Germination and predation of *Acacia karroo* seeds on acid mine drainage polluted soils

A research report submitted to the Faculty of Science at the University of Witwatersrand, Johannesburg, in partial fulfilment of the requirements for a degree of Master of Science.

Prepared by

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Declaration

I declare that this thesis is my own, unaided work. It is being submitted for the Master of Science degree at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree for examination at any other university.

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(Signature of candidate)

02 day of June 2016

Abstract

The study aims to assess the impacts of Acid Mine Drainage (AMD) polluted soils on *Acacia karroo* seed germination and viability, seed dry mass and predation, in comparison with trees from the same provenance growing on non-polluted soils.

The study was undertaken within the Vaal River Operations mining rights area. This area is bisected by the Vaal River which separates the polluted area from the non-polluted area. Contamination of soils on the northern section of the Vaal River is a result of mining operations, historical tailings spillage as well as an existing pollution plume which has resulted in AMD polluted soils.

The rehabilitation of disturbed land is often hindered due to low seedling establishment. The success of germination is one of the most important first steps for seedling establishment and growth and hence towards establishing a self-sustaining vegetation cover over disturbed areas.

Dry seed mass was slightly higher from trees in non-polluted (0.051±0.009g) compared to the polluted areas (0.046±0.009g), however no significant difference was found. Seeds collected from the non-polluted area had highest proportion of seeds in the seed mass class 0.0455-0.0904g, compared to the seeds from the polluted areas which were highest in the smaller seed mass class 0.0155-0.454g. At the tree level, the Coefficient of Variation (CV) for dry seed mass was higher for seeds collected from the polluted area (20.5%) compared to the non-polluted area (17.9%), however, no significant difference was found. However, percentage seed predation was significantly lower in the polluted (35±15.76%) relative to the non-polluted areas (48±14.69%). Percentage seed germination was significantly higher in the non-polluted (92±9.35%) compared to the polluted areas (81±20.42%), with a significantly more rapid germination rate of 4.2±0.19 days compared to 4.7±0.45 days, respectively.

In conclusion, despite their lower dry seed mass, seeds collected from AMD polluted soils still had high percentage germination, while exhibiting a lower percentage of seed predation compared to those growing on unpolluted soils. Due to *A. karroo*'s apparent tolerance to the poor conditions on the AMD polluted soils and its regeneration capabilities, it is likely to be a good species for rehabilitation of AMD polluted sites.

Further studies should aim to determine seedling performance from those seeds collected from polluted areas in terms of seedling establishment, rates of growth and survival over time when established in AMD polluted soils as well as non-polluted soils, to determine their likely success.

Keywords

Acacia karroo, germination, predation, pollution, rehabilitation, seed mass

Acknowledgements

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Abbreviations

AMD	Acid mine drainage	Acid mine drainage occurs due to the exposure of sulphide minerals found in metal mines, to water and air, which together react to form sulfuric acid. This acid is then released into the environment having severe impacts on animals and plants.
NP	Non-polluted	The study area that has not been subjected to acid mine drainage
PP	Polluted	The study area that has been subjected to acid mine drainage as a result of mining operations

List of symbols

°C	degree Celsius
m	metre
mg	milligram
g	grams
mm	millimetre
%	percent
μm^2	square micrometre

Chapter 1

1.1 Literature review

Mining in South Africa

Approximately 7% of the land in South Africa is disturbed by mining activities (The Chamber of Mines, 2007; Watson et al., 2014). Mining activities can result in a number of negative impacts on the surrounding environment due to mining operations and their mineral waste deposits that remain after closure (Watson et al., 2014).

The Witwatersrand Basin is one of the most famous gold production areas in the world and is situated in the Gauteng Province of South Africa (Naicker et al., 2003). Mining activities in this region began in 1886 and have resulted in the conurbation of Johannesburg providing large revenue for the economy (Naicker et al., 2003). The Witwatersrand super group extends in an east-west direction over a strike length of approximately 45 km (Naicker et al., 2003). The ore mined underground from the Witwatersrand super group is brought to the surface where it is processed using complex metallurgical processes to obtain the gold resource (Naicker et al., 2003). As a result of the gold processing, a waste by-product is produced which is known as tailings material and is disposed of onto a tailings facility (Ritcey, 2005). These tailings facilities consist of high levels of pyrite and other harmful wastes and metals which can have significant impacts on the surrounding environment (Straker et al., 2007).

The main environmental impacts of tailings is Acid Mine Drainage (AMD) which can result in the pollution of surface and groundwater systems (Straker et al., 2007). The AMD is created due to weathering of the tailings facilities resulting in the exposure of the tailings material to oxygenated rainfall causing oxidation of the pyrite and other sulphides in the material (Bakatula et al., 2008; Naicker et al., 2003). Oxidation of the pyrite acidifies the water moving through the facility, which then enters the groundwater regime beneath the tailings facility. This acidic water is believed to be entering into local surface water systems along the Witwatersrand, such as the Vaal River (Naicker et al., 2003; McCarthy, 2011).

Tailings residue dust has also been highlighted as having negative impacts on the surrounding environment in the Witwatersrand basin as it is a nuisance to humans (Sutton & Weiersbye, 2008). Tailings material has poor physical composition, high levels of salts and heavy metals, are nutrient deficient and are often finely ground unconsolidated sands and clay, that when mobilized by wind, sandblast and bury plants (Lacy, 2005).

Although tailings are localised, the transport of tailings materials by wind and water into soils, water courses and groundwater systems can result in concentrations of sulphur, chloride and other harmful metals accumulating in these sink areas (Sutton & Weiersbye, 2008). Erosion and AMD from tailings facilities can cause physical stress to vegetation due to high levels of sulphates which have severe impacts on nutrient cycling as well as the establishment and regeneration of vegetation over these areas, making them difficult to rehabilitate (Weiersbye et al., 2006).

Rehabilitation

A number of studies have been undertaken in various countries on AMD contamination in soils due to mining activities and on the rehabilitation and amelioration of tailings contaminated soils (Weiersbye et al., 2006). Tailings facilities are often inhospitable to vegetation establishment and growth due to their chemical and physical properties including high levels of erosion, low levels of nutrients (nitrogen and phosphorus), low surface water availability, low natural organic matter and associated microbial populations (Straker et al., 2007). As already mentioned, tailings facilities often contain high levels of salts and heavy metals that can act as phytotoxic elements limiting vegetation establishment (Lacy, 2005).

To ensure successful rehabilitation of tailings facilities, plants must be capable of tolerating saline conditions and still be capable of regeneration. According to Weiersbye & Witkowski (2002), the use of indigenous species already growing on tailings facilities and AMD polluted soils for rehabilitation are expected to result in a higher probability of regeneration to create a persistent vegetation cover (Weiersbye & Witkowski, 2002). The establishment of vegetation over these areas is important as it stabilises the facilities and ultimately contains possible movement of contamination (Weiersbye & Witkowski, 2002).

Based on previous studies undertaken by Witkowski & Weiersbye (1998; 2003), vegetation found on tailings storage facilities and AMD polluted soils showed that the harsh environments negatively affected all aspects of reproductive biology, including seed production, nutrient content, metal content, seed viability and germination. It was further shown that the herbaceous plants including grasses and weedy species were most severely affected, whereas the woody plants appeared to be more tolerant (Witkowski & Weiersbye, 2003). These woody species exhibit tolerances to salinity, acidity, nutrient deficiencies, ion imbalances, as well as bioaccumulation of various heavy metals and radionuclides. This is advantageous in terms of establishment of woody species on AMD polluted soils (Witkowski & Weiersbye, 2003).

Acacia karroo

Of these woody species identified by Witkowski & Weiersbye (1998; 2003) to grow and persist on AMD polluted areas, *Acacia karroo* (Family: Fabaceae; Sub family: Mimosoideae) was one of the most common species. In this study the generic name *Acacia* is used in its traditional broad sense. Although the scientific name was recently changed to *Vachellia karroo* (Hayne) Banfi & Galasso (Robbertse et al., 2014), it is still generally referred to as *Acacia karroo* in South Africa.

A. karroo is commonly found within the Savanna and Grassland Biome, specifically within the Highveld region of the Witwatersrand basin (Witkowski & Weiersbye, 2003). *Acacia's* are significant contributors to nutrient cycling (Witkowski, 1991) and provide a source of food and habitat for wildlife and livestock as well as livelihood goods to rural people (Shackleton et al., 2007). The environmental benefits of *A. karroo* include shade, a deep root system which obtains water and nutrients from depth and its ability to fix nitrogen, which encourages the development of perennial grasses (Barnes et al., 1996). The shading from *A. karroo* may also reduce soil surface temperatures and evaporation, making conditions more suitable for drought susceptible grasses (Baskin & Baskin, 2014).

Based on studies undertaken by Witkowski & Weiersbye (2003) at Anglo Ashanti Gold's Vaal Operations and West Wits Operations located in Northwest Province of South Africa along the Witwatersrand Basin, *A. karroo* illustrated relatively good regeneration potential on the tailings facilities compared with that of other species growing on the tailings, despite the poor growing conditions and AMD polluted soils (Witkowski & Weiersbye, 2003).

Based on the above environmental benefits of *A. karroo* and its apparent ability to tolerate stressful conditions, *A. karroo* is considered a promising species for rehabilitation of infertile, degraded or saline environments (Witkowski & Weiersbye, 2003).

Seed size and germination

The success of germination is contingent on a number of factors (Esler et al., 1989). Viable seeds of many species require complex sets of cues in order to germinate (Baskin & Baskin, 2014). Seeds of most savannah trees are dormant, and physical dormancy is the most common type. According to Baskin & Baskin (2014), *A. karroo* is one of these species with the characteristic of physical dormancy. Physical dormancy can be broken through mechanical scarification by means of acid, boiling water or dry heat (Baskin & Baskin, 2014).

Ingestion by animals (O'Connor, 1995) and fire has been observed to promote the germination of viable *A. karroo* seeds (Baskin & Baskin, 2014). It is not uncommon for veld fires to occur in the Savanna and Grassland Biomes where there is a good grass cover to burn each year. Fires create favourable

conditions for seed germination causing seeds with physical dormancy to become non-dormant (Baskin & Baskin, 2014). Fire has been observed to promote the germination of *A. karroo* seeds (Baskin & Baskin, 2014). Based on studies undertaken by Weiersbye & Witkowski (2002), a screening exercise was undertaken to determine the most successful method for the 'practical bulk germination' of *A. karroo* for use in large scale tree planting trials on slimes dams and along polluted aquifers. Germination was highest in *A. karroo* from the heat point method using a soldering iron to break seed dormancy (Weiersbye & Witkowski, 2002). Once the physical dormancy of a seed is broken seed reserves are used for energy for germination.

Seed size has been considered to be an important trait that affects the establishment of many plants, directly impacting on germination time, percentage germination and seedling vigour (Souza & Fagundes, 2014). According to Baskin & Baskin (2014), survivorship of seedlings from large seeds is often higher than that of seedlings from smaller seeds. This is due to the larger seeds providing seedlings with a store of provisions that improve their chances of becoming established under unfavourable conditions (Venable & Brown, 1988). Larger seeds gain a competitive advantage over smaller seeds as they are able to germinate earlier and at a more rapid rate (El-ahmir et al., 2015). This is contrary to Westoby et al. (1996), that larger seeds generally have slower germination rates. This may be due to slower respiration rates which gives them longer survivorship under various environmental conditions (Westoby et al., 1996).

There are a number of factors that influence seed size, including resource availability, environmental conditions and plant growth form (El-ahmir et al., 2015). Studies undertaken by Westoby et al. (1996), showed that larger seeds are able to survive better in disturbed environments during early growth as larger seeds hold a higher percentage of seed reserves to support respiration and / or repair of potential damage due to harsh environments. Larger seeds also give rise to larger seedlings immediately after germination which allows the seedlings to reach deeper into the soil to obtain water supplies or to grow higher to obtain more sunlight for photosynthesis (Westoby et al., 1996).

Seeds contain a very large proportion of the most limiting nutrients, especially Nitrogen (N) and Phosphorus (P) (Witkowski & Lamont, 1996). According to Witkowski & Lamont (1996), the allocation of limiting nutrients is more equated with fitness than dry mass in nutrient impoverished environments. Witkowski & Lamont (1996), identified the important role of P and to a lesser extent N in limiting plant growth in nutrient poor soils. It was shown that a large proportion of a plants relative allocation of the most limiting resources, in this case P and N, was allocated to seeds to ensure seedling establishment in these nutrient impoverished environments (Witkowski & Lamont, 1996). In disturbed environments, the essence of resource allocation to a plant is limited so that available resources are divided among the plant parts in such a way as to maximise fitness (Witkowski & Lamont, 1996). This

may also lead to the allocation of resources to plant parts such as roots for access to water and nutrients rather than producing larger seeds. These trade-offs are seen as adaptions to reduce impacts of environmental variation in disturbed environments (Venable & Brown, 1988).

Seed size versus seed number trade-offs have also been seen as an adaptive mechanism in disturbed environments. Contrary to Venable & Brown (1988), Esler et al. (1989), discussed how higher seed nutrient content could be associated with enhanced seedling establishment in infertile soils due to a possible trade-off between seed size and number of seeds produced, where the more nutrient poor soils would produce larger seeds with higher N and P, but a lower annual seed production than those plants growing on more nutrient rich soils to ensure seedling establishment (Esler et al., 1989). In areas where there is high resource availability, this usually leads to greater seed production in terms of number of seeds (El-ahmir et al., 2015). Esler et al. (1989) further suggest that these seeds can be smaller since seedlings would be less dependent on seed reserves of these minerals for successful establishment due to the high resource availability. Seed size, however, varies between and within plant species and populations, sometimes by several orders of magnitude (Souza & Fagundes, 2014).

Leishman et al., (2000), discussed how bet-hedging can determine seed size where the higher the level of bet-hedging via one mechanism (for example better dispersal to other sites), the weaker the selection for other mechanisms (for example seed size). Venable & Brown (1998), regarded large seeds as contributing to bet-hedging by permitting establishment under a wider range of seedling establishment conditions.

Predation

Seeds of leguminous plants such as *A. karroo* have high nutritive contents and are usually attractive to seed predators (Witkowski & Weiersbye, 2003). O'Connor et al. (2010) found that high production of *A. karroo* seeds was counterbalanced by a host of agents that killed the seeds before they germinated, which greatly reduced the seed abundance. Many species of the family Bruchidae parasitize seeds of the *Acacias* including *A. karroo* which can seriously affect reproduction. Predation can destroy the viability of up to 90% of the seeds produced by a plant (Barnes et al., 1996)..

The females of bruchid beetles oviposit on the tree pods or seeds when still green. Re-infestation following emergence may occur in the mature, dry pods on the canopy or ground (Miller, 1996). According to Miller (1996), the degree of bruchid beetle infestation may differ between pods on the canopy and on the ground as well as between *Acacia* populations. Miller (1996), found when assessing predation of *Acacia* seeds by bruchid beetles in an African savanna ecosystem, percentage predation of seeds within pods from the ground exceeded that of canopy held seeds.

The larvae develop and feed inside the seeds, on the endosperm and embryo (Abdullah & Abulfaith, 1995). Bruchid beetles leave a large hole in the seed coat upon emergence. The adult emerges after consuming most of the seed content (Baskin & Baskin, 2014). Limited studies have been undertaken to determine the likely impact of heavy metals being absorbed by plants on the success of bruchid beetle emergence.

It was shown by Witkowski & Weiersbye (2003) that effects of seed predation appeared to be much lower on plants growing in AMD polluted soils compared to off AMD polluted soils. Witkowski & Weiersbye (2003), suggested that this may be due to microclimate changes with proximity to tailings facilities and / or tree densities being lower on polluted soils which may contribute towards lower predator population densities as well as harsh environments in which trees are located (Witkowski & Weiersbye, 2003).

1.2 Motivation for this study

Rehabilitation of disturbed areas is often hindered by low seed germination and poor seedling establishment (Abari et al., 2012). Distribution and abundance of plants could be associated with variations in seed germination and seedling recruitment between various habitats and environmental conditions (Souza & Fagundes, 2014). As already mentioned, disturbed environments could negatively affect all aspects of reproductive biology. Range expansion of plants requires successful germination and seedling establishment (O'Connor, 1995).

Understanding the response of *A. karroo* seed germination and predation on AMD polluted soils is important in informing rehabilitation management and the use of *A. karroo* as a species for rehabilitation. The success of seed germination from *A. karroo* growing on AMD polluted soils provides an indication of whether this species is capable of sustainable regeneration through successful seedling recruitment within a disturbed area.

1.3 Aim

This study aims to determine whether seed germination and predation of *A. karroo* is negatively affected when growing in AMD polluted soils compared to non-polluted soils.

1.4 Objectives

The objectives of this study are as follows:

- To investigate the level of seed predation of *A. karroo* growing on non-polluted soils compared to those growing on polluted soils.
- To investigate the impacts of polluted soils on *A. karroo* seed germination percentage and rate of germination;
- To determine the relationship between dry seed mass and germination on polluted and non-polluted sites; and
- To determine seed size variation between seeds collected from non-polluted soils compared with those collected from polluted soils.

1.5 Probable Expectations

The following are expected results for this study:

- Seeds produced on *A. karroo* trees growing on AMD polluted soils are expected to have a lower percentage germination and slower germination rates compared to those growing on the non-polluted soils;

- Seeds with a larger mass are expected to have higher percentage germination;
- Seeds with a larger mass are expected to have a slower rate of germination; and
- Seed predation is expected to be lower on the polluted soils compared to the non-polluted soils due to the hostile environment in which the polluted samples are growing, affecting the seed predators more than the host trees.

Chapter 2

2.1 Materials and methods

2.1.1 Study area

The Vaal River Operations are situated to the south and north of the Vaal River and located at the boundary between the North-West and Free State Provinces, approximately 18 km North West of the town of Klerksdorp in the Witwatersrand Basin (Figure 1) (AngloGold Ashanti Ltd, 2009).

The mean annual precipitation for the area is approximately 650 mm which occurs during the summer period mainly in the form of thunderstorms (AngloGold Ashanti Ltd, 2009). Maximum and minimum temperatures range between 25°C in the summer and 0°C during winter, respectively (AngloGold Ashanti Ltd, 2009). Frost occurs on average for approximately 34 days of the year during the winter period (AngloGold Ashanti Ltd, 2009).

The Vaal River Operations is situated on doleritic and sandy soils situated in Vaal Reefs Dolomite Sinkhole Woodland (Gh12) and Vaal Vet Sandy Grassland (Gh10) within the Grassland Biome, transitional to the Savanna Biome (Mucina & Rutherford, 2006). The soil profile towards the Vaal River consists mainly of Quaternary alluvium soils (1-5 m) and weathered dolomite or wad (5-10m) on dolomite bedrock.

The mine lease area is generally flat to gently undulating terrain. The area is situated at an elevation of approximately 1270 – 1340 m above sea level. Man-made structures including shaft headgears, tailings facilities and waste rock dumps alter the general topography of the surrounding landscape (AngloGold Ashanti Ltd, 2009).

The area in which sampling was undertaken is bisected by the Vaal River which separates the polluted area north of the Vaal River from the non-polluted area south of the Vaal River. Coordinates of the *A. karroo* sampled on both non-polluted and polluted areas are provided in Table 1 and illustrated in Figure 1. Figure 2 illustrates the catchment area within the study area whereby the drainage moves towards the Vaal River in a southerly direction. Two main pollution sources were identified as part of this study

contributing to the contamination of the surrounding environment, including the main pollution source whereby historical tailings spillage has taken place and the minor pollution source (Figure 1). Soil surveys conducted by Red Earth CC (2015), confirmed that a perched aquifer exists on the polluted site, which causes salts to be deposited onto the surface (Red Earth CC, 2015). Groundwater mapping of polluted aquifers by Anglo Gold, further confirmed that these areas were polluted (AngloGold Ashanti Ltd, 2009).

Seeds were collected from *A. karroo* located within the Vaal Reefs Dolomite Sinkhole Woodland vegetation unit. The majority of the *A. karroo* trees were growing in Tukulu soil form, especially those trees located in close proximity to the Vaal River. Trees sampled further away from the Vaal River, within close proximity to the pollution source, were found to be growing in Oakleaf soil form (Red Earth CC, 2015). Oakleaf soil form generally consists of a dark organic rich topsoil of 30 - 40 cm, with a deep yellow or red neocutanic subsoil. The Tukulu soil form is different only in that there are signs of short periods of wetness in the deeper soil profiles.

Table 1 Coordinates of *Acacia karroo* trees sampled on both the non-polluted and polluted areas

Non-polluted samples	Coordinates	Polluted samples	Coordinates
NP1	S 26°58.866; E 26°42.671	PP1	S 26°58.274; E 26°43.178
NP2	S 26°58.933; E 26°42.614	PP2	S 26°58.278; E 26°43.149
NP3	S 26°59.634; E 26°42.539	PP3	S 26°57.546; E 26°41.707
NP4	S 26°59.548; E 26°42.569	PP4	S 26°57.100; E 26°41.613
NP5	S 26°59.420; E 26°42.572	PP5	S 26° 58.191; E 26° 43.123
NP6	S 26°58.850; E 26°42.866	PP6	S 26° 57.892; E 26° 43.685
NP7	S 26°58.474; E 26°43.612	PP7	S 26° 57.383; E 26° 43.622

*As indicated in Figure 1

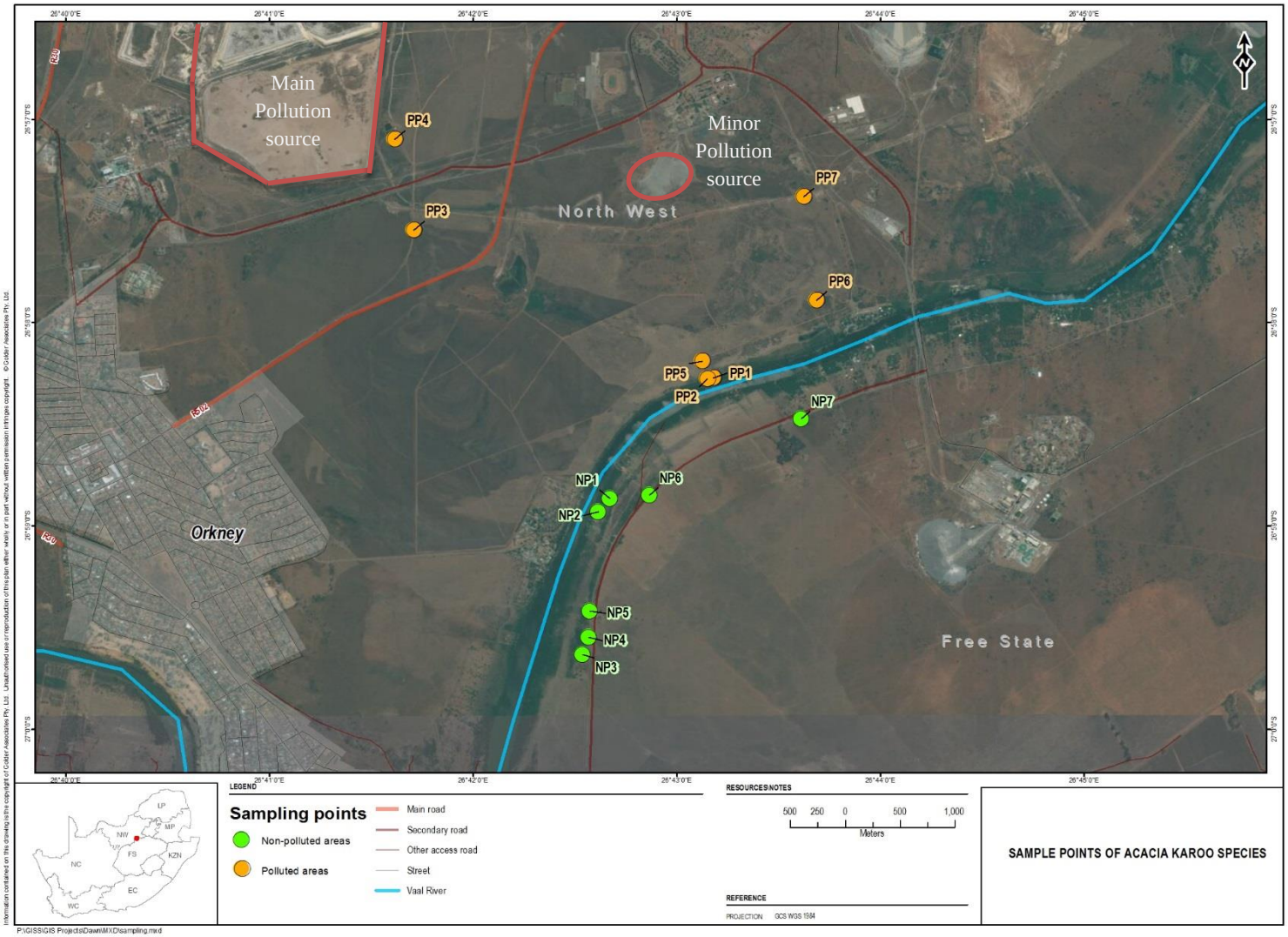

Figure 1 Location of *Acacia karroo* trees from which seeds were collected growing on polluted soils (north of the Vaal River) and non-polluted soils (south of the Vaal River).

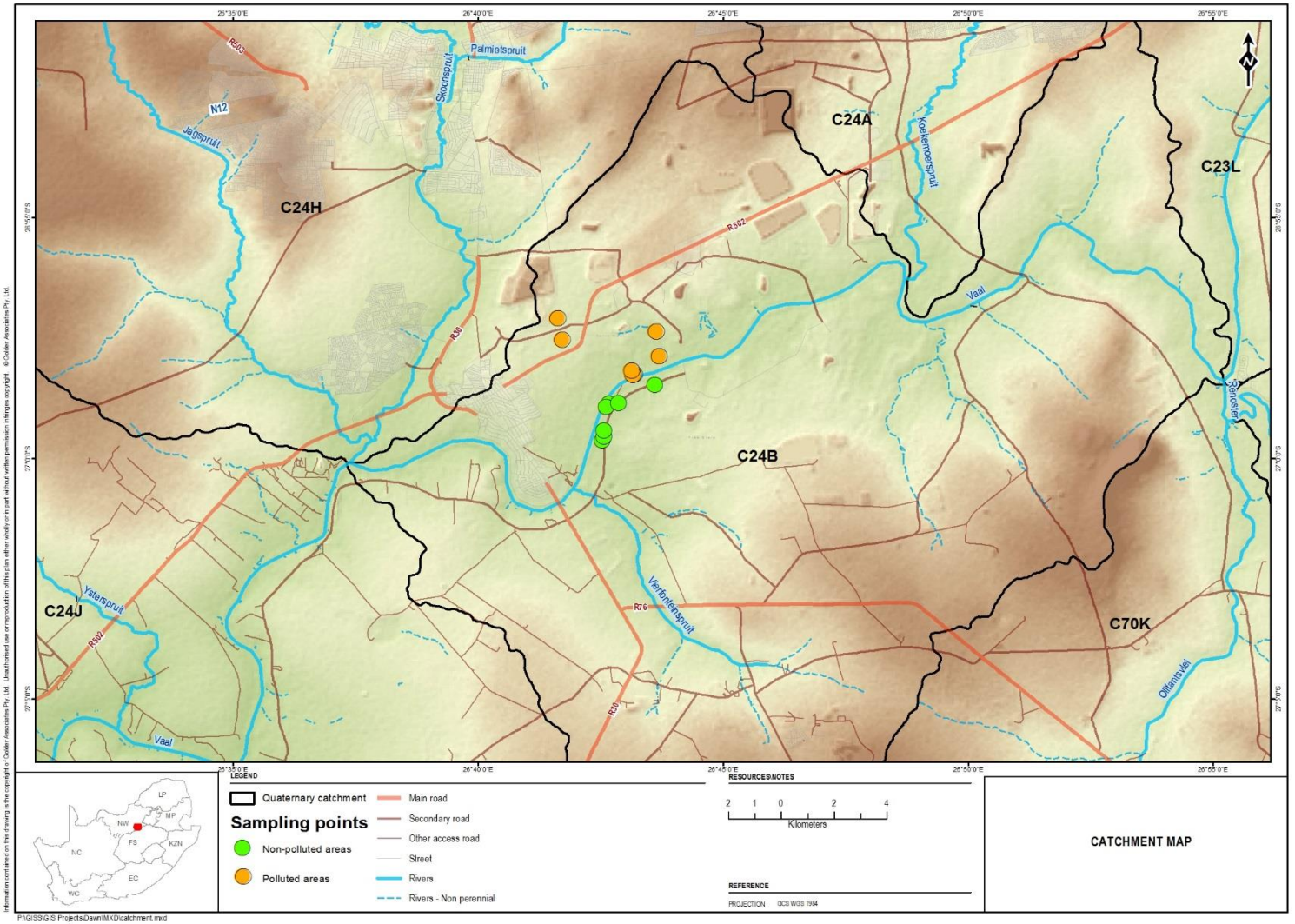

Figure 2 Location of *Acacia karroo* trees in relation to the Vaal River and surrounding catchment system.

2.1.2 Study species: *Acacia karroo*

Acacia karroo is one of the most abundant and widespread *Acacias* in southern Africa. Dense populations of *A. karroo* occur along the Witwatersrand Basin within the transition between the Savanna and Grassland Biomes (Mucina & Rutherford, 2006).

Acacia karroo have a long taproot system which enables the plant to use water and nutrients from deep within the soils. Due to this and its ability to fix nitrogen, grasses and other plants establish underneath their canopies (O'Connor, 1995).

Flowers are borne in spherical flower heads and the stipules are straight or slightly curved spines (Robbertse et al., 2014).. *A. karroo* have dehiscent fibrous pods which ripen between February and June which is when the pods open on the tree and seeds are suspended by a thin thread like funicle, which become brittle as it dries allowing the pod to drop to the ground (Barnes et al., 1996)..

The *A. karroo* seeds have physical dormancy which is usually broken in nature as a result of ingestion by animals or the heat of veld fires. The heat created in the soils as a result of a fire, breaks the dormancy allowing water to move in and for imbibition (Wilson & Witkowski, 1998) and germination to take place (Baskin & Baskin, 2014).

2.1.3 Seed collection and preparation

Seed pods were collected by hand from 14 randomly selected *A. karroo* individuals in June 2014, growing within the Vaal River Operations surface rights area (Figure 1). Seed pods were collected from seven randomly-selected *A. karroo* trees growing on polluted soils and seven from trees growing on non-polluted soils. The *A. karroo* individuals sampled were located within dense stands, especially along the Vaal River, or as isolated individuals surrounded by grassland. These dense stands of *A. karroo* were less common further away from the Vaal River.

For each *A. karroo* tree (14 individual trees), seed pods were collected by hand to fill half of a 30 cm brown bag, from the ground directly beneath the canopy and then separately from the canopy itself (28 samples in total) (Figure 3). Only pods that were split open were collected, as this indicated that the seeds within the pods were mature.

Seed were extracted from the pods and stored in a laboratory in brown bags under ambient conditions in a cool, dry place out of direct sunlight for approximately 3 months prior to undertaking germination tests.

Figure 3 (a) Tailings storage facility at Vaal River Operations, (b) *Acacia karroo* individual growing in natural habitat, (c) *A. karroo* seed pods within tree canopy, (d) collecting seeds on the ground under the canopy, and (e) collecting seeds on the tree. Photographer: Dawn Lagerwall

2.1.4 Predation

Seeds extracted from all the pods collected were categorised as either intact or predated. Predated seeds had bruchid beetle exit holes, and were partially or wholly eaten inside the seed compared with the large undamaged, swollen intact seeds (Wilson & Witkowski, 2003; Weiersbye & Witkowski, 2007).

Predated seeds were further assessed to determine the success of bruchid beetle emergence from the seeds. Each of the predated seeds were individually analysed and recorded as either 'successful emergent', where there is no bruchid remaining in the seed and the bruchid has already vacated the seed, or 'unsuccessful emergent', where the bruchid is still within the seed and was not able to vacate. This might be due to the exit hole being too small, the bruchid being unable to create a hole to escape or due to the bruchid beetle still developing with seeds being collected before the bruchid beetles had enough time to grow to full size to emerge due to oxygen starvation during storage (Figure 4).

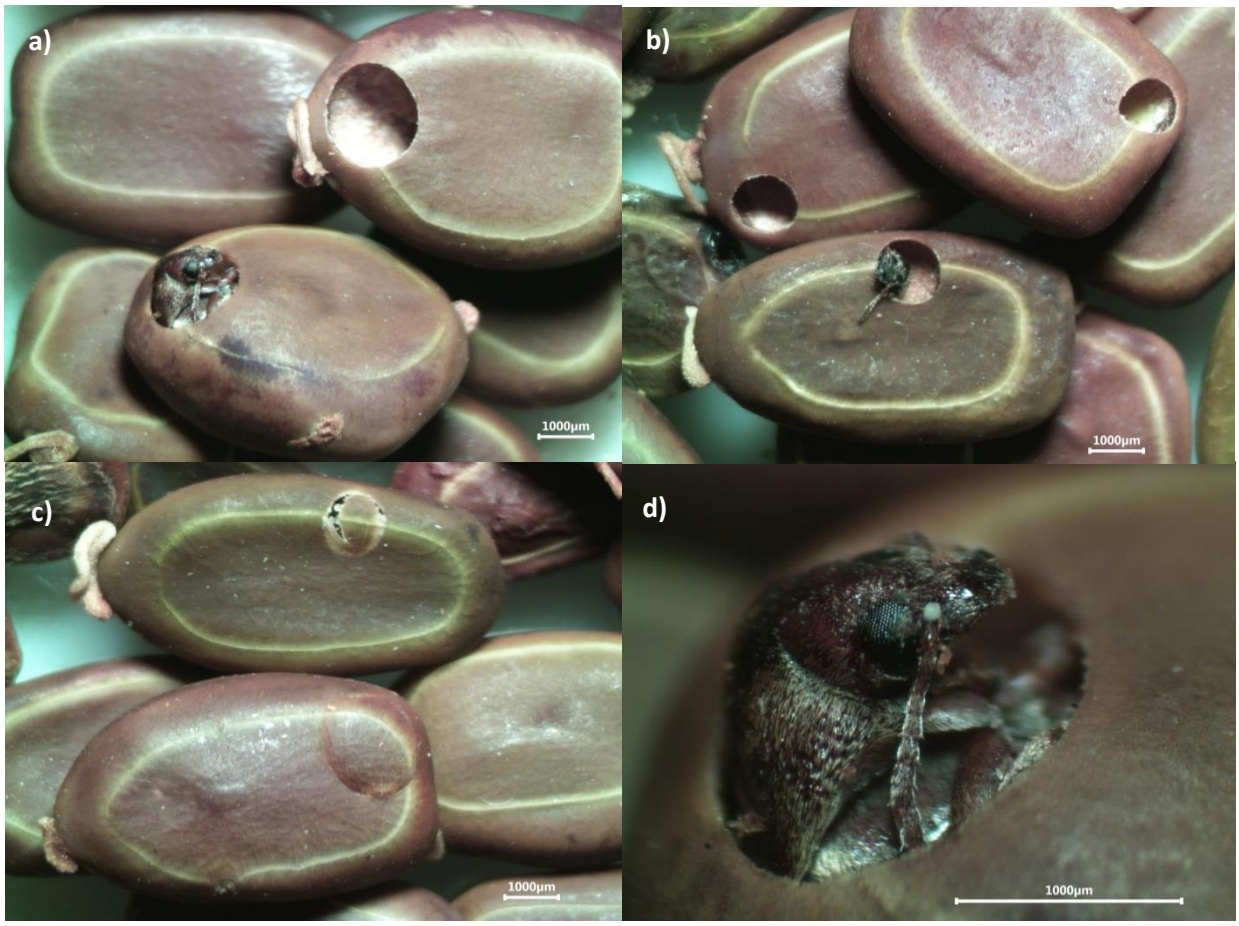

Figure 4 Success of bruchid beetle emergence: a) & b) unsuccessful (bruchid beetle still within the seed) and successful bruchid beetle emergence (exit hole after successful emergence), c) bruchid unable to create an exit hole, and d) close up of unsuccessful emergence of bruchid beetle. Photographer: Alex Gumovsky

2.1.5 Dry seed mass

A sub-sample (replicate) of seeds from those pods collected on the ground and from the canopy were selected at random from each tree sampled and placed into 4 repli-dishes (a sub-sample of 96 seeds from the canopy and 96 seeds from the ground) per tree.

These seeds were weighed one month after collection during the winter period as the relative humidity during this time is low on the Highveld region (Johannesburg). Each individual seed was weighed on a Precisa 92SM-202A scale (Dietikon, Switzerland) correct to four decimal places and recorded (total of 2688 seeds were weighed).

2.1.6 Seed germination

The intact *A. karroo* seeds weighed (a sub-sample of 96 seeds from the canopy and 96 seeds from the ground per tree) were pre-treated by scarifying the seed coat using the heat point method by lightly touching the seed coat using a soldering iron for less than 2 seconds (Figure 5). This was shown by Weiersbye & Witkowski (2002) to be successful in terms of breaking the physical dormancy and thereafter germination of *A. karroo,* with 94%±0.6 of their seeds germinating.

Germination of seeds was undertaken by placing each of the seeds that had been weighed on tap-water moistened cotton wool within repli-dishes with the lids on to reduce water loss by evapotranspiration (Schroder et al., 2013). The repli-dishes were placed onto an electric blanket at a temperature of ±32°C, receiving ±1.5 mm of water daily at 16h00 using a pipette (Wilson & Witkowski, 1998).

Seeds that imbibed within the first hour were assessed for predation. These seeds were found to all contain bruchid beetles that had not yet emerged and thus the number of intact and predated seeds was modified at this stage (see Appendix A).

Germination was recorded daily as the time in which the emergent radicle measured at least 2 mm over a period of 30 days (Weiersbye & Witkowski, 2002; Schroder et al., 2013). The number of seeds that germinated per sample was expressed as a percentage of the total number of seeds tested (excluding those seeds that were removed as a result of predation). The germination rate was assessed by determining how many seeds germinated each day over the 30 day observation period. Germination rate was the mean number of days it took for that seed batch to germinate.

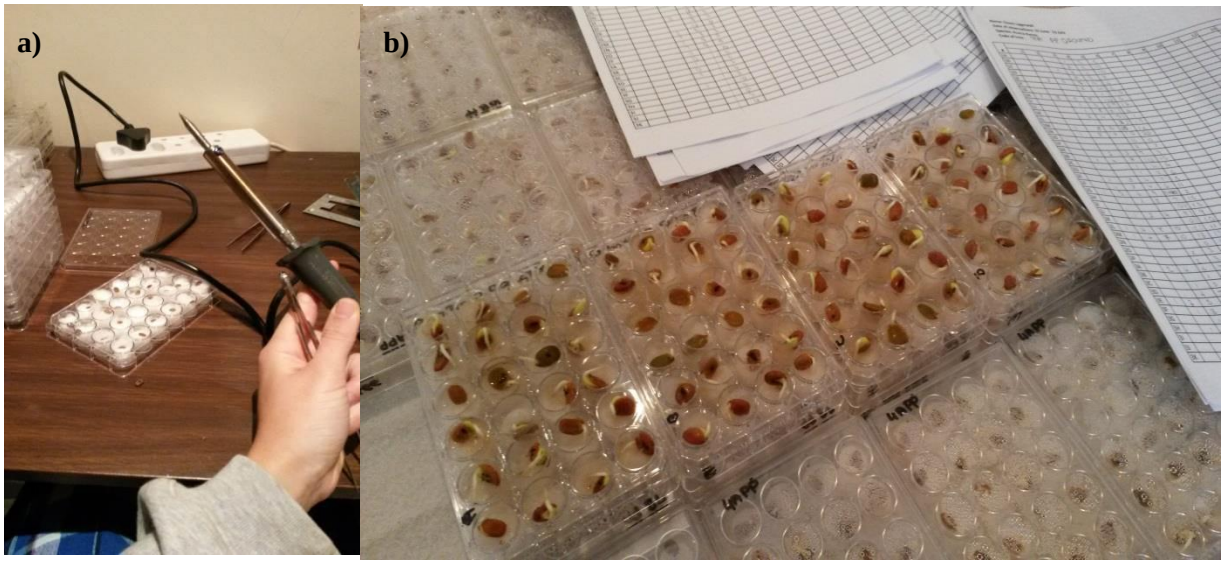

Figure 5 Scarification and germination of *Acacia karroo* seeds: a) Scarification of *A. karroo* seed coats by means of the heat point method using a soldering iron, b) Germination of *A. karroo* seeds in repli-dishes on tap-water moistened cotton wool. Photographer: Dawn Lagerwall

2.2 Data and Statistical analysis

To determine the differences in *A. karroo* seeds from trees growing on the polluted area versus those growing on the non-polluted area in terms of dry seed mass, percentage germination and percentage predation between the sites, a number of comparison tests were undertaken. Where necessary data were transformed, including arcsin transformations for percentage and coefficient of variation (CV) data. The data were tested for normality using a Shapiro Wilk test, with seed mass, percentage predation and germination rates being normally distributed (P= 0.703; 0.373, 0.193 respectively), and hence parametric tests can be used, whereas percentage germination and the CV of seed mass for each tree were not normally distributed (P< 0.0001), requiring nonparametric tests to be used.

The percentage of total number of seeds predated and total number of intact seeds was readjusted based on the findings from the germination experiment where further seeds were discovered to have been predated.

Contingency table $\chi 2$ tests were used to test associations between, 1) actual predation and soil pollution 2), actual predation and level from which the seeds were collected (i.e. canopy or ground), 3) successful emergence of bruchid beetles and non-successful emergence, 4) five seed size categories (see Results) and soil pollution, 5) five seed size categories and level from which the seeds were collected for non-polluted and polluted area, 6) five seed size categories and actual germination, 7) actual germination, polluted versus non-polluted soils, and 8) actual germination and level from which the sample were collected.

Mann Whitney U-tests were used to compare CVs of seed mass and percentage germination between the polluted and non-polluted sites. A Wilcoxon signed test was used to compare seeds collected from the canopy as opposed to the ground (paired or dependent data) between trees growing within the non-polluted area and then between trees growing in the polluted area for: (a) percentage predation, (b) mean seed mass/tree, (c) percentage germination and, (d) germination rate.

Independent t-tests were used to compare between the polluted and non-polluted sites for: (i) percentage predation, (ii) seed mass (iii) the success of emergence of bruchid beetles and, (iv) germination rate.

Chapter 3

3.1 Results

3.1.1 Predation

Percentage predation at the tree level was significantly higher in seeds collected from trees on the non-polluted soils 48% (±14.69) as opposed to the polluted soils 35% (±15.76)(t=2.394, df=26, P=0.0241) (Table 2). This was further confirmed at the seed level when comparing predation of seeds collected from the non-polluted area compared to the polluted area (2x2 contingency table $\chi 2$ with Yates correction) ($\chi 2$=602.47, df=1, P<0.0001) (Table 3).

Table 2 Comparison of total seed germination, mean days to germination, seed predation, and seed mass of seeds collected from *Acacia karroo* growing on non-polluted areas and polluted areas.

Comparison	Non-polluted		Polluted		Statistical analysis
	Mean±SD	CV (%)	Mean±SD	CV (%)	
Percentage germination	92±9.35	10.2	81±20.42	25.2	U=51.5, df=26, P=0.0345
Rate of germination (days)	4.2±0.19	4.6	4.7±0.45	9.4	t= 4.39, df=26, P<0.0001
Percentage predation	48±14.69	30.8	35±15.76	45.4	t=2.394, df=26, P=0.0241
Seed mass (g)	0.051±0.009	17.9	0.046±0.009	20.5	t=1.819607, df=26, P=0.0803

Refer to Appendix A for the full summary table

At the tree level, there was no significant difference in seed predation of seeds collected from the ground compared to seeds collected from the canopy when comparing between 1) non-polluted area canopy and ground (N=7, df=6, P=0.7353), 2) polluted area canopy and ground (N=7, df=6, P=0.3980) and, 3) all trees sampled between the canopy and ground (N=14, df=13, P=0.6377). At the seed level, 2x2 contingency table $\chi 2$ analyses comparing seed predation of ground versus canopy seeds, was significantly different for the polluted area ($\chi 2$=208.78, df=1, P<0.0001), however was not significantly different for the non-polluted area ($\chi 2$=0.19, df=1, P=0.6591) (Table 3).

There was no difference shown for the success of bruchid beetle emergence for seeds collected from non-polluted area compared to those seeds collected on the polluted area (t=0.745, df=26, P=0.4628). At the seed level, a 2x2 contingency table $\chi 2$ analyses comparing emergence of bruchid beetles from seeds collected from non-polluted area compared to polluted area, revealed a significant difference ($\chi 2$=6.15, df=1, P=0.0110) (Table 4).

Table 3 Results of 2x2 contingency table $\chi 2$ with Yates correction for predation compared to non-predated for *Acacia karroo* growing on non-polluted versus polluted soils and on the canopy only and on the ground only between polluted and non-polluted areas.

Area of collection	Predated	Non-predated	$\chi 2$ with Yates correction
Non-polluted	5827	6241	$\chi 2$=602.47, df=1, P<0.0001
Polluted	4691	9378	
NP Canopy	3654	3887	$\chi 2$=0.19, df=1, P=0.6591
NP Ground	2174	2353	
PP Canopy	2799	6730	$\chi 2$=208.78, df=1, P<0.0001
PP Ground	1892	2648	

Table 4 Results of a 2x2 contingency table $\chi 2$ with Yates correction for successful emergence compared to un-successful emergence of bruchid beetles for *Acacia karroo* growing on non-polluted versus polluted soils.

Area of collection	Successful emergence	Un-successful emergence	$\chi 2$ with Yates correction
Non-polluted	5571	161	$\chi 2$=6.15, df=1, P=0.0110
Polluted	4536	94	

3.1.2 Variation in seed dry mass

There is a higher number of seeds in the seed dry mass class between 0.0455 – 0.0904 g for *A. karroo* growing on the non-polluted area and a higher number of seeds on the polluted area within the smaller seed dry mass class, 0.0155 – 0.0454g (Figure 6).

At the tree level, there was no significant difference in dry seed mass of seeds collected from 1) non-polluted area between canopy versus ground (N=7, df=6, P=0.3980), 2) polluted area canopy versus ground (N=7, df=6, P=0.7353) and, 3) when comparing all trees sampled between the canopy versus ground (N=14, df=13 P=0.6377).

At the seed level, a significant difference was shown when doing a 5x2 $\chi 2$ contingency table, comparing seed size class distribution of seed mass for seeds collected on the canopy from the non-polluted and polluted area ($\chi 2$ =114.0, df=4, P<0.0001) (Table 5) and seed size class distribution of seed mass for seeds collected from the ground from the non-polluted and polluted area ($\chi 2$ =34.0, df=4, P<0.0001) (Table 5).

At the tree level, the overall dry seed mass tended to be higher in non-polluted (0.051±0.009g) as opposed to polluted samples (0.046±0.009g), however no significant difference was shown (t=1.819607, df=26, P=0.0803) (Table 2).

At the seed level, when doing a 5x2 $\chi 2$ contingency table, comparing seed size class distribution of seed mass between polluted and non-polluted areas, a significant difference was shown ($\chi 2$=119.5, df=4, P<0.0001) (Table 6).

The coefficient of variation (CV) describes the extent of variability in seed dry mass in relation to the mean of each of the samples (Table 2). At the tree level, the CV for dry seed mass tends to be higher for seeds collected from the polluted area (20.5%) compared to non-polluted area (17.9%), however no significant difference was found (U=56, df=26, P=0.056).

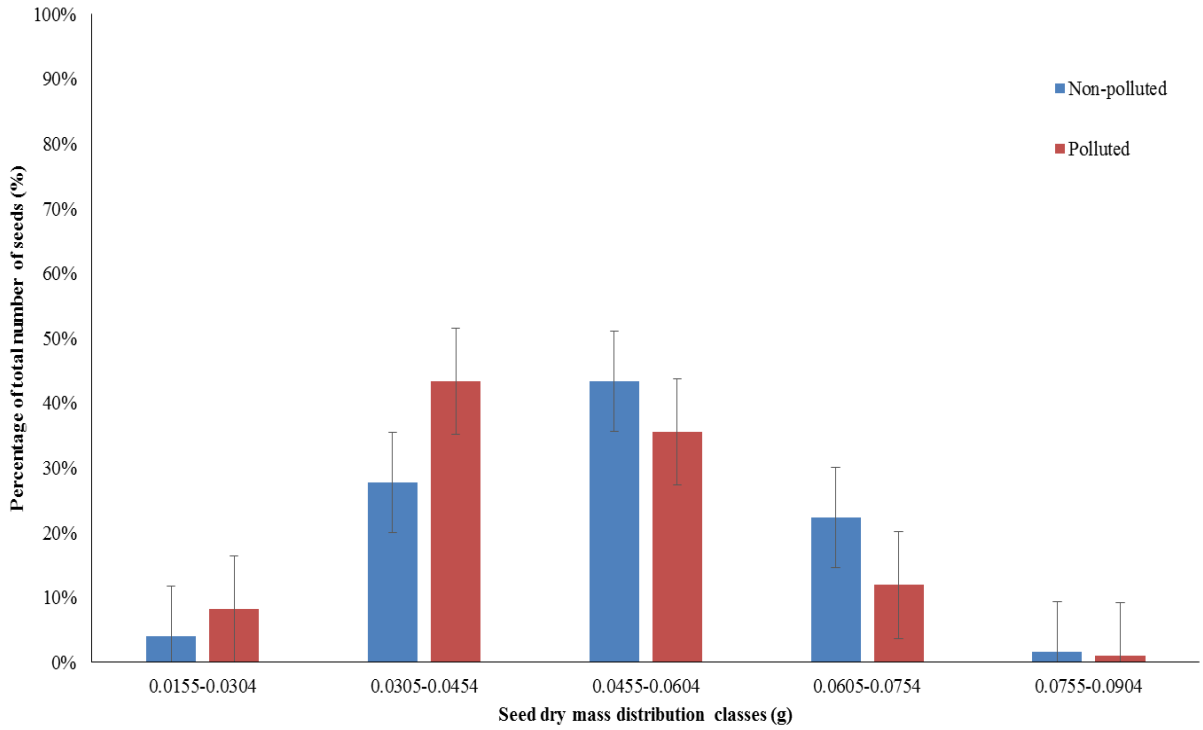

Figure 6 Comparison of the seed dry mass distribution classes (±SE) of *Acacia karroo* seeds between non-polluted versus polluted areas.

Table 5 Results of 5x2 contingency table $\chi 2$ for: 1) canopy, and 2) ground seed mass classes between non-polluted versus polluted areas.

Seed size class	Non-polluted	Polluted	$\chi 2$
1) Canopy			
0.0155 – 0.0304g	9	59	$\chi 2$ =114.0, df=4, P<0.0001
0.0305 – 0.0454g	189	298	
0.0455 – 0.0604g	280	236	
0.0605 – 0.0754g	169	65	
0.075 – 0.0904g	15	7	
2) Ground			
0.0155 – 0.0304g	44	50	$\chi 2$ =34.0, df=4, P<0.0001
0.0305 – 0.0454g	181	280	
0.0455 – 0.0604g	299	238	
0.0605 – 0.0754g	129	94	
0.075 – 0.0904g	7	7	

Table 6 Results of 5x2 contingency table $\chi 2$ squared for seed mass classes between non-polluted and polluted areas.

Seed size class	Non-polluted	Polluted	$\chi 2$
0.0155 – 0.0304g	53	109	$\chi 2$=119.5, df=4, P<0.0001
0.0305 – 0.0454g	370	578	
0.0455 – 0.0604g	579	474	
0.0605 – 0.0754g	298	159	
0.075 – 0.0904g	22	14	

3.1.3 Seed germination

Percentage germination at the tree level was significantly higher in seeds collected from the non-polluted area at an average of 92% (±9.35) compared to the polluted area with an average of 81% (±20.42) (U=51.5, df=26, P=0.0345) (Table 2). This was further confirmed at the seed level when comparing percentage germination of seeds collected from the non-polluted area compared to the polluted area (2x2 contingency table $\chi 2$ with Yates correction) ($\chi 2$=218.52, df=1, P<0.0001) (Table 7).

At the tree level, there was no significant difference shown in percentage germination of seeds collected from 1) non-polluted area canopy and ground (N=7, df=6, P=0.1762), as well as, 2) polluted area canopy and ground (N=7, df=6, P=0.0629). However a significant difference was found when comparing all of the trees sampled between the canopy and ground (N=14, df=13 P=0.0219), with percentage germination being higher for the seeds collected from the canopy (Figure 7).

At the seed level, a 2x2 contingency table $\chi 2$ analyses comparing seed germination of ground versus canopy seeds, were significantly different for both polluted and non-polluted areas ($\chi 2$=6.22, df=1, P<0.0126; $\chi 2$=64.38, df=1, P<0.0001; respectively) (Table 7).

Table 7 Germination success of *Acacia karroo* on (1) non-polluted versus polluted soils and (2) on tree versus ground. Statistics using 2x2 contingency table $\chi 2$ with Yates correction.

	Germinated	Non-germinated	Chi-squared with Yates correction
Non-polluted	1217	14	$\chi 2$=218.52, df=1, P<0.0001
Polluted	1079	255	
NP Canopy	623	39	$\chi 2$=6.22, df=1, P<0.0126
NP Ground	594	65	
PP Canopy	596	69	$\chi 2$=64.38, df=1, P<0.0001
PP Ground	483	186	

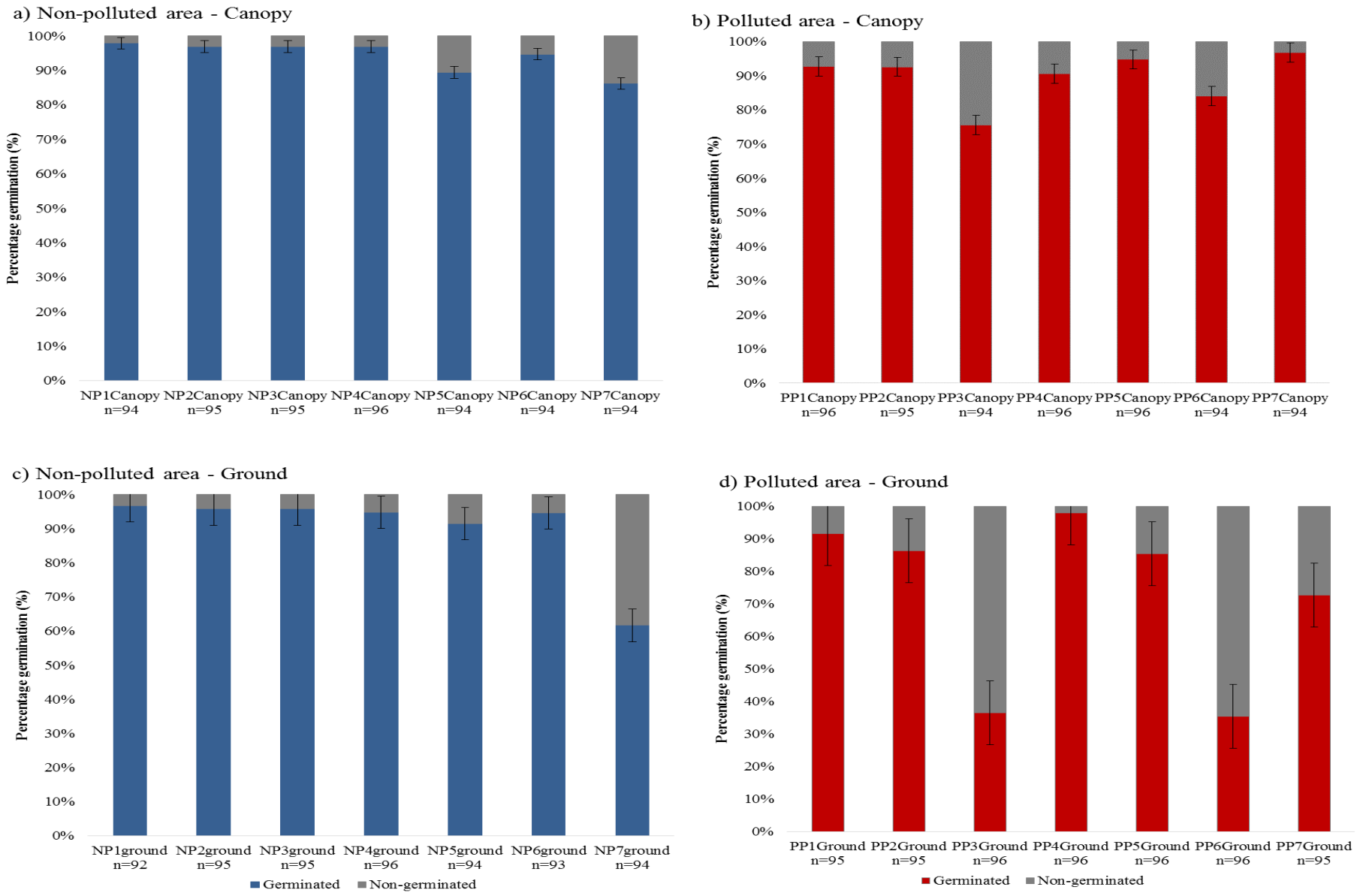

Figure 7 Comparison of total percentage germination (±SE) of *Acacia karroo* for seeds collected from: a) non-polluted area from the canopy, b) polluted area from the canopy, d) non-polluted area from the ground and, d) polluted area from the ground.

A positive linear relationship was shown between dry seed mass and percentage germination for both the polluted and non-polluted areas (P<0.05) (Figure 8).

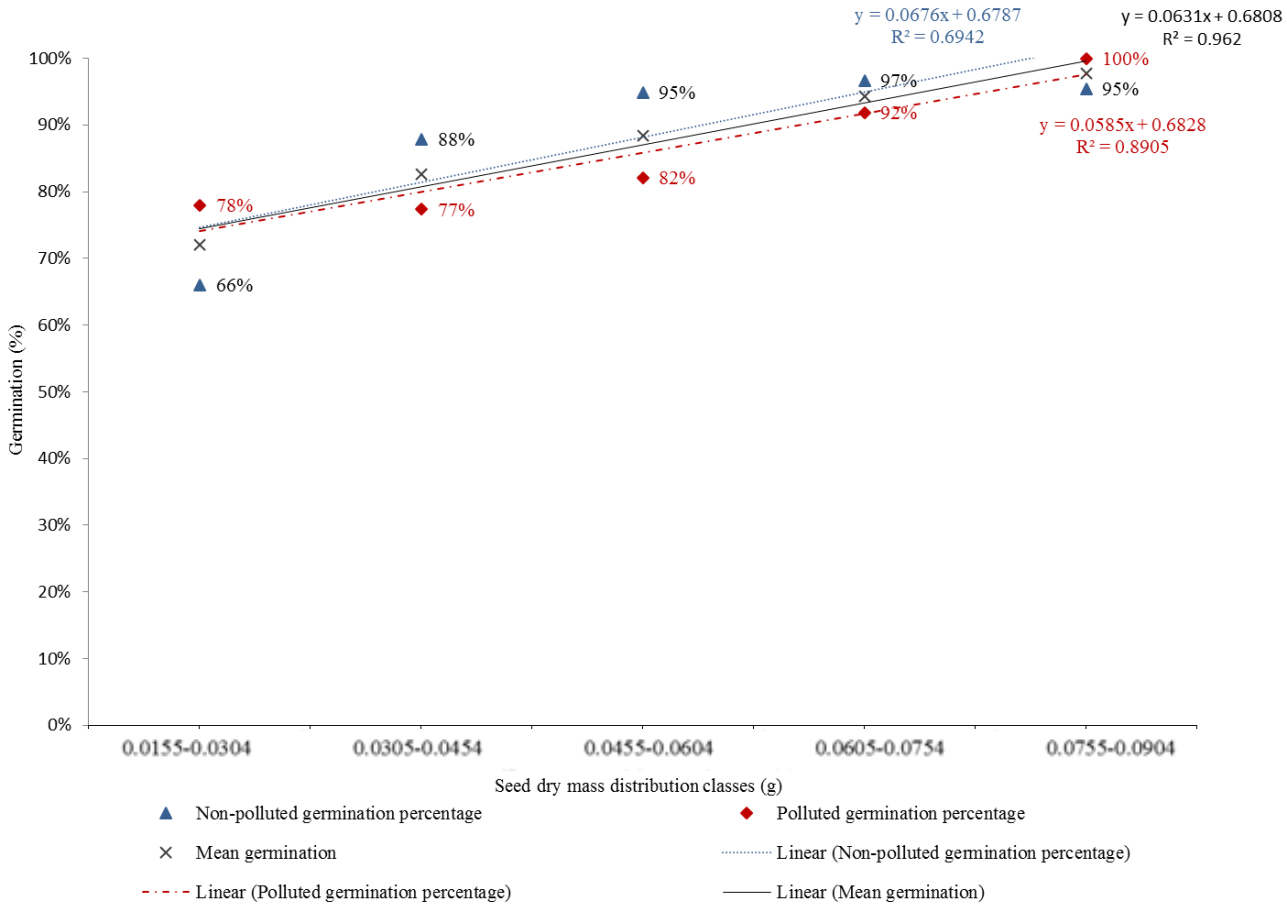

Figure 8 Linear relationships of percentage germination as a function of the mid-point of the five seed dry mass classes for *Acacia karroo* seeds collected from trees on (i) non-polluted compared (P<0.05) with (ii) polluted areas (P<0.05).

At the seed level, comparing germination between the seed size distribution classes, significant differences were shown for both the non-polluted and polluted areas (5x2 contingency table $\chi 2$) ($\chi 2$=73.68; df=4; P<0.0001; $\chi 2$=21.46; df=4; P<0.0001; respectively) (Table 8 and Table 9).

Table 8 Results of 5x2 contingency table $\chi 2$ squared for germinated and non-germinated seeds per seed size classes for the non-polluted area.

Seed size class	Germinated	Non-germinated	$\chi 2$
0.0155 – 0.0304g	35	18	$\chi 2$=73.68; df=4; P<0.0001
0.0305 – 0.0454g	325	45	
0.0455 – 0.0604g	549	30	
0.0605 – 0.0754g	288	10	
0.075 – 0.0904g	21	1	

Table 9 Results of 5x2 contingency table $\chi 2$ squared for germinated and non-germinated seeds per seed size classes for the polluted area.

Seed size class	Germinated	Non-germinated	$\chi 2$
0.0155 – 0.0304g	85	24	$\chi 2$=21.46; df=4; P<0.0001
0.0305 – 0.0454g	447	131	
0.0455 – 0.0604g	389	85	
0.0605 – 0.0754g	146	13	
0.075 – 0.0904g	14	0	

There was no significant difference in germination rate of seeds collected from 1) non-polluted area canopy versus ground (N=7, df=6, P=0.6456) and, 2) polluted area canopy and ground (N=7, df=6, P=0.7452), and 3) when comparing between the canopy and the ground of all trees sampled (polluted and non-polluted) (N=14, df=13 p=0.9894).

However, the germination rate was significantly faster for seeds collected from non-polluted area at 4.2±0.19 days compared to polluted area of 4.7±0.45 days (t= 4.39, df=26, P<0.0001) (Table 2) (Figure 9).

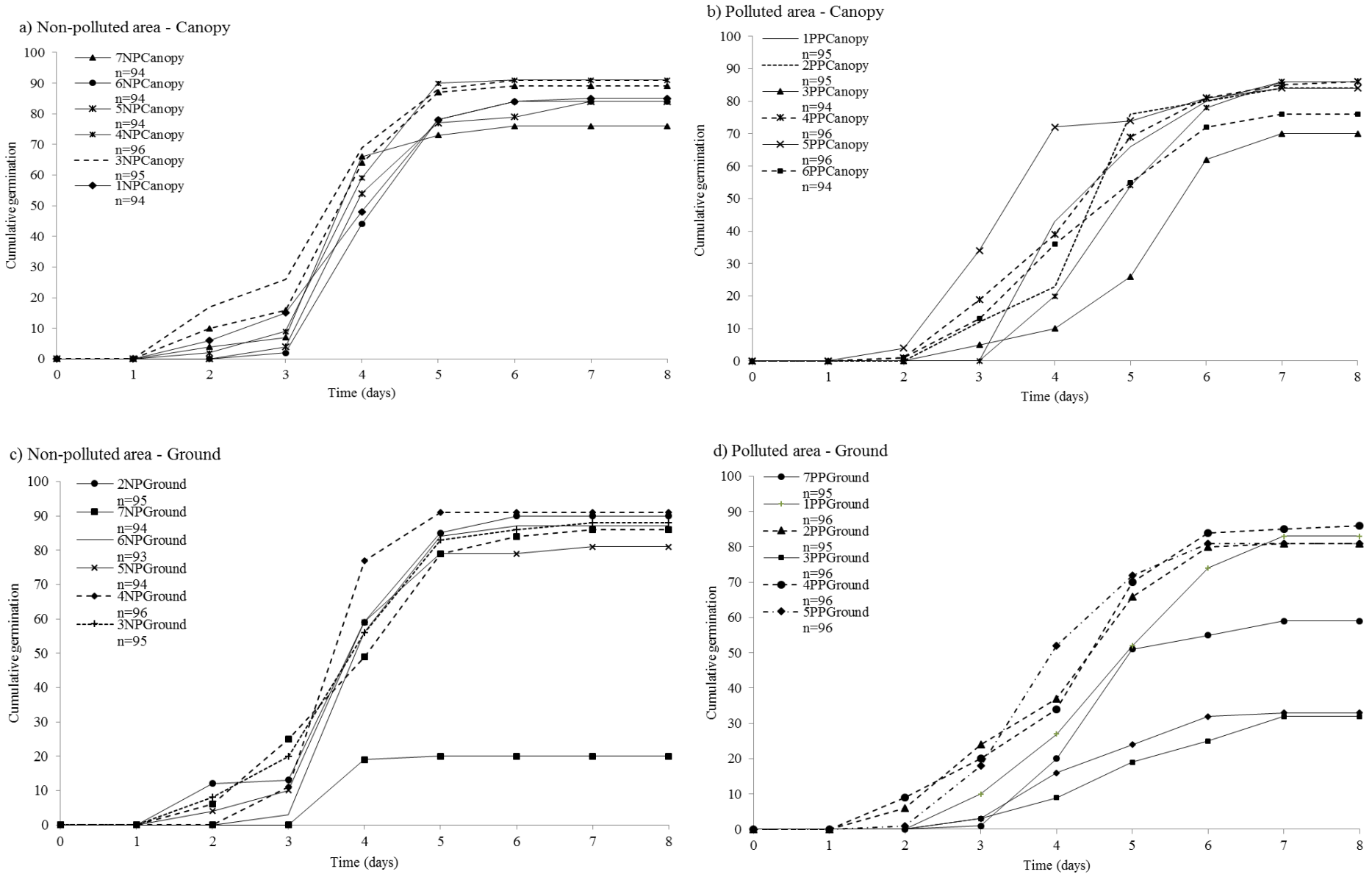

Figure 9 Cumulative germination count of *Acacia karroo* seeds per day collected from 7 trees off non-polluted soils compared with 7 trees on polluted soils for seeds collected off the tree and those that were shed onto the ground.

A weak positive linear relationship was shown between dry seed mass and rate of germination for both the polluted (P<0.05) and non-polluted areas (P<0.05) (Figure 10).

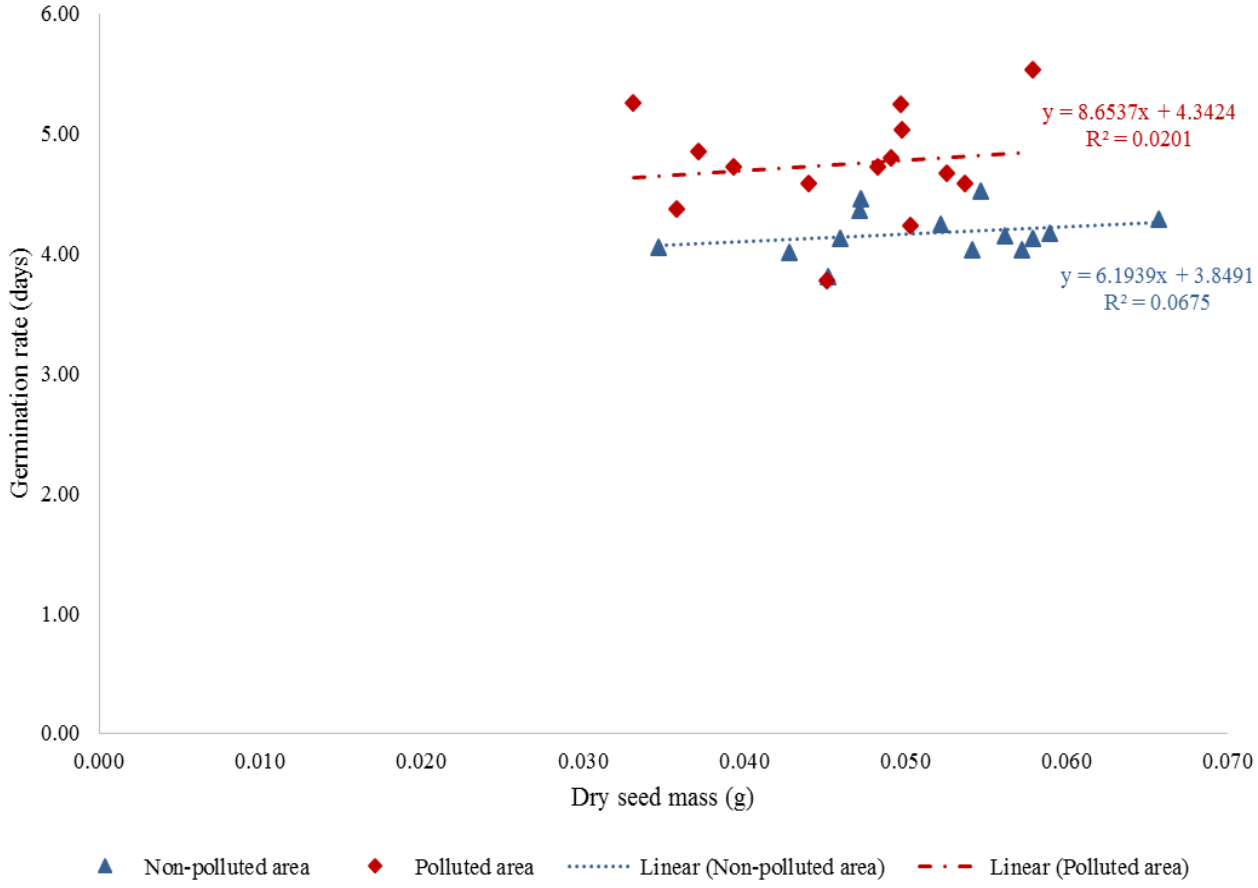

Figure 10 Very weak relationships between seed mass and germination rate of *Acacia karroo* seeds collected from non-polluted (P<0.05) and polluted areas (P<0.05).

The Vaal River bisects the non-polluted area from the polluted area, providing a buffer between the two areas. Table 10 provides the distance of the main and minor pollution source as illustrated in Figure 1 from *A. karroo* growing in the polluted area as well as the percentage germination for each of these trees. Those trees marked with an (*) were thought to be affected by the minor pollution source due to the drainage of the site being in a southerly direction and proximity of these trees to the minor pollution source.

Table 10 Distance of *Acacia karroo* trees growing in the polluted area from main and minor pollution sources and their percentage seed germination.

***A. karroo* growing in polluted soils**	**Distance from main pollution source where there is historical spillage (m)**	**Distance from minor pollution source (m)**	**Percentage germination**
$PP1^{Ground}$	3291	1710	92%
$PP1^{Canopy}$	3291	1710	93%
$PP2^{Ground}$	3279	1639	86%
$PP2^{Canopy}$	3279	1639	93%
$PP3^{Ground}$	589	1844	36%
$PP3^{Canopy}$	589	1844	76%
$PP4^{Ground}$	160	2007	98%
$PP4^{Canopy}$	160	2007	91%
$PP5^{Ground}$	3141	1505	85%
$PP5^{Canopy}$	3141	1505	95%
$*PP6^{Ground}$	3725	1460	35%
$*PP6^{Canopy}$	3725	1460	84%
$*PP7^{Ground}$	3458	958	73%
$*PP7^{Canopy}$	3458	958	97%

Chapter 4

4.1 Discussion

The main points from this study are the following. Predation of *A. karroo* was shown to be consistently higher for seeds collected on the non-polluted area (48±14.69) compared to those collected from the polluted area (35±15.76). Secondly, percentage germination was also found to be higher for seeds collected from the non-polluted area (92±9.35), compared to the polluted area (81±20.42). In addition, the rate of germination was faster for seeds collected from the non-polluted (4.2±0.19 days) compared to the polluted area (4.7±0.14 days).

The seed size distributions, as shown by seed dry mass, were significantly different between the polluted and non-polluted areas. Seeds collected from the polluted areas showed a higher number of seeds within the smaller dry seed mass class ranging between 0.0155 – 0.0454g, whereas the seeds collected form the non-polluted area had a higher number of seeds within the larger dry seed mass class 0.0455 – 0.0904 g.

Seed predation

The higher percentage predation of seeds collected from non-polluted compared to polluted areas was consistent with Witkowski & Weiersbye's (2003) findings, where percentage predation in *A. karroo* growing on non-polluted soils was higher (12.8±1.7) than for those trees growing on polluted soils (6.8±1.5), although their percentages were much lower than in this study. A consistent difference in levels of predation between the non-polluted areas and polluted areas was found by Witkowski & Weiersbye (2007) between 1998 and 2005 on *Acacia* seeds of several species, including *A. karroo*. This suggests that AMD pollution limits predation on *A. karroo* and that there is a there is a preference by bruchid beetles for seeds that are developed in non-hostile environments (Weiersbye & Witkowski, 2007).

This may be due to factors such as higher dust loads, reduced palatability and likely seed nutrient content as well as high level of heavy metals (Witkowski & Weiersbye, 2003) deterring predation. Witkowski & Weiersbye (2007) suggest that this could also be a result of other plant population factors such as varying spatial distributions, tree densities, phenology, pollination or fruiting between polluted and non-polluted areas. Further, possible alternative explanations include AMD-induced chemical or physical changes in foliage as well as toxicity to developing larvae of bruchid beetles which in turn limits the number of predators.

Percentage seed predation is ultimately determined by the interaction between seed predator and the environment in which the host plant has developed (van Klinken & White, 2014). The lower levels of predation at the polluted sites could be advantageous as the seed abundance is not reduced as much as for those trees growing on the non-polluted area, if one assumes that seed production does not differ between the two. The larger seed abundance allows for a higher probability of seeds to germinate from the polluted trees (van Klinken & White, 2014). Seed predation could also be related to seed size (Miller, 1996). This was found in leguminous species by Szentesi & Jermy (1995), where the larger the seed volumes, the higher the probability of bruchid infestations. Seed dry mass was shown to be lower in seeds collected from the polluted area as opposed to the non-polluted area. According to Miller (1996), it may not be economically profitable for bruchid beetles to compete for smaller seeds owing to the limited space and food resource.

There was no significant difference in seed predation of seeds collected from the ground opposed to those seeds collected from the canopy. Miller (1996), however, found that predation was much higher for seeds collected from the ground opposed to the canopy. This was thought to be due to seeds on the ground being older and, therefore had a longer time for larval development and re-infestation once emerged (Miller, 1996). Further studies comparing *A. karroo* seed predation on the ground compared to seeds collected from the canopy should be undertaken using a larger data set to determine whether there is a significant difference.

Due to heavy metals being absorbed by trees growing in the polluted area, the seed testa permeability could possibly be reduced (Weiersbye & Witkowski, 2007). Although no literature could be sourced, it is postulated that this could possibly affect oviposition by female bruchid beetles within the seed as well as the success of bruchid emergence. Due to the absorption of metals, the seed integuments become hard and hence, only allow some beetles, generally the stronger ones, to eat through the seed coat. The success of bruchid emergence could also, however, be attributed to the heavy metals affecting the growth of the bruchid beetles, by slowing down their performance and likely success of emergence from the seed. No evidence could be found to support this notion. It is suggested that further studies be undertaken to analyse the chemical composition of the seed testa collected from the non-polluted area and polluted area to determine the likely impact on bruchid beetles. Due to the extent to which bruchid beetles can destroy *Acacia* seeds, it is necessary to adopt effective seed collection and storage practices in order to use the seeds effectively for rehabilitation purposes (Miller, 1996).

Seed size and germination

The higher percentage germination on non-polluted area compared to the polluted area was again consistent with Witkowski & Weiersbye (2003) findings where percentage germination for *A. karroo* seeds collected from non-polluted soils was 100% compared to 94.4% (±5.6) for seeds collected from polluted soils. Witkowski & Weiersbye (2003) explain that this may be due to the seed coat acting as a buffer between the embryo and the saline conditions due to higher tissue content of calcium (Ca) and potassium (K). *Acacia* species have been shown to allocate nutrients differently, especially Ca and K, between the embryo and the testa when established under varying salt conditions (Rehman et al., 2000).

Although a significant difference was shown for percentage germination between the polluted and non-polluted areas, the percentage of germination was still shown to be greater than 80% for both areas in this study as well as by Witkowski & Weiersbye (2003). Seed size and number are determined by a wide range of biotic and abiotic selective forces (Esler et al., 1989). Germination is generally closely related to seed size, with the larger seeds having a higher percentage germination compared with smaller seeds (Souza & Fagundes, 2014). This is due to the larger seeds providing seedlings with a higher store of resources (Venable & Brown, 1988).

Although no significant difference was found between seed size and pollution at the tree level in my study, a significant difference was shown between the various seed size classes and pollution at the seed level. Based on studies undertaken by Weiersbye & Witkowski (2007), it was found that *A. karroo* growing on AMD polluted soils had a lower mean dry seed mass compared with non-polluted soils. This suggests that in stressful environments, a possible trade-off exists between the number of seeds produced and seed size - a larger number of smaller seeds is selected for, rather than fewer bigger seeds (Souza & Fagundes, 2014). A larger production of smaller seeds produced equals more opportunity for germination. However, smaller seeds generally have lower potential for germination than larger seeds due to limited energy reserves stored within the seed.

Conversely Esler et al. (1989) suggested that those plants growing in disturbed environments generally have larger seeds with higher N and P in order to establish in infertile soils such as found in the fynbos biome. Souza & Fagundes (2014), however, explain that dry seed mass is directly related to the amount of resource reserves that will be allocated to initial seedling growth. Plants respond to their environment in such a way as to optimize their resource use (Witkowski & Lamont, 1996). Souza & Fagundes (2014) explain that according to the resource optimization hypothesis, plants allocate relatively less resource to their root system when nutrient availability increases.

The percentage of germination when compared to seed size class may be a useful indicator of *A. karroo* preferentially allocating limited resources to other plant organs such as its roots to obtain nutrients at depth when growing under stressful conditions (Souza & Fagundes, 2014). In stressful environments,

the essence of resource allocation in a plant is limited so that these will be divided among the plant parts in such a way as to maximise fitness (Witkowski & Lamont, 1996). This is seen as an adaptive mechanism in harsh environments (Eriksson, 1999).

It was shown that seeds collected from the polluted soils were slower to germinate than seeds from non-polluted soils. This was also shown by Weiersbye & Witkowski (2007). The rate of germination, as for percentage germination, may be attributed to seed size (Venable & Brown, 1988).

Souza & Fagundes (2014) found during an experiment with Fabaceae species, that small seeds had a higher germination percentage and germinated faster when compared to larger seeds. Conversely, El-ahmir et al. (2015) explains that larger seeds generally gain a competitive advantage over the smaller seeds due to their earlier germination. This is consistent with this study where the non-polluted seeds were generally larger and had a faster rate of germination than seeds collected from the polluted area. Weiersbye & Witkowski (2002), showed that seeds collected from *A. karroo* with a high seed dry mass were generally more viable and were quicker to germinate. This may be due to the larger seeds having greater energy reserves, whereas the smaller seeds have less energy reserves that are sparingly used over a longer period of time to ensure survival (Venable & Brown, 1988).

Faster germination observed by Rehman et al. (2000) in salt-tolerant species as a mechanism to allow the seedling to escape salt injury. Rehman et al. (2000) showed that *Acacia* species established in soils of high salt levels, tended to have slower germinating seeds compared to those species growing in soils of low salt levels. Studies undertaken by Witkowski & Weiersbye (2003) on elemental composition of seeds found that the testa of viable legume seeds from AMD polluted soils contained very high concentrations of Fe and Ca, which may influence the ion balance within the plants, water uptake as well as radicle emergence.

Trees located in close proximity to the major and minor pollution source (Table 10) generally showed a low percentage germination. This was also shown by Weiersbye & Witkowski (2007), where it was found that the origin of seeds had a marked effect on viability and germination. This may be due to contaminants being more concentrated around the pollution sources, which then declines with distance due to the attenuation by soils and organics (Weiersbye & Witkowski, 2007).

Further studies should aim to determine seedling performance from those seeds collected from the polluted area in terms of seedling establishment, rates of growth and survival over time.

4.2 Conclusion

Percentage seed predation was significantly higher in non-polluted (48±14.69) compared with the polluted areas (35±15.76). This results in a higher seed abundance for dispersal and germination within the disturbed area.

Percentage germination was higher for seeds collected from the non-polluted area (92±9.35) compared to the polluted area (81±20.42). This may be a result of the non-polluted trees producing larger seeds with higher levels of N and P for growth. This would explain the higher rate of germination in the non-polluted seeds (4.2±0.19 days), compared with the polluted seeds (4.7±0.45 days).

Despite the lower seed mass, the polluted samples exhibited many advantageous characteristics which would aid survival and regeneration of *A. karroo* on AMD polluted soils due to their higher percentage germination as well as lower vulnerability to predation.

A. karroo trees have several traits that make them ideal for rehabilitation, including long life spans, a tolerance of poor soil conditions and high regeneration capacities.

Seed germination and seedling establishment are critical stages of the plant life cycle, influencing the distribution and number of plants within an area. By understanding the germination and predation of *A. karroo* seeds growing on polluted soils compared with non-polluted soils, appropriate species for rehabilitation can be planted that are likely to regenerate, contributing towards achieving a self-sustaining vegetation cover over these disturbed areas.

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Appendix A – Summary of result

Table 11 Summary table of seeds sampled from *Acacia karroo* growing on non-polluted and polluted areas and assessed in terms of (a) percentage of germination, mean days to germination and peak day of germination, (b) percentage of predation, mean predation and coefficient of variation (CV), (c) mean seed dry mass and CV

Tree	Seed sample size (n)	a) Germination (%)	Adjusted germination percentage after removal of predated seeds (%)	Mean days to germination	Standard deviation	b) Initial predation percentage (%)	Adjusted predation percentage after germination (%)	c) Mean seed dry mass (g)	Standard deviation	CV (%)
NP1^{ground}	92	93	97	4.174	1.129	41	43	0.059	0.010	16.4
NP1^{Canopy}	94	96	98	4.282	1.031	69	70	0.066	0.009	13.9
NP2^{ground}	95	95	96	4.122	1.026	35	35	0.058	0.008	13.3
NP2^{Canopy}	95	96	97	4.011	0.935	42	42	0.043	0.008	17.9
NP3^{ground}	95	95	96	4.125	1.070	59	59	0.046	0.011	23.1
NP3^{Canopy}	95	96	97	3.802	1.077	29	30	0.045	0.007	15.3
NP4^{ground}	96	95	95	4.033	0.526	41	41	0.057	0.009	15.1
NP4^{Canopy}	96	97	97	4.242	0.705	30	30	0.052	0.009	17.1
NP5^{ground}	94	90	91	4.452	0.870	51	52	0.047	0.009	19.7
NP5^{Canopy}	94	88	89	4.148	0.853	61	62	0.056	0.009	16.1
NP6^{ground}	93	92	95	4.356	1.044	36	38	0.047	0.010	20.2
NP6^{Canopy}	94	93	95	4.524	0.667	67	67	0.055	0.010	17.6
NP7^{ground}	94	60	62	4.050	0.224	66	66	0.035	0.012	35.5
NP7^{Canopy}	94	84	86	4.026	0.711	30	31	0.054	0.010	18.7
	Mean (total) germination for non-polluted areas			**±SD**	**CV (%)**	**Mean (total) predation on non-polluted areas**			**±SD**	**CV (%)**
	92			**9.353**	**10.2**	**48**			**14.693**	**30.8**
	Mean days to germination			**±SD**	**CV (%)**	**Mean seed mass**			**±SD**	**CV (%)**
	4.2			0.191	**4.6**	**0.051**			**0.009**	**17.9**

Tree	Seed sample size (n)	a) Germination (%)	Adjusted germination percentage after removal of predated seeds (%)	Mean days to germination	Standard deviation	b) Initial predation percentage (%)	Adjusted predation percentage after germination (%)	c) Mean seed dry mass (g)	Standard deviation	CV (%)
PP1^{Ground}	95	91	92	5.036	1.184	34	35	0.050	0.011	21.6
PP1^{Canopy}	96	93	93	4.798	0.956	18	18	0.049	0.010	20.8
PP2^{Ground}	95	85	86	4.370	1.249	40	41	0.036	0.007	20.6
PP2^{Canopy}	95	92	93	4.726	0.936	26	26	0.039	0.009	23.5
PP3^{Ground}	96	36	36	5.250	1.270	47	47	0.050	0.012	24.9
PP3^{Canopy}	94	74	76	5.529	1.032	58	59	0.058	0.010	17.7
PP4^{Ground}	96	98	98	4.581	1.203	34	34	0.054	0.010	18.8
PP4^{Canopy}	96	91	91	4.581	1.203	44	44	0.044	0.008	18.5
PP5^{Ground}	96	85	85	4.235	0.952	69	69	0.050	0.011	22.7
PP5^{Canopy}	96	95	95	3.778	1.149	15	15	0.045	0.006	14.3
PP6^{Ground}	96	35	35	4.727	1.039	26	26	0.048	0.011	22.4
PP6^{Canopy}	94	82	84	4.671	1.182	21	23	0.053	0.009	16.9
PP7^{Ground}	95	72	73	4.847	0.847	21	22	0.037	0.010	27.1
PP7^{Canopy}	94	95	97	5.256	0.919	25	26	0.033	0.006	16.7
	Mean (total) germination for polluted areas			**±SD**	**CV (%)**	**Mean (total) predation on polluted areas**			**±SD**	**CV (%)**
	81			20.422	25.238	35			15.765	45.405
	Mean days to germination			**±SD**	**CV (%)**	**Mean seed mass**			**±SD**	**CV (%)**
	4.742			0.449	9.47407	0.046			0.009	20.5