentage germination (mean ± SE) of seeds derived from phreatophyte plants growing `Off' versus `On' AMD for all study regions combined in 1998. * indicates significant difference between On & Off (*t*-test, *P*<0.05), with the greater of the two values in bold

Species & Origin	*N* plants	% Intact	% Aborted	% Predated	% Viable (of total)	% Viable (of intact)	% Germin (of total)
Legumes with hard-coated seed (species ranked from highest to lowest seed mass):							
Elephantorrhiza elephantina:							
Off	10	97.5±12.3	0.0±0.0	2.5±2.3	97.5±12.3	100±0.0	93.2±11.1
On	10	95.6±14.2	**4.4±1.3***	0.0±0.0	95.6±14.2	100±0.0	95.6±14.2
Acacia erioloba:							
Off	9	30.1±4.3	22.2±3.6	**47.6±7.2***	11.1±1.0	**36.9±3.0***	11.1±1.0
On	5	**41.7±4.8***	23.1±3.7	35.2±4.9	9.9±1.5	19.0±2.1	9.9±1.5
Acacia hebeclada ssp. *hebeclada:*							
Off	12	29.2±4.4	37.5±5.2	**33.2±2.2***	26.3±5.2	**89.9±18.0***	22.4±5.2
On	16	**38.6±4.5***	34.7±4.8	26.7±3.1	19.0±6.3	49.1±11.6	14.8±8.1
Acacia hereroensis:							
Off	10	25.0±3.4	46.1±1.9	**28.9±3.6***	**29.2±1.1***	87.2±3.2	**18.3±1.5***
On	25	**33.4±3.1***	45.2±3.0	21.4±1.6	20.0±2.0	80.0±8.0	13.5±1.7
Acacia robusta ssp. *robusta:*							
Off	6	79.0±2.7	3.7±4.1	**17.3±3.7***	**79.0±2.7***	**100.0±0.0***	**71.3±11.2***
On	6	**88.4±4.1***	2.8±3.9	8.8±2.9	51.5±6.2	58.3±11.6	48.3±9.6
Acacia karroo:							
Off	15	77.4±2.6	9.3±1.1	**12.8±1.7***	**74.9±4.0***	**89.3±4.8***	**74.9±4.0***
On	30	83.9±1.7	9.8±1.8	6.8±1.5	58.3±5.0	75.3±6.4	55.0±3.2
Mundulea sericea:							
Off	7	65.1±4.2	11.3±4.0	**23.6±2.0***	54.8±9.9	**84.2±1.9***	**54.8±9.9***
On	5	**79.3±2.0***	7.3±2.0	13.5±2.2	42.9±2.3	54.1±2.7	29.1±6.7
Non-legumes (species ranked from highest to lowest seed mass):							
Diospyros lycioides ssp. *lyciodes:*							
Off	15	76.8±6.5	1.42±0.70	21.8±6.7	**76.8±6.5***	**100.0±0.0***	**76.8±6.5***
On	15	78.9±4.0	0.7±0.4	20.4±3.8	54.6±5.9	69.2±6.3	31.5±12.3
Ziziphus zeyheriana:							
Off	5	65.6±7.5	26.4±8.8	8.0±3.7	57.7±6.3	**88.0±1.2***	**48.3±9.2***
On	5	58.7±5.0	22.7±6.4	8.6±4.8	36.6±7.1	62.4±3.3	2.7±1.9
Ziziphus mucronata:							
Off	10	75.4±5.8	18.8±5.3	5.8±2.3	70.9±8.0	**94.0±6.0***	**70.9±8.0***
On	10	79.5±4.0	13.8±3.2	6.7±3.3	40.6±6.4	51.0±8.2	38.3±13.4
Rhus pyroides var. *pyroides:*							
Off	5	**93.7±2.9***	0.0±0.0	6.3±2.9	**85.9±8.4***	**93.8±9.2***	**53.7±6.1***
On	5	69.0±11.1	0.0±0.0	**31.3±12.9***	32.2±6.3	46.8±9.7	24.1±4.2
Rhus lancea:							
Off	10	92.7±2.9	0.0±0.0	7.3±2.9	87.5±11.4	**94.4±7.2***	**68.9±13.9***
On	10	87.5±4.1	0.0±0.0	12.5±4.1	52.5±35.0	60.0±36.4	31.5±19.2
Eucea crispa var. *crispa*:							
Off	5	86.9±6.0	0.0±0.0	**13.1±5.0***	**31.3±3.0***	**36.0±4.3***	**15.5±6.3***
On	5	84.1±5.3	**6.2±2.7***	9.7±3.1	17.6±4.2	20.9±2.8	5.2±4.3
Grewia flava:							
Off	5	**61.1±5.0***	22.1±3.1	16.8±5.1	**30.6±2.3***	**50.0±7.1***	**29.3±2.3***
On	10	41.2±2.8	16.7±2.8	**42.1±4.9***	18.6±4.1	45.2±6.3	17.3±4.4
Gymnosporia buxifolia:							
Off	20	**83.3±1.7***	9.0±1.3	7.6±1.2	**80.0±11.3***	**86.4±15.1***	**22.7±10.4***
On	20	73.1±3.5	**15.0±3.9***	11.9±2.9	27.3±10.2	37.4±8.8	6.4±8.3

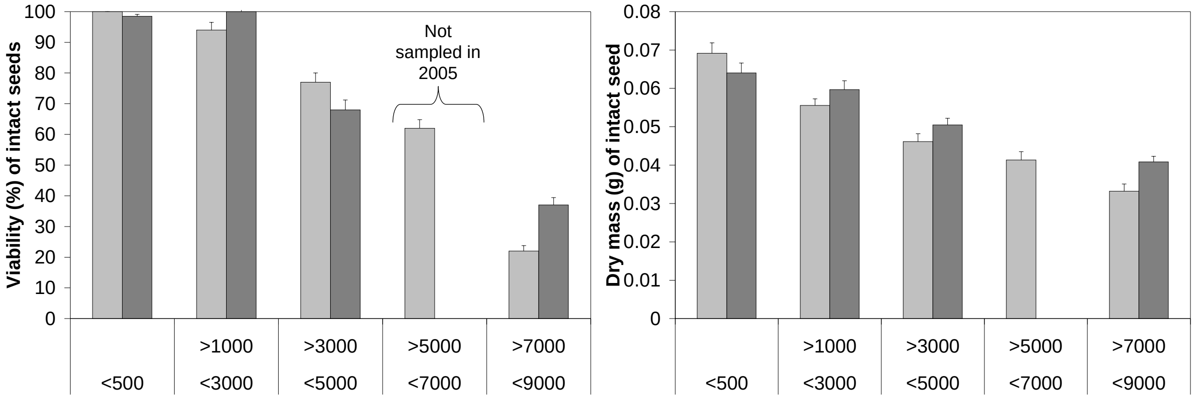
Acacia hebeclada* ssp. *hebeclada:



Acacia hereroensis:



Acacia karroo:



Diospyros lycioides* ssp. *lycioides:

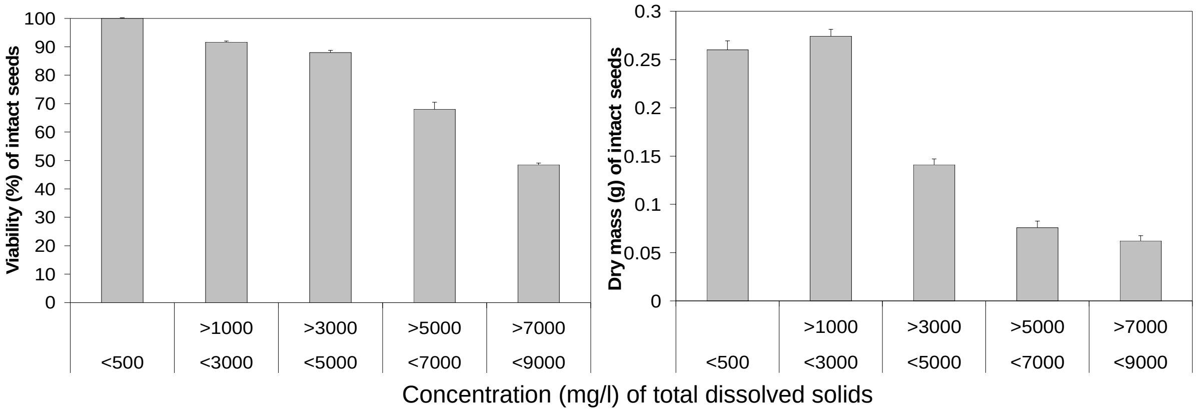
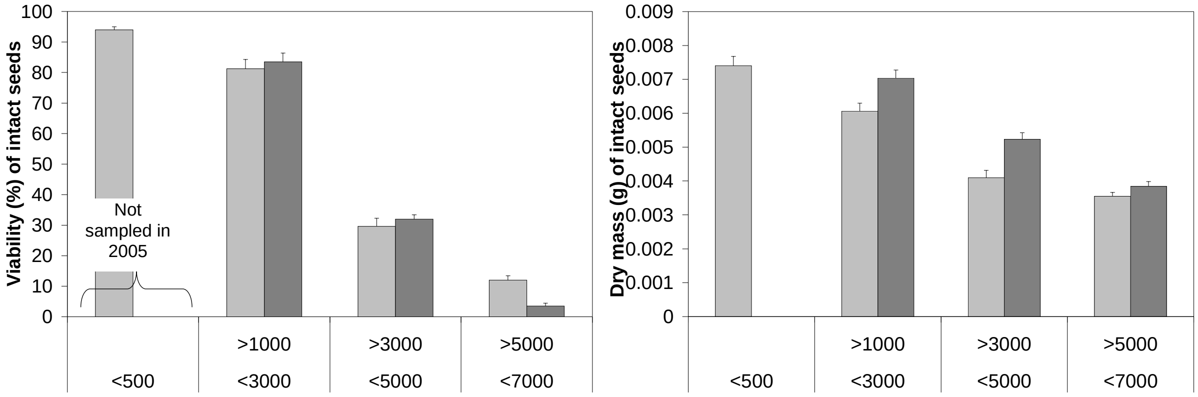
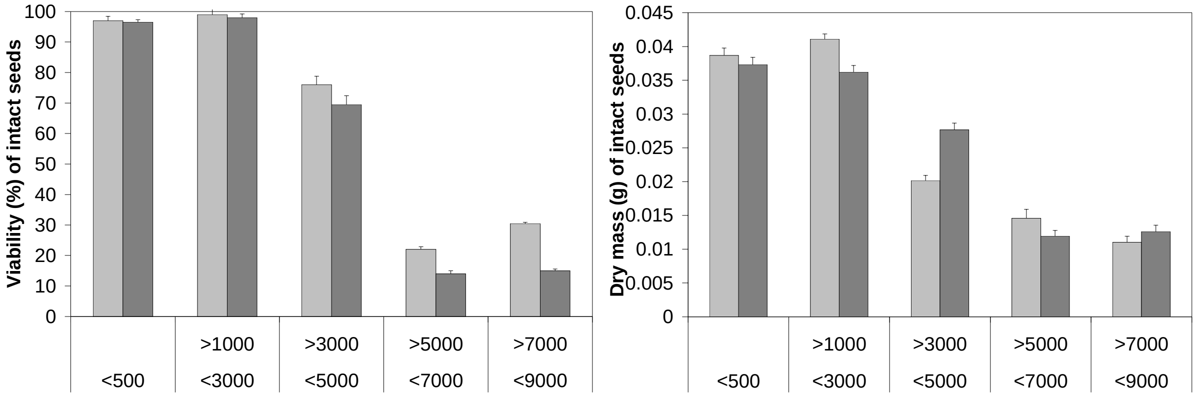


Figure 2. The influence of phreatophyte tree growth in acid mine drainage on the percentage viability and dry mass of intact seed (means + SE) in 1998 ☐ and 2005 ■.

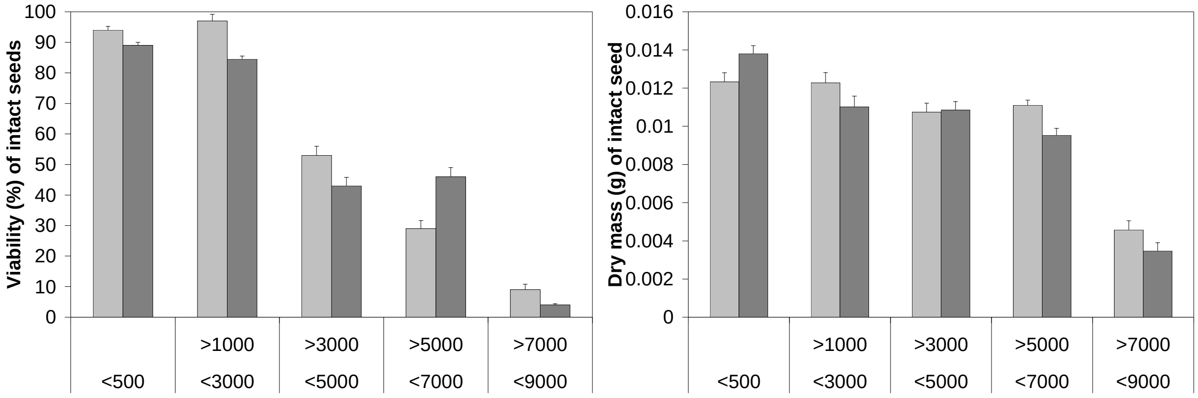
Gymnosporia buxifolia:



Rhus lancea:



Rhus pyroides* var. *pyroides:



Ziziphus mucronata:

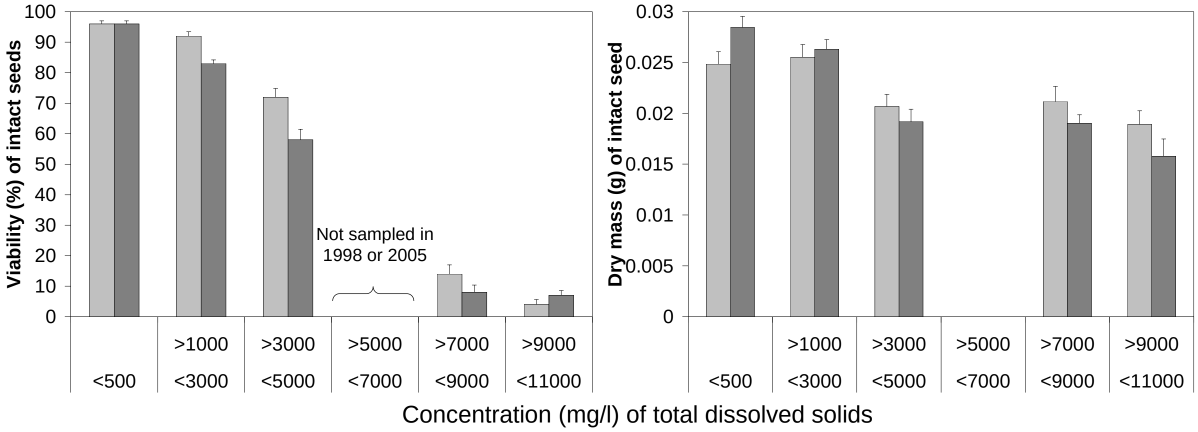


Figure 2.(Continued): the influence of phreatophyte tree growth in acid mine drainage on the percentage viability and dry mass of intact seed (mean ± SE) in 1998 ☐ and 2005 ■.

Table 2. Imbibition and germination (mean ± SE) in a subset of seeds from `Off' versus `On' AMD

		IMBIBITION OF VIABLE SEED:			GERMINATION OF VIABLE SEED:		
Spp & Origin	Time to imbibe (days)	Peak imbibition (days)	% Germinated in tests	Time to germinate (days)	Peak germination (days)	Germination Lag (days)	t_{50} (days)
Legumes with hard-coated seed (species ranked from highest to lowest seed mass):							
Elephantorrhiza elephantina:							
Off	2.0	-	95.6	4.0	6.0	-	-
On	2.0	-	100	7.0	9.0	-	-
Acacia erioloba:							
Off	2.0	2.5	100	3.2±0.5	4.0	2.7±1.3	6.3±1.4
On	1.0	1.5	100	3.7±0.4	4.0	3.3±1.0	6.9±1.6
Acacia hebeclada ssp. *hebeclada:*							
Off	1.0±0.0	1.0±0.0	100.0	11.0±0.0	11.0±0.0	11.0±0.0	11.0
On	2.1±0.0	1.5±0.4	66.7±3.3	11.6±0.3	14.0±0.0	5.0±2.4	10.5±2.9
Acacia hereroensis:							
Off	3.2±0.7	3.0±0.0	80.3	4.3±0.4	4.0±0.0	3.5±0.5	4.0±0.0
On	3.0±0.3	2.6±0.2	57.9	4.6±0.4	3.9±0.4	3.2±0.2	4.2±0.2
Acacia robusta ssp. *robusta*							
Off	2.0	-	90.3	2.5	2.5	2.0	-
On	1.0	-	93.8	2.5	3.5	-	-
Acacia karroo:							
Off	2.1±0.0	1.0±1.0	100	8.0±1.4	7.5±1.0	4.0±1.0	4.0
On	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
Mundulea sericea:							
Off	-	-	100	-	2.5	-	-
On	-	1.0	67.8	-	2.0	2.0	2.0
Non-legumes (species ranked from highest to lowest seed mass):							
Diospyros lycioides ssp. *lycioides:*							
Off	-	-	100	18.9±5.0	21.0±4.9	9.0±1.6	21.0±4.9
On	-	-	57.7	20.5±1.9	22.2±2.8	22.2±3.2	23.8±2.5
Ziziphus mucronata:							
Off	1.0±0.0	1.0	100	4.4±0.5	4.0	2.0	4.0
On	1.0±0.0	1.0	83.7	7.0±0.0	7.0	7.0	7.0
Rhus lancea:							
Off	6.0±0.0	6.0	78.7	28.9±2.1	25.0	12.0	25.0
On	4.0±0.0	3.0	60.0	-	22.0	22.0	22.0
Gymnosporia buxifolia:							
Off	2.5±0.4	2.5±0.4	34.6	21.0±1.0	18.7±3.3	17.3±2.9	18.7±3.3
On	2.8±0.0	3.0	23.4	-	26.0	26.0	26.0

4. Discussion

Depending on pollutant attenuation in a landscape, the impact of mining activities on groundwater quality can be remote from the pollution point source. Large-scale impacts are well established worldwide for nitrate contamination of groundwater by agricultural activities, and acidification of watersheds by plantation forests. However, South African land-use planning in the vicinity of mines only considers the property dimensions, and the dimensions of obvious pollution point sources within it (TSFs & other infrastructure). On the highveld it is common to see pollution-sensitive land-uses such as grazing, edible crops and housing with orchards and vegetable patches close to the base of TSFs, all of which activities rely on the integrity of ecosystem services and could therefore result in increased liabilities for land owners (Sutton *et al.,* 2006). Land-use planning for activities that rely, directly or indirectly, on the chemical quality of sub-soils and groundwater must therefore also consider the below-ground `footprint' of mining, industrial and agricultural activities.

Seed production in savanna trees is seasonal, and generally varies between years. More nutrients are required by plants after pollination has occurred, during anthesis. Unless the plant can acquire adequate nutrients and exclude excess amounts of salt ions and trace elements, seed development could be impaired. Studies on the effects of tailings run-off and AMD-polluted soils on regeneration by 227 plant species found that seed viability and germinability was reduced by seed ontogeny in polluted environments, with a high proportion of germinating seeds in acacia species having lethal developmental abnormalities (Witkowski & Weiersbye, 1999; Weiersbye & Witkowski, 2003). In this study we found that growth in AMD resulted in reduced shelf life for all seed with physical dormancy (data not shown). This was manifested as increases in seed lipid oxidation during storage, and more rapid declines in viability over time. Elevated lipid oxidation is expected for tissues enriched in transition metals (Cuypers *et al.*, 1999).

We found that the origin of seeds had a marked effect on viability and germinability. Even where seeds from AMD-grown plants had substantial viability, the germinability was often much lower. It is unclear what endogenous seed factors prevent germination of viable AMD-grown seeds. Studies on seed elemental composition found that the testa of viable legume seeds from TSFs contains excessively high concentrations of Fe and Ca, which may influence ion balance, water uptake or radicle emergence (Weiersbye & Witkowski, 2003; Weiersbye, Przybylowicz, Witkowski & Mesjasz-Przybylowicz, unpublished data). Additional factors that could inhibit the germination of viable seeds from AMD, are the low levels of seed P and high levels of many transition elements in the cotyledons and embryos. P is essential nutrient stored by seeds, and through phytates, contributes to the immobilization of potentially reactive elements in seed tissues.

Seed predation was highest in the *Acacia* species, excepting *A. karroo* and *A. robusta*. Seeds of leguminous plants have high nutritive contents and are usually attractive to seed predators (Liptay & Arevalo, 1998). However, seed predation was lower for most species growing on AMD. Ernst *et al.* (1990) observed for *Acacia*'s, that in years with large seed crops, percentage bruchid infestation was lower. However, we found lower percentage bruchid infestation to be associated with the smaller seed crops of AMD-grown trees, and higher percentage infestation for the larger seed crop's of trees off AMD. The average 19% seed predation for all *Acacia* species combined `On' AMD is well below the range of 47-96% reported for various *Acacia* species in a range of unpolluted sites in southern and East Africa (Seymour & Milton, 2003). Bruchid infestation of *Acacia* seeds is known to vary between individual trees and between years. Hence the consistent pattern that we observed across all species in both 1998 and 2005 suggests that AMD-related factors are indeed discriminating against predation. This could be an indirect consequence of differences in plant spatial distribution, tree density, phenology, pollination or fruiting between AMD and unpolluted rangelands, or it could be directly due to AMD-induced chemical or physical changes in foliage and seed nutrient contents that result in lower attractiveness to granivores, toxicity to insect eggs and developing larvae, or even due to dust loads on plants. All of the former have been identified as factors that can influence pollination, disease and predation in general for plants growing in low fertility soils, metaliferous soils and polluted soils (Heil *et al*., 2000; Loreau & de Mazancourt, 1999; Chapin *et al*., 1986).

In addition to the production of viable seed, germination limits the establishment of plants under saline conditions and even salt-tolerant plants are most sensitive at the germination and seedling stage (Catalan *et al.*, 1994). Although it is well established that seed germination % and rate is decreased by increasing external salts (Lovato *et al.*, 1994; Rehman *et al.*, 2000), there is less information on the effects of internal seed elemental concentrations as a consequence of seed ontogeny in AMD. The impact of sodium chloride-rich AMD is not greater than the impact of calcium sulphate-rich AMD in terms of seed percentage viability (data not shown), but does have a significant impact on seed germination and subsequent seedling mortality in unpolluted soils under containerised conditions. This may be due to internal osmotic effects during seed ontogeny. 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, previous studies showed that higher, and not lower, K concentrations were associated with acacia seeds from AMD polluted sources (Weiersbye & Witkowski, 2003). This latter finding is in accord with what is known about the role of stressful parental growing conditions on seed mineral nutrient accumulation during ontogeny (Liptay & Arevalo, 1998). Whereas nutrients that are non-limiting will be accumulated by seeds, there is also evidence from plants grown in silicate versus calcareous soils that less available elements are accumulated preferentially in seeds (at the cost of foliar concentrations) in order to serve as a starting source for seedling growth (Tyler & Zohlen, 1998). Preferential allocation of nutrients in limited supply to legume seed cotyledons was found to occur in three African *Acaci*a spp. on AMD (Weiersbye & Witkowski, 2003). The results of this earlier study support the general view (Rehman *et al.*, 2000), that the success of acacia seedling establishment in hostile environments is assisted by their ability to accumulate limiting nutrients in the seed, and for the seed testa to act as a sink for excess ions. Higher seed nutrient (especially Ca and K) contents would also be expected to facilitate seedling survival in AMD-impacted environments (Bewley & Black, 1994).

Faster germination by salt-tolerant species is also a strategy that may allow seedlings to escape salt injury (Rogers *et al.*, 1995). External salt solutions have been reported to affect the germination rate more than the germination percentage (Lovato *et al.*, 1994; Rehman *et al.*, 2000). For most species, imbibed seeds derived from On-AMD plants were slower to germinate than those from Off-AMD. Slower germination results in a longer time period before the emerging radicle anchors the seed to the substrate, and hence rates of germinant loss due to erosive forces could be higher. The resultant extended period of imbibed seed vulnerability to dehydration and predation would further disadvantage regeneration of plants on AMD. The proportion of viable seeds that subsequently germinated may be a useful indicator of a species ability to preferentially allocate limited resources to seeds when trees are growing under stress (On AMD) conditions.

Despite overall lower seed production, seed masses, percentage seed viability and percentage seed germination by trees growing on AMD, the majority were still able to achieve similar proportional germination of viable seed compared to con-specifics off AMD. Within the legumes, increasing seed size was also associated with higher maintenance of germinability by AMD-grown viable seed.

The South African gold fields are largely situated on the Witwatersrand Basin within the grassland and savanna biomes, often within an agricultural matrix of rangelands and cropping, as well as urbanisation. The non-consumptive value of many trees in rangelands and croplands is seldom fully acknowledged or known (Seymour & Milton 2003). The long-term ecological implications of impaired phreatophyte regeneration on AMD in the gold fields therefore merits investigation as rangelands and croplands might be fundamental to livelihoods in these regions after mine closure. Seymour & Milton (2003) indicate that acacia trees in arid grasslands and savannas take decades to establish, and then improve grazing quality. Large woody species deliver a variety of ecosystem services, beneficially impacting on soil quality and safe sites through shading, organic matter replenishment and nutrient enrichment, preventing soil erosion on steep slopes, sequestering carbon, reducing the rate of nutrient leaching and mitigating soil degradation, as well as producing forage, fruit and fuel wood. Large *Acacia* species contribute to livestock forage during drought via pod production, and may contribute to increased grain crop yields, livestock weight gain and increased milk production (reviewed by Seymour & Milton, 2003; and Shackleton *et al.*, 2007).

It can be expected that the primary determinant of phreatophyte survival post-seedling establishment (when rainfall would be important) is access to adequate deep water of suitable quality. Due to the difficulties inherent in examining tree response to groundwater for which only imprecise information was available for the rooting zone, there were constraints in this study. Initially, in 1998, we assessed the impact of AMD for plants growing on transects from point sources (assuming higher to lower salt concentrations with distance from point sources). However, the results of foliar analysis showed there was not a linear relationship between distance from point source, and concentration gradients for mobile ions in groundwater that contribute most to TDS (i.e. sulphates and chlorides) (Weiersbye *et al.*, 2007). This was confirmed by other studies on groundwater in the region (P.F. Labuschagne, S. Labuschagne, J.J.P. Vivier and H.J. Van Rensburg, unpublished), and is due to the influence of preferential flow pathways (faults and paleochannels), and secondary contamination plumes in evaporative sink zones, which can be remote from the original point sources. However, the foliar analysis confirmed that there was a linear to exponential relationship between distance from point source and the concentrations of less mobile elements in AMD, such as many metals and radionuclides. The latter contaminants are more concentrated around the point source but decline rapidly with distance due to attenuation by soil and organics. Heavy metal toxicity is known to be exacerbated in the presence of high salts, and this may have been a confounding factor for plants growing closest to point sources (TSFs).

There has been extensive historic clearing of woody plants for fuelwood, mining developments, grazing land and cropping in the study regions, and we could not assess the impact of AMD on species that are now less common or absent. By sampling common species it is probable that we only sampled the most tolerant fraction of the woody vegetation in these areas, i.e. those species remaining. Natural densities of woody plants are high in the seepage and run-off zones around the base of old TSFs (Weiersbye *et al.*, 2006), although the riparian zone of the Vaal River appears highly impoverished in both woody biomass and species due to land-use disturbances, including fire. On highveld gold mines, extensive artificial wetlands and *Phragmites* reed-beds are associated with AMD seepage zones from TSFs. Many of the seepage zones discharge into rivers, and burning of the reedbeds is associated with major destruction of the surrounding riparian zone vegetation. Riparian woody species are known to be fire-sensitive, and the generally negative impact of growth in AMD on seed production, viability and germinability reported in this study could impede the natural recovery of riparian zones in AMD-impacted regions. This is of concern since riparian vegetation stabilizes river banks, and represents a sink for terrestrial contaminants, including AMD.

Our experimental design could also not take cognisance of potential influences of locally tolerant ecotypes on species response to AMD – which may have been the only fraction we could harvest seed from on AMD-contaminated lands. However, we are monitoring the performance and regeneration of a range of trees and shrubs in matched site-species trials on AMD across the Witwatersrand Basin (the Mine Woodlands Research Project). These trials include cross-planting experiments for local provenances in order to test the home-site advantage hypothesis (*viz.* the Mine Woodlands Trials, Weiersbye *et al.*, 2002).

5. Conclusions and recommendations

The overall study (of which this report forms a part) found that the regeneration of all plants, and in particular shallow-rooted herbaceous species and grasses, was severely impaired through exposure to tailings spillages and AMD. Deep-rooted woody species in fact formed the most tolerant component of the vegetation and maintained the highest levels of regeneration potential under these conditions (Witkowski &

Weiersbye, 1999). This report confirms that the potential regeneration of a range of phreatophyte species, from sub-shrubs to trees, is still negatively affected by growth in AMD-contaminated groundwater, and that these impacts can be widespread and remote from the point sources. We found that the viability and germination of such seed as is produced on AMD is compromised, although viability levels remain surprisingly high and a previous study also found that the actual long-term survival rate of acacia seedlings derived from AMD-grown seed is higher than that for non-AMD grown seed when planted out into acidic tailings or polluted soil (Weiersbye & Witkowski, 2003). These findings indicate that there are strong pressures for selection of AMD-tolerant land-races, both through the demise of non-tolerant plants, and the limits set to successful seed germination and seedling establishment in the remaining individuals. Selection for AMD-tolerant land-races of phreatophyte species may therefore be contributing to impoverishment of woody biomass in general, and to reduced genetic diversity within the surviving plant populations on AMD-contaminated land and groundwater. Selection for salt-, acid- and metal-tolerant flora is well documented for analogous natural environments and old mining regions all over the world. Use of such tolerant local flora for the rehabilitation of salinized lands, contaminated soils and mine tailings has been considered a `proven technology' for decades (Bradshaw, 1970). Similarly, there is evidence for the emergence of AMD-tolerant flora on gold / uranium mines in South Africa (Weiersbye *et al.* 2006), and recent studies on water-use by *Phragmites* reedbeds (Jarmain *et al.* 2007) and *Rhus lancea* trees on AMD have found plants to be healthy and water-use apparently unaffected (Dye *et al*. 2007).

In terms of area affected, although the relative impact of tailings spillages and AMD on the regeneration of herbaceous and grass species is more severe than that of AMD on woody species (Witkowski & Weiersbye, 1999), these impacts are localized to the receiving soils around point sources such as TSFs. In contrast, phreatophyte vegetation is vulnerable over a larger area due to the widespread nature of AMD-contaminated groundwater on the Witwatersrand Basin (L. Croucamp, unpublished information). As well as phreatophytes, shallow-rooted species can also be exposed to AMD-polluted groundwater at great distances from the point source, as salts percolate to surface soils on the highveld when evapotranspiration exceeds precipitation (Naiker *et al*., 2003; Tutu *et al*., 2005; Weiersbye *et al.*, 2007).

A recent report discounted the ability of South African mining to play any meaningful role in the mainstreaming of biodiversity conservation in the grassland biome due to the high negative impact of mining (O'Connor & Kuyler, 2007). The highest impacts of mining in the biome are considered to be for grassland, rocky grassland, riparian, wetland and aquatic ecosystems. Ecosystem services for humans were not assessed, but intimated to be "even less flattering". The severe impact of underground gold mining in the grassland biome arises from the high proportion of individual properties that are lost to transformation, the fragmentation and loss of connectivity in the remaining area, impaired geometry and severely impaired hydrological functioning. An 80% loss of property is estimated for underground mining, such as gold mines, due to these activities. The impact of mining on carbon storage and water quality is the highest of all land-uses in the grassland biome, and soil erosion is one of the highest (O'Connor & Kuyler, 2007).

However, we recommend that strategic interventions must still be taken to encourage mining to contribute to biodiversity conservation and ensure the integrity of ecosystem services on and around their properties. Our rationale is that, by virtue of emissions such as AMD contaminated water, mining also indirectly controls and influences future land-use options on areas beyond their own property boundaries. A preliminary and conservative estimate of the value of the flow of ecosystem services in South African grasslands is in the order of R9.7 billion per annum, or R29 000/km^2 of grassland (De Witt & Blignaut, 2006). Since rehabilitation of the transformed grasslands is considered impossible, these interventions may have to be creative and indirect approaches to render them achievable by the mining industry. Offsets and trade-offs appear to be a sound and workable option. In another approach, our research focuses on the use of indigenous vegetation for AMD control and conversion of TSFs and polluted soils to meaningful ecological functions, such as carbon sequestration in tailings, and cropping of indigenous plant products. It is envisaged that the conversion of waste to resources can divert development pressures from other lands, and indirectly contribute to protecting ecosystem services on the gold fields. As another example, the South African mining industry employs ± 400 000 people, or ± 9% of the national workforce (MSSD, 2002). This workforce in turn supports dependents across the SADC region, and may also influence dependents viewpoints on health and safety as a consequence of the mine induction training process. Education on sound land-use practices for contaminated or degraded lands can be mainstreamed into this process.

However, the ultimate challenge is for mines to implement designs and practices that minimize their above-ground and hydrological footprints, and to target already degraded areas for future developments rather than undisturbed lands. When assessing the economic and environmental viability of future land-uses in mining regions, regulatory agencies and developers must consider the potential for latent impacts and impaired ecosystem functioning long after mine closure. Failure to foster compatible land-uses could exacerbate the liabilities of the closing mines, and render sub-economic the activities of subsequent land-users where their livelihoods depend upon the integrity of ecosystem services.

Acknowledgements

This study is part of the Ecological Engineering & Phytoremediation programme (*Mine Woodlands Project*) at Wits University and AngloGold Ashanti. We are grateful for funding to the Environmental Management Dept. of AngloGold Ashanti, FreeGold (now Harmony Ltd), National Research Foundation, Dept of Trade & Industry (THRIP), and Wits University. We thank farm-owners around mines for access, and the following staff and volunteers for assistance: M.J. Kekana, T. Regnier, J. Dini, M.L. Magagula, S. Seeletsi, N. Thothobola, C. Groenewald, A. Groenewald, B. Oageng, J. Mashlangu, N. Procter, P. Belebese, I. Monei, R. Nel, T. Zuma, C. Hitge, T. Parsons, M. Jansen, M. Sutton, L. Witkowski, G. Angove, T. Hilditch, R. Sutton, B. Wilson and B. Whitfield at Wits University; J. Selobogo, P. van der Grijp, D. Benson, A. Groenewald, D. Abrahms, S. Mnisi and his team at FreeGold; D. Deva (Umphakathi Nursery), J. Mans (DWAF, Upington), M. Procter (DWAF, Bloemfontein), P. Modiane (Modula Qhowa Nursery) and D. Human (Lifestyle Seeds).

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