Investigation on selected biotic and abiotic factors in the maintenance of the "fairy circles" (barren patches) of southern Africa

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ABSTRACT

The fairy circles are an intriguing and unexplained feature of the pro-Namib in Namibia and northwestern parts of South Africa. The presence of hundreds of almost circular patches where no plants grow were first mentioned in scientific literature in 1971 and since then scientists have tried to find an explanation for the origin of these circles. Although there are many hypotheses regarding the origin of these circles not one of these can explain the existence of these circles satisfactory. In this study several aspects of the fairy circles were investigated to improve the characterization of the phenomenon.

Total element analysis of the soil from the different microhabitats (inside the circle, on the edge of the circle and between the circles, referred to hereafter as the matrix) and at different depths in these microhabitats were performed by inductively coupled plasma mass spectrometry (ICP-MS) and analyzed by principal component analysis (PCA). No patterns emerged regarding the concentration of the elements in the respective microhabitats. The occurrence of vesicular arbuscular mycorrhizae (VAM) in the roots of plants collected from the different microhabitats was also investigated. It was shown that VAM occurred in most of the roots of plants collected in the matrix and on the edge but no VAM were found in plants collected inside the circles. The succulent plant *Euphorbia damarana* has also been implicated in the origin of the circles. The presence of germination inhibiting compounds in this species was investigated. No such compounds were found. The extracts of it inhibited the growth of radicles of lettuce seeds at a concentration of 25 mg/ml.

Bio-assays were also performed on soil collected in the different microhabitats using a dominant grass of the area, *Stipagrostis uniplumis,* as bioindicator. The fresh an dry shoot mass of plants harvested from soil collected on the edge and in the matrix grew much better than the shoots grown in soil collected from the inside of the circles ($p = 0.0007$). The dry shoot mass showed the same trend as the fresh shoot mass. The fresh root mass showed a marked increase in the roots collected from soil on the edge of circles when compared to plants grown in soil collected from the inside and the matrix ($p = 0.013$). There was a significant difference in the length of shoots measured in plants grown in the soils collected from the different microhabitats with the shoots measured from plants grown in the soil collected from the edge showing stimulation in growth when compared to the plants grown in soil from the matrix and inside the circles ($p = 0.00004$). The difference in shoot length between grasses grown in soil collected from the edge and the matrix was also significant ($p = 0.00004$) with the edge samples showing a stimulation in growth.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

The round, concave patches devoid of any vegetation (Figure 1.1), which occur mainly in the western part of Namibia, are a fascinating and inexplicable phenomenon and are generally known as fairy circles. At present no irrefutable scientific evidence can explain the origin of the fairly circles.

Figure 1.1. Fairy circles in the Marienfluss (photo by N van Rooyen).

Tinley (1971) made the earliest scientific reference to the possible formation of these rings, suggesting that they are the remains of fossil termitaria from wetter times. Since then research on various aspects concerning these rings was done sporadically.

Theron (1979) suggested that an allelopathic substance released from *Euphorbia damarana* Leach upon the death of the plants while Moll (1994) reasoned that termites was the cause of the circles. According to Becker & Getzin (2000), the Himba people from Kaokoland in Namibia, believe that the fairy circles were made by their god and have been there ever since. This is but one of many beliefs existing today, regarding the origin of these mysterious rings. Explanations range from impact points of meteorites, rolling spots for zebras, possible locations of Ovahimba huts, unknown flying objects (UFOs) (Albrecht *et al.*, 2001). Radioactivity has also been implicated as the cause of these barren patches (Karaerua, 2000), while Danin & Orshan (1995) suggested that the formation of the circles was due to a specific type of vegetative multiplication. This vegetation multiplication occurs when the older central parts of original clumps of grass dies, younger clumps on its periphery then makes up circles of plants detached from each other. These circles can then be up to 15 m in diameter.

Seely & Hamilton (2003) contented that the regular spacing suggests a competitive interaction of some settling organism, whether termite or microorganism and suggested that fairy circles have a biological rather than a physical origin. They did not however propose a specific origin for the circles.

1.2 Distribution and characteristics

Fairy circles mainly occur in the pro-Namib zone, which is the transition zone between the Namib Desert and the great escarpment in Namibia on the west coast of southern Africa (Becker & Getzin, 2000). These circles are also found in the Richtersveld and Bushmanland regions of the Northern Cape Province of South Africa. Moll (1994) generally described the distribution of these rings as stretching in a broken belt extending from southern Angola in the north, through Namibia to just south of the Orange River in South Africa (Figure 1.2). The circles are located mostly between the 50 and 100 mm isohyet with some between the 100 and 150 mm isohyet with altitudes ranging from 500 to 1 000 m (Van Rooyen *et al.*, 2004). Rainfall in northwest Namibia regions occurs mainly from mid-summer to autumn (Eicker *et al.*, 1982) while in the south (Richtersveld) winter rain occurs. In northwest Namibia (Kaokoland) the majority of the fairy circles are located in the Hartmann's Valley, the Marienfluss and the Giribes Plains which are found between 60 and 120 km inland from the coast (Becker & Getzin, 2000; Van Wyk & Smith, 2001; Van Rooyen *et al.* 2004).

In general, fairy circles decrease in size from north to south throughout their entire distribution range (Van Rooyen *et al.*, 2004). In the Richtersveld in South Africa the circles have a mean diameter of 2 m and the circles on the farm Wolwedans (Keerweder), which forms part of the Namibrand Nature reserve (number 8 on Figure 1.2), has a mean diameter of 6 m (Albrecht *et al.*, 2001). In the Hartmann's Valley the circles are on average the largest with a mean diameter of 10 m (Van Rooyen *et al.*, 2004). The density of the fairy circles range from 11 circles per hectare in Marienfluss (Theron, 1979) to 36 to 47 per hectare in the Giribes Plains (Moll, 1994).

The vegetation in between the barren patches (henceforth called the matrix) depends on the location of the sites and is generally scant, except during the rainy season in good rainfall years. In the Giribes Plains, Hartmann's Valley and Marienfluss the vegetation is dominated by *Stipagrostis* Nees species. The matrix vegetation consists mainly of *Stipagrostis uniplumis* (Licht.) De Winter, while either *S. giessii* Kers. or *S. hochstetteriana* (Beck ex Hack.) De Winter, both perennial species inhabits the edges of the circles.

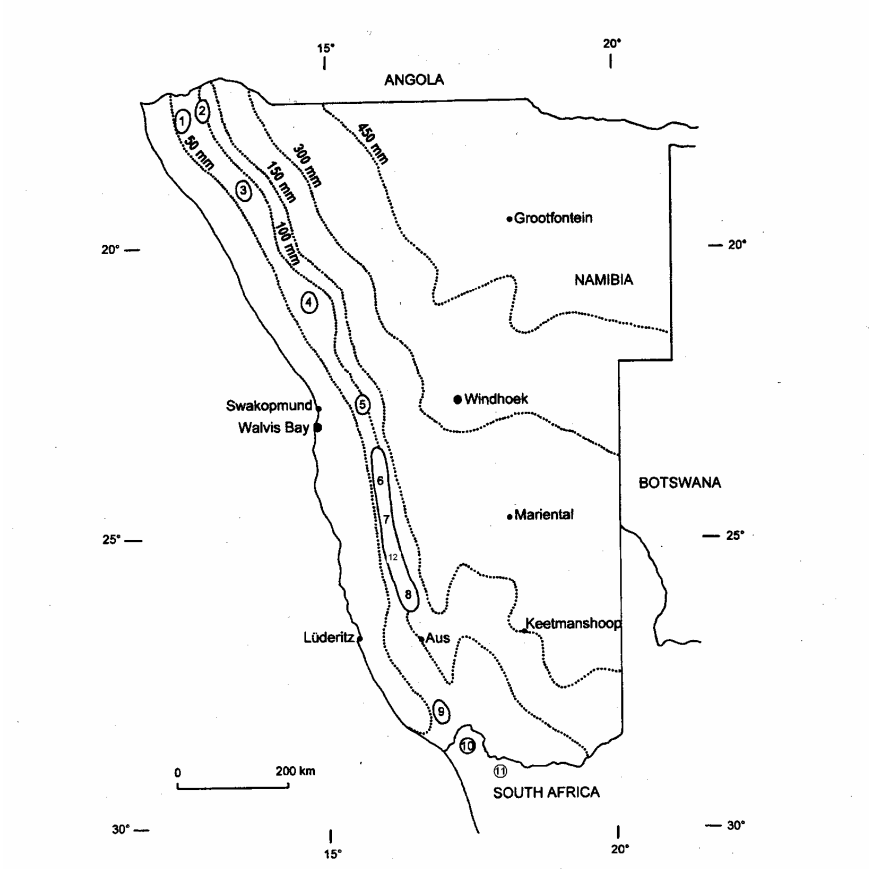

Figure 1.2. Map of where the fairy circles occur in Namibia and South Africa. (1) Hartmann's Valley, (2) Marienfluss, (3) Giribes plain, (4) Brandberg, (5) Khan, (6) Escourt Experimental Farm, (7) Sesriem, (8) Namib Guest Farm, (9) Rooiduin, (10) Kubus, (11) Amam in Bushmanland, (12) Keerweder. Rainfall gradients are indicated by ....lines (adapted from Van Rooyen *et al.*, 2004).

Becker & Jürgens (2000) classified the Kaokoveld broadly as *Mopane* Savanna and then into four major vegetation units. Generally the western part of Kaokoveld receives less rain than the eastern part and the species richness thus decreases. The western parts are thus regularly dominated by ephemeral grasslands. They characterise the Marienfluss Valley and the plains of the pro-Namib to the west (Giribes Plains and Hartmann's Valley) *Colophospermum mopane* (J. Kirk ex Benth) J. Kirk ex J. Léonard Savanna. In contrast the regions such as the Marienfluss, dominated by deep sandy deposits, (where the fairy circles occur) have *S. uniplumis* as the characteristic species (Becker & Jürgens, 2000).

South of the Kuiseb River *S. uniplumis* is replaced by *S. ciliata* (Desf.) De Winter on the edges and *S. obtusa* (Delile) Nees in the matrix. In some cases *S. obtusa* (Delile) Nees forms both the edge and the matrix vegetation (Van Rooyen *et al.*, 2004). A distinction is made between the grasses in the matrix and on the edge (Figure 1.3), not only because they are sometimes different species, but because the grasses growing on the edge are usually lusher than those in the matrix.

In Bushmanland some of the barren patches can be found in Bushmanland Sandy Grasslands, (Mucina *et al.*, 2005) with *S. obtusa* and *S. brevifolia* (Nees) De Winter as the dominant species. In other places in Bushmanland they can be found in sourgras, *Schmidtia kalihariensis* Stent vegetation (personal observation).

The geology of the Bushmanland Sandy Grasslands is classified as mostly Quaternary sediments (sand, calcrete) with some contribution of the pre-Pleistocene Kalahari Group sediments in the east. Typically the surface is covered by red sands about 300 mm deep, forming dunes in places (Mucina *et al.*, 2005). In the Richtersveld and far southern part of Namibia, fairy circles also occur in succulent shrub land with almost no grass (Van Rooyen *et al.*, 2004).

Figure 1.3. The well defined edges of a circle in Marienfluss (Photo by N. van Rooyen).

Becker & Getzin (2000) proposed possible reasons for the abundance of grass on the edges of these circles. Firstly, the circles are nearly devoid of any other plants, resulting in less competition for water and nutrients for the individuals inhabiting the edge. This contributes to lush plant growth. The same phenomenon is observed along edges of tar and gravel roads where the vegetation benefits from run-off water.

Secondly, Becker & Getzin (2000) proposed that the concave shape of the circles as a result of wind erosion and the lack of vegetation, cause the circles to collect more water. Percolated water was found to a depth of 300 mm in the matrix as opposed to a depth of 800 mm in the circles themselves.

The third and last reason might be contributed to the fact that the grasses on the edge are good buffers for organic litter that get blown about by the wind and would thus accumulate more litter than the grass tufts in the matrix, making the grasses on the edge grow better.

1.3 Aim of the study

In order to improve the characterization of the circles it was decided to investigate some biotic and abiotic factors in the maintenance of the fairy circles. The following investigations were carried out:

- Bioassay of the soil from the different microhabitats (matrix, edge of circles, inside of circle) using a grass species from the region;
- Total elemental analysis of the soil from the different microhabitats;
- The occurrence of vesicular arbuscular mycorrhiza (VAM) in roots of plants collected from the different microhabitats;
- Inhibition of germination and growth of *Stipagrostis* seeds and seedlings by *Euphorbia damarana* Leach extracts and compounds.

Some of the experiments were done to answer specific questions while others were done to characterize the circles better. The questions raised during this study were as follows:

- Would there be less mycorrhizal infection in roots collected from plants growing inside the circle than in roots collected from plants growing in the matrix?
- Would extracts made from *Euphorbia damarana* inhibit germination and / or growth of *Stipagrostis* seedlings *in vitro*?
- Would a total element analysis of the soil show any distinct differences in soil elements from the different microhabitats?

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Unvegetated circular sand patches surrounded by strongly growing grass are a prominent feature of the eastern Namib Desert dune grasslands (Seely & Hamilton, 2003). Over the years many people have investigated the fairy circles to try to discover the origin of these circles, but no irrefutable evidence has been brought forward to support any of these fully. These hypotheses are discussed below.

2.2 Hypotheses

2.2.1 Termite related hypotheses

Tinley (1971) was the first person to propose that the fairy circles were the remains of fossil termitaria from a period when rainfall was much higher. The fairy circles have been linked to the mima mounds or 'heuweltjies' found in the Succulent Karoo and Nama Karoo Biomes in South Africa and they are regarded by some researchers as the northern counterpart of the mounds (Van Rooyen *et al.*, 2004) although there is controversy regarding the possibility that the 'heuweltjies' is the counterpart of the fairy circles.

In the Richtersveld, the southernmost distribution of fairy circles and the northernmost distribution of the fairy circles overlap and the ‘heuweltjies’ are also sometimes mistaken for fairy circles as they are sometimes devoid of vegetation and do not always have the mound shape (Van Rooyen *et al.*, 2004).

Most of the hypotheses on the origin of the ‘heuweltjies’ are biological and involves among others soil translocation by termites or bathyergid mole-rats or both of these animal groups (Cox *et al.*, 1987). Lovegrove & Siegfried (1986) and Moore & Picker (1991) found the harvester termite, *Microhodotermes viator* inside the ‘heuweltjies’ (earth mounds). They supported the research further by showing the remains of fossil termite tunnels in the calcrete and mentioned that they were probably between 2 000–4 000 years old. Midgley *et al.* (2002) challenged the possibility that *M. viator* was responsible for the ‘heuweltjie’ formation due to the fact that (i) the spatial concurrence between the occurrence of the ‘heuweltjies’ and the distribution of *M. viator* is poor, (ii) *M. viator* does not make large, sandy mounds and (iii) *M. viator* only rarely makes small nests above ground level. According to $\delta^{13}C$ and ^{14}C dating done by Midgley *et al.* (2002) the mounds are also considerably older than suggested by Moore & Picker (1991). Carbon dating suggested that they were in the region of 25 000 - 30 000 years old and that ‘heuweltjies’ were fossil features from the Late Pleistocene era and not from the mid-Holocene period as previously suggested. It must, however, be mentioned that Lovegrove & Siegfried (1986) also linked the formation of the mounds by *M. viator* to activity of the mole-rat *Cryptomys hottentotus* as this mole-rat has a tendency to nest within small termite mounds.

Moll (1994) and Becker & Getzin (2000) proposed that *Hodotermes mossambicus* was the causative agent of the fairy circles. Moll (1994) matched the distribution maps of Namibian termites with the occurrence of fairy circles and the closest matches were with

Psammotermes allocerus and *H. mossambicus*. By way of deductive reasoning he suggested that *H. mossambicus* was probably the causative agent based on its behaviour. He did, however, not collect any specimens while investigating the fairy circles. He collected two other species on his field trip, but concluded that *Baucaliotermes hainsei* and *Psammotermes allocerus* were unlikely candidates for the formation of the circles. Later Becker & Getzin (2000) collected *H. mossambicus* and formulated a hypothesis directly linking the termites to the formation of the circles. They proposed that the termites harvest only in the immediate vicinity of the breeding hive which is in the centre of the nest system when the conditions are good (average rainfall) causing circular denuded patches. *Hodotermes mossambicus* constructs numerous inter-connected hives all but a few of which are supplementary structures not occupied by functioning reproductives (Coaton & Sheasby, 1975). *Hodotermes mossambicus* is an efficient harvester and it is estimated that the area of an average fairy circle (5 – 8 m in diameter) can be denuded in approximately seven days. If the grass becomes scarce they would move and build new galleries and foraging ports. This would result in new circles forming while the old ones would recover (Lovegrove, 1993).

Grube (2002) refuted their statement by pointing out that lower termites like *H. mossambicus* tend to create polydomous or polycalic nest systems (i.e. the colony may be divided among subunits) and those subterranean termite colonies occupy one or more feeding sites. Grube (2002) also observed that harvesting behaviour of the termites did not indicate that they would harvest in circular patches.

The dispersion index for the fairy circles in Wolwedans (Namib Rand Nature reserve) closely matches those of the ‘heuweltjies’ (Lovegrove, 1993). Becker & Getzin (2000) also explained the variation in the size of the diameter of the circles with regards to the foraging behaviour of the termites in relation to the temperature. The termites are

very sensitive to temperature and in hot areas they would forage in the cooler hours but the low night temperatures would limit the termite's surface activity thus limiting the size of the circles. Grube (2002) refuted this statement by mentioning that these termites did not cover long distances from their foraging holes to potential food sources and would rather increase the number of foraging holes when food became scares making them less vulnerable to extreme temperatures. The lethal maximum temperature cited by Becker & Getzin (2000) was also shown to be inaccurate as Grube (2002) observed *H. mossambicus* searching for food at temperatures exceeding 44°C without showing signs of heat distress.

Albrecht *et al.* (2001) associated the fairy circles with a semi-volatile chemical associated with viable termite nests situated directly under the fairy circles which causes dehydration-stress in *Stipagrostis* species. This would cause the plants on top of the nests to die. The death of the plants would mean that more water would be available to the termites thus promoting the survival of the species. They proposed that the factor that caused the circles (a factor associated with the termites) would diffuse in a cone shape to the surface, thus causing the circles.

Van Rooyen and co-authors (2004) summarised the shortcomings of the termite hypothesis as follows:

- The notion that the circles are dynamic and that they disappear after a number of years of poor rainfall is questioned. In 1978 a site on the Giribes Plain in the Koakoveld was selected and some fairy circles marked permanently with steel droppers at a depth of one meter. After 22 years (in 2000) all marked patches were still visible.
- Moll (1994) stated that the termites harvested all the grass seeds in the vicinity of their nests. Van Rooyen *et al.* (2004) stated that after good rainfall many seedlings

are noticed in the barren patches, indicating that a lack of seeds is not the cause of the barren patches. One can also find quite a number of seeds in these patches but almost no termite activity (personal observation).

- Observations in the Kaokoveld on actively foraging termites did not reveal that they harvested in circular patches and in areas with dense grass cover active foraging left hardly visible irregular bare areas (Grube, 2002). Grube (2002) also mention that termites do not eat whole plants and in general no plants or plant remains can be seen in the barren patches.
- Albrecht *et al.* (2001) hypothesised that the barren patches act as water traps and found that the soil within the patches contained more water than outside the patches. Van Rooyen *et al.* (2004) investigated many barren patches, in the Giribes Plain and Marienfluss and found that the water distribution pattern was not consistent and Moll (1994) also indicated that water infiltrated slower into the barren patches than into soil from the matrix.
- The hypothesis described by Albrecht *et al.* (2001) relies on the termite nests beneath the barren patches to induce susceptibility to dehydration on the plants. When the plants are subjected to dehydration they will eventually die and use less water thereby providing the termites with more water. However in potting trails done by Van Rooyen *et al.* (2004) plants showed restricted growth even when well watered. Albrecht *et al.* (2001) could not isolate and identify the semi-volatile substance.

Coaton & Sheasby (1975) mentioned that the characteristic damage usually takes the form of patches of denuded veld fringed by vegetation in which grass stalks have been cut of horizontally leaving stubs with finely serrated edges. This was, however, not observed during field work done for the present study.

2.2.2 Allelopathy hypothesis

Allelopathy has been defined as the effect of plants on neighbouring plants through the release of chemical compounds into the environment (Inderjit & Weiner, 2001). That definition is, however, so broad that it can include almost all interactions among plants in the environment. It can also be more narrowly defined as the suppression of growth of one plant species by another due to the release of toxic substances. This would thus be a direct influence (Inderjit & Weiner, 2001). The definition need not be limited to plants and some authors define allelopathy as the chemical inhibition of one species by another (Abell *et al.*, 2006). One can thus conclude that the definition of allelopathy is broad and is adapted to fit the organisms under study. One of the oldest examples of allelopathy is the inhibitory effect that walnut (*Juglans nigra* L.) has on associated plant species. This is caused by the compound juglone (5-hydroxy-1, 4-naphthoquinone that has been isolated from many plant species from the Juglandaceae. Juglone is found in the plant in a colourless, non-toxic form called hydrojuglone and when exposed to some oxidizing agent (such as air) the hydrojuglone is oxidized to its toxic form juglone (Kocacaliskan & Terzi, 2001).

Theron (1979) proposed that the succulent *Euphorbia damarana* Leach released an allelopathic compound that killed other plant species especially grass species and grass seedlings. *Euphorbia damarana* is a large stem succulent and in the Giribes Plains these plants and fairy circles co-occur. The mean size of an *E. damarana* and the fairy circles in the Giribes Plains was also shown to be similar with a mean diameter of 6.4 m and 6.2 m respectively (Theron, 1979). Theron also mentioned that an organic allelopathic substance might not be in the soil long enough to maintain the circles but an inorganic compound on the other hand might be able to stay in the soil long enough to maintain the

circles for long periods of time. Theron (1979) thus proposed that the plants removed inhibitory inorganic material from deep in the soil and upon dying released the inhibitory material into the upper layers of soil. Precipitation in these regions is very low, thus preventing the leaching of these inorganic materials to a large extent. Some preliminary experiments done by Theron (1979) showed that one or more inorganic compounds in *E. damarana* had growth inhibitory properties and he speculated that the compound might be a cation.

This hypothesis has a few shortcomings. Van Rooyen *et al.* (2004) pointed out that *E. damarana* prefers a stony habitat whereas most fairy circles are found in valleys with sandy soil. In the Giribes Plains the occurrence of *E. damarana* is unusual as they grow in sandy soils there. Even if the allelopathic compounds decomposed very slowly, it seems unlikely that the effect would show after long periods and according to van Rooyen *et al*. (2004) no *E. damarana* plants can be found in any other location co-occurring with the fairy circles. However on the farm Keerweder some *Euphorbia* species were observed on the hills in the same location as the fairy circles (personal observation). Lastly, Van Rooyen *et al.* (2004) did bioassays with the soil collected under live and dead *E. damarana* using *Lolium multiflorum* Lam. as bio-indicator and found stimulation in plant growth from plants grown in soil collected under dead *E. damarana*, when compared with plants grown in soil collected in the matrix.

2.2.3 Radioactivity hypothesis

Namibia is characterised by a complex geology and can be divided into several geotectonic and lithologic domains. The sedimentary and volcanogenic succession of the

Neoproterozoic Damara Belt comprises more than 60% of Namibia's rock outcrops (Van Straaten, 2002) and nearly half of the country's surface area is bedrock exposure, while the remainder is covered by young surficial deposits of the Kalahari and Namib Deserts (Van Straaten, 2002).

According to Karaerua (2000) sand in the plains originating from the Damara geosynclines often contains radioactive elements. Experiments have shown that chronic radiation produced lethal zones where all plants died and 'effect zones' where some plant species showed increased and others decreased growth (Fraley, 1987). When combining the effect zone hypothesis of Fraley (1987) and Karaerua's (2000) assertion of the radioactive origin of the sand, the circles with the enhanced growth on the edge makes some sense. However, upon investigation Van Rooyen *et al.* (2004) found no increase in radiation inside the fairy circles.

2.2.4 Vegetation patterns

The fairy circles are by no means the only strange vegetation pattern known to scientists. In arid and semi-arid areas one often finds banding patterns with bands of more closely spaced trees alternating with bands of sparser vegetation (Mabbutt & Fanning, 1987). According to Mabbutt & Fanning (1987) there are patterns known as 'brousse tigrée' roughly translated as 'bands of a tiger' in northern Africa. Tiger bush is named like that because from the air the pattern of trees forming stripes resembles the stripes of a tiger. In the 1950s curiosity about these striking geometric natural landscapes led to a lot of isolated studies and similar patterns were described in Sahel, East Africa, southern Africa, Mexico and Australia. All possible hypotheses on the origin and dynamics of such

patterns, involved the role of soil heterogeneity (texture or nutrient content), wind erosion, water circulation, amongst others. (Tongway *et al.*, 2001). Vegetation punctuated by bare spots referred to as 'termitaria-peppering' in Somalia and as *brousse tachetée* (spotted bush) in West Africa has also been reported (Couteron & Lejeune, 2001) and they sometimes occur in the immediate vicinity of tiger bush but has received far less attention.

Mathematical models have been successfully applied to describe vegetation patterns (see, e.g. Couteron & Lejeune, 2001; Lejeune *et al.*, 2004; Van de Koppel & Rietkerk, 2004). According to Couteron & Lejeune (2001) one does not need environmental heterogeneity (such as a slope) to produce a regular vegetation pattern. One must, however, be able to show that there is (i) a positive influence of the vegetation on the reproduction and (ii) the competitive interactions must occur over a larger range than the positive influence in (i). There will thus be a trade-off between the positive influence (facilitation) and competition. According to Callaway & Walker (1997) facilitation and competition co-occurring within the same community produces complex and variable effects. Couteron & Lejeune (2001) predict that a range of periodic spotted patterns such as spots of bare soil arranged on a hexagonal lattice or stripes alternating with bare soil can emerge in the absence of any environmental heterogeneity such as a slope. No relation has yet been made between fairy rings and the spotted patterns, but according to Van Rooyen *et al.* (2004) spatial self-organization has also been used to describe patterns in arid ecosystems and these patterns might be able to explain why *Stipagrostis* species form barren patches when rainfall is less that 100 mm annually but grow well in other grasslands where rainfall is more than 100 mm a year.

2.2.5 Fungi as causative agents

Fairy rings are caused by a fungal mycelium which grows redially through soil and produces fruiting bodies near its outer edge. They are familiar features of grassland areas particularly on poor soil where their effect on the vegetation is often very apparent (Edwards, 1984). Fairy rings can be classified according to their effects on vegetation. Type one kills or badly damages the vegetation, type two only stimulates the vegetation and type three causes no apparent effect on the native vegetation (Edwards, 1984). From this it is apparent that the fungus does not necessarily kill the vegetation but can also stimulate the vegetation for better growth.

Some scientists associate the circles with the 'fairy rings' created by various mushroom species in temperate, more humid regions. The mushrooms in these rings initially grow as a closed colony but soon move outwards and form a circle which gets bigger in diameter every year (Günster, 1993). Although they are more common in wet environments, fungi also occur in deserts.

The question then arises if a fungus could be causing the fairy circles? It could be possible that some fungus caused the circles in a time period when conditions would have been more favourable (more humid) for the formation of circles as big as the fairy circles. The fungus that caused the circles might not be there anymore and maintenance of the circles may be due to a completely different factor such as the lack vesicular arbuscular mycorrhiza.

Studies to determine the presence of fungi and bacteria have been undertaken by Eicker *et al.* (1982). They tested for mesophilic and termophilic bacteria as well as fungi. They concluded that the soil from the bare patches was relatively poor regarding the population density of the microorganisms, compared to the soil from the matrix and the

edge. The soil from the bare patches has more anaerobic bacteria than the soil from the edge and matrix. They stated that all three the sub-habitats had coarse sand with very little clay rendering the habitat low in water-holding capacity and very well oxygenated, making the high concentration anaerobic bacteria difficult to explain. They also found only two termophilic fungi, *Humicola grisea* and *H. lanunginosa*. In the end they could not provide an explanation for the origin and maintenance of the circles.

CHAPTER 3

BIOASSAY OF SOIL USING

STIPAGROSTIS UNIPLUMIS

3.1 Introduction

The vegetation on the sandy plains where the fairy circles occur is typical arid grassland and most of the year the vegetation is scant (Van Rooyen *et al.*, 2004). The grassland is usually composed of *Stipagrostis* species which cover the plains after good rains (Viljoen, 1980). Van Rooyen *et al.* (2004) stated that seedlings of the *Stipagrostis* species become established inside the fairy circles after good rains, but most of them die before reaching maturity.

3.2 Literature review

Theron (1979) did a bioassay using soil collected from the different microhabitats and stated that *Eragrostis tef* (Zuccs) Trotter seedling growth was inhibited when grown in soil collected from the inside of the circles when compared to plants grown in soil collected from the matrix and the edge of the circles. Moll (1994) and Albrecht *et al.*

(2001) did germination experiments and all concluded that germination of species such as alfalfa and *Cynodon dactylon* (L.) Pers. was not significantly inhibited in pot experiments with fairy circle soil. Van Rooyen *et al.* (2004) stated that seedlings do establish after good rain but most of them then die off. It would thus seem that the germination potential is not influenced by die soil but that growth is inhibited to such an extent that the plants die.

Van Rooyen *et al.* (2004) collected soil from various locations of the most prominent locations of the fairy circles in the Kaokoveld namely Giribes Plain, Hartmann's Valley and Marienfluss. The soil was used in a bioassay to determine the inhibitory or stimulating effect of the soil on *Lolium multiflorum* Lam. growth. They concluded that shoot and root growth were severely inhibited in the topsoil from the centre of the barren patches and stimulated in the soil from the edge of the fairy circle. They also showed that soil collected from a depth of 0.5 m in the centre of the barren patch still inhibited shoot as well as root growth.

In the soil bioassay experiments done by Van Rooyen *et al.* (2004), *Lolium multiflorum* was used as a bio-indicator. *Lolium multiflorum* was used because it has small seeds, with few reserves, germinates well and grows quickly, making it an ideal plant for bioassays. It could, however, be that this species reacts differently to the factors maintaining the circles than the *Stipagrostis* species growing there naturally. Van Rooyen *et al.* (2004) stated that indigenous species often show dormancy patterns making them less suitable for germination experiments.

3.3 The genus *Stipagrostis*

The genus *Stipagrostis* Nees comprises an ecologically important group of species that grows in desert dunes and related habitats (Danin, 1996). Skinner (1964) did an ecological and physiological study on *S. obtusa* and *S. ciliata*. He concluded that both *S. obtusa* and *S. ciliata* seeds had an after ripening period of at least six months and that the germination of the seeds were influenced by temperature. In a comprehensive article regarding *Stipagrostis* adaptations to desert dunes Danin (1996) described the morphology and distribution of the genus with emphasis on the adaptations they have to the dry and harsh conditions of the arid environment they grow in. There is, however, not a lot of information regarding the germination potential of the seeds and the physiology of the plants.

Stipagrostis is an 'Old World' genus of about 50 species found mainly in desert areas with 29 species occurring in southern Africa (Fish, 2003). This genus includes the first colonisers of mobile dunes, stabilizing dunes and sand sheets which were once mobile (Danin, 1996).

In the Giribes Plains the vegetation between the barren patches (matrix) is composed mainly of *S. uniplumis* and the edges of the barren patches composed of either *S. giessii* or *S. hochstetteriana* (Van Rooyen *et al.*, 2004).

Danin (1996) divided the *Stipagrostis* species into different categories according to their specific growth forms. He grouped *S. uniplumis*, *S. giessii* and *S. hochstetteriana* into the *S. obtusa* group, which he described as species confined to stable or slightly eroded sandy plains (thus low sand mobility). They also have a diaspore (Figure 3.1) with a feathery awn and are mostly perennials. They are classified as mostly perennials

because some of the *Stipagrostis* species can switch between perennial or annual according to the conditions they grow in.

Figure 3.1. Diaspore of *Stipagrostis uniplumis* (Van Oudtshoorn, 2004).

Stipagrostis diaspores can be divided into two morphological types i.e. the feathery type, which 84.4% of the taxa have and the less abundant type, the pappus-like type (Danin, 1996). The feathery type glides above the ground and is anchored into the ground by the sharp pointed tip of the diaspore. This is the main way of dispersing seeds in *Stipagrostis*. All the *Stipagrostis* species referred to in this study belong to the more abundant feathery type. Diaspore is a technical term used for dispersal structures in plants. Although the *Stipagrostis* species all have diaspores the term ‘seed’ will also be used with the understanding that the structure is technically a diaspore.

No literature is available on the dormancy and germination of the *Stipagrostis* species of the Giribes Plains, but Skinner (1964) did an intensive study of the dormancy and germination patterns of *S. ciliata* and *S. obtusa*. He concluded from his study that seeds of *S. obtusa* had an after-ripening period of three to six months and that they had a water soluble germination inhibitor that lost its efficacy with time. He also stated that

germination could occur over a wide range of temperatures, but his results clearly showed that germination was the best at temperatures from 20ºC to 25ºC, and that seeds germinated better when planted at a depth of 10 mm. He also showed that when the conditions for germination were favourable, germination occurred in five days and germination potential decreased when seeds were heated although the effect differed for *S. ciliata* and *S. obtusa*.

3.4 Aim of this chapter

The aims of this chapter were as follow:

- To determine the germination potential of the collected *Stipagrostis uniplumis* seeds collected in the Giribes Plains.
- To establish whether a soil bioassay using *Stipagrostis uniplumis* could reproduce the fairy circle phenomenon in an artificial environment.

3.5 Materials and methods

3.5.1 Collection of diaspores and soil

One field trip was undertaken in June 2005 to collect the diaspores and soil samples for the study. All the soil used for the bioassays were collected in the Giribes Plains by using a soil auger 150 mm long x 70 mm in diameter. One circle was chosen and enough soil

collected from the different microhabitats of that specific circle to perform the bioassay. Diaspores were also collected in the Giribes Plains by gently removing the whole diaspore from the soft soil. The diaspores were stored at ambient temperature in a dry environment until the germination experiments were performed in February of 2006. *Stipagrostis uniplumis* is the predominant species in this region and the diaspores collected were identified as those of *S. uniplumis*.

3.5.2 Seed viability testing

The awns of the diaspore were removed from the spear - shaped seed bearing tip. To test the viability of the seeds, 20 seeds were soaked in distilled water, cut longitudinally and incubated in a 0.05% 2,3,5-triphenyltetrazolium chloride solution (TTC) (Mian & Coffey, 1968) for 3 hours in the dark at 25ºC. After incubation the seeds were removed and checked for viability.

3.5.3 Seed germination testing

Due to a limited number of seeds collected, germination trails were done on a small scale. Germination trails were conducted by placing the seeds on Whatman nr 3 filter paper, moistened with 5 ml distilled water in glass Petri dishes sealed with parafilm. Ten seeds were used per treatment and each treatment was germinated at two temperatures, 20°C and 25°C and under light and dark conditions. The Petri dishes of the dark treatments

were sealed in cardboard boxes and examined under a green light every second day. The following treatments were examined:

- scarification by lightly rubbing the seed with sand paper until the coat was removed for at least half the seed;
- leaching of the seeds in running tap water for 24 hours;
- a combination of scarification and leaching and
- control

This experiment was repeated every two months until satisfactory germination occurred after eight months.

3.5.4 Bioassay of soil

Bioassays where conducted on the soil using *S. uniplumis* as bio-indicator. Five replicates for each microhabitat (matrix, edge and inside) were used. The pots with a volume of about 600 ml were filled with soil and 20 seeds planted at a depth of 10 mm. Plants were watered with 25 or 50 ml of distilled water every day depending on the amount of moisture present in the soil. After one week seedlings started emerging. Seedlings were thinned to six plants per pot after 21 days. Leaf lengths of the plants were measured after six weeks as a non-destructive measure of growth. This was done by measuring the longest leaf of each plant in a pot to the nearest millimetre (Tucić *et al.*, 2006). After 10 weeks the plants were harvested and the fresh and dry mass of the aboveground parts and the fresh mass of the belowground parts were determined. The dry mass of the different parts was measured after drying the plant material at 60ºC for four days. Data were

analyzed using the Microsoft Exel program and Statistica v7 with the Tukey pos hoc test (Statsoft, Tulsa).

3.6 Results

3.6.1 Seed viability testing

Colourless 2, 3, 5-triphenyltetrazolium chloride solution is converted to red triphenyl formazan in the presence of the dehydrogenase enzyme present in viable seeds (living seeds) (Main & Coffey, 1968). After incubation the seed-halves were examined under a dissection microscope. The embryo could be seen at the lower tip of the diaspore and all the embryos were stained pink indicating 100% viability.

3.6.2 Seed germination testing

To determine the germination potential of the seeds collected in the Giribes plain the seeds were treated and germinated in the light in the dark and at two different temperatures of 20°C and 25°C. The germination results are shown in Table 3.1. The last seeds to be tested showed good germination potential and all the seeds that were leached germinated at 20°C and 25°C in the dark whereas 90% of the leached seeds germinated at 20°C in the light and all of them germinated at 25°C in the light. The number of seeds was not enough to do proper statistical analysis but scarification and leaching seem to have a small effect on the germination potential of the seeds.

Table 3.1. Mean germination of *Stipagrostis uniplumis* in different treatments

Date of experiment	Treatment	Germination percentage Light		Germination percentage Dark	
		20°C	25°C	20°C	25°C
Aug-05	Leaching	0	0	0	0
	Scarification	0	0	0	0
	Scarification & leaching	0	0	0	0
	Control	0	0	0	0
Oct-05	Leaching	0	0	0	0
	Scarification	0	0	0	0
	Scarification & leaching	0	0	0	0
	Control	0	0	0	0
Dec-05	Leaching	0	10	20	20
	Scarification	20	20	20	30
	Scarification & leaching	20	20	30	20
	Control	0	0	0	0
Feb-06	Leaching	90	100	100	100
	Scarification	100	80	80	90
	Scarification & leaching	70	90	100	100
	Control	80	70	60	80

3.6.3. Bioassay of soil

Results from the biomass analysis of *S. uniplumis* are given in Table 3.2. The fresh and dry mass of the shoots harvested from the plants grown in soil collected from the matrix and the edge showed no significant difference but the shoots collected from plants grown in soil from the inside of the circles differed significantly when compared to the matrix and edge masses ($p = 0.0007$). The fresh root mass showed a different trend than the shoots. The roots collected from plants grown in soil from the matrix and the inside of the circle showed no significant difference in mass whereas the roots collected from plants

grown in soil collected from the edge showed a marked increase in mass when compared to plants grown in the matrix and the inside soil ($p = 0.013$).

The shoots from plants grown in the soil collected from the inside were shorter when compared to the plants grown in soil from the shoots of the matrix and the edge of the circles ($p = 0.00004$). The difference in shoot length between grasses grown in soil collected from the edge and the matrix was also significant ($p = 0.00004$) with the edge samples showing a stimulation in growth. The shoot length differences were apparent already after 34 days of growth (Figures 3.2-3.4).

Table 3.2. Biomass and shoot length analysis of *Stipagrostis uniplumis* planted in soil from different microhabitats. Values with the same superscript do not differ significantly at $\alpha = 0.05$

Microhabitat		**Std. Dev.**
	Fresh shoot mass (g)	
Matrix	0.49[b]	0.07
Edge	0.51[b]	0.12
Inside	0.27[a]	0.03
All groups	0.41	0.14
Analysis of Variance p = 0.0007		
	Dry shoot mass (g)	
Matrix	0.24[b]	0.04
Edge	0.24[b]	0.06
Inside	0.12[a]	0.02
All groups	0.20	0.07
Analysis of Variance p = 0.0006		
	Fresh root mass (g)	
Matrix	0.18[a]	0.03
Edge	0.25[b]	0.03
Inside	0.18[a]	0.03
All groups	0.20	0.04
Analysis of Variance p = 0.013		
	Shoot length (mm)	
Matrix	156[b]	11.62
Edge	184[c]	17.88
Inside	122[a]	10.13
All groups	154	29.34
Analysis of Variance p = 0.00004		

Figure 3.2. *Stipagrostis uniplumis* after 10 days of growth with the differences between the edge and inside not clearly visible.

Figure 3.3. *Stipagrostis uniplumis* after 25 days. The plants growing in the soil from the middle of the fairy circles are shorter than the matrix and edge soil plants.

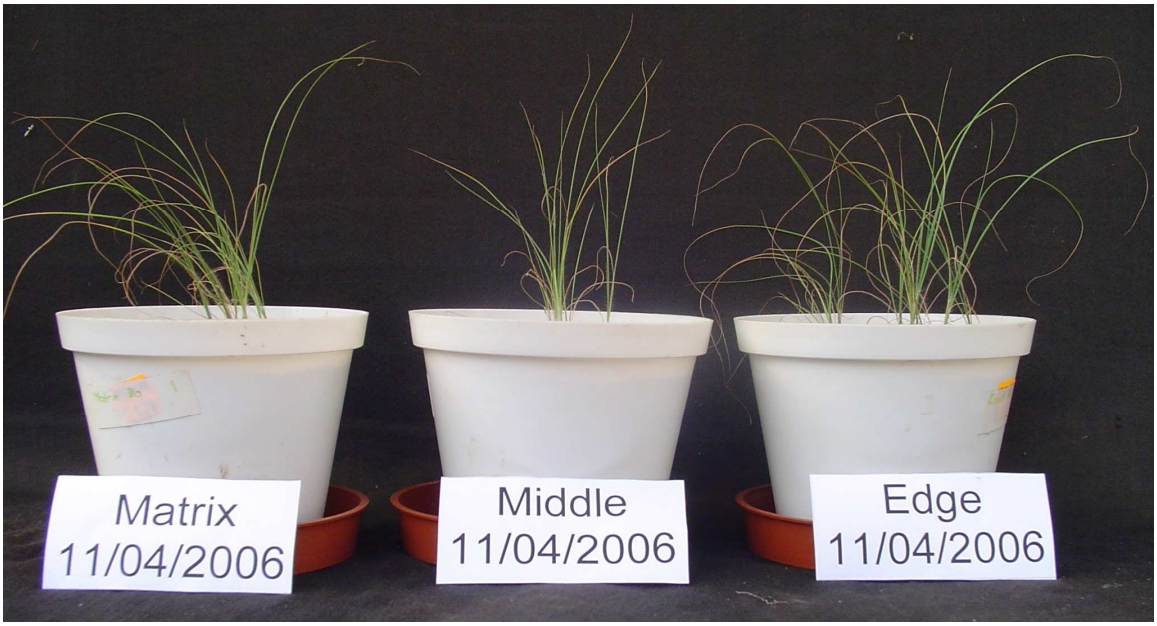

Figure 3.4. *Stipagrostis uniplumis* at 44 days. The plants growing in the soil from the middle of the fairy circles are not as big as the plants from the edge and the matrix.

3.7 Discussion

3.7.1 Viability and seed germination

All the seeds tested in the tetrazolium test were viable and seed production in *Stipagrostis* was therefore probably successful. However, the seeds did not germinate readily, giving an indication that dormancy might be a factor. The seed germination results showed much the same pattern as the experiment done by Skinner (1964). There seem to be an after-ripening period of six to eight months for the seeds. Unlike the *S. obtusa* seeds described by Skinner (1964) temperature do not seem to have a big influence on the germination rate of *S. uniplumis.* The germination experiments should be repeated in future with more seeds for good statistical analyses to confirm the effects of temperature, leaching and scarification on the germination percentage.

3.7.2 Bioassays

This bioassay confirmed the results of the only other described pot experiment of Van Rooyen *et al.* (2004) done with *L. multiflorum*. There was a significant difference between grasses grown in the soil collected from the inside of the circle and the matrix. This gives an indication that the factor responsible for the maintenance of the circles might be transferable to an artificial environment. If transference of the factor responsible is possible it would exclude theories such as the termite theory proposed by Moll (1994) and Becker & Getzin (2000). It would however support theories such as allelopathy (Theron, 1979) and radioactivity (Karaerua, 2000).

3.8 Conclusion

I would seem that an after-ripening period of at least six months is necessary for *S. uniplumis* seeds to germinate and that the germination is almost 100% after eight months. The factors maintaining the fairy circles can be transferred to an artificial environment as shown in this bioassay with *S. uniplumis* and the previous experiment with *L. multiflorum* reported by van Rooyen *et al*. (2004).

CHAPTER 4

THE OCCURRENCE OF VESICULAR ARBUSCULAR MYCORRHIZAE (VAM) IN ROOTS COLLECTED FROM PLANTS OCCURRING IN AND AROUND THE FAIRY CIRCLES

4.1 Introduction

During the latter half of the 19th century, several researchers noted the presence of fungi in plant roots without any apparent disease and necrosis (Schenck & Trappe, 1984). In 1885 the term mycorrhiza was used for the first time to describe the relationship between fungi and plant roots. Mycorrhiza literally means fungus root and can be classified into five types (Janerette, 1991; Lombaard, 1993):

- Ectomycorrhizae, where the fungal associate does not penetrate the root cells of the host but only develops a Hartig net around the root cells.
- Ectendomycorrhizae or arbitiod mycorrhizae where the fungus forms a Hartig net as well as penetrates the root cells.
- Ericoid endomycorrhizae are confined to the family Ericaceae and septated hyphae fungi penetrate the host roots to colonise the cortex cells by forming coils.
- Orchid endomycorrhizae are like Ericoid endomycorrhizae but are confined to the Orchidaceae.

- Vesicular arbuscular mycorrhizae (VAM), where non-septate hyphae of the fungal partner penetrate the host cortex to form characteristic intercellular and intracellular vesicles and intracellular arbuscules. An external hyphal system which extends into the soil is also formed. No Hartig net is formed (Lombaard, 1993).

VAM can be classified into a single family (Endogonaceae) and species are separated according to their manner of spore formation (Schenk & Trappe, 1984).

The VAM association is considered to be the most common mycorrhizal association in nature (Lombaard, 1993) and the associations between VAM and plant roots are usually non-specific in terms of fungus-plant associations that are compatible (McGonigle & Fitter, 1990). They are all obligate symbionts and the symbiosis between VAM and vascular plants occurs in 80% of plant species (Veenendaal *et al.*, 1992). The associations are extremely ancient, and VAM have been identified in fossils of early Devonian land plants (Harrison, 1997). The beneficial influence that VAM can have on plants has been shown extensively and growth responses in plants mostly depend on three factors; the degree of infection of a plant, how effective the symbiosis is and the nutrient status of the soil (Hayman & Tavares, 1985). Different species and strains of VAM show different benefits to the host plant (Hayman & Tavares, 1985). VAM have been shown to increase the uptake of nutrients in plants (Veenendaal *et al.*, 1992), to improve water relations and to reduce pathogenic infection (Muthukumar & Udaiyan, 2002).

4.2 Anatomy and morphology of VAM

In most cases the anatomical and cytological changes caused by VAM infection in the host do not induce root alterations recognisable with the naked eye (Powell & Bagyaraj, 1984). This means that one would generally need to stain the roots and observe them under a microscope to detect infection.

According to Powell & Bagyaraj (1984) one would need a light microscope to grasp the complexity of the plant / mycorrhizal fungus interaction.

VAM form thick-walled inter- or intracellular vesicles, while arbuscules are produced progressively within cells of the inner cortex (Brown & King, 1984). The infection does not penetrate the endodermis and is therefore not present in the central vascular cylinder or the meristematic region. The infection is thus located in the outer regions of the root only (Figure 4.1).

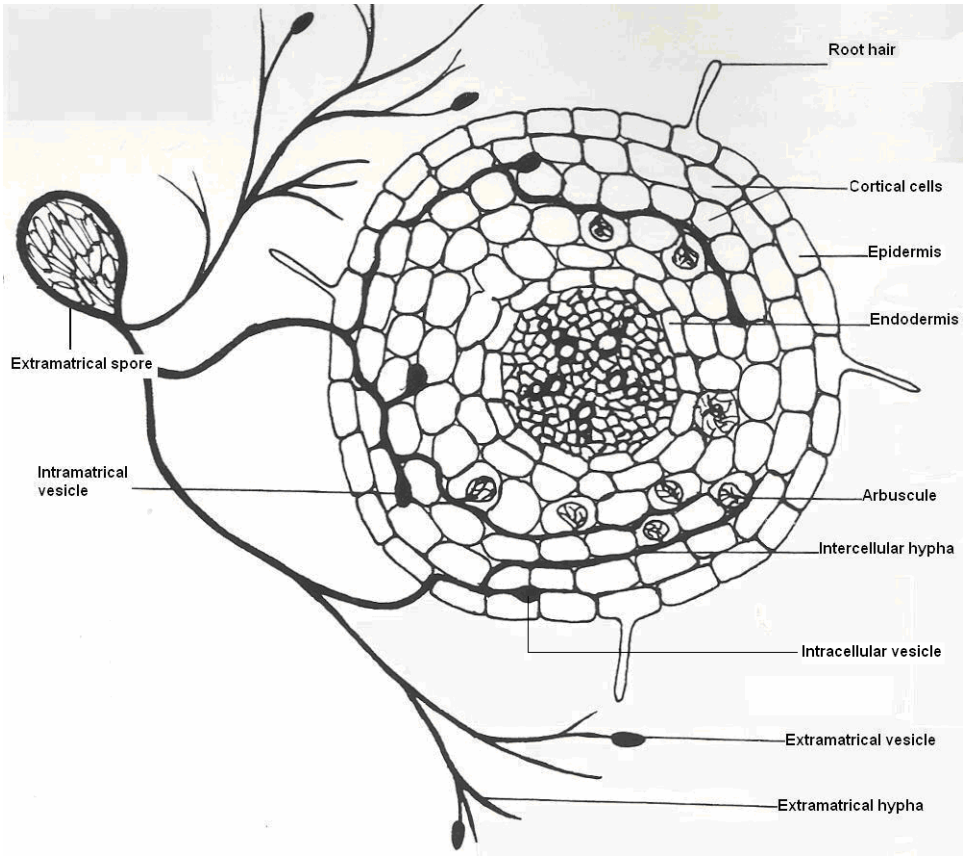

Figure 4.1. A representation of a transverse section of a root colonized by VAM (adapted from Lombaard, 1993).

Arbuscules form in the inner layers of the cortical parenchyma where intercellular hyphae penetrate the cortical cells giving rise to a complex hyphal branching system, like ‘small bushes’ and are the most significant structure in the VAM complex. The arbuscules are considered to be the functional unit of VAM symbiosis, thus the place where the host plant and the fungus really interact with each other symbiotically to transfer nutrients between each other (Lombaard, 1993).

Vesicles on the other hand can be intercellular or intracellular and can be found in the inner and outer layers of the cortical parenchyma. Not all VAM fungi form vesicles within roots and the cytological organization of the vesicles and the fact that their numbers increase in the old and dead roots suggest that they are mainly resting organs (Powell & Bagyaraj, 1984).

4.3 Factors influencing the occurrence of vesicular arbuscular mycorrhizae

VAM fungi occur in almost all soils and the few instances where these fungi may be absent are usually in disturbed soil. The disturbances include eroded soils; fumigated soils or the removal of the topsoil such as in mining (Abbott & Robson, 1991). The relationship between soil chemical and physical properties and VAM colonisation are markedly variable (Abbott & Robson, 1991). There are, however, differences between different species regarding soil properties such as pH, soil phosphate level and salinity (Haymann & Tavares, 1985; Abbott & Robson, 1991). Nutrient content of the soil can play a very important role in the infectivity and subsequent colonisation of VAM fungi in host plants. High levels of phosphorus and nitrogen decreases VAM fungi colonisation and subsequent sporulation. However, if the phosphorus content is low and the zinc and nitrogen content is high, there will be an increase in colonisation (Lombaard, 1993). Sodium and chloride in saline soils inhibit spore germination and hyphal growth (Bagyaraj, 1991). The pH of the soil has a great influence on the fertility of the soil, because changes in pH can change the diffusibility of nutrients in the soil. The pH of the soil has a definite influence on the germination of VAM fungal spores but the effect differs in different species (Lombaard, 1993). Mycorrhizal associations are widely spread throughout the vascular plant kingdom including major crops. However they are rare or totally absent in the Brassicaceae, Cyperaceae, Commelinaceae, Juncaceae and Proteaceae as well as in some

members of the Capparaceae, Polygonaceae, Resedaceae, Urticaeae and herbaceous members of the Caryphyllales (Lombaard, 1993).

4.4 Mycorrhizae in grasslands and arid ecosystems

There are very few studies on arbuscular mycorrhizae from Namibia and most focus on the arid regions (Jacobson 1997; Stutz *et al.*, 2000). VAM fungi benefit their host by improving uptake of certain minerals, such as phosphorus, and water and they improve drought tolerance in plants (Bohrer *et al.*, 2003).

Veenendaal *et al.* (1992) investigated the VAM in grass seedlings in degraded semi-arid savannah of Botswana and a few studies have been done on coastal sand dunes, which are classified as a habitat with low nutrient status (Ernst *et al.*, 1984) and possibly a low water status. VAM have been shown to improve both water status and nutritional status of the host plants, making their occurrence of particular interest at sites that suffer from drought most of the year (Uhlmann *et al.*, 2004). Albrecht *et al.* (2001) noted that seedlings of *Cynodon dactylon* (L.) Pers. grown in the soil from the inside of the circle died when subjected to cycles of hydration and dehydration but that the plants when grown in the soil from the matrix survived. They then went on to say that a biological factor in the soil from the inside inhibits dehydration resistance which may be the reason for this and implicated termites in this regard. They did not consider that it might be the lack of a biological factor such as a microorganism that could cause this phenomenon. In this regard VAM symbiosis may be of particular importance for plants colonizing disturbed, infertile or dry habitats with a poor nutrient status (Veenendaal *et al.*, 1992).

4.4.2 C_4 - and C_3- photosynthesis

Plant species have different photosynthetic pathways known as C_4, C_3 and Crassulacean acid metabolism (CAM) photosynthesis. Veenendaal *et al.* (1992) showed that tallgrass prairie C_4 plant species growing in a nutrient poor environment were more dependent on mycorrhizal symbiosis than C_3 plant species, especially at the seedling stage and during regrowth from rhizomes. *Stipagrostis* spp. are exclusively C_4 plant species (Gibbs Russel *et al.*, 1990) and mycorrhizal association would thus benefit them greatly.

The differences in photosynthetic pathways are broadly reflected in geographical distributions and ecological ranges of grass subfamilies, genera and species, where the C_4 species are concentrated in the tropics and subtropics and in dry, saline environments (Gibbs Russel *et al.*, 1990). They dominate the grass flora throughout south western Africa (Ellis, 1980). C_4 grasses are also characterized by 'Kranz anatomy'. Kranz anatomy is distinguished by the vascular bundles which are surrounded by a distinct, thick-walled sheath (Kranz in German) of cells containing specialized chloroplasts which differ in shape, size and number from the chloroplast in the mesophyll (Vogel *et al.*, 1978).

It would thus seem that the different *Stipagrostis* species, in the regions where fairy circles occur, would exclusively make use of the C_4 pathways and that VAM would benefit these plants regarding their water and nutrient uptake abilities.

4.4.3 Techniques for quantifying mycorrhizae colonisation

There are many different techniques for quantifying mycorrhizae in roots and these methods can be categorized into several distinct procedures. They are, however, not comparable and many researchers have modified these methods to serve their purposes (Kormanik & McGraw, 1984). The

basic conclusion of many researchers was that one should decrease the number of root segments studied, but increase the number of observations per segment.

The evaluation of VAM in roots can be expressed and quantified according to the study-objectives and can be divided into three broad groups. McGonigle & Fitter (1990) divided the procedures for visual determination of root length colonised into three groups; (i) subjective estimation, (ii) calculation of the percentage of root segments or microscope field of view that contains any colonisation and (iii) gridline intersect methods. There are also biochemical estimations which are of limited value. It should be noted that all the methods rely heavily on the researcher doing an unbiased selection of the root segments to be studied.

4.5 Aim of this chapter

The aim of this investigation was to determine if there was a difference in root colonisation by VAM from plants collected inside the fairy circles, on the edge of the circles and in the matrix.

4.6 Materials and methods

4.6.1 Field collection of roots

Plant roots and soil samples were collected in four of the regions where fairy circles occur (see distribution map, Figure 1.2). These regions were Amam in Bushmanland (South Africa), Keerweder (Namib Rand Nature Reserve), Giribes plain and Marienfluss, in Namibia. At each site three circles were chosen at random. Roots were taken from plants inside the circle, on the edge and in the matrix

near the sampled circle. Additionally at each site three soil samples were taken, 7 cm in diameter and 15 to 20 cm deep, right next to the plant. The roots were then removed and fixed in formaldehyde acetic acid alcohol (FAA) (5 ml formaldehyde, 5 ml acetic acid, 90 ml 50% ethanol) (Gaur *et al.*, 1999). In instances where only small plants were available (inside the circles) the whole plant was removed from the soil and all the roots fixed in FAA.

4.6.2 Bioassay root collection

Roots from the bioassay described in Chapter 3 were also collected. The roots of the five repeats of each microhabitat done for the bioassay were collected. All the roots from the pots were collected, washed in water to remove the soil and fixed in FAA immediately after washing. A subsample of these roots was stained for colonisation assessment.

4.6.3 Staining of roots for visualisation

Before staining, the roots were removed from the FAA, washed in tap water and cut into lengths of 1 cm. Roots from plants growing in the matrix were coated with a net of mycelia and sand whereas the roots from inside had very little sand and mycelia attached to them. Albrecht *et al.* (2001) concluded that the sand particles were attached by adventitious root hairs. The outer layer of sand was carefully removed from the roots by hand before clearing. The roots were then cleared by boiling in 10% KOH (w/v) for approximately 10 minutes initially. This was done because grass roots are very hard and short clearing times would not have given sufficient clearing to show colonisation satisfactory. Hebert *et al.* (1999) cleared the roots of the grass *Paspalum notatum* Stapf for 10 minutes, thus

giving a good indication of the time needed for clearing. In some instances where the roots were very fine, boiling was done for a period of five to seven minutes. The cleared roots were rinsed with water and bleached with alkaline hydrogen peroxide (3 ml 25% NH_4OH in 30 ml 3% H_2O_2). The duration in the bleaching reagent was determined by the intensity of the root pigments. After bleaching the roots were washed with water and then transferred to the staining solution. The roots were stained with 0.05% tryphan blue in acidic glycerol (50:45:5 glicerol: water: 1% HCl) and heated for five to ten minutes depending on the thickness of the roots and the time it took to bleach the roots properly. The staining solution was then carefully removed and the roots stored in a distaining solution of acidic glycerol prior to assessment.

4.6.4 Assessment of percentage root colonisation

Semi-permanent slides of all the root segments of 1 cm each were made with acidic glycerol as a mounting medium. First an assessment of the roots was made by scoring the absence or presence of arbuscules and vesicles under 100 X or 250 X magnification (Kormanik & McGraw, 1984). The percentage root colonisation was calculated by dividing the number of infected roots by die total number of roots observed. An attempt was made to estimate the proportion of the root length colonised in each sample using a compound microscope and the technique described by McGonigle & Fitter (1990). Sometimes only small pieces of roots were infected and this presented problems to accurately quantify the proportion of colonisation. It was then decided to use the method described by Kormanik & McGraw (1984). All segments of the root, approximately 10 mm long were arranged lengthwise on the slide with a needle and forceps and covered with a 22 X 40 cover slip, gently

tapping the slide to remove air bubbles and squashing the roots. Length of cortical colonisation was then assessed in millimeters for each root segment, averaged over all the root segments and expressed as a percentage of total root length (Table 4.1).

4.7 Results

All the roots from the field collection that showed the presence of VAM only had vesicles (Figure 4.2) and no arbuscles. There were also some fungal hypha present, but fungal hypha on roots do not necessarily indicate the presence of internal arbuscular mycorrhizae as ectomycorrhizae will also form fungal structures on the outside of the root. In the experiment, where only the absence or presence of VAM structures was assessed, one could clearly see a trend between the roots taken from inside the circles and the roots from the matrix or the edge (Table 4.1). No roots from plants inside the circles showed any infection. The degree of infection of plants from the edges and matrix differed greatly. In the roots collected from Marienfluss, only one subsample of roots collected from the edge of one circle showed any infection while no other sample had vesicles. In comparison the samples collected in Bushmanland, showed consistent infection in the roots collected from the outside of the circles. Samples collected in Giribes also showed consistent infection of plants growing on the edges and matrix, except for one sample collected from the edge.

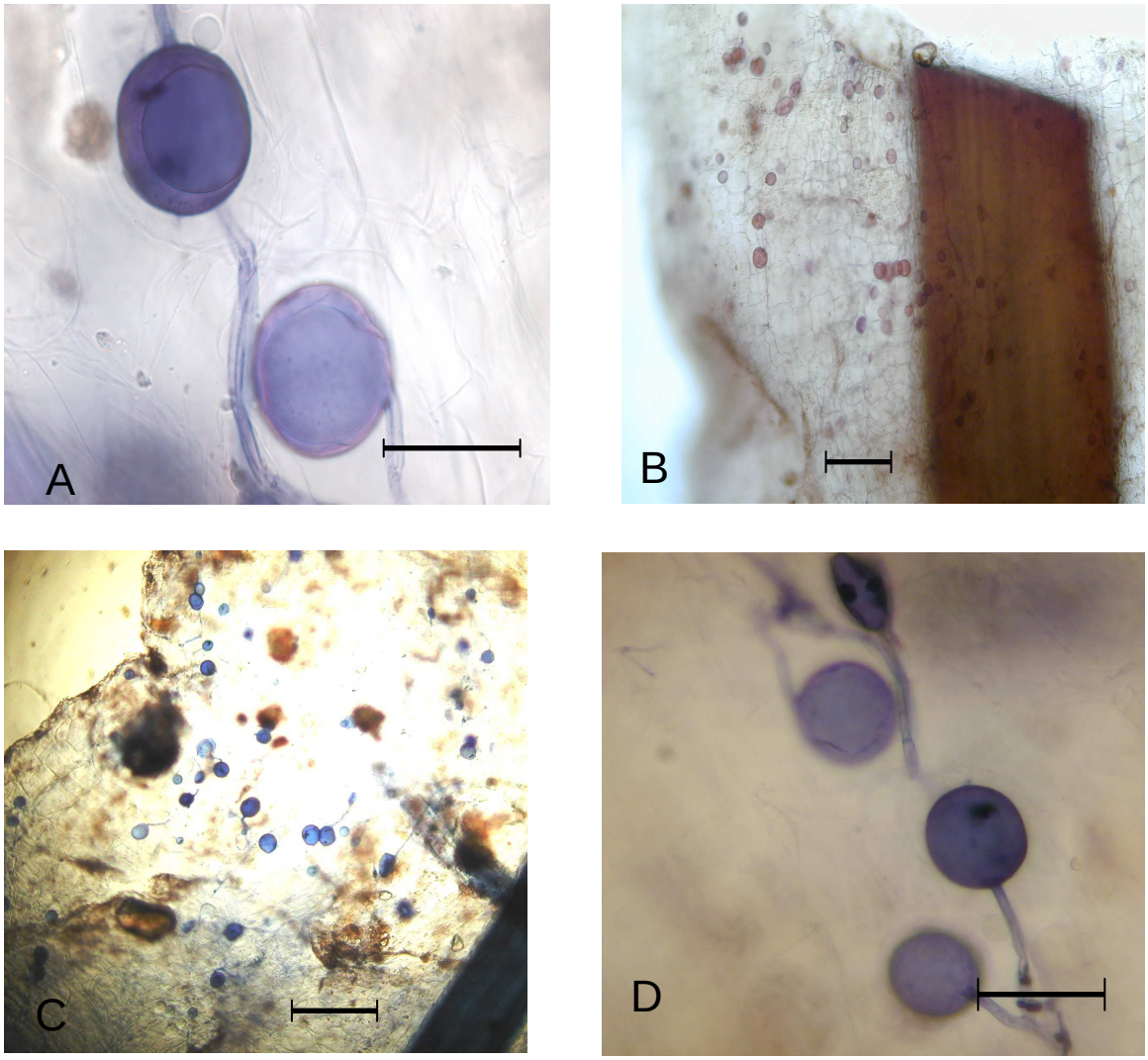

Figure 4.2. Vesicles found in roots of *Stipagrostis uniplumis* collected from the different locations. A) Bushmanland (matrix), B) Giribes (edge), C) Bushmanland (matrix), D) Keerweder (matrix). Scale bars in A & D represent 50 µm and in B & C 100 µm.

The roots collected from the soil bioassay experiment (Chapter 3) looked quite different from the roots collected in the field. The roots where thin and had no external sheaths as described previously. Upon examination no fungal structures were found in any of the samples. This experiment will be repeated later. As there was no colonisation no table regarding the results are added for comparison with the field collected samples.

Table 4.1. The absence or presence of fungal structures in the roots examined from the four different localities where roots of grass plants were collected. All the structures recorded were vesicles. The percentage colonisation is provided, as well as the length of cortical colonisation in millimetres expressed as a percentage of total root length

Sample number	Locality	Microhabitat	Fungal structure absent/ present	% Colonisation *	Standard Error	Length of cortical colonisation in millimetres expressed as a percentage	Standard Error
1	Giribes	Inside	Absent	0.0		0.0	
2	Giribes	Inside	Absent	0.0		0	
3	Giribes	Inside	Absent	0.0		0.0	
					0.0		**0.0**
1	Giribes	Edge	Present	40.0		25.0	
2	Giribes	Edge	Present	100.0		50.0	
3	Giribes	Edge	Absent	0.0		0	
					29.0		**10.2**
1	Giribes	Matrix	Present	40.0		44.0	
2	Giribes	Matrix	Present	100.0		47.0	
3	Giribes	Matrix	Present	66.0		33.0	
					17.4		**4.3**
1	Marienfluss	Edge	Absent	0.0		0	
2	Marienfluss	Edge	Present	20.0		25.0	
3	Marienfluss	Edge	Absent	0.0		0	
					6.7		**8.3**
1	Marienfluss	Inside	Absent	0.0		0	
2	Marienfluss	Inside	Absent	0.0		0	
3	Marienfluss	Inside	Absent	0.0		0	
					0.0		**0.0**
1	Marienfluss	Matrix	Absent	0.0		0	
2	Marienfluss	Matrix	Absent	0.0		0	
3	Marienfluss	Matrix	Absent	0.0		0	
					0.0		**0.0**
1	Keerweder	Inside	Absent	0.0		0	
2	Keerweder	Inside	Absent	0.0		0	
3	Keerweder	Inside	Absent	0.0		0	
					0.0		**0.0**
1	Keerweder	Edge	Present	20.0		11.0	
2	Keerweder	Edge	Absent	0.0		0	
3	Keerweder	Edge	Present	67.0		30.0	
					19.9		**7.8**
1	Keerweder	Matrix	Absent	0.0		0	
2	Keerweder	Matrix	Present	40.0		27.0	
3	Keerweder	Matrix	Present	87.0		36.0	
					25.1		**3.7**
2	Bushmanland	Inside	Absent	0.0		0	
3	Bushmanland	Inside	Absent	0.0		0	
4	Bushmanland	Inside	Absent	0.0		0	
5	Bushmanland	Inside	Absent	0.0		0	
					0.0		**0.0**
2	Bushmanland	Matrix	Present	67.0		48.0	
3	Bushmanland	Matrix	Present	71.0		38.0	
4	Bushmanland	Matrix	Present	50.0		55.0	
5	Bushmanland	Matrix	Absent	0.0		0	
5	Bushmanland	Matrix	Present	80.0		38.0	
					14.3		**3.7**

* **Number of roots segments colonised/ total number of segments observed**

4.8 Discussion

The fact that only roots from the matrix or the edge showed any presence of VAM is interesting. Unfortunately only vesicles were observed. There could be quite a few reasons why only vesicles were observed. Typically vesicles are produced most abundantly in the outer cortical region of older infection units, but in some instances they appear to form rather prolifically without extensive prior development of arbuscules (Brown & King, 1984), although the reason for this is not known. They are also thought to be dormant structures and as the roots were collected at the end of the growing season, the mycorrhizae might have been in a resting stage at the time of collection. The experiment will be repeated earlier in the rainy season when new seedlings are emerging and infection would be in the early stages making the possibility of observing arbuscules better.

The absence of vesicles in the roots from the inside of the fairy circle indicate that some unknown agent might have caused either the death of VAM spores or inhibited the germination of the spores, if they were present. If one assumes that the seedling roots are normal at the time of germination, it is safe to say that infection of the roots by VAM did not occur in the early stages of development. C_4 grasses benefit from the presence of VAM (Veenendaal *et al.*, 1992) and members of the *Stipagrostis* genus exclusively follows the C_4 photosynthetic pathway (Gibbs Russel *et al.*, 1990). VAM also gives plants resistance to drought stress (Bohrer *et al.*, 2003) and this would mean that if the seedlings did not have any VAM in the symbiotic relationship, they would not be able to cope with the infrequent rains as well as the other plants. One could also argue that the VAM did not colonise the plants because of possible abnormal root formation of the seedlings. Albrecht *et al.* (2001) noted that plants from the outside had roots tenaciously coated with sand and a mycelium structure and roots from plants collected from inside had little sand attached. The observation was also personally made.

Another way to investigate the occurrence of VAM is to quantify and identify the spores in the soil from the inside, edges and the matrix. One would then be able to determine if (i) there were any fungal spores in the soil from the inside of the circles and (ii) if the total number of spores differed greatly from location to location. Careful investigation into the germination possibility of spores from the different soil types would also give an indication of the possibility that VAM might be a factor in the maintenance of the circles. The absence of VAM might be the cause of the plants not growing properly, but some other factor must be the cause of the VAM not infecting the roots in the first place. VAM releases spores into the surrounding soil and this would mean that there would be spores in at the least the outer parts of the circles, which would cause the circles to get smaller over time and eventually disappear completely. This is not the case as shown by Van Rooyen *et al.* (2004) as the circles marked in 1978 were still there in 2000.

The results of the mycorrhizal colonisation raise some questions regarding the obvious edge effect one sees in some of the localities where the fairy circles occur, especially in the Marienfluss valley. The grasses on the edge of the circles in Marienfluss grows much better than those in the matrix but only one of the samples collected from the edge showed mycorrhizal infection, however none of the samples collected in the matrix showed any infection either. As mentioned earlier the edge effect might be due to factors such as accumulation of organic litter or the availability of more water to the plants growing on the edge.

Some plant families like Brassicaceae, Cyperaceae and Commelinaceae are known for not forming mycorrhizal associations (Lombaard, 1993). One could reason that the absence of mycorrhiza and grasses within the circles could form a favourable environment for non-mycorrhizal plant species to grow as there would be no competition from other plants that need mycorrhiza to grow. This would mean that any plant that does not need mycorrhiza associations to grow in a dry environment would grow inside the circles unless some substance kills these plants as well. As mentioned previously grass seeds sometimes germinate in these circles but die before reaching

maturity and some other plant species from the Asteraceae family have also been observed inside the circles (personal observation). One would think that these plants would be able to overgrow the circles in the absence of competition from the grasses or other plants but that does not happen. Becker & Jürgens (2000) stated that the arid parts of Namibia are very species poor. It is thus possible that the plants that do grow inside the circles occur outside of the circles in the same frequency and that they do have the ability to grown inside the circles as well as outside but that they will not grow prolifically inside circles due to environmental factors such as severe heat and drought that affect them in the normal environment as well. No data exist which compare the distribution of other species inside and outside of the fairy circles.

Investigation of the grass roots from the bioassay experiment (Chapter 3) indicated that there was no VAM infection in any of the roots. It this is the case it raises questions regarding the difference in growth observed in the trials between seedlings grown in soil from the different microhabitats. If VAM were responsible for maintaining the fairy circles there should be no difference in growth in a pot experiment if the roots of the edge and matrix plants were not infected with mycorrhiza. In Marienfluss there was almost no infection except for one sample. This could be due to the harvesting time, as this happened very late in the season or due to sampling methods. More experiments need to be done to answer these questions regarding the influence of VAM on the overall growth of the plants and the influence that the mycorrhizae will have on seedling establishment. Although VAM is not species specific some species seem to have a preference for infecting a specific plant species (Lombaard, 1993) it would thus be interesting to investigate the possibility of the VAM being species specific.

4.9 Conclusion

The VAM hypothesis might be worth further experimentation. One must keep in mind that the lack of VAM will not explain the cause of the circles but only its maintenance. More studies need to be done to determine all the factors influencing the physiology of VAM in the area where the fairy circles occur. Very little has been done on the influence of VAM in the arid and semi-arid regions and our understanding of the importance of these organisms to help plants survive is not complete. A lot of questions arise with the preliminary VAM data and no definite conclusions can be made as such but a new avenue for investigation has been opened to characterise possibly the fairy circles better.

CHAPTER 5

SOIL ANALYSIS BY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

5.1 Introduction

Soil supports and provides nutrients for plants and thus influences every aspect of plant growth. Soil is also highly variable and complex (Page *et al*., 1982) both in chemistry and biology.

The type of analysis of soil done depends largely on the nature of the study and the answer needed for best interpretation. The analysis of soil ranges from total elemental analysis to the availability of a specific element in the soil. Other analyses include determining the biological content of the soil, including total count of microorganisms as well as algae, protozoa and nematodes.

5.2 Literature review

5.2.1 History of soil research related to the fairy circles

Eicker *et al*., (1982) did a microbiological investigation of the soil from the edge, matrix and inside of the circles. They described the soil of all three subhabitats as mainly coarse sand, with low clay content and very low water holding capacity. Moll (1994) analyzed the soil's major and trace

nutrients, as well as soil compaction and rate of water percolation. His results showed that the major and trace element contents were always higher in the soil from the inside than in the soil from the matrix although the concentration sometimes differed by as little as 1 ppm. He also showed that the compaction of soil inside the circles was a little more than that in the matrix and he mentioned that water percolated through the soil more slowly on the inside. He stated that termites cement soil particles together with saliva and it is this organic additive that inhibits percolation. According to Moll (1994) the accumulation of elements such as Ca, Mg and Na and a few trace elements together with the slow percolation are in accordance with termite activity.

Van Rooyen *et al.* (2004) also reported on chemical analysis of the soils and they showed that resistance of the soil was the only factor that correlated positively with the growth patterns in the different microhabitats, with the resistance being the highest inside the circle. They did mention however, that the differences were not statistically significant. They tested pH and the concentration of phosphorus, calcium, sodium, magnesium and nitrogen, and concluded that no trends could be seen in these factors to result in the marked response in plant growth.

5.2.2 Inductively coupled plasma mass spectrometry (ICP-MS) analysis of soil

Atmospheric pressure inductively coupled plasmas (ICPs) are flamelike electrical discharges that have revolutionized the practice of elemental and isotopic analyses (Montaser *et al*., 1998). There are many ICP-based methods and in all of the analyses the test sample is typically converted to an aerosol and transported into the plasma where a desolvation-vaporization-atomisation-excitation process occurs. The ICP-MS and ICP-AES (inductively coupled plasma atomic fluorescence spectrometry) are the most powerful methods for rapid, multi-element analysis (Montaser *et al*., 1998). A short description of how ICP-MS works is given in the book specifically written on the

method by Montaser *et al.* (1998) and is briefly described below. With ICP-MS the tailflame of the plasma is extracted into the low pressure interface containing both a sampler and a skimmer. The ions are then focused and transmitted to a mass analyser (from there the MS) via an ion lens system prior to detection by either an ion counter or an analogue detector (Figure 5.1).

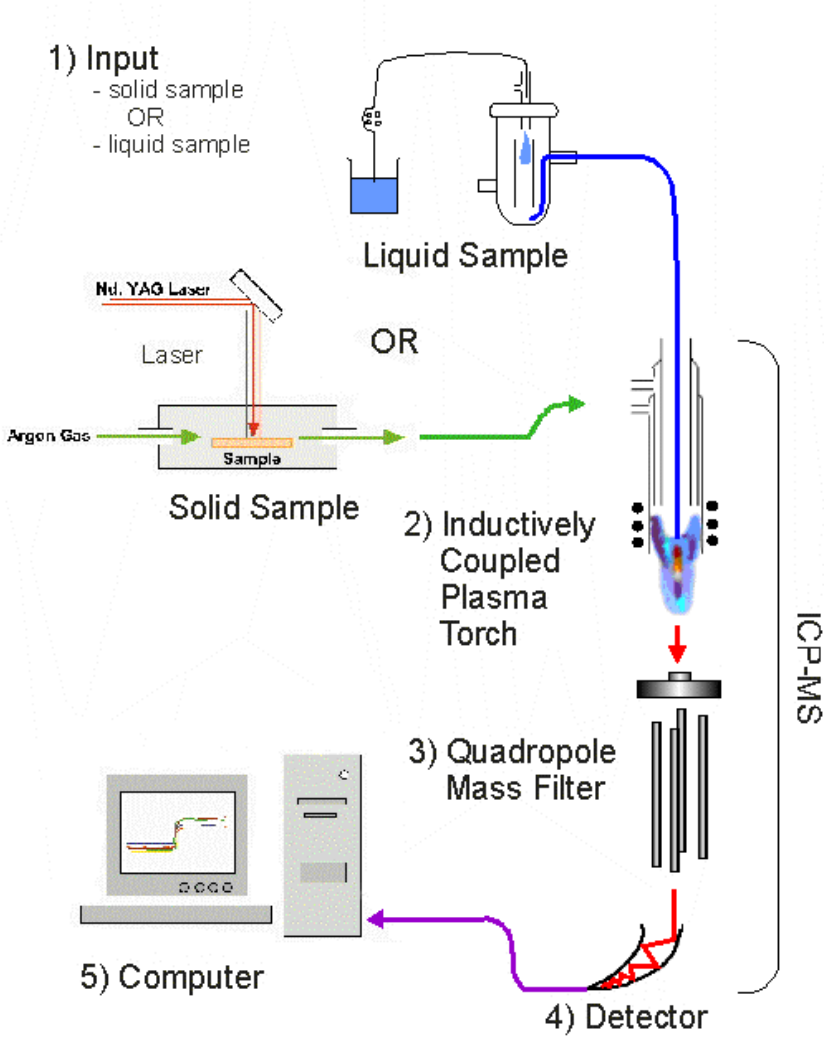

Figure 5.1. A schematic representation of an ICP-MS system (http://web.uvic.ca/~icpmslab).

The advantages of ICP-AES and ICP-MS, have led ICP to becoming the preferred method for the multi-element analysis of samples in solution (Sharp, 1991). The ICP is suitable for the determination of most elements in the periodic table, except the halides, the inert gases and atmospheric gases,

although by excluding air from the plasma, nitrogen may be determined (Sharp, 1991). ICP-MS has some distinct advantages.

- High sensitivity for most elements in the periodic table (except those mentioned above) covering the range 0.001 ng/ml to > 10 ng/ml.
- The ability to carry out isotope and isotope ratio determinations.
- Produces greatly simplified spectra compared with optical techniques.
- Fast quantitative analysis for the majority of the elements.

However, ICP-MS analysis also has some disadvantages, such as:

- Reduced sensitivity of some elements like calcium and iron because of isobaric interferences from the plasma gas.
- Restricted use of some acids and some solvents. Nitric acid is the preferred medium.
- The total dissolved content of the samples should be kept below 1 000 µg/ml to reduce clogging of the sample orifice.
- High cost per analysis.

Naturally there are other methods for determining the elemental composition of soil, but they are generally limited to specific elements and/or elemental availability to plants. The method of analysis depends greatly on the type of research being conducted and the specificity of the answer needed. ICP-MS seems to be the best choice to analyse the most elements in a sample in one analysis. Although a lot of elements are analysed with ICP-MS the analyses do not take into account factors such as pH and the form which the element occurs in inside the soil, thus disregarding the availability of the elements to the plants.

5.2.3 Acid digestion elemental analysis of soils by ICP-MS

For total element analysis of soil samples, all the elements must be dissolved. High salt content is not suitable for ICP-MS and all the problems associated with the fusion methods make the use of strong acids essential. Digestion with strong acids is thus used in ICP-MS analysis (Yamasaki, 1995). Many different acids can be used for digestion and acids are classified according to their major role in the digestion reaction. Non-oxidizing agents include hydrochloric, hydrobromic, hydrofluoric, phosphoric, dilute sulfuric and dilute perchloric acids and they dissolve metals with greater negative reduction potentials than hydrogen. Oxidizing acids include nitric acid, hot perchloric acid, concentrated sulfuric acid or mixtures of acids. Nitric acid is the most commonly acid used and will dissolve most metals. Its oxidizing strength can be increased by the addition of chlorate, permanganate, hydrogen peroxide or bromine. Gold and platinum are not oxidized by nitric acid and a mixture of acids will solve the problem. Hydrochloric acids dissolve some metals (Au, Cd, Fe, Sn) but dissolution of the metals is increased by addition of other acids. While hydrochloric acid reacts with most metals, it is frequently combined with a second acid. *Aqua regia*, a mixture of 3:1 hydrochloric and nitric acids reacts to form the following more reactive components:

$$3HCl + HNO_3 \rightarrow NOCl + Cl_2 + 2H_2O$$

This enables the mixture to dissolve several additional metals, including the noble metals not dissolved by the individual acids (Kingston & Walter, 1998).

5.3 Aim of this chapter

The aim of this research was to analyse the total elemental content of the soil samples to determine whether there are distinct differences in element composition between the microhabitats i.e. the inside of the fairy circles, the edge and the matrix.

5.4 Materials and methods

5.4.1 Soil collection

Soil samples were collected in Giribes Plains, Marienfluss, Amam (Bushmanland) and Keerweder where fairy circles occur (see distribution map, Chapter 1). At each site circles were selected at random and soil samples collected within the circles, on the edge of the circles and in the matrix near the chosen circle. Surface samples were collected up to a depth of 150 mm with a soil auger the dimension of 150 mm long x 70 mm. Soil samples collected at depths of 0.5 m and 1 m involved digging down to the desired depths respectively, after which samples of the same volume were collected using the soil auger. Samples were sealed in ziplock bags and stored at ambient temperatures for six days and then at 4°C until extraction.

As the circles in Bushmanland do not have the noticeable edge effect only samples from inside the circle and the matrix were collected. Soil samples in Bushmanland were collected at two different locations within three circles selected from each location. Samples one to six were collected in one location and samples seven to 12 in the other.

In Keerweder, the Giribes Plains and Marienfluss samples were collected by selecting three circles at random at each location. Soil samples were collected at three different depths, namely soil surface, 0.5 m and 1 m. In Keerweder, however, the majority of samples were collected on the surface, except for two samples (49 & 52) which were collected at a depth of 0.5 m. One sample (47) was collected in a circle which seems to be in the process of disappearing.

At Marienfluss, two samples were taken from a circle at a depth of 0.5 m (63 & 66). A further four samples were collected from a different circle at various depths (55-58) in the same location. Similar soil sampling was done on the Giribes Plains, however, additional samples were collected from the different microhabitats of one specific circle (i.e. inside, edge and matrix) at different places inside the circle and at various depths (Figure 5.2).

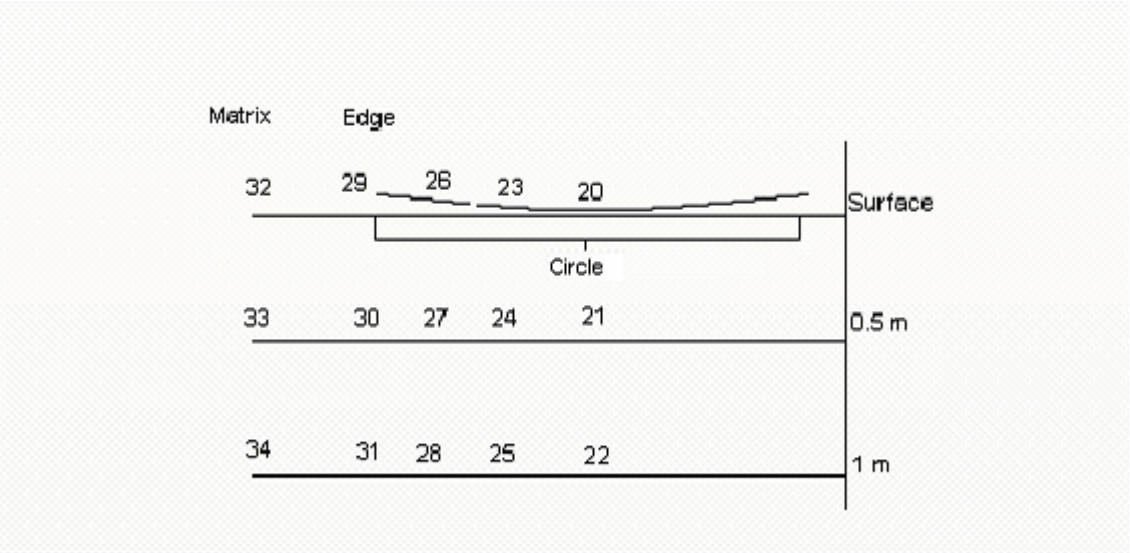

Figure 5.2. Collection of samples at different depths and at different places in one circle in the Giribes Plains. Each number represents a sample as presented in Figure 5.4 and 5.5.

5.4.2 Extraction

Soil was placed in Petri dishes in a drying oven at 80°C, until a constant mass was obtained. The dried soil was then accurately weighed and 15.0 g added to nitric acid: hydrochloric acid (3:1) for extraction (Kingston & Walter, 1998) for 36 hours. After extraction the acid was filtered under vacuum through a Whatman nr 42 filter paper and the filtrate made up to a total volume of 250 ml with distilled water. The extract was analysed by the Council of Geosciences (Pretoria, South Africa) using an ICP-MS (Perkin Elmer, Elan 6000).

5.4.3 Analysis of data

Analysis of the data was performed using the statistical program CANOCO version 4.5. The data were transformed using ln transformation before analysis. A detrended correspondence analysis (DCA) was performed to determine whether a principal component analysis (PCA) or a correspondence analysis (CA) should be performed. This is determined (Ter Braak & Smilauer, 2002) by looking at the length of the gradient in the DCA. If one of the axes is longer than four units, unimodal methods such as DCA or CA are recommended. If the length of the longest axis does not exceed three units PCA is recommended. In addition to the PCA the means and p-value for all the elements in the different microhabitats were determined along with the standard deviation for each location. The percentage difference for each element was also calculated using the matrix value as the standard.

5.5 Results

After performing the DCA on all data, it was found that a PCA would be sufficient. Consequently the data are presented as follows. For each region the PCA values of the length of the gradient of the first four axes is given together with the cumulative percentage variance of the element data (Figure 5.3-5.7). The Eigen values, as well as the sum of the Eigen values are given. The total number of elements were too numerous to add to the biplot of the PCA and it was thus decided to add only the 10 elements that have the most influence on the ordination to the plot, adding the significant inclusion percentage as an indication of the relevance of the added elements. The mean, standard deviation and p-value for all the elements at the different locations and in different microhabitats were calculated (Tables 5.1-5.5).

The PCA done on soil collected in Bushmanland did not show any discernable pattern (Figure 5.3). No distinction can be made between soil collected from the inside of the circle or from the matrix. Three samples collected on the inside (1, 3 & 5) of those circles and one collected in the matrix (2) are clearly separated from the other samples in the ordination. The other two samples collected in the first location (4 & 6) are grouped together with rest of samples, collected in the second location. On analysis of statistical significance of individual elements between the different microhabitats within a specific location (Table 5.1) only four elements showed a statistically significant difference. These elements are Lithium ($p = 0.01$), Niobium ($p = 0.002$), Tellurium ($p = 0.04$) and Tungsten ($p = 0.04$). These elements were quite different from the elements that were included in the PCA as the 10 most important elements for the specific biplot. There was no element in common for the PCA's 10 most relevant elements and the elements that were statistically significant different.

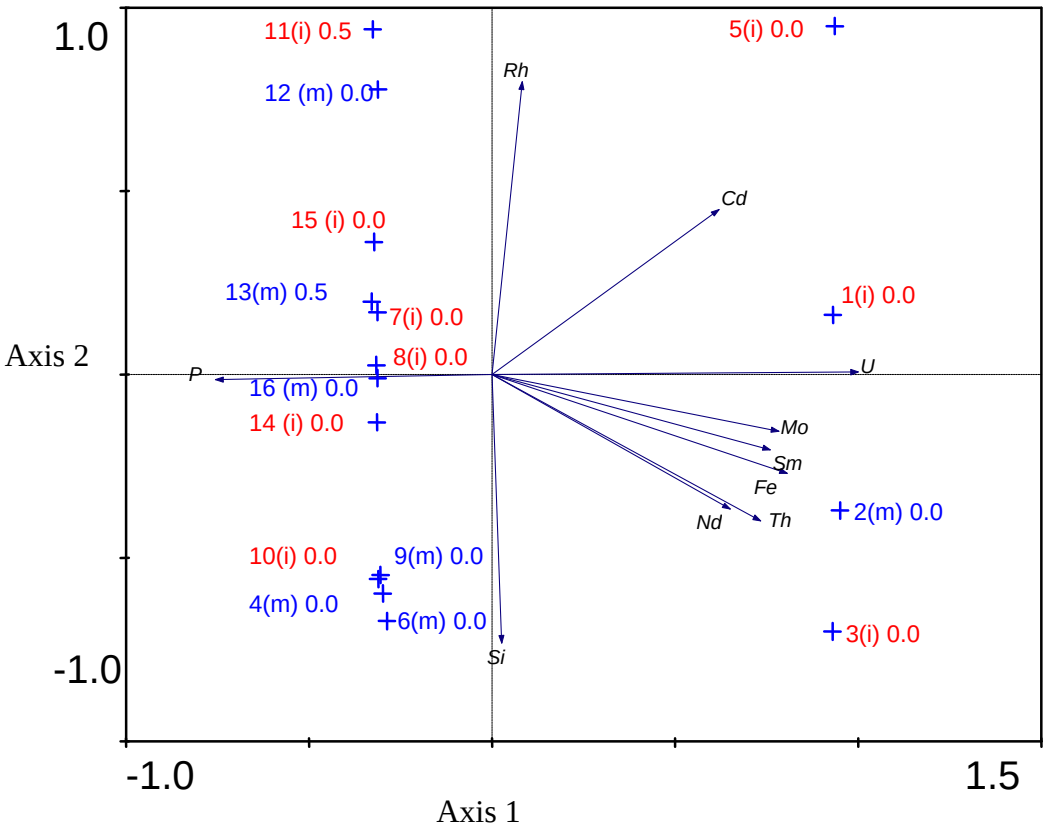

Figure 5.3. Principal component analysis biplot of samples and elements collected in Bushmanland and the 10 most important elements (relevance of inclusion set at 53%). Each sample is marked as follows: the sample number is given as well as the microhabitat where the sample was collected. The abbreviations are as follows: i = inside, m = matrix with the depth given in meters as a sample taken at 0.0 and 0.5 m.

PCA values

Axes	**1**	**2**	**3**	**4**
Eigen values	0.822	0.052	0.029	0.02
Sum of Eigen values	1			
Cumulative percentage variance of species data	82.2	87.4	90.2	92.3

Table 5.1. Mean and standard deviation for all elements from soil collected in Bushmanland broken down into the different microhabitats and given as a mean over all microhabitats in ppm. As only two samples were collected at a depth of 0.5 m they were omitted and results are given for surface samples only

Elements	Inside			Matrix			All	
	Mean	Std dev	Percentage*	Mean	Std dev	p -value	Mean	Std dev
Li	0.10	0.30	7.10	1.40	1.30	0.01	0.70	1.10
Be	8.30	2.20	105.10	7.90	1.90	0.65	8.10	2.00
B	97.40	16.40	103.90	93.70	15.00	0.65	95.80	15.40
Na	5151.40	356.10	94.70	5438.10	504.40	0.20	5276.90	437.00
Mg	19973.60	2447.20	93.30	21396.70	2472.00	0.27	20596.20	2483.90
Al	28508.80	2135.90	95.90	29723.70	3439.70	0.40	29040.30	2748.30
Si	2504.40	2333.70	114.20	2193.60	1165.50	0.75	2368.40	1863.70
P	4992.70	1165.80	91.10	5479.40	1275.70	0.44	5205.60	1199.20
K	11394.20	1062.30	93.40	12198.40	1530.10	0.23	11746.10	1306.90
Ca	23406.70	5584.50	82.40	28395.60	13157.80	0.32	25589.30	9613.40
Sc	12.40	1.30	102.50	12.10	1.20	0.65	12.30	1.30
Ti	1506.60	79.90	96.50	1560.70	82.60	0.21	1530.30	83.10
V	1004.70	79.10	96.50	1041.00	60.10	0.33	1020.60	71.60
Cr	288.90	23.60	94.10	307.00	13.50	0.09	296.80	21.40
Mn	2047.80	230.20	94.70	2162.90	349.50	0.44	2098.10	283.90
Fe	161000.70	33429.50	93.70	171772.60	29157.40	0.51	165713.40	31089.20
Co	49.80	6.30	95.80	52.00	5.70	0.48	50.80	6.00
Ni	180.70	95.20	124.00	145.70	31.10	0.37	165.40	74.50
Cu	247.20	237.30	155.50	159.00	30.00	0.35	208.60	180.10
Zn	598.80	135.30	104.20	574.40	86.10	0.69	588.10	113.50
Ga	28.60	3.90	95.70	29.90	2.30	0.45	29.10	3.30
Ge	1.60	0.50	114.30	1.40	0.50	0.64	1.50	0.50
As	269.90	23.80	98.00	275.30	13.10	0.60	272.30	19.40
Se	29.00	6.40	101.40	28.60	3.30	0.87	28.80	5.10
Br	2501.10	1291.50	108.60	2302.70	497.60	0.71	2414.30	999.50
Rb	53.90	3.70	98.50	54.70	4.90	0.70	54.30	4.10
Sr	146.00	13.20	91.50	159.60	31.30	0.26	151.90	23.10
Y	271.80	23.30	98.30	276.60	18.70	0.66	273.90	20.80
Zr	29.80	5.20	103.10	28.90	4.50	0.72	29.40	4.80
Nb	10.30	0.70	86.60	11.90	0.90	0.00	11.00	1.10
Mo	8.20	3.30	103.80	7.90	2.10	0.80	8.10	2.80
Ru	0.10	0.30		0.00	0.00	0.40	0.10	0.30
Rh	0.90	1.50	100.00	0.90	2.30	0.97	0.90	1.80
Pd	0.30	1.00		0.00	0.00	0.40	0.20	0.80
Ag	1.30	0.50	76.50	1.70	0.50	0.15	1.50	0.50
Cd	0.60	1.30		0.00	0.00	0.29	0.30	1.00
Sn	21.00	12.40	96.80	21.70	11.10	0.91	21.30	11.50
Sb	1.20	0.40	120.00	1.00	0.00	0.21	1.10	0.30
Te	0.40	0.50		0.00	0.00	0.04	0.30	0.40
I	3.80	2.00	88.40	4.30	3.00	0.69	4.00	2.40
Cs	3.30	0.50	97.10	3.40	0.50	0.72	3.40	0.50
Ba	861.80	75.10	98.40	876.10	138.70	0.79	868.10	103.70
La	788.90	55.40	96.40	818.10	50.40	0.29	801.70	53.70
Ce	1334.40	120.80	96.90	1377.40	99.30	0.46	1353.30	110.50
Pr	188.90	14.10	97.00	194.70	13.70	0.42	191.40	13.80
Nd	674.00	61.80	97.10	694.10	41.10	0.47	682.80	53.10

Elements	Inside		Matrix				All	
	Mean	Std dev	Percentage*	Mean	Std dev	p -value	Mean	Std dev
Sm	112.30	12.60	99.40	113.00	6.80	0.90	112.60	10.20
Eu	12.00	0.70	99.20	12.10	0.90	0.73	12.10	0.80
Gd	102.20	11.00	97.10	105.30	8.20	0.55	103.60	9.70
Tb	13.30	1.20	97.10	13.70	0.80	0.48	13.50	1.00
Dy	68.70	6.80	96.90	70.90	5.90	0.51	69.60	6.30
Ho	12.10	1.40	96.00	12.60	1.00	0.46	12.30	1.20
Er	31.10	3.20	99.00	31.40	2.10	0.82	31.30	2.70
Tm	4.20	0.40	102.40	4.10	0.40	0.71	4.20	0.40
Yb	25.20	1.90	100.80	25.00	2.40	0.84	25.10	2.10
Lu	3.60	0.50	100.00	3.60	0.50	0.95	3.60	0.50
Hf	1.00	0.00	111.10	0.90	0.40	0.27	0.90	0.30
Ta	1.00	0.00	100.00	1.00	0.00		1.00	0.00
W	9.30	0.90	90.30	10.30	0.80	0.04	9.80	0.90
Re	0.00	0.00		0.00	0.00		0.00	0.00
Os	0.00	0.00		0.00	0.00		0.00	0.00
Ir	0.00	0.00		0.00	0.00		0.00	0.00
Pt	0.30	0.50	100.00	0.30	0.50	0.85	0.30	0.50
Au	0.00	0.00		0.00	0.00		0.00	0.00
Hg	0.40	0.70	57.10	0.70	1.10	0.57	0.60	0.90
Tl	0.00	0.00	0.00	0.10	0.40	0.27	0.10	0.30
Pb	175.90	23.00	95.10	184.90	13.40	0.38	179.80	19.40
Bi	3.30	0.50	91.70	3.60	0.50	0.37	3.40	0.50
Th	6968.10	1927.10	97.90	7114.10	2187.30	0.89	7032.00	1974.80
U	1104.40	1657.90	193.20	571.60	1512.20	0.52	871.30	1566.90

* Percentages calculated using the matrix as the control

In the first PCA done for the soil collected in the Giribes Plains all samples were used (Figure 5.4). Samples from the inside of the circle collected at different depths (21-28) grouped together relatively well except for sample numbers 20, 37 and 40 which was also collected inside the circle on the surface. The rest of the samples did not show any pattern as the samples collected on the edge of the circles (29-31, 36 & 39) and in the matrix (32-35 & 38) did not group together.

Another PCA was performed using the samples collected at different depths for only one circle (Figure 5.2 & 5.5). The samples collected on the inside of the circle (21-28) grouped together relatively well with sample number 20 once again not being part of this grouping of soils collected from inside the circle. The only element common to both the analyses for the Giribes Plains was Gallium (Ga).

The analysis to see the statistical difference of individual elements between different microhabitats showed that there were quite a few elements that differed between the different microhabitats when all the samples collected in the Giribes Plains were analyzed (Table 5.2). These elements are Iridium ($p = 0.02$), Barium, Holmium and Terbium ($p = 0.03$), Germanium, Tungsten and Mercury ($p = 0.04$) and Gallium, Silver, Europium and Thulium ($p = 0.05$). The differences were always significant between samples collected from the inside and the matrix with the edge samples having no effect on the significant difference. The analysis of the samples collected on the surface (Table 5.3) showed that there was only a significant difference between the different microhabitats in two elements, namely Nickel ($p = 0.05$) and Palladium ($p = 0.03$).

It would seem that the depth at which the samples were collected had an influence as the analysis of the elements collected in one circle at different depths (Table 5.4) also showed a few elements where there was a significant difference between elements in samples collected from the different microhabitats. In this case Barium and Magnesium ($p = 0.01$), Palladium and Cadmium ($p = 0.002$) and Tin (0.05) was the only elements that had any significant difference for samples collected at a depth of one meter. At a depth of 0.5 meter a different set of elements showed significant differences, these were Arsenic ($p = 0.01$), Lead and Tin ($p = 0.02$), Iodine ($p = 0.03$) and Gallium ($p = 0.05$).

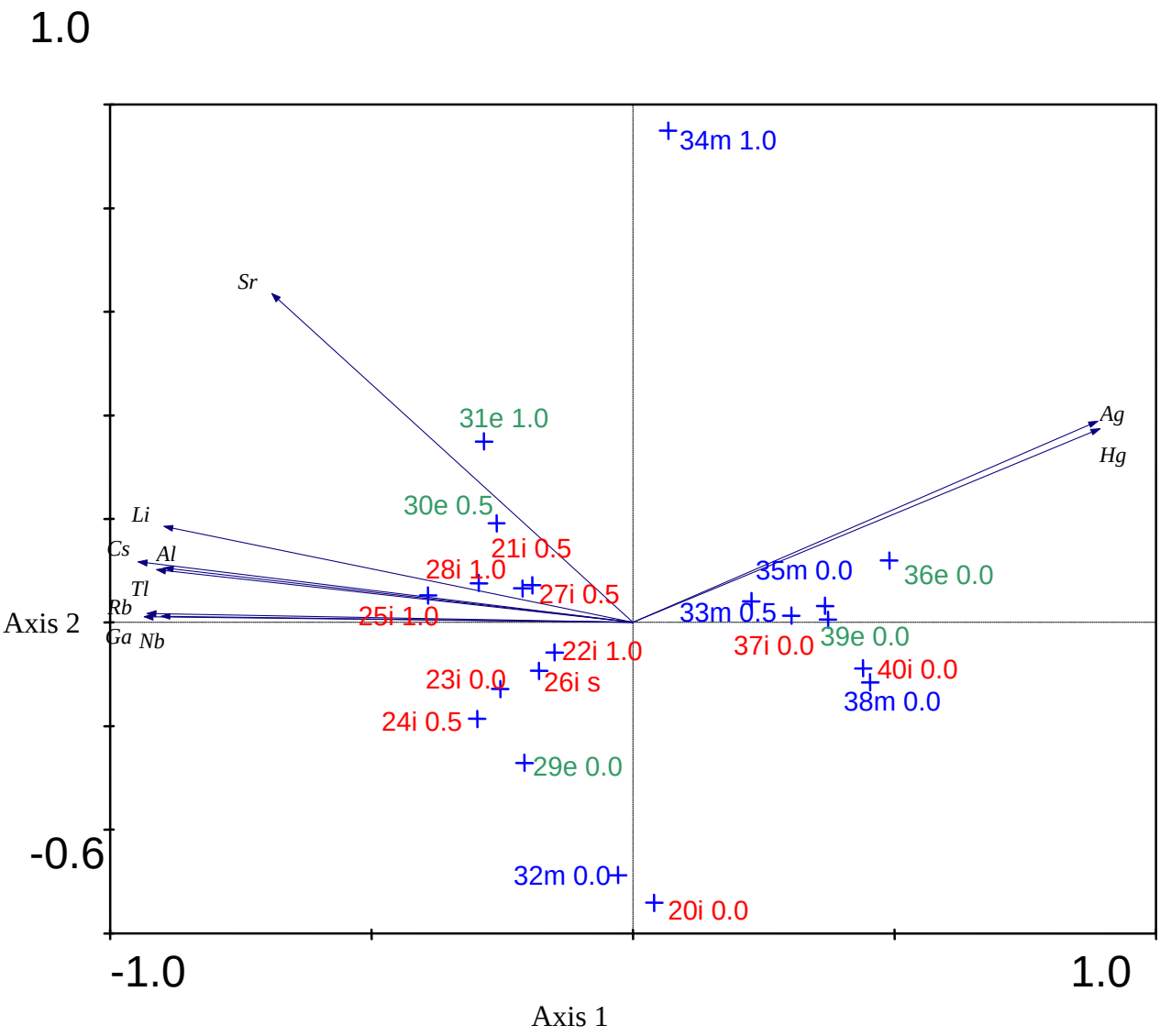

Figure 5.4. Principal component analysis biplot of all samples and elements collected in the Giribes Plains and the 10 most important elements relevance of inclusion set at 81%. The legend of samples is as follows: The sample number, i= inside, m = matrix and e = edge with the depths given as 0.0 = surface, 0.5 = 0.5 m and 1.0 = 1.0 m (e.g. 35 m 0.0 = sample number 35 collected in the matrix on the surface).

PCA values

Axes	**1**	**2**	**3**	**4**
Eigen values	0.388	0.19	0.159	0.105
Sum of Eigen values	1			
Cumulative percentage variance of species data	38.8	57.8	73.7	84.1

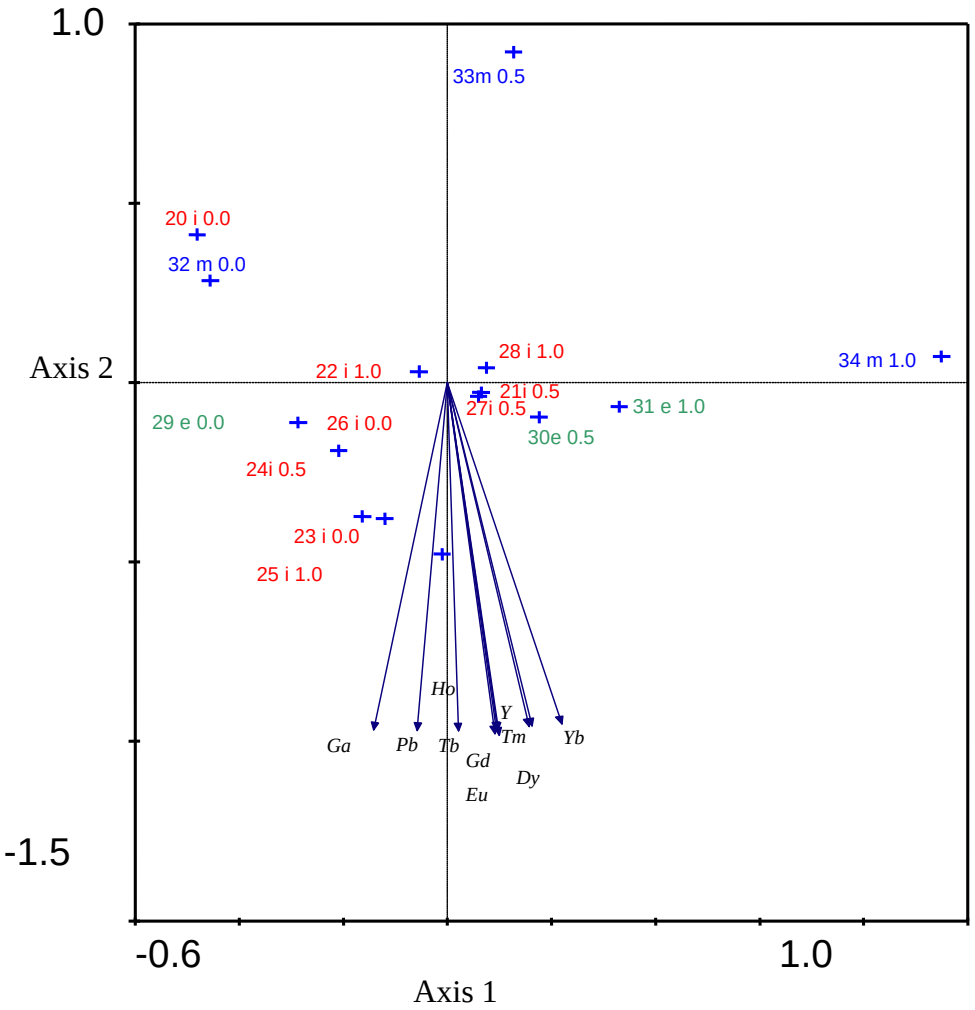

Figure 5.5. Principal component analysis of samples and elements collected in the Giribes Plains in one fairy circle and the surrounding edge and matrix at different places and at different depths in the circle (see Figure 5.2). Relevance of inclusion of the 10 most important elements set at 94%. The samples number, i= inside, m = matrix and e = edge with the depths given as 0.0 = surface, 0.5 = 0.5m and 1.0 = 1.0 m (e.g. 35 m 0.0 = sample number 35 collected in the matrix on the surface).

PCA values

Axes	**1**	**2**	**3**	**4**
Eigen values	0.355	0.238	0.187	0.073
Sum of Eigen values	1			
Cumulative percentage variance of species data	35.5	59.3	78	85.3

The PCA in Keerweder (Figure 5.6) once again showed no groupings for samples collected from one specific microhabitat or at a specific depth. Only one element (Palladium, p = 0.04) showed any statistically significant difference (Table 5.5).

In Marienfluss (Figure 5.7) a clear distinction could be seen in samples collected at the different locations as the samples collected for the one circle (55-58) grouped together. All the other samples, except for one (68) collected from the three different circles grouped together. The analysis of the statistically significant differences between the different microhabitats showed that there were a lot of elements that differed significantly in concentration between the different microhabitats when all the samples were analysed. There was however no significant difference when only surface samples were analysed (Table 5.6). The elements that showed significant differences between the different microhabitats in the analysis of all the samples were Barium, Scandium and Iron (p = 0.01), Titanium, Zirconium and Molybdenum (p = 0.03), Europium, Beryllium and Aluminium (p = 0.04) and Vanadium, Cobalt and Gallium (p = 0.05). None of these elements were present in the PCA where only the 10 most important elements were included.

Table 5.2. Mean and standard deviation for all elements from soil collected in the Giribes Plains broken down into the different microhabitats and given as a mean over all microhabitats for all depths in ppm

	Inside			Edge			Matrix		All		
Elements	Mean All depths	Std dev	Percentage*	Mean	Std dev	Percentage*	Mean	Std dev	Mean	Std dev	p-value
Li	302.70	103.80	127.50	293.00	115.50	123.40	237.40	92.30	284.90	102.50	0.51
Be	15.40	5.40	120.30	15.40	3.90	120.30	12.80	8.20	14.80	5.70	0.70
B	304.60	66.50	70.40	332.80	77.90	77.00	432.40	107.80	341.80	92.60	0.03
Na	6917.10	7440.30	86.70	7258.80	4487.10	91.00	7974.40	8613.30	7250.20	6836.40	0.96
Mg	202977.00	66331.20	131.50	181970.40	58690.00	117.90	154358.20	60725.70	186399.50	63553.50	0.38
Al	267620.10	86801.00	137.90	239270.60	72999.70	123.30	194131.60	64234.40	243373.00	81190.80	0.25
Si	9054.30	10219.30	178.80	4903.60	1390.60	96.90	5062.60	1704.70	7115.60	7584.80	0.49
P	32363.70	8716.90	155.20	30884.20	8981.90	148.10	20855.00	6690.00	29271.30	9311.10	0.06
K	109568.50	30610.10	132.80	105338.40	28487.10	127.70	82494.20	18444.60	102115.00	28779.00	0.22
Ca	446021.50	508081.10	109.30	517215.40	613844.00	126.70	408091.40	518937.10	453941.40	509758.90	0.95
Sc	73.70	17.40	102.60	70.40	7.90	98.10	71.80	13.30	72.50	14.20	0.91
Ti	15481.60	5670.30	147.80	13566.80	5697.10	129.50	10473.60	4272.30	13833.30	5527.40	0.25
V	2595.00	658.20	129.70	2324.20	481.80	116.10	2001.20	561.10	2389.10	622.90	0.21
Cr	1352.30	272.90	112.80	1308.80	159.70	109.10	1199.20	258.50	1305.50	244.40	0.53
Mn	9682.60	2112.20	131.30	9360.20	1930.60	127.00	7372.40	819.10	9055.80	2014.50	0.09
Fe	594624.40	173668.80	141.20	524471.40	148787.30	124.50	421186.60	115621.80	536626.60	165530.20	0.15
Co	372.50	98.30	137.50	300.60	79.80	110.90	271.00	116.10	331.20	104.30	0.15
Ni	722.30	281.90	131.20	600.20	96.20	109.00	550.40	171.60	652.30	231.20	0.34
Cu	1033.20	315.90	131.00	853.00	288.10	108.20	788.60	487.40	932.00	355.40	0.40
Zn	1497.60	448.40	138.40	1545.60	363.80	142.80	1082.40	269.80	1410.20	420.90	0.13
Ga	130.10	41.90	166.40	111.40	38.20	142.50	78.20	26.10	113.30	42.00	0.06
Ge	3.70	0.80	142.30	3.40	0.50	130.80	2.60	0.90	3.40	0.90	0.04
As	308.30	72.10	142.20	283.60	47.90	130.80	216.80	49.70	280.60	70.70	0.05
Se	49.10	17.30	83.80	53.80	24.50	91.80	58.60	27.30	52.50	20.80	0.71
Br	4495.70	2749.40	72.40	6169.80	3764.70	99.30	6213.80	2927.70	5303.40	3013.70	0.46
Rb	721.70	243.40	155.10	651.60	245.90	140.10	465.20	159.60	644.00	241.10	0.14
Sr	1208.50	786.10	113.20	1453.40	1091.30	136.10	1068.00	975.70	1233.40	869.90	0.79
Y	304.50	74.00	141.90	282.80	44.90	131.80	214.60	54.20	277.90	71.60	0.06
Zr	94.80	59.80	168.70	59.60	30.80	106.00	56.20	26.00	77.20	49.70	0.24
Nb	21.10	6.00	150.70	18.60	6.60	132.90	14.00	5.30	18.80	6.40	0.12
Mo	25.70	9.70	88.60	30.20	11.80	104.10	29.00	11.50	27.60	10.30	0.70
Ru	0.10	0.30		0.00	0.00		0.00	0.00	0.00	0.20	0.66
Rh	0.20	0.40	16.70	2.80	6.30	233.30	1.20	2.20	1.00	3.20	0.32
Pd	3.70	1.70	57.80	3.60	1.10	56.30	6.40	6.50	4.30	3.40	0.31
Ag	0.40	0.80	22.20	0.80	1.10	44.40	1.80	1.30	0.80	1.10	0.05
Cd	4.30	3.60	5.80	6.60	3.90	8.90	73.80	155.60	21.40	75.80	0.22
Sn	52.80	25.80	161.00	140.60	145.60	428.70	32.80	14.50	69.00	79.80	0.06
Sb	2.50	0.90	125.00	2.40	0.50	120.00	2.00	0.70	2.30	0.80	0.58
Te	0.50	1.00	62.50	0.60	1.30	75.00	0.80	1.10	0.60	1.10	0.92
I	28.20	34.10	116.50	35.40	34.70	146.30	24.20	26.80	29.00	31.30	0.86
Cs	35.50	12.20	161.40	29.40	11.40	133.60	22.00	10.00	30.80	12.30	0.12
Ba	3283.10	801.90	144.00	3164.00	546.50	138.80	2280.00	800.90	3015.90	830.50	0.07
La	1070.50	378.90	159.10	912.00	126.90	135.50	673.00	282.90	938.10	344.10	0.09
Ce	2024.30	772.60	162.00	1641.60	316.00	131.40	1249.40	484.30	1748.70	687.20	0.10
Pr	205.90	65.10	152.70	177.60	23.40	131.80	134.80	49.40	182.20	59.90	0.08
Nd	716.40	213.50	151.60	644.40	91.70	136.40	472.60	169.80	641.20	201.10	0.07
Sm	106.90	27.60	152.70	96.00	14.60	137.10	70.00	22.90	95.50	27.60	0.04
Eu	17.50	4.10	143.40	16.00	2.20	131.10	12.20	3.80	15.90	4.10	0.05
Gd	111.40	29.20	145.80	99.60	19.40	130.40	76.40	25.70	100.20	29.00	0.08
Tb	12.50	3.00	148.80	11.40	1.30	135.70	8.40	2.70	11.30	3.10	0.03

	Inside			Edge			Matrix		All		
Elements	Mean	Std dev	Percentage*	Mean	Std dev	Percentage*	Mean	Std dev	Mean	Std dev	p-value
	All depths										
Dy	56.10	14.20	141.00	51.80	7.50	130.20	39.80	12.40	51.20	13.80	0.08
Ho	9.10	2.10	146.80	8.80	1.10	141.90	6.20	1.90	8.30	2.20	0.03
Er	22.40	5.50	136.60	21.00	2.90	128.00	16.40	4.40	20.60	5.20	0.10
Tm	2.90	0.70	145.00	2.80	0.40	140.00	2.00	0.70	2.70	0.70	0.05
Yb	17.10	4.00	137.90	16.20	3.10	130.60	12.40	3.40	15.80	4.00	0.09
Lu	2.60	0.70	130.00	2.40	0.50	120.00	2.00	0.70	2.40	0.70	0.22
Hf	1.50	0.80	250.00	0.80	0.40	133.30	0.60	0.50	1.10	0.80	0.07
Ta	0.00	0.00		0.00	0.00		0.00	0.00	0.00	0.00	
W	6.60	1.00	126.90	6.00	0.70	115.40	5.20	1.10	6.10	1.10	0.04
Re	0.00	0.00		0.00	0.00		0.00	0.00	0.00	0.00	
Os	0.00	0.00		0.00	0.00		0.00	0.00	0.00	0.00	
Ir	2.60	1.60	260.00	1.00	0.00	100.00	1.00	0.00	1.90	1.40	0.02
Pt	1.70	0.60	77.30	2.00	1.00	90.90	2.20	0.80	1.90	0.80	0.52
Au	0.00	0.00		0.00	0.00		0.00	0.00	0.00	0.00	
Hg	1.60	3.60	9.10	3.60	4.90	20.50	17.60	22.10	5.90	12.40	0.04
Tl	5.00	1.70	166.70	4.20	2.00	140.00	3.00	1.40	4.30	1.80	0.12
Pb	273.30	62.60	145.10	259.60	51.90	137.80	188.40	59.90	249.80	67.00	0.05
Bi	6.50	1.50	61.30	1448.00	3223.30		10.60	8.80	350.70	1572.60	0.21
Th	243.80	110.50	151.10	177.60	45.60	110.00	161.40	51.90	208.40	92.40	0.18
U	21.80	6.30	122.50	19.60	6.30	110.10	17.80	9.50	20.30	7.00	0.57

* Percentages calculated using the matrix as the control

Table 5.3. Mean and standard deviation for all elements from soil collected on the surface only of the microhabitats in the Giribes Plains. Values given in ppm

Elements	Inside			Edge			Matrix		
	Mean	Std dev	Percentage*	Mean	Std dev	Percentage*	Mean	Std dev	p-value
Li	243.20	116.00	133.80	242.00	129.70	133.20	181.70	71.00	0.73
Be	12.80	5.50	182.90	15.30	5.50	218.60	7.00	2.60	0.16
B	325.80	88.60	85.80	378.70	56.80	99.70	379.70	93.80	0.59
Na	4183.00	1022.30	145.30	4480.70	726.80	155.60	2879.00	319.80	0.09
Mg	169980.20	81146.10	146.30	161522.70	72916.60	139.10	116149.70	43576.30	0.59
Al	226817.80	103157.40	144.60	214092.00	90975.90	136.50	156819.30	53974.70	0.58
Si	10188.00	10470.40	184.50	5776.00	921.60	104.60	5520.70	2121.00	0.62
P	28418.20	10688.00	140.30	28234.00	7921.70	139.40	20248.30	4377.00	0.44
K	101754.60	39018.70	132.30	103990.30	39863.50	135.20	76928.30	20789.80	0.59
Ca	111446.00	31966.00	121.40	127252.30	28059.20	138.60	91784.30	8074.50	0.32
Sc	75.60	19.40	120.60	75.00	6.20	119.60	62.70	5.10	0.46
Ti	13656.60	7575.00	162.20	12515.00	7776.60	148.70	8417.30	4535.00	0.60
V	2205.80	725.20	136.60	2093.00	511.20	129.60	1615.00	265.00	0.42
Cr	1261.00	295.10	124.60	1326.00	217.50	131.10	1011.70	21.10	0.27
Mn	10079.40	2783.60	131.90	9910.00	2463.90	129.70	7639.70	937.60	0.38
Fe	545188.00	230902.60	146.10	499701.70	203456.70	133.90	373203.00	118919.70	0.53
Co	333.20	127.40	148.90	267.70	89.70	119.70	223.70	60.70	0.39
Ni	581.00	88.20	132.30	602.30	65.70	137.20	439.00	53.40	0.05
Cu	870.80	345.40	149.50	777.70	379.00	133.60	582.30	217.20	0.51
Zn	1386.80	331.80	136.40	1659.30	464.70	163.20	1016.70	73.80	0.12
Ga	113.00	54.70	154.80	98.00	47.10	134.20	73.00	27.80	0.54
Ge	3.60	0.90	156.50	3.70	0.60	160.90	2.30	0.60	0.09
As	261.80	66.60	135.20	253.70	34.80	131.00	193.70	19.80	0.23
Se	50.80	20.20	108.80	60.70	22.70	130.00	46.70	26.10	0.74
Br	5449.60	3823.30	88.70	7746.00	4315.10	126.10	6144.00	3913.00	0.74
Rb	612.20	299.10	147.60	570.70	308.90	137.60	414.70	187.40	0.63
Sr	646.20	218.90	127.30	736.70	147.50	145.10	507.70	56.10	0.32
Y	278.60	94.00	140.70	260.00	39.40	131.30	198.00	34.50	0.34

Elements	Inside			Edge			Matrix		
	Mean	Std dev	Percentage*	Mean	Std dev	Percentage*	Mean	Std dev	p-value
Zr	134.40	67.90	201.50	76.30	29.00	114.40	66.70	29.70	0.20
Nb	18.40	8.00	157.30	17.00	8.70	145.30	11.70	5.50	0.50
Mo	31.40	6.50	101.30	36.70	10.10	118.40	31.00	11.30	0.68
Ru	0.00	0.00		0.00	0.00		0.00	0.00	
Rh	0.40	0.50		4.70	8.10		0.00	0.00	0.32
Pd	5.40	0.90	145.90	4.30	0.60	116.20	3.70	0.60	0.03
Ag	0.80	1.10	47.10	1.30	1.20	76.50	1.70	1.50	0.63
Cd	6.20	3.60	124.00	6.30	5.50	126.00	5.00	4.40	0.91
Sn	34.20	12.40	126.70	39.30	12.50	145.60	27.00	10.60	0.48
Sb	2.00	1.00	100.00	2.30	0.60	115.00	2.00	0.00	0.82
Te	1.00	1.40	142.90	1.00	1.70	142.90	0.70	1.20	0.94
I	8.40	3.20	70.00	14.30	3.10	119.20	12.00	1.70	0.06
Cs	27.60	12.50	145.30	23.70	11.60	124.70	19.00	8.70	0.61
Ba	2944.80	930.20	127.90	3063.00	746.60	133.00	2302.30	336.70	0.45
La	1134.20	559.80	172.10	901.30	163.50	136.80	659.00	109.00	0.33
Ce	2176.60	1136.40	176.20	1665.70	379.40	134.90	1235.00	222.80	0.34
Pr	213.20	95.90	159.10	176.70	30.80	131.90	134.00	19.70	0.35
Nd	734.40	314.30	157.00	640.70	125.70	137.00	467.70	68.30	0.34
Sm	106.20	39.50	152.40	96.00	20.00	137.70	69.70	11.10	0.30
Eu	16.60	5.60	141.90	15.30	2.90	130.80	11.70	1.50	0.33
Gd	109.40	41.60	150.50	94.00	25.20	129.30	72.70	10.50	0.35
Tb	12.20	4.40	147.00	11.00	1.70	132.50	8.30	1.50	0.33
Dy	54.20	19.30	142.60	48.70	8.00	128.20	38.00	5.00	0.36
Ho	8.20	2.60	136.70	8.30	1.20	138.30	6.00	1.00	0.30
Er	20.40	6.10	129.90	19.30	2.10	122.90	15.70	2.50	0.40
Tm	2.60	0.90	130.00	2.70	0.60	135.00	2.00	0.00	0.45
Yb	15.80	5.40	139.80	15.00	3.60	132.70	11.30	1.50	0.39
Lu	2.40	0.50	120.00	2.30	0.60	115.00	2.00	0.00	0.54
Hf	2.00	1.00	285.70	1.00	0.00	142.90	0.70	0.60	0.09
Ta	0.00	0.00		0.00	0.00		0.00	0.00	
W	6.40	1.10	136.20	6.00	1.00	127.70	4.70	0.60	0.11
Re	0.00	0.00		0.00	0.00		0.00	0.00	
Os	0.00	0.00		0.00	0.00		0.00	0.00	
Ir	2.60	2.10	260.00	1.00	0.00	100.00	1.00	0.00	0.26
Pt	2.00	0.70	100.00	2.70	0.60	135.00	2.00	1.00	0.47
Au	0.00	0.00		0.00	0.00		0.00	0.00	
Hg	3.60	4.90	16.90	6.00	5.20	28.20	21.30	30.30	0.33
Tl	4.40	2.10	163.00	3.30	2.30	122.20	2.70	1.20	0.49
Pb	259.40	82.30	137.00	258.00	70.50	136.30	189.30	30.40	0.38
Bi	6.40	1.10	42.70	2409.70	4160.70	16064.70	15.00	9.20	0.29
Th	274.60	159.80	206.00	176.70	52.70	132.60	133.30	45.70	0.28
U	18.80	8.20	160.70	16.00	5.60	136.80	11.70	3.80	0.39

* Percentages calculated using the matrix as the control

Table 5.4. Mean and standard deviation for all elements from soil collected in the Giribes Plains broken down into the different microhabitats and given as a mean over all microhabitats for the depths of 0.5m and 1m in ppm. For samples collected in the edge and the matrix only one sample was collected at a depth of 1.0m and 0.5m, thus no standard deviations are given

	0.5m							1m						
Elements	Inside			Edge		Matrix		Inside			Edge		Matrix	
	Mean	Std dev	Percentage*	Mean	Percentage*	Mean	p-value	Mean	Std dev	Percentage*	Mean	Percentage*	Mean	p-value
Li	366.70	42.80	121.40	359.00	121.40	302.00	0.53	338.00	90.80	99.40	380.00	111.80	340.00	0.92
Be	16.70	2.50	72.60	16.00	72.60	23.00	0.27	18.30	6.70	91.50	15.00	75.00	20.00	0.87
B	304.70	53.80	68.00	296.00	68.00	448.00	0.25	269.30	22.10	46.80	232.00	40.30	575.00	0.01
Na	13512.70	13535.90	159.30	8140.00	159.30	8482.00	0.92	4878.30	1143.30	21.40	14712.00	64.70	22753.00	0.01
Mg	240450.00	23735.50	114.30	210567.00	114.30	210356.00	0.51	220498.70	54566.70	103.50	214717.00	100.80	212986.00	0.99
Al	316439.00	30052.80	132.30	279024.00	132.30	239234.00	0.28	286805.00	84834.70	109.90	275053.00	105.40	260966.00	0.96
Si	4114.70	618.90	112.70	4000.00	112.70	3650.00	0.83	12104.30	15772.10	237.30	3190.00	62.50	5101.00	0.86
P	32824.30	3936.70	243.90	26359.00	243.90	13460.00	0.10	38479.00	6373.50	128.00	43360.00	144.20	30070.00	0.47
K	122464.30	9267.50	154.30	112567.00	154.30	79353.00	0.11	109695.70	33940.80	107.20	102154.00	99.80	102333.00	0.97
Ca	391646.00	48730.20	81.90	674896.00	81.90	478401.00	0.07	1058022.70	663482.20	82.20	1529424.00	118.90	1286703.00	0.83
Sc	73.70	5.50	89.90	66.00	89.90	82.00	0.32	70.70	26.50	79.40	61.00	68.50	89.00	0.77
Ti	17925.00	1346.40	129.50	15683.00	129.50	13847.00	0.21	16080.00	5134.80	121.20	14606.00	110.10	13269.00	0.89
V	3027.00	160.00	117.00	2720.00	117.00	2587.00	0.23	2811.70	599.90	109.20	2622.00	101.90	2574.00	0.93
Cr	1468.00	111.40	101.70	1334.00	101.70	1444.00	0.65	1388.70	377.90	91.50	1232.00	81.20	1517.00	0.87
Mn	9612.00	545.50	147.20	9036.00	147.20	6530.00	0.08	9092.00	2363.90	122.60	8035.00	108.40	7413.00	0.82
Fe	672073.30	57679.90	156.20	585707.00	156.20	430227.00	0.13	599569.30	160316.00	107.80	537545.00	96.70	556097.00	0.94
Co	408.70	39.30	185.80	375.00	185.80	220.00	0.10	402.00	84.70	86.60	325.00	70.00	464.00	0.60
Ni	765.00	93.60	123.40	716.00	123.40	620.00	0.52	915.00	514.60	112.30	478.00	58.70	815.00	0.79
Cu	1166.30	106.60	201.10	999.00	201.10	580.00	0.08	1170.70	357.70	72.40	933.00	57.70	1616.00	0.51
Zn	1364.30	132.60	164.60	1361.00	164.60	829.00	0.13	1815.70	747.30	118.40	1389.00	90.60	1533.00	0.87
Ga	151.30	12.70	236.40	137.00	236.40	64.00	0.05	137.30	33.50	127.10	126.00	116.70	108.00	0.77
Ge	3.70	0.60	185.00	3.00	185.00	2.00	0.24	4.00	1.00	100.00	3.00	75.00	4.00	0.71
As	351.00	10.60	174.60	324.00	174.60	201.00	0.01	343.00	83.90	113.60	333.00	110.30	302.00	0.92
Se	47.00	16.60	78.30	21.00	78.30	60.00	0.41	48.30	19.50	51.90	66.00	71.00	93.00	0.33
Br	3946.70	1188.40	79.40	3166.00	79.40	4972.00	0.63	3455.00	1672.70	45.10	4445.00	58.00	7665.00	0.30
Rb	862.30	73.00	186.60	804.00	186.60	462.00	0.08	763.70	222.90	123.20	742.00	119.70	620.00	0.86
Sr	1201.70	145.60	113.60	1870.00	113.60	1058.00	0.09	2152.70	919.00	78.00	3187.00	115.50	2759.00	0.65
Y	326.70	28.90	182.50	294.00	182.50	179.00	0.09	325.30	76.30	108.40	340.00	113.30	300.00	0.93
Zr	73.30	27.50	152.70	35.00	152.70	48.00	0.54	50.30	18.10	152.40	34.00	103.00	33.00	0.66
Nb	24.00	1.70	160.00	20.00	160.00	15.00	0.09	22.70	4.00	113.50	22.00	110.00	20.00	0.86
Mo	23.30	11.70	155.30	25.00	155.30	15.00	0.81	18.70	9.30	50.50	16.00	43.20	37.00	0.37
Ru	0.00	0.00		0.00		0.00		0.30	0.60		0.00		0.00	0.83
Rh	0.00	0.00	0.00	0.00	0.00	5.00		0.00	0.00	0.00	0.00	0.00	1.00	
Pd	2.00	0.00	66.70	3.00	66.70	3.00		2.70	0.60	15.00	2.00	11.10	18.00	0.00

Elements	0.5m							1m						
	Inside			Edge		Matrix		Inside			Edge		Matrix	
	Mean	Std dev	Percentage*	Mean	Percentage*	Mean	p-value	Mean	Std dev	Percentage*	Mean	Percentage*	Mean	p-value
Ag	0.00	0.00	0.00	0.00	0.00	1.00		0.00	0.00	0.00	0.00	0.00	3.00	
Cd	3.70	3.20	185.00	7.00	185.00	2.00	0.61	1.70	2.90	0.50	7.00	2.00	352.00	0.00
Sn	67.30	28.70	240.40	354.00	240.40	28.00	0.02	69.30	24.80	126.00	231.00	420.00	55.00	0.05
Sb	2.70	0.60	270.00	3.00	270.00	1.00	0.21	3.00	1.00	100.00	2.00	66.70	3.00	0.71
Te	0.00	0.00		0.00		0.00		0.30	0.60	15.00	0.00	0.00	2.00	0.21
I	21.30	2.30	163.80	40.00	163.80	13.00	0.03	68.00	48.50	94.40	94.00	130.60	72.00	0.90
Cs	44.30	4.50	260.60	39.00	260.60	17.00	0.07	39.70	10.70	110.30	37.00	102.80	36.00	0.95
Ba	3547.00	377.70	304.20	3275.00	304.20	1166.00	0.06	3583.00	903.80	107.70	3356.00	100.90	3327.00	0.96
La	949.00	124.70	306.10	999.00	306.10	310.00	0.08	1086.00	206.90	100.70	857.00	79.50	1078.00	0.67
Ce	1746.70	215.10	279.90	1837.00	279.90	624.00	0.08	2048.00	425.70	106.80	1374.00	71.60	1918.00	0.51
Pr	187.70	20.40	272.00	191.00	272.00	69.00	0.07	212.00	40.80	104.40	167.00	82.30	203.00	0.69
Nd	663.30	63.90	265.30	681.00	265.30	250.00	0.06	739.30	142.70	104.10	619.00	87.20	710.00	0.79
Sm	103.00	11.50	257.50	101.00	257.50	40.00	0.08	112.00	21.90	110.90	91.00	90.10	101.00	0.73
Eu	17.70	2.10	221.30	17.00	221.30	8.00	0.11	18.70	3.80	103.90	17.00	94.40	18.00	0.93
Gd	111.30	12.70	231.90	109.00	231.90	48.00	0.09	114.70	24.70	98.90	107.00	92.20	116.00	0.96
Tb	12.70	1.20	254.00	12.00	254.00	5.00	0.06	13.00	2.00	108.30	12.00	100.00	12.00	0.87
Dy	57.00	7.80	219.20	53.00	219.20	26.00	0.14	58.30	13.70	98.80	60.00	101.70	59.00	0.99
Ho	10.00	1.00	250.00	9.00	250.00	4.00	0.07	9.70	2.10	107.80	10.00	111.10	9.00	0.94
Er	23.70	4.00	197.50	22.00	197.50	12.00	0.24	24.30	6.70	105.70	25.00	108.70	23.00	0.98
Tm	3.00	0.00	300.00	3.00	300.00	1.00		3.30	0.60	110.00	3.00	100.00	3.00	0.83
Yb	18.30	1.50	183.00	17.00	183.00	10.00	0.08	18.00	3.60	100.00	19.00	105.60	18.00	0.97
Lu	3.00	0.00	300.00	2.00	300.00	1.00		2.70	1.20	90.00	3.00	100.00	3.00	0.95
Hf	1.00	0.00		1.00		0.00		1.00	0.00	100.00	0.00	0.00	1.00	
Ta	0.00	0.00		0.00		0.00		0.00	0.00		0.00		0.00	
W	7.30	1.20	146.00	6.00	146.00	5.00	0.37	6.30	0.60	90.00	6.00	85.70	7.00	0.56
Re	0.00	0.00		0.00		0.00		0.00	0.00		0.00		0.00	
Os	0.00	0.00		0.00		0.00		0.00	0.00		0.00		0.00	
Ir	3.00	1.70	300.00	1.00	300.00	1.00	0.56	2.30	1.20	230.00	1.00	100.00	1.00	0.56
Pt	1.30	0.60	65.00	1.00	65.00	2.00	0.56	1.70	0.60	56.70	1.00	33.30	3.00	0.24
Au	0.00	0.00		0.00		0.00		0.00	0.00		0.00		0.00	
Hg	0.00	0.00	0.00	0.00	0.00	9.00		0.00	0.00	0.00	0.00	0.00	15.00	
Tl	6.00	0.00	300.00	6.00	300.00	2.00		5.00	1.70	100.00	5.00	100.00	5.00	1.00
Pb	280.30	15.30	259.50	282.00	259.50	108.00	0.02	289.30	69.90	108.80	242.00	91.00	266.00	0.85
Bi	6.00	1.00	150.00	5.00	150.00	4.00	0.38	7.00	2.60	175.00	6.00	150.00	4.00	0.67
Th	206.00	31.10	92.80	216.00	92.80	222.00	0.90	230.30	64.50	124.50	142.00	76.80	185.00	0.57
U	23.70	1.50	74.10	24.00	74.10	32.00	0.08	25.00	4.60	113.60	26.00	118.20	22.00	0.82

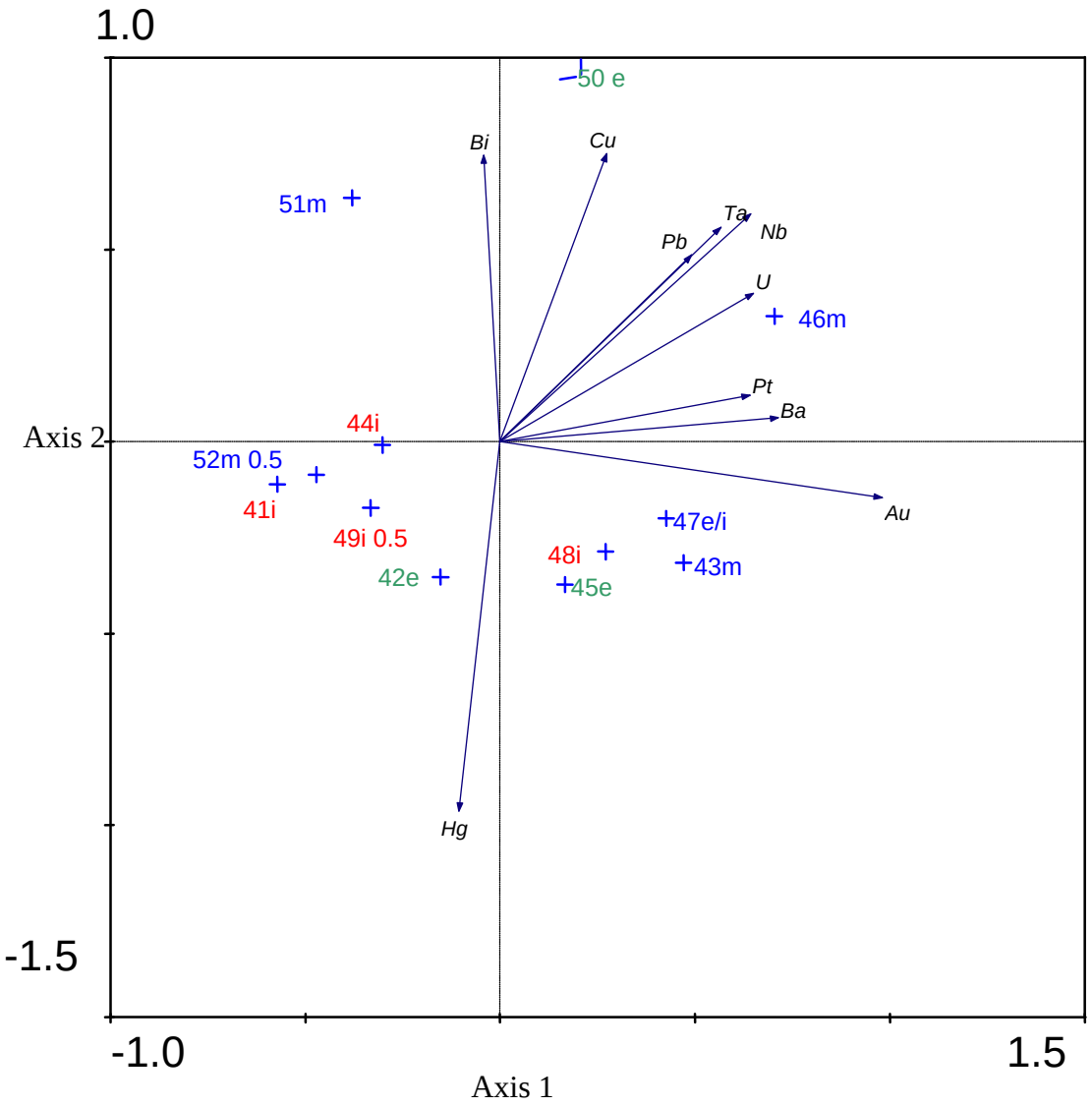

Figure 5.6. Principal component analysis biplot of samples collected on the farm Keerweder and the 10 most important elements relevance set at 40%. Legend of samples is as follows: The samples number, i= inside, m = matrix and e = edge with the depths given as 0.5 = 0.5m and one sample with the number 47(e/i) collected at a circle that was in the process of disappearing.

PCA values

Axes	**1**	**2**	**3**	**4**
Eigen values	0.386	0.177	0.133	0.09
Sum of Eigen values	1			
Cumulative percentage variance of species data	38.6	56.3	69.6	78.7

Table 5.5. Mean and standard deviation for all elements from soil collected in Keerweder broken down into the different microhabitats and given as a mean over all microhabitats. All values given as ppm. As only two samples were collected at a depth of 0.5 m they were omitted and results are given for surface samples only

Elements	Inside			Edge			Matrix			All	
	Mean	Std dev	Percentage*	Mean	Std dev	Percentage*	Mean	Std dev	p-value	Mean	Std dev
Li	36.30	4.00	93.80	33.30	9.80	86.00	38.70	1.50	0.51	34.50	4.40
Be	12.30	1.50	100.00	11.50	3.50	93.50	12.30	1.50	0.53	12.30	1.50
B	153.00	39.90	122.70	127.50	37.60	102.20	124.70	34.00	0.91	129.30	33.50
Na	4863.00	4159.40	127.60	3051.50	1390.70	80.10	3810.70	1572.30	0.60	4040.50	2222.50
Mg	28980.70	3705.00	98.50	28125.30	8017.10	95.60	29414.70	890.30	0.98	28151.00	3397.90
Al	54388.70	7201.30	97.90	52787.50	14945.90	95.00	55537.70	2252.40	0.96	52327.90	6970.40
Si	6434.00	3980.90	147.50	4425.50	805.60	101.50	4361.70	238.50	0.48	4815.30	1986.60
P	4194.30	826.70	105.40	3848.50	1045.60	96.80	3977.70	284.30	0.91	3787.80	653.30
K	15748.30	2094.80	88.70	16880.30	4728.30	95.10	17747.70	1010.80	0.47	15981.80	2391.80
Ca	36537.70	2944.60	83.70	39457.80	11746.00	90.40	43650.00	10637.40	0.50	38649.30	6155.20
Sc	56.30	4.90	100.50	51.50	14.90	92.00	56.00	3.00	0.95	52.50	7.10
Ti	6796.70	647.30	97.60	6813.30	1933.30	97.80	6963.70	464.10	0.94	6757.80	531.50
V	2197.00	234.90	99.30	2175.80	620.00	98.40	2212.30	145.50	0.99	2176.40	177.60
Cr	1467.30	146.40	98.60	1448.80	412.20	97.30	1488.70	71.60	0.99	1443.70	140.60
Mn	3702.00	575.40	92.30	3554.30	1049.00	88.60	4010.30	195.20	0.85	3601.50	561.60
Fe	410760.00	44533.90	97.50	391676.30	113781.40	93.00	421169.30	34118.00	0.92	398834.90	45503.00
Co	93.30	12.70	93.30	90.00	26.80	90.00	100.00	4.40	0.87	91.80	13.60
Ni	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Cu	184.30	26.30	90.70	348.50	184.80	171.40	203.30	16.70	0.37	240.70	178.20
Zn	942.30	239.40	86.40	1146.00	415.00	105.10	1090.30	282.40	0.82	1051.10	357.20
Ga	43.70	4.50	97.10	42.30	12.10	94.00	45.00	2.00	0.95	42.20	5.10
Ge	2.30	0.60	115.00	2.30	0.60	115.00	2.00	0.00	0.62	2.20	0.40
As	204.70	26.10	97.20	223.30	61.30	106.00	210.70	3.80	0.35	213.60	16.10
Se	30.70	6.00	91.10	29.00	10.20	86.10	33.70	9.30	0.47	30.00	7.90
Br	4198.30	1060.70	81.80	4270.00	1367.70	83.20	5133.70	104.50	0.25	4372.30	961.70
Rb	92.00	10.10	97.10	90.30	25.70	95.40	94.70	7.40	0.96	90.10	9.50
Sr	354.30	62.40	85.60	374.30	107.70	90.40	414.00	22.60	0.52	361.90	60.30
Y	196.30	18.50	99.00	190.30	54.20	96.00	198.30	11.60	0.99	191.20	14.70
Zr	116.30	9.70	145.40	76.00	29.00	95.00	80.00	13.70	0.11	88.30	29.20
Nb	26.00	5.00	92.90	27.80	8.30	99.30	28.00	6.10	0.87	26.60	5.50
Mo	12.00	1.00	109.10	11.00	3.50	100.00	11.00	2.00	0.83	11.40	2.20
Ru	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Rh	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Pd	2.00	0.00	153.80	1.30	0.50	100.00	1.30	0.60	0.04	1.50	0.50
Ag	2.30	0.60	135.30	1.80	0.60	105.90	1.70	0.60	0.18	1.80	0.60
Cd	3.00	1.70	69.80	2.50	1.50	58.10	4.30	0.60	0.54	3.10	1.50
Sn	19.30	2.50	32.70	47.80	39.60	81.00	59.00	61.50	0.48	38.40	39.40
Sb	3.30	0.60	82.50	3.80	5.50	95.00	4.00	1.00	0.32	5.10	5.40
Te	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
I	7.00	1.70	90.90	9.30	4.50	120.80	7.70	2.90	0.87	9.50	4.30
Cs	5.30	0.60	100.00	5.30	1.50	100.00	5.30	0.60	1.00	5.30	0.50
Ba	747.70	118.10	84.80	764.00	225.50	86.70	881.70	108.50	0.45	769.90	111.00
La	427.30	39.70	102.50	411.30	114.60	98.60	417.00	28.60	0.94	407.90	34.30
Ce	541.70	57.00	102.90	508.50	142.50	96.60	526.30	25.40	0.84	511.60	48.10
Pr	100.70	10.70	101.40	99.00	27.50	99.70	99.30	8.10	0.92	97.50	8.20
Nd	372.00	35.40	102.80	361.50	100.40	99.90	361.70	26.50	0.86	356.90	27.50

Elements	Inside			Edge			Matrix			All	
	Mean	Std dev	Percentage*	Mean	Std dev	Percentage*	Mean	Std dev	p-value	Mean	Std dev
Sm	69.30	9.60	103.00	68.00	18.60	101.00	67.30	6.40	0.71	66.70	6.60
Eu	7.70	0.60	96.30	7.00	2.00	87.50	8.00	0.00	0.69	7.30	0.80
Gd	74.00	6.90	100.40	72.30	20.20	98.10	73.70	5.10	0.93	71.50	5.90
Tb	9.00	1.00	103.40	8.80	2.40	101.10	8.70	0.60	0.81	8.50	1.00
Dy	40.70	4.90	97.60	40.00	11.30	95.90	41.70	2.50	0.98	40.00	3.20
Ho	6.30	0.60	94.00	6.30	1.80	94.00	6.70	0.60	0.75	6.30	0.50
Er	16.00	2.00	100.00	16.30	4.50	101.90	16.00	1.00	0.71	15.80	1.40
Tm	2.00	0.00	100.00	2.00	0.60	100.00	2.00	0.00		2.00	0.00
Yb	13.00	1.00	102.40	12.00	3.40	94.50	12.70	0.60	0.80	12.30	1.00
Lu	2.00	0.00	100.00	1.80	0.60	90.00	2.00	0.00	1.00	1.80	0.50
Hf	1.70	0.60	170.00	0.80	0.30	80.00	1.00	0.00	0.10	1.10	0.50
Ta	0.00	0.00	0.00	0.50	2.40	18.50	2.70	4.60	0.49	0.80	2.30
W	5.30	1.20	112.80	4.30	1.30	91.50	4.70	1.20	0.60	4.60	1.00
Re	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Os	0.00	0.00		0.00	0.00		0.00	0.00		0.00	0.00
Ir	0.30	0.60		0.00	0.10		0.00	0.00	0.41	0.10	0.30
Pt	0.30	0.60	30.00	0.80	0.40	80.00	1.00	0.00	0.75	0.70	0.50
Au	6.30	10.10	18.50	17.00	18.70	50.00	34.00	28.60	0.34	15.90	19.20
Hg	108.30	18.70	174.70	127.30	60.40	205.30	62.00	49.90	0.39	106.50	56.30
Tl	1.30	0.60	130.00	1.00	0.20	100.00	1.00	0.00	0.41	1.10	0.30
Pb	262.00	55.40	45.90	364.80	219.00	63.90	570.70	378.60	0.42	374.80	213.40
Bi	8.30	3.50	92.20	14.50	7.90	161.10	9.00	3.50	0.57	11.00	7.70
Th	166.00	16.10	109.90	142.00	39.50	94.00	151.00	6.20	0.59	146.30	20.30
U	11.70	0.60	88.00	11.30	3.50	85.00	13.30	3.20	0.60	11.70	1.90

*Percentages calculated using the matrix as the control

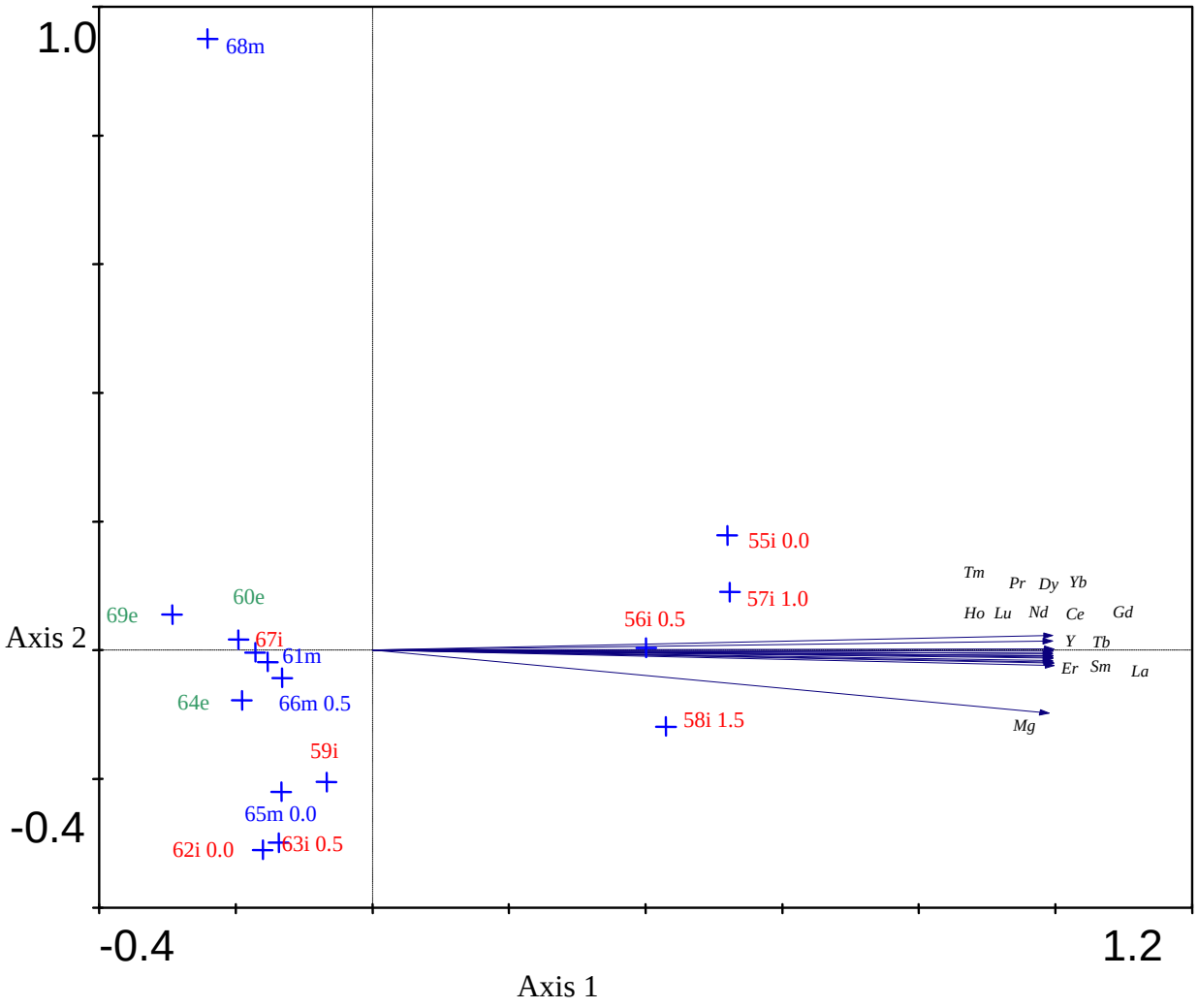

Figure 5.7. Principal component analysis biplot of samples collected in Marienfluss and the 15 most important elements relevance set at 98%. Legend of samples is as follows: The samples number, i= inside, m = matrix and e = edge with the depths given as s = surface, 0.5 = 0.5m, 1.0 = 1.0 m and 1.5= 1.5m.

PCA values

Axes	**1**	**2**	**3**	**4**
Eigen values	0.774	0.086	0.041	0.024
Sum of Eigen values	1			
Cumulative percentage variance of species data	77.4	86	90.1	92.6

Table 5.6. Mean and standard deviation for all elements from soil collected in Marienfluss broken down into the different microhabitats and given as a mean over all microhabitats and splitting the data into samples collected on the surface only and all samples collected at the different depths. All values given in ppm

	Surface only											All depths					
Elements	Inside			Edge			Matrix		All			Inside			Matrix		
	Mean	Std dev	Percentage*	Mean	Std dev	Percentage*	Mean	Std dev	Mean	Std dev	p-value	Mean	Std dev	Percentage*	Mean	Std dev	p-value
Li	61.30	44.10	168.90	29.70	7.00	81.80	36.30	5.50	53.60	33.20	0.37	71.40	37.50	198.30	36.00	4.50	0.07
Be	28.80	21.80	288.00	10.70	3.10	107.00	10.00	1.70	25.90	22.50	0.21	39.00	24.30	354.50	11.00	2.40	0.04
B	107.50	49.30	238.90	64.70	26.30	143.80	45.00	3.60	86.70	44.10	0.12	114.60	41.10	242.30	47.30	5.40	0.01
Na	2075.50	575.00	97.30	2870.70	1668.30	134.60	2132.00	531.50	2356.00	790.50	0.57	2232.80	468.60	100.70	2216.50	465.70	0.48
Mg	49192.00	28146.60	153.40	28290.30	3271.00	88.20	32075.70	6178.70	46298.50	23961.70	0.34	59490.60	26431.20	178.00	33420.30	5716.90	0.06
Al	102719.80	47608.20	141.10	62387.00	7943.30	85.70	72816.30	13729.20	94501.50	38777.90	0.29	116721.60	41103.70	157.40	74147.30	11521.50	0.04
Si	3440.30	469.80	101.80	3358.00	476.90	99.40	3379.00	63.50	3401.10	441.30	0.96	3487.10	528.40	106.90	3261.50	240.70	0.72
P	11415.80	10431.30	209.60	5569.30	557.00	102.20	5447.00	459.50	7887.70	5720.90	0.45	10118.90	7276.00	196.00	5164.00	679.00	0.29
K	54649.00	25101.50	135.10	35554.70	5210.10	87.90	40461.70	5518.40	49306.30	18064.30	0.35	59051.90	19956.80	147.20	40128.80	4554.70	0.07
Ca	40559.80	29940.90	148.80	26086.70	2092.30	95.70	27251.30	2961.00	53070.30	56081.70	0.58	76104.10	70618.10	279.40	27240.50	2417.70	0.25
Sc	70.00	17.70	130.40	45.00	8.90	83.80	53.70	10.10	62.30	15.30	0.11	72.10	12.10	129.20	55.80	9.30	0.01
Ti	13540.50	1917.90	115.40	10558.30	1285.00	90.00	11731.00	1868.40	12549.90	1776.70	0.14	13502.60	1313.70	111.20	12138.00	1729.10	0.03
V	1641.00	306.70	119.40	1337.30	201.90	97.30	1374.30	251.50	1606.90	355.20	0.31	1809.40	345.80	128.80	1404.30	213.90	0.05
Cr	510.80	131.90	117.60	441.30	32.00	101.60	434.30	44.20	505.00	99.90	0.50	556.30	110.50	123.50	450.30	48.10	0.10
Mn	9950.50	5687.50	150.10	4747.70	1294.90	71.60	6630.30	1173.60	9527.10	5889.80	0.26	12723.40	6520.90	189.40	6719.30	974.60	0.06
Fe	848432.50	150354.80	117.00	613091.70	77031.20	84.60	724934.00	129926.60	789334.70	154099.10	0.12	880255.90	128191.90	119.00	739674.80	110105.00	0.01
Co	111.00	66.90	147.40	56.70	9.20	75.30	75.30	12.50	104.70	57.90	0.32	136.60	63.50	177.40	77.00	10.70	0.05
Ni	0.00	0.00		0.00	0.00		0.00	0.00	0.00	0.00		0.00	0.00		0.00	0.00	
Cu	368.30	232.30	202.00	177.70	8.10	97.50	182.30	8.10	272.10	145.60	0.23	351.00	164.50	189.70	185.00	8.50	0.07
Zn	1490.80	1007.40	63.70	1389.00	402.30	59.30	2340.70	2151.00	1688.60	1056.70	0.64	1605.00	784.20	77.10	2080.50	1831.80	0.69
Ga	142.00	60.10	132.70	88.30	10.70	82.50	107.00	21.30	133.70	51.90	0.28	162.50	55.30	147.70	110.00	18.40	0.05
Ge	6.30	3.20	170.30	4.30	0.60	116.20	3.70	0.60	6.00	3.00	0.31	7.60	3.40	190.00	4.00	0.80	0.07
As	247.50	112.50	133.80	268.30	157.90	145.00	185.00	41.10	257.50	111.90	0.66	286.40	117.90	149.30	191.80	36.20	0.41
Se	27.50	16.60	132.90	22.30	0.60	107.70	20.70	12.60	31.90	20.30	0.77	41.40	23.60	203.90	20.30	10.30	0.16
Br	810.30	191.00	110.80	1007.00	207.90	137.70	731.30	158.70	841.50	166.10	0.24	822.90	135.10	109.00	754.80	137.80	0.12
Rb	638.80	384.60	148.10	368.70	40.30	85.50	431.30	75.50	615.30	323.50	0.39	791.00	359.80	176.20	449.00	71.10	0.06
Sr	300.80	252.20	156.10	167.30	20.00	86.80	192.70	9.10	311.30	244.90	0.55	425.10	296.60	221.60	191.80	7.60	0.16
Y	527.00	489.30	200.60	226.30	24.00	86.10	262.70	38.60	521.90	445.90	0.44	758.80	509.10	281.20	269.80	34.50	0.08
Zr	202.80	107.30	251.30	59.00	10.60	73.10	80.70	17.50	136.40	79.60	0.06	182.60	78.10	179.00	102.00	45.00	0.03
Nb	96.80	54.20	133.90	59.00	7.90	81.60	72.30	9.50	97.70	51.90	0.42	124.40	59.50	169.70	73.30	8.00	0.09
Mo	29.00	13.00	163.80	17.00	3.60	96.00	17.70	4.20	27.10	12.80	0.20	34.90	13.00	183.70	19.00	4.30	0.03
Ru	0.00	0.00		0.00	0.00		0.00	0.00	0.00	0.00		0.00	0.00		0.00	0.00	
Rh	0.50	1.00		0.00	0.00		0.00	0.00	0.10	0.50	0.53	0.30	0.70		0.00	0.00	0.68
Pd	2.50	1.90	250.00	1.00	0.00	100.00	1.00	1.00	1.70	1.20	0.30	2.10	1.50	161.50	1.30	1.00	0.32
Ag	2.00	1.40	200.00	1.00	0.00	100.00	1.00	0.00	1.60	1.00	0.31	2.10	1.10	210.00	1.00	0.00	0.08

Elements	Surface only											All depths					
	Inside			Edge			Matrix		All			Inside			Matrix		
	Mean	Std dev	Percentage*	Mean	Std dev	Percentage*	Mean	Std dev	Mean	Std dev	p-value	Mean	Std dev	Percentage*	Mean	Std dev	p-value
Cd	3.30	2.10	62.30	2.70	0.60	50.90	5.30	5.80	3.80	2.70	0.61	3.80	1.80	79.20	4.80	4.90	0.63
Sn	49.00	30.90	136.10	33.70	22.00	93.60	36.00	28.60	44.80	26.70	0.74	55.40	27.50	173.10	32.00	24.70	0.28
Sb	2.80	1.70	140.00	1.70	0.60	85.00	2.00	1.00	2.50	2.10	0.54	3.10	2.70	172.20	1.80	1.00	0.46
Te	0.00	0.00		0.00	0.00		0.00	0.00	0.10	0.30		0.00	0.00	0.00	0.30	0.50	0.27
I	16.80	5.90	129.20	10.70	2.50	82.30	13.00	2.60	28.30	26.50	0.24	40.90	31.40	250.90	16.30	6.80	0.14
Cs	14.80	8.80	152.60	9.00	1.00	92.80	9.70	1.50	15.10	8.70	0.41	19.90	9.80	193.20	10.30	1.70	0.07
Ba	1501.30	974.70	121.20	857.70	95.80	69.30	1238.30	405.20	1409.30	723.80	0.50	1734.60	841.10	148.00	1172.30	356.30	0.15
La	1451.50	1079.10	176.20	763.30	72.80	92.70	823.70	94.90	1394.80	951.00	0.40	1907.80	1076.30	226.40	842.50	86.20	0.07
Ce	2532.80	2240.90	202.70	1151.30	143.60	92.10	1249.70	183.50	2443.10	1983.90	0.42	3506.50	2254.00	272.90	1285.00	165.60	0.07
Pr	315.80	244.20	177.10	164.30	15.20	92.10	178.30	21.70	303.10	210.20	0.42	415.50	239.10	227.70	182.50	19.60	0.07
Nd	1132.00	874.80	179.90	576.30	60.90	91.60	629.30	82.10	1079.30	752.70	0.41	1485.00	851.70	230.20	645.00	74.00	0.07
Sm	184.80	160.30	201.50	86.30	8.70	94.10	91.70	12.10	170.30	129.20	0.42	239.80	146.60	253.80	94.50	11.40	0.07
Eu	15.30	5.30	127.50	10.30	1.20	85.80	12.00	1.70	14.30	4.70	0.25	17.00	4.90	141.70	12.00	1.40	0.04
Gd	188.50	161.30	198.40	85.00	9.20	89.50	95.00	13.90	178.50	140.10	0.40	254.00	158.70	260.50	97.50	12.40	0.07
Tb	22.50	21.00	210.30	9.70	1.50	90.70	10.70	1.50	21.10	17.60	0.43	30.50	20.10	277.30	11.00	1.40	0.08
Dy	98.30	96.50	203.50	40.70	4.00	84.30	48.30	6.40	96.30	85.10	0.46	141.00	97.70	290.70	48.50	5.20	0.08
Ho	16.50	16.30	226.00	6.30	0.60	86.30	7.30	1.20	15.90	14.50	0.42	23.60	16.50	314.70	7.50	1.00	0.07
Er	41.50	43.70	234.50	15.30	2.10	86.40	17.70	3.20	40.00	38.10	0.44	60.30	43.50	335.00	18.00	2.70	0.08
Tm	5.30	5.90	196.30	2.00	0.00	74.10	2.70	0.60	5.30	5.00	0.52	7.90	5.80	282.10	2.80	0.50	0.10
Yb	33.50	35.00	239.30	11.30	1.20	80.70	14.00	1.70	31.80	30.50	0.42	48.10	34.80	331.70	14.50	1.70	0.07
Lu	4.50	4.40	225.00	2.00	0.00	100.00	2.00	0.00	4.50	4.10	0.44	6.60	4.70	330.00	2.00	0.00	0.08
Hf	2.50	1.90	192.30	1.00	0.00	76.90	1.30	0.60	1.80	1.20	0.32	2.40	1.40	184.60	1.30	0.50	0.14
Ta	0.30	0.50	8.10	0.00	0.00	0.00	3.70	6.40	0.90	2.80	0.38	0.30	0.50	10.70	2.80	5.50	0.32
W	5.80	6.80	252.20	2.00	0.00	87.00	2.30	1.20	5.60	6.00	0.50	8.60	7.10	373.90	2.30	1.00	0.11
Re	0.00	0.00		0.00	0.00		0.00	0.00	0.00	0.00		0.00	0.00		0.00	0.00	
Os	0.00	0.00		0.00	0.00		0.00	0.00	0.00	0.00		0.00	0.00		0.00	0.00	
Ir	0.00	0.00		0.00	0.00		0.00	0.00	0.00	0.00		0.00	0.00		0.00	0.00	
Pt	0.30	0.50		0.00	0.00		0.00	0.00	0.10	0.40	0.53	0.30	0.50		0.00	0.00	0.42
Au	10.50	20.30	1500.00	3.70	5.50	528.60	0.70	0.60	7.00	13.70	0.64	11.10	17.90	853.80	1.30	1.30	0.48
Hg	84.30	70.70	182.10	54.30	18.90	117.30	46.30	37.10	64.70	43.20	0.60	80.40	50.30	196.10	41.00	32.10	0.32
Tl	3.50	3.00	205.90	1.70	0.60	100.00	1.70	0.60	3.30	2.50	0.42	4.60	2.80	255.60	1.80	0.50	0.07
Pb	258.80	180.90	53.10	216.70	75.50	44.40	487.70	526.40	302.30	251.30	0.53	284.10	154.50	70.50	403.00	462.00	0.63
Bi	4.00	2.70	133.30	2.30	0.60	76.70	3.00	1.00	4.00	2.40	0.53	5.30	2.70	189.30	2.80	1.00	0.09
Th	342.50	305.10	208.50	137.30	21.70	83.60	164.30	21.00	307.30	250.60	0.38	444.60	281.00	277.40	160.30	19.00	0.06
U	24.30	26.50	162.00	8.70	2.30	58.00	15.00	3.60	23.90	22.20	0.53	34.80	26.10	252.20	13.80	3.90	0.12

*Percentages calculated using the matrix as the control

5.6 Discussion

In most analyses done no definitive groupings could be seen relating a specific microhabitat to one or more element. The elements related most strongly to the groupings of the samples varied between different sampling locations, although there were some elements that were relevant in more than one location. In the Giribes Plains ordination where all samples from one circle at different depths were analysed (Fig. 5.5) and the Marienfluss ordination (Fig. 5.7) samples from the same circle grouped together well. In the other ordinations (Fig 5.3, 5.4, 5.6) the samples were randomly distributed. In some ordinations one can see the samples from the same circle (inside, edge and matrix) grouping together. This is especially true in the ordination of all the samples collected in the Giribes Plains (Fig. 5.4) where samples 20-34 were collected in and around one circle, as well as one circle for 35-37 and 38-40 respectively. It thus seems that there is more variation in a specific location (e.g. Giribes Plains) than between the microhabitats (matrix, edge and inside). In two of the ordinations where some form of segregation could be seen some common elements showed up in the ordination (Figure 5.5 & 5.7) and the means for the elements for each habitat and depth are given to indicate quantity of these elements in the soil (Table 5.6). The common elements are Terbium (Tb), Ytterbium (Yb), Yttrium (Y), Gadolinium (Gd), Lutetium (L), Holmium (Ho) and Thulium (Tm) all of which are part of the rare earth elements (REE) except for Yttrium. The analysis of statistically differences of the concentations of the different elements did not highlight a specific pattern or a specific element as one that could be a factor in the maintenance of the circles. In the analysis of all the samples taken in the Giribes Plains

the significant difference for the elements that had a p-value smaller or equal to 0.05 was always between samples taken from the matrix and samples taken from the inside of the circle. This pattern was however not repeated in any of the other analysis allthough most of the differences was between samples taken from the inside of the cirle and the matrix.

In all ordinations there was always the presence of one or more of the rare earth elements (REE) among the species contributing most to the ordination. They were, however, more abundant in the samples collected at different depths as can be seen in the ordination of Giribes (Fig. 5.5) and Marienfluss (Fig. 5.7 samples 55-58). In Marienfluss the elements contributing most the ordinations were always higher on the inside of the circle than on the outside (Table 5.5).

The rare earth elements are the elements numbered from 57-71 (La – Lu) in the periodic table (Fig. 5.8) and include elements such as Europium (Eu) and Samarium (Sm) which have radioactive isotopes. These elements all have the same chemical properties and although called rare earth elements, they are not rare and are distributed widely in nature, even if they are not abundant. The rare earth elements are so called because of their unusual chemical properties. They are all chemically quite active, form salts readily (because of the presence of a d-orbital), and react violently with acids and even with water to form basic solutions (Stwertka, 1998).

While rare earth elements are abundant, their concentration in plants is usually low. It is quite possible that these elements are both beneficial and toxic to plants, similar to the properties of trace metals (Shtangeeva & Ayrault, 2007). It has been shown that REE stimulate the synthesis of chlorophyll, promote seedling development and improve the bioavailability of Ca and Mn in soil (Shtangeeva & Ayrault, 2007). There is however

little known about the uptake of these elements in plants and although these elements are more abundant inside the circles than in the matrix it might not have any effect on the plants. Yttrium on the other hand is not part of the rare earth element group but is grouped into this class because of its similar chemical properties (Kamijo *et al.*, 1998).

Even if one of the elements in the ordinations is known to have an effect on the plant, the question of bioavailability remains unanswered. Bioavailability of the major elements is easy to determine, but methods for trace metals and rare elements are not readily available and not always reliable (Ehlken & Kirchner, 2002). This would make interpretation of any purely elemental data difficult.

5.7 Conclusion

Total element analysis will in some cases indicate if a toxic substance is present or absent in the soil. In the case of the fairy circles, the distribution of the elements in the different microhabitats was too varied to give any indication of elemental difference, in the soil. This does not mean that there are no differences in the soil, as the method of analysis might not be appropriate to detect the difference that influences the existence of the circles.

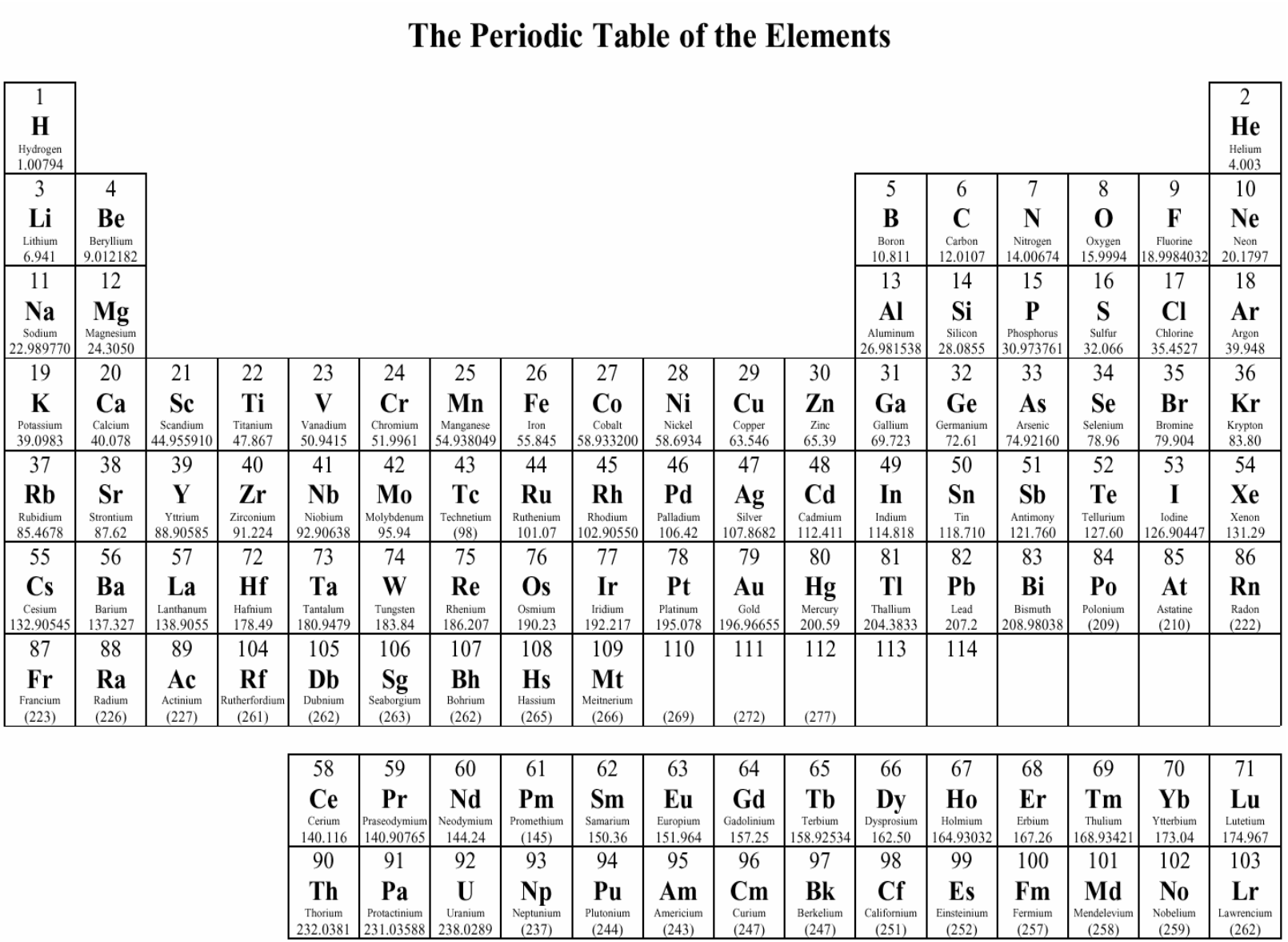

Figure 5.8. The periodic table of elements.

CHAPTER 6

INVESTIGATING THE POSSIBLE ALLELOPATHIC EFFECTS OF *EUPHORBIA DAMARANA* AND THE ISOLATION OF TWO COMPOUNDS

6.1 Introduction

Allelopathy is a subject of current fascination and controversy among plant physiologists and ecologists. Allelopathy is derived from the Greek *allelon*, 'of each other' and *pathos*, 'to suffer'; hence it means: the injurious effect of one upon another. The term broadly denotes that body of scientific knowledge which concerns the production of biomolecules by one plant, mostly secondary metabolites, that can induce suffering in, or give benefit to, another plant (Rizvi *et al.*, 1992).

Literature to illustrate the beneficial effects that plants might have on each other is rare but one good example is the example of weeds in cropping systems. Weeds in cropping systems are most often considered to be detrimental. However, the interaction of weeds with crops may be positive. In a study where controlled densities of wild mustard (*Brassica campestris* L.) were interplanted with broccoli (*Brassica oleracea* var. Premium crop), crop yield increased by as much as 50% compared with broccoli planted

alone (Jimenez-Osornio & Gliessman, 1987). The most accepted definition of allelopathy is any direct or indirect effect of one plant on another, through the production of chemical compounds that escape into the environment (Willis, 1985).

More recently some authors have proposed plant responses can be better understood in terms of allelochemical interactions with soil ecological processes than the classical concept of direct plant-plant allelopathic interference (Inderjit & Weiner, 2001). This has led to the broadening of the concept of allelochemical interference to include the effect that allelochemicals have on abiotic components of the soil. This would include the availability and accumulation of inorganic ions, and the activities of allelochemicals that are influenced by ecological factors such as nutrient accumulation and availability, light regimes and moisture deficiency to name but a few (Inderjit & Weiner, 2001).

Allelopathic interactions obviously have an evolutionary history and according to Sinkkonen (2006) there are three different possibilities in natural circumstances based on the evolutionary history of the donor species. First, chemicals released for non-allelopathic purposes may reduce the fitness of an unintentional target, or the unintentional release of phytochemicals, for example due to physical damage may result in growth alterations in plants. This is known as unintentional allelopathy. Parallel allelopathy, on the other hand, may be spatial or temporal and occurs when the same compound is used for different purposes in different parts of the plant. The third is when chemicals are released mainly or solely for their allelopathic activity. According to the author this is rare as most phytochemicals have multiple roles in nature. As the ecological roles of these three types of compounds would differ the experimental designs for each type would be different.

6.2 Literature review

6.2.1 The origin of the allelopathy hypothesis

The succulent *Euphorbia damarana* grows in the region where the fairy circles occur in the Giribes Plains although this is not true for most of the other regions where the fairy circles occur. The size and distribution patterns of *E. damarana* plants on the Giribes Plains are very similar to those of the fairy circles. These facts made Theron (1979) propose the existence of an allelopathic substance that inhibits the growth of plants. He contended that *E. damarana* might be responsible for the allelopathic action. He furthermore proposed that a deep rooted *E. damarana* could take up an inorganic compound from deep in the earth and store it in aboveground parts. Upon dying the compound would be released in the topsoil having an allelopathic type action

There are a few problems with this hypothesis, the main one being that *E. damarana* prefers stony soils and not the sandy plains where most of the circles occur. The Giribes Plains and some other isolated areas where the circles and the *E. damarana* can be seen together is the exception (Van Rooyen *et al.,* 2004). The other problems mentioned by Van Rooyen and co-workers (2004) were that the allelopathic compounds were unlikely to have the longevity to sustain the circles, especially when no *E. damarana* plants were currently found in the region.

6.2.2 Euphorbia damarana

Euphorbia damarana was first described by Leach in 1975 and is most closely related to *E. gregaria* Marloth. It differs mostly from *E. gregaria* in the fruit and leaves, which in the case of *E. damarana* are more globular (fruit) and erect (leaves) than those of *E. gregaria*. Both species are restricted almost entirely to desert areas north of the Tropic of Capricorn in Africa (Leach, 1975).

Very little is known about the chemistry of *E. damarana* and the other *Euphorbia* species of the desert and dry regions of Africa, although patents have been awarded for making fibres from the stems of *E. gregaria*, *E. damarana*, *E. gummifera* Boiss, and *E. tirucalli* L. by liberating the fibres from the stems and treating them either chemically or microbiologically (Van Zyl, 2000).

The different species in the *Euphorbia* genus seem to have a wide range of secondary compounds, as can be seen by their different uses and toxic properties. In Namibia *E. monteiroi* Hook. is used for toothache and as a purgative and *E. prostrata* Aiton is used as a prophylactic against stinging insects and snakes. On the other hand *E. virosa* Willd., better known as the ‘gifboom’ or ‘noorsdoring’, is very poisonous and is used to make arrow poison. The latex is known to burn the skin and can lead to temporary blindness and the plant contains carcinogenic substances (Van Koenen, 2001).

The Dictionary of Natural Products contains 811 compounds isolated and identified from the genus *Euphorbia* (Chapman & Hall, 2006) but none of them have been recorded to have any allelopathic effects.

6.2.3 Proving allelopathic interactions

The concept of plants waging chemical warfare is an attractive concept and may partly explain the diverse nature of secondary compounds. It is, however, difficult to prove allelopathic effects of one plant species on another. The inhibitory effect of black walnut (*Juglans nigra*) on associated plant species is one of the oldest examples of allelopathy. The chemical responsible for walnut allelopathy is juglone (5-hydroxy-1, 4-naphthoquinone) and has been isolated from many plants in the walnut family (Kocacaliskan & Terzi, 2001). Allelopathy has an impact on agriculture (see e.g. Chon *et al*., 2005; Hao *et al*., 2007) and this has led to the investigation of many crop species and the production of allelopathic compounds by these crop species. It has even been shown that crops, such as watermelon, have autotoxicity as well as toxicity towards other plants (Hao *et al*., 2007) so the possibility of plants poisoning themselves over a period of time is also a reality when investigating allelopathy.

The methodology of allelopathic research states that six criteria must be confirmed or proven to establish that allelopathy is present (Willis, 1985). These criteria are as follows:

- A pattern of inhibition needs to be established of one plant species on another;
- The aggressor plant must produce a toxin;
- There must be a mode of toxin release in the plant;
- There must be a mode of toxin transport and/or accumulation in the environment;
- The ‘victim’ plant must have some mode of toxin uptake;
- The observed pattern of inhibition must not be solely explained by physical or biotic factors such as competition and herbivory.

Very few studies satisfy all these criteria and according to Willis (1985) most articles in the literature only satisfy three or four of these criteria. He also stated that all too often the allelopathic potential of plants is based on laboratory experiments carried out in semi-sterile, artificial environments with the tests done on plant species not even present in the natural environment of the plant.

In an ecological and evolutionary context, it is however hardly necessary to assume that all these steps always remain distinguishable once evolved. If allelopathy has been evolutionary significant for both counterparts of the interaction an evolutionary arms race should exist between these two. The outcome of this race would depend largely on environmental conditions, and it may change as new adaptations evolve. It is in this case important to study the allelopathic interaction from either the perspective of the donor species or from the perspective of the target species as methods of study would be different (Sinkkonen, 2006).

Bioassays are generally designed to test a hypothesis with laboratory studies used as a tool to understand a particular component of the allelopathic interaction and the field trials used to understand if different components are operating together as a unit in field situations (Inderjit & Nilsen, 2003). It could thus be said that field trials would be ideal to prove or disprove the existence of the allelopathic effect, but they are time consuming, expensive and the environmental variables are sometimes uncontrollable. Stowe (1979) challenged the validity of a bioassay in allelopathic research and concluded that there is often little concordance between bioassay results and the pattern of vegetation observed in the field.

Aside from the primary research objective (to find an allelopathic compound) there are several criteria that should be considered when selecting a bioassay. These would include sensitivity, specificity, response time, reproducibility, plant material, cost, labour, equipment and space requirements (Hoagland & Williams, 2004).

Even with all the so-called negative aspects of allelochemical research, it is a widely researched field and some researchers have isolated germination and growth inhibiting compounds from plants and isolated the same chemicals from soil surrounding the plants (Inoue *et al.*, 1992). This is a good indication of the allelopathic potential of the plant species and might not prove that the 'victim' plant species can take up the toxin but it does prove that the aggressor plant produces and releases the toxin and that the toxin accumulates in the environment. That should be enough to make reasonable assumptions about the potential of allelopathic properties of a plant species.

6.3 Aim

The aim of this part of the study was to investigate if an allelopathic compound is present in *E. damarana* plants.

6.4 Materials and methods

6.4.1 Plant collection

A field trip was undertaken in June 2005 and dead and alive *E. damarana* plant material was collected in the Giribes Plain, an area where *E. damarana* and the fairy circles occur together. The plant material was air dried in the shade at ambient temperatures until extraction.

6.4.2 Euphorbia damarana extraction

The dried stems (7 kg) of *E. damarana* were milled and extracted in 100% absolute ethanol with occasional agitation for three days. After three days the ethanol extract of the stems was filtered using Whatman nr 42 filter paper and the extraction with ethanol repeated. After filtration the extract was dried in a Büchi rota-evaporator under reduced pressure at 45°C until no more solvent evaporated. The filtrate was stored in a sealed glass container at 4°C until further use (Figure 6.1).

6.4.3 Thin layer chromatography (TLC) agar plate bioassay

A thin layer chromatography assay was carried out using the method described in Meyer *et al*. (2007). The extract, fractions and controls were dissolved in ethanol to a concentration of 1 mg/ml and spotted on 80 mm x 50 mm normal phase silica gel aluminium plates (Merck, Kieselgel 60 F_{254}). The spotting was not quantitative and although the concentration of the fractions was 1 mg/ml the amount of extract on the plate would probably not have been as much. The plates were covered with a 2 mm 1% agar layer and left to cool. As a control, the germination inhibitor 5-hydroxy-1-4-naphthoquinone (juglone) (Sigma-Aldrich) was used at a concentration of 1 mg/ml. The lack of sufficient numbers of *S. uniplumis* seeds to do the bioassay prompted the use of lettuce seeds, *Lactuca sativa* L. The seeds were arranged on the agar on the plate and incubated at 20°C in the dark for two days, before the germination percentage was calculated (Kato-Noguchi, 2003).

6.4.4 Growth and germination inhibition bioassay

A growth inhibition bioassay was carried out in duplicate using the method described by Kato *et al.* (1977). Ethanol crude extracts at different concentrations where added to filter paper and placed in Petri dishes. The ethanol was then left to evaporate after which the

paper was saturated with aqueous tween-80 (100 ppm). Fifteen lettuce seeds were then placed on the filter papers, and incubated at 20°C for 48 hours.

Germination percentage was determined by counting the number of seeds that germinated and dividing the number by the fifteen. The experiment was repeated twice.

To determine the growth inhibition the radicle lengths was measured and the mean and standard deviation calculated. As control ethanol was added to the plates instead of the crude extract.

6.4.5 Isolation of compounds

Liquid-liquid fractionation was used initially to separate a large quantity of compounds to make isolation of pure compounds easier. Using equal amounts (v/v/v) of crude extract: water: ethyl acetate a liquid-liquid extraction was performed (Figure 6.1). The ethyl acetate and water layers were separated and the ethyl acetate layer dried until a powder was obtained. The water layer was extracted three times with butan-1-ol, after which the water layer was discarded and the butan-1-ol layer dried. The water layer was not used due to the fact that water soluble compounds would probably leach out of the soil over a period of time. The ethyl acetate and butan-1-ol fractions where then analysed in a germination inhibition test, once again using juglone as a control.

Using the ethyl-acetate (308 g) fraction obtained from the liquid-liquid chromatography a dry silica gel column (silica 60) was run using a hexane: ethyl acetate gradient starting with 100:0 and ending with 100% ethyl acetate. Methanol was used to

elute compounds that were still trapped in the column. The fractions were subsequently concentrated and evaluated by means of thin layer chromatography (TLC) on silica gel 60 plates. Similar fractions were combined and those that appeared to have the least amount of compound in used for further purification. Two fractions formed white precipitates. The precipitates were washed with hexane and subjected to TLC in an 8:2 hexane: ethyl acetate mobile phase to check the purity and then subjected to nuclear magnetic resonance for identification.

Fractions 28 – 36 (5 g) were combined, dissolved in ethyl acetate and subjected to another dry silica gel 60 column using 80:20 hexane: ethyl acetate as mobile phase. Fractions were collected and evaluated using TLC with a mobile phase of 70:30 hexane: ethyl acetate as the mobile phase. Similar fractions were combined, dried and re-evaluated using TLC.

Combined fractions 4 and 5 yielded 2.1 g of dried material and was once again subjected to a silica column using hexane: ethyl acetate in the following ratios, starting with 100% hexane then 70:30 hexane: ethyl acetate, and ending with 100% ethyl acetate . From this column fractions were combined, dried and evaluated by TLC using 2% methanol: dichloromethane as mobile phase. Two of the fractions were combined and subjected to preparative TLC (20 cm x 20 cm, 250 µm layer, glass TLC plates RP 18 F) to obtain one pure compound (6.4 mg).

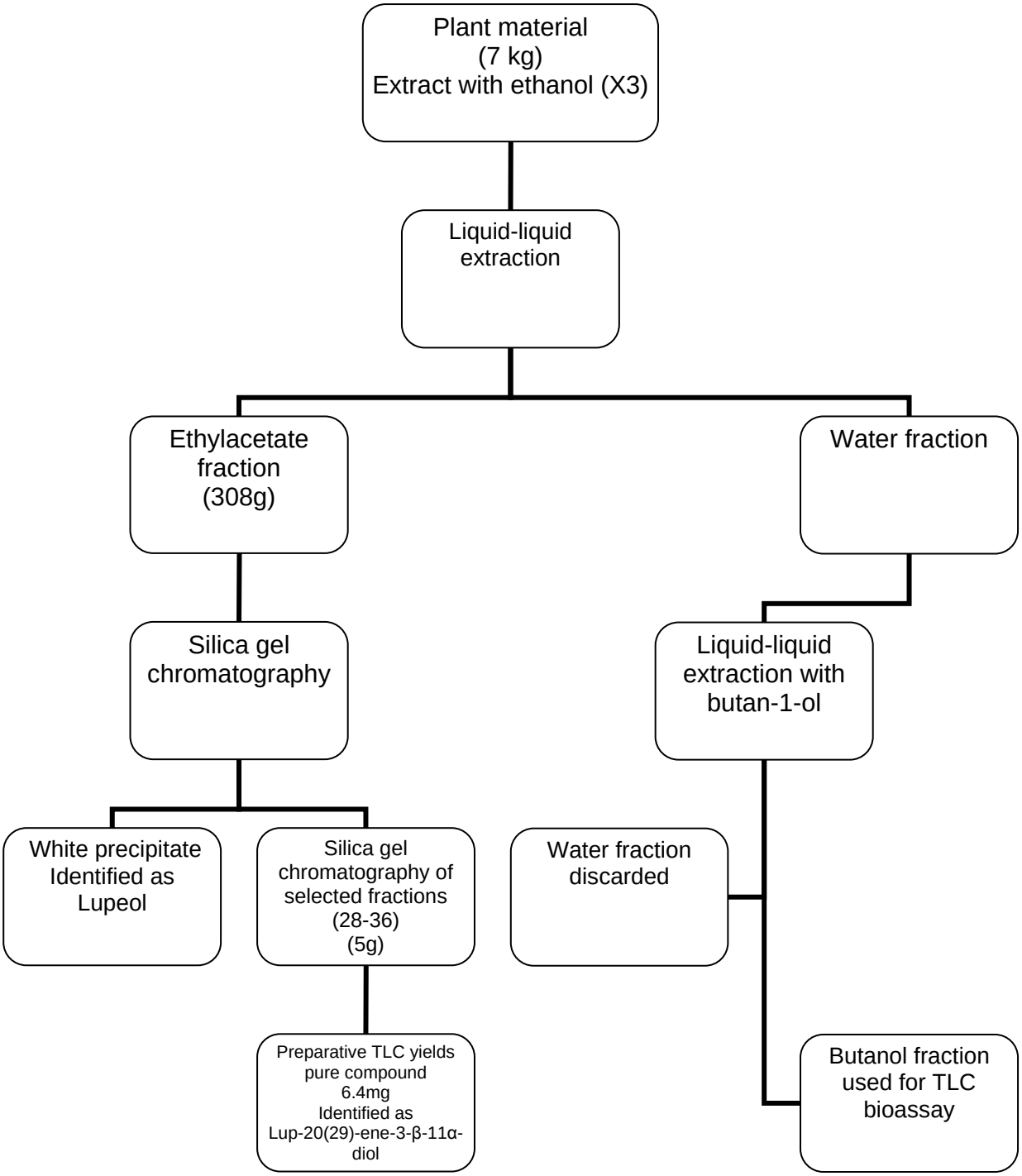

Figure 6.1. A simplified schematic representation of the extraction procedure for isolation of compounds.

6.5 Results

6.5.1 TLC agar plate bioassay

The positive control, juglone completely inhibited the germination of the lettuce seeds (Figure 6.2) but the seeds germinated on all the *E. damarana* extracts, possibly because of the very low concentrations on the TLC plates.

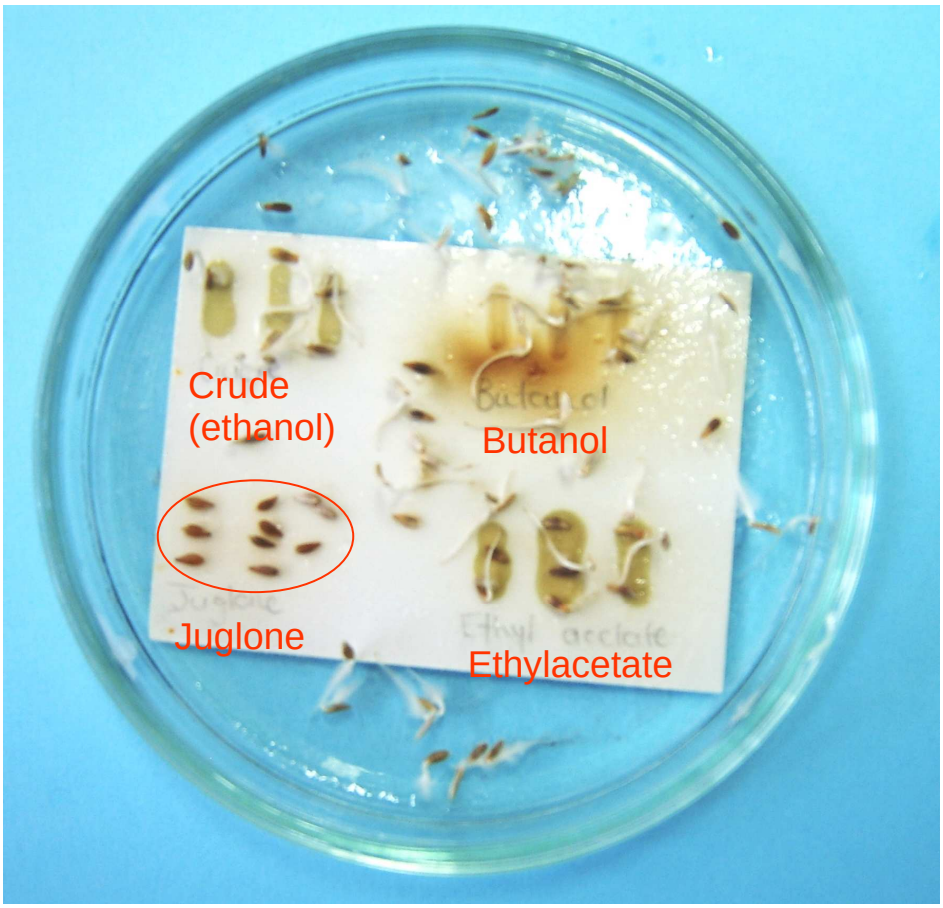

Figure 6.2. Thin layer chromatography bioassay agar plate showing the germination of lettuce seeds on the different extracts from *Euphorbia damarana* with juglone as positive control.

6.5.2 Growth and germination inhibition bioassay

Growth and germination inhibition was determined in the same experiment using different concentrations of crude ethanol extract on filter paper (Figure 6.3, Tables 6.1 & 6.2). Seeds geminated less well at higher concentrations of the crude extract and germination improved concentrations got lower but the differences were not significant. There was however a significant difference in germination percentage of seeds germinated on the 25 mg/ml concentration when compared to the control ($p = 0.02$). For some unknown reason more seeds germinated at 1.0 mg/ml than at the two lowest concentrations of 0.5 and 0.1 mg/ml.

The length of the radicles showed a decrease with an increase in concentration but the radicle length at 0.5 mg/ml was higher than those at the lower concentration of 0.1 mg/ml although the difference was not statistically significant. There was a significant difference between the radicle lengths measured on a concentration of 25 mg/ml when compared to the control ($p = 0.004$) and to concentrations of 0.5 mg/ml ($p = 0.0003$) and 1.0 mg/ml ($p = 0.005$).

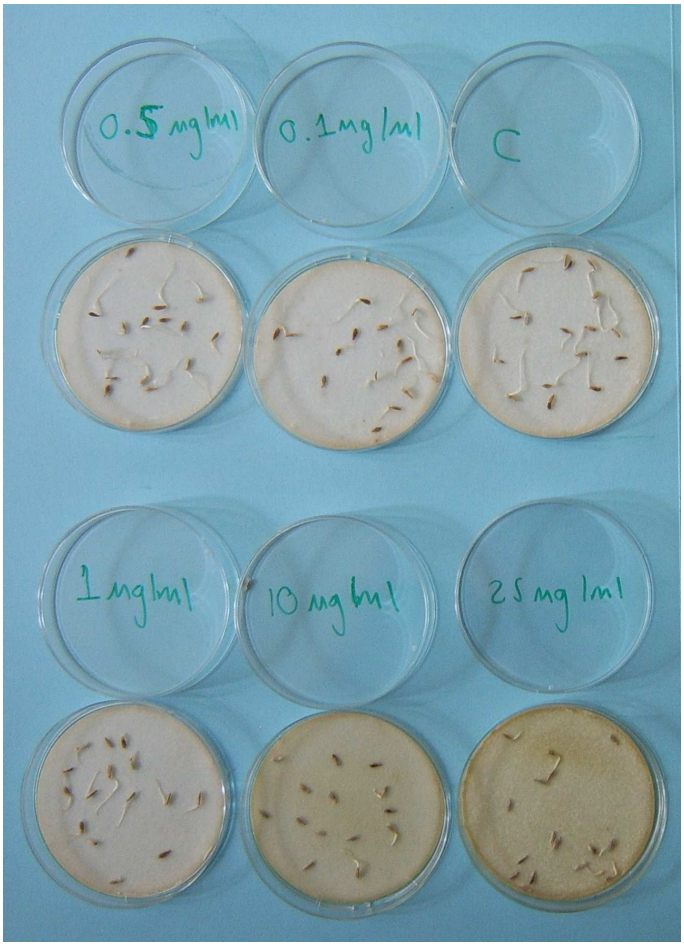

Figure 6.3: Inhibition of radicle growth by *Euphorbia damarana* ethanol extracts with 100% ethanol added to the filter paper as a control. No seeds germinated at the 50 mg/ml concentration.

Table 6.1. Percentage germination in different concentrations of ethanol crude extract of *Euphorbia damarana*

Concentration in mg/ml	50.0	25.0	10.0	1.0	0.5	0.1	Control
Germination percentage	0.0	30.0	50.0	83.3	63.3	63.3	100.0
Standard Error	0	16.8	10.0	16.7	3.3	3.3	0

Table 6.2. Radicle length of lettuce seedlings at different concentrations of *E .damarana* crude extract. Values with common superscripts do not differ significantly at α = 0.05

Concentration (in mg/ml)	Mean length in mm	Std. Dev.	Length of radicle as percentage of control
50.0	0.0	0.0	0.0
25.0	3.3[a]	2.4	40.7
10.0	3.7[a]	2.1	45.7
1.0	7.8[b]	4.2	96.3
0.5	9.2[b]	3.7	113.6
0.1	6.5[a]	3.1	38.3
Control	8.1[b]	3.8	100.0
All groups	7.2	3.9	
Analysis of variance p <0.05			

6.5.3 Isolation and identification of pure compounds from ethanol extract

Two pure compounds were isolated from the ethanol crude extract using chromatographic procedures and identified on the basis of nuclear magnetic resonance (NMR) data.

The NMR spectra (Appendix A) obtained from the white precipitate (Figure 6.1) proved to be lupeol (Figure 6.4).

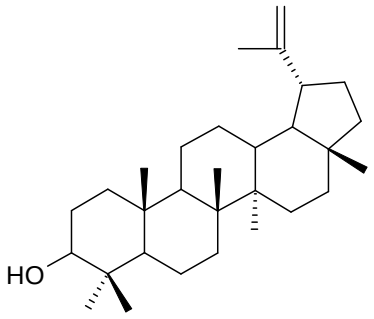

Figure 6.4. Structure of lupeol.

The signals exhibited in the ^{1}H-NMR spectrum of the compound is characteristic of lupane type triterpenes (Alves *et al*., 2000). Seven signals of three proton at δ 0.74, 0.77, 0.81, 0.92, 0.94, 1.01 and 1.66 ppm, one proton signal at δ 2.12 ppm, *m*, which assigned to H-19, one proton signal at δ 3.4 ppm, *dd*, assigned to 3α–H, and two proton signals at δ 4.54, 4.66 ppm, one of them shows long range coupling with one methyl (at δ 1.66), those last two protons assigned to isopropenyl olefinic protons. All the above data indicate that, the isolated compound is the commonly found triterpene, lupeol (Figure 6.4).

Another compound (Appendix B) was also isolated from the hexane fraction, which was purified using preparative TLC. The pure compound also showed signals characteristic of a lupane skeleton triterpene with seven signals of three protons singlets at δ 0.73, 0.77, 0.84, 0.92, 0.97, 1.23 and 1.70 ppm; one proton signal at δ 2.16, *m* [H-19], one proton at δ 3.21, *dd* [H-3α], two proton signals at δ 4.81, 4.90 ppm, in addition to a triplet one proton signal at 3.99. The above data indicated the presence of a lupeol skeleton with a proton on the oxygenated carbon atom. Careful revision of the literature (Alves *et al.*, 2000) and comparing the obtained data of ^{1}H- and ^{13}C-NMR indicated that the compound is lup-20(29)-ene-3-β–11α-diol (Figure 6.5) which was isolated for the first time from *Nepeta hindostana* (Roth) Haines and several *Salvia* species (Chapman & Hall, 2006).

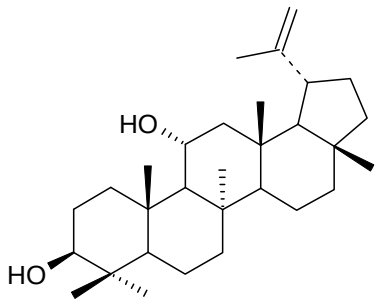

Figure 6.5. Structure of lup-20(29)-ene-3β–11α-diol.

6.6 Discussion

6.6.1 TLC agar plate bioassay

The results from the thin layer chromatography plate bioassay (Figure 6.2) indicated that there is no compound in the crude ethanol extract that inhibits germination at the low concentrations tested. Upon column fractionation and the consequent relative increase in the concentration of the compounds, no germination inhibition was still observed on TLC. The fact that fractions of a plant do not cause inhibition *in vitro* does not necessarily mean that there is no possibility of the plant having allelopathic actions in a natural environment. As previously mentioned allelopathic experiments should ideally include the species implicated in the allelopathy present in the environment. This was not the case in these experiments due to a lack of sufficient amounts of *S. uniplumis* seeds. This experiment should be repeated using *S. uniplumis* seeds or other species growing in the vicinity of the fairy circles.

6.6.2. Germination and growth inhibition bioassay

In contrast to the thin layer chromatography plate bioassay, the germination inhibition assay showed that germination of the lettuce seeds was completely inhibited by a concentration of 50 mg/ml and only 30% and 50% of the seeds germinated on the 25 mg/ml and 10 mg/ml respectively. Only 63.3% of the seeds germinated on the 0.5 mg/ml

concentration, compared to the higher concentration of 1.0 mg/ml where 83.3% seeds germinated (Figure 6.3 & Table 6.1).

There was a significant difference between radicle lengths of the control when compared to the concentrations of 50 mg/ml, 25 mg/ml, 10 mg/ml and 0.1mg/ml. There are not many literature reports describing the use of crude extracts in bioassays to get an indication of the allelopathic potential of plants. Chon and co-workers (2005) used fractions of crude extract at concentrations of between 0.025 mg/ml and 0.1 mg/ml. The concentrations used in the *E. damarana* experiments were to orders of magnitude higher but only crude extracts were used and no fractions. The experiment gives an indication that there might be growth inhibiting compounds in *E. damarana*. Whether this compound, if present, is in the soil and in a form that could be taken up by the plant is a question that has to be answered before any conclusions can be drawn about the possibility that *E. damarana* might be a factor in the origin of the fairy circles.

6.6.3 Isolated compounds

The two compounds isolated were rather common compounds. Lupeol occurs in many plant species, e.g. *Ficus* spp and *Manilkara* spp. It was first isolated in 1889 from *Lupinus luteus* L. and is one of the most widespread of the pentacyclic triterpenes. The other isolated compound is a common derivative of lupeol and is found in *Nepeta hindostana* and *Salvia* spp. The common name of the compound is nepeticin and it has

antibiotic and blood cholesterol reducing properties (Chapman & Hall, 2006). No allelopathic action has been shown previously for these compounds.

6.7 Conclusion

When Theron (1979) came forth with the hypothesis that *E. damarana* might be responsible for the creation of the fairy circles it was not an unfounded one, especially when looking at the fairy circles that occur in the Giribes Plains. As mentioned in the introduction, it is very difficult to prove allelopathic action satisfactorily and most researchers can only satisfy three or four of the criteria to prove allelopathic action. Germination inhibition and growth inhibition is a good starting point to validate full allelopathic studies. Although seeds do germinate in the fairy circles they die before reaching maturity (Van Rooyen *et al.*, 2004). The results indicate that germination might not be inhibited, but that growth might be retarded and that plants might die because of this factor, thus keeping the circles bare.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

7.1 Introduction

Many papers written on the fairy circles attempted to explain the origin. They do not, however, always give data solely collected for the purpose of experimentation regarding the conditions prevailing in and around the fairy circles currently. It is however important to have a broad overview of the factors influencing the existence of the circles at present while doing research on the possible origin.

The results from the bioassay with *Stipagrostis uniplumis* indicated that the factor causing the inhibition of growth of the plants can be transferred to an 'artificial' environment.

The mycorrhizae experiments gave very promising results with regards to the possible maintenance of the circles but more research needs to be done to determine the extent of the infection and the different species responsible for the infection. It would also be helpful to determine the different mycorrhizal species present in the soil. It must be kept in mind that VAM will probably need to be linked to another factor to be able to explain the maintenance of the fairy circles as the VAM alone cannot account for the existence of these circles.

The bioassay with *Stipagrostis* species confirmed the results of van Rooyen *et al.* (2004) with *Lolium multiflorum*. This would mean that it is indeed possible to transfer the factor that causes inhibition of growth to an artificial environment and that it is possible to use a different species and reproduce the phenomenon in an artificial pot experiment. It is however not always the case as other authors, using different bioindicators did not get the same results (Theron, 1979; Moll, 1994 and Albrecht *et al.*, 2001). If at all possible a species such as *Stipagrostis* that occur in the same environment as the fairy circles should be used in repeat experiments.

The total element analysis of the soil using ICP-MS did not show any definite patterns as to a specific difference in any elements when comparing the inside of the circles to that of the edge and the matrix. It does not however mean that there is not an elemental difference in the soil. Soil is complicated, with many factors influencing the availability of elements to the plants on a macro- and microhabitat scale. The extractions done could have influenced the results in such a way that the difference was not obvious. The results could also be refined by adding parameters such as soil moisture, and pH from the different microhabitats into the calculations. This would give one a better view of the overall conditions prevalent in the soil.

Allelopathy is difficult to prove but carefully designed experiments can prove or disprove allelopathic effects. Proper experiments will be able to put the role, if any, that *E. damarana* plays in the fairy circles into perspective. The possibility of isolating allelopathic compounds from *E. damarana* should also be investigated further as the ethanol extract showed some growth inhibition at 25 mg/ml. As with the mycorrhizal

experiments many factors should be taken into account when investigating the possibility of allelopathy, keeping in mind that the origin and the maintenance might differ greatly.

7.2 The competitive exclusion principle

In 1934 Gause stated that two species with similar ecology cannot indefinitely coexist (Hardin, 1960). The statement was then rephrased by Hardin (1960) to be more specific into the following: 'if two non-interbreeding populations occupy precisely the same ecological niche and if species A multiplies faster that species B, ultimately B will completely disappear.'

In the case of the fairy circles a hypothetical scenario on the origin of the circle could include the possibility that the competitive exclusion principle might be in action in the microbial environment. In a semi-arid environment resources are low and the competition for these resources would be intense. Assuming that the factor responsible for the origin and the factor responsible for the maintenance of the circles is not the same, one could argue that after the initial disturbance of the microbial population, the population re-established at a different equilibrium. This could have resulted in the exclusion of the vesicular arbuscular mycorrhizae. The lack of VAM would the cause the seedlings that germinate inside the circles to die upon dehydration where the plants in the matrix would be resistant to the dehydration to some extent.

7.3 Conclusion

The absence of vesicular arbuscular mycorrhizae in the plants from the inside of the circles and the growth inhibition properties of *E. damarana* show promising results to possibly explain the fairy circle phenomenon. Further research into these aspects of the fairy circles combined with more bioassay studies and a better understanding of the growth patterns and germination requirements of the different *Stipagrostis* species that occur in the areas where the fairy circles occur might give us the answer to this mysterious phenomenon. Until then we will have to accept it as the one of the mysteries of science and believe that fairies are responsible for them.

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APPENDIX A

NMR SPECTRA OF ISOLATED LUPEOL

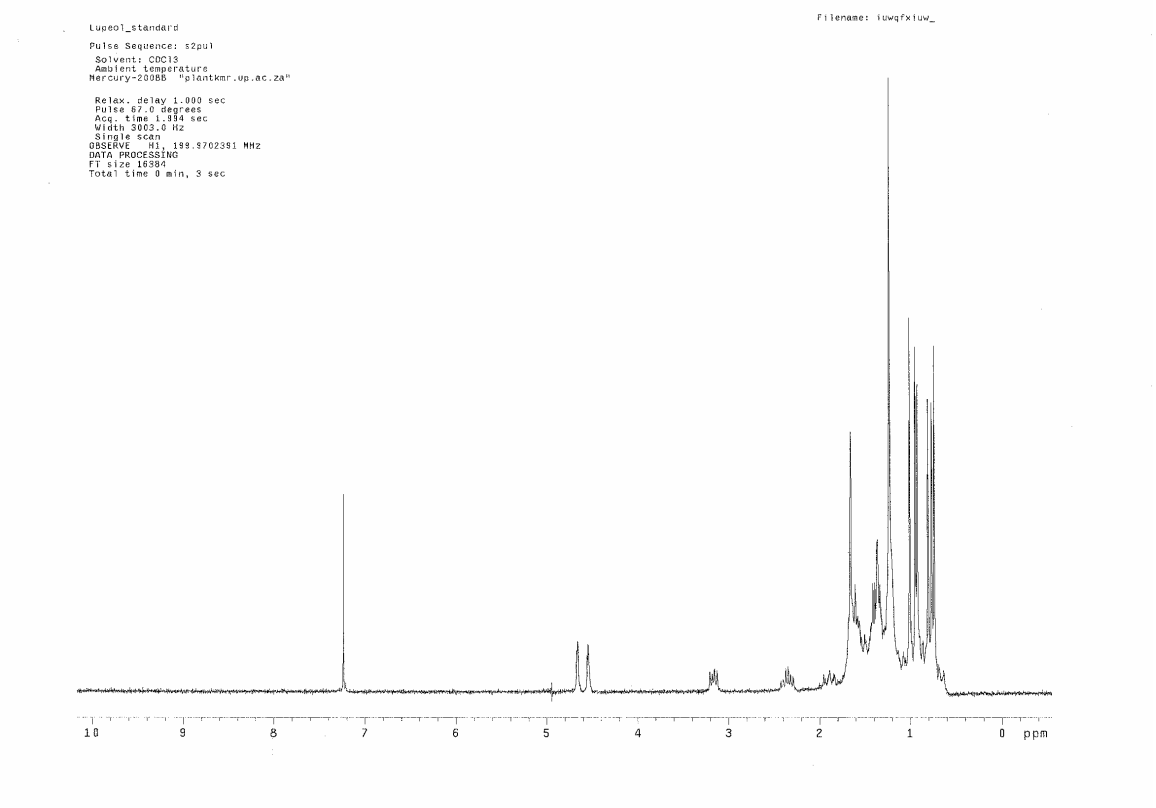

Figure A.1. ^{1}H-NMR spectrum.

APPENDIX B

NMR SPECTRA OF ISOLATED AS LUP-20(29)-ENE-3, 11-DIOL

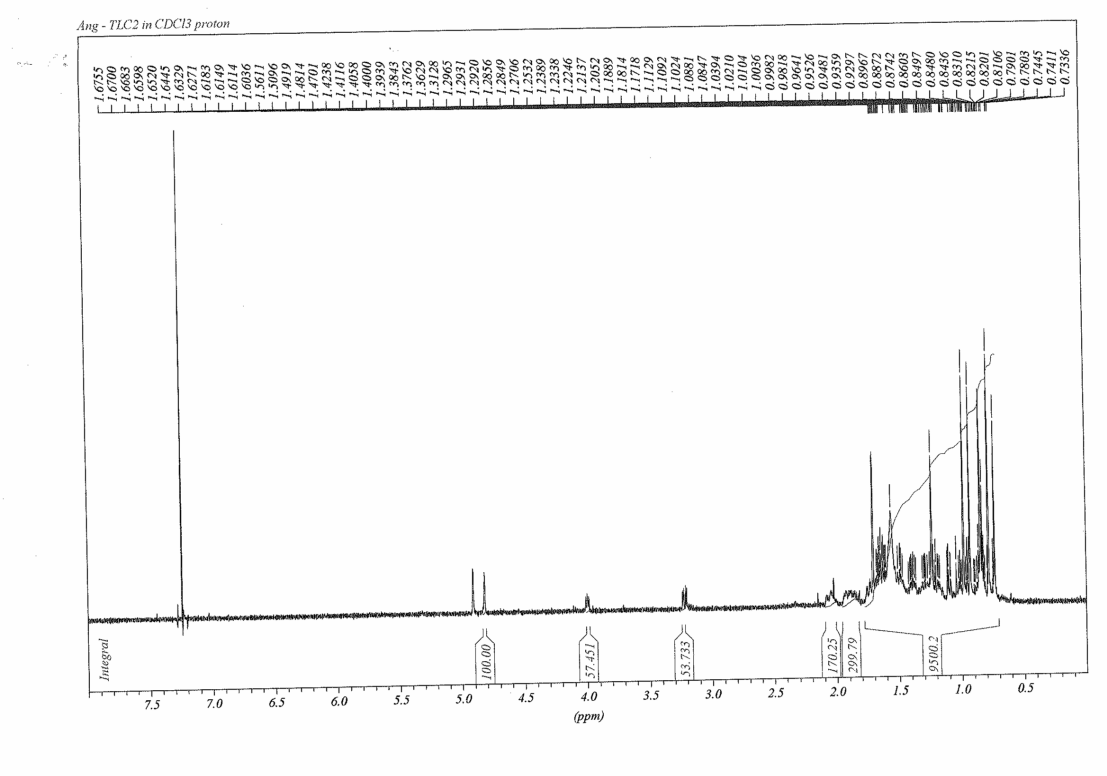

Figure B.1. ^{1}H-NMR spectrum.

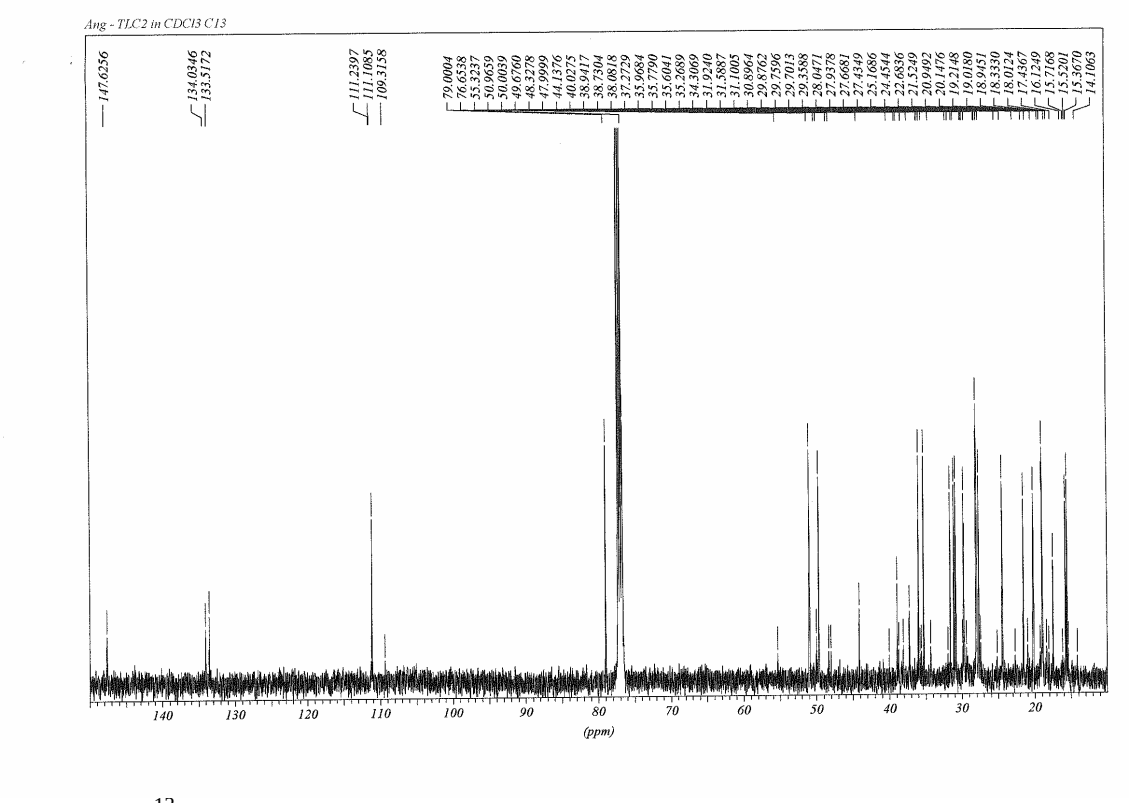

Figure B.2. ^{13}C-NMR spectrum.

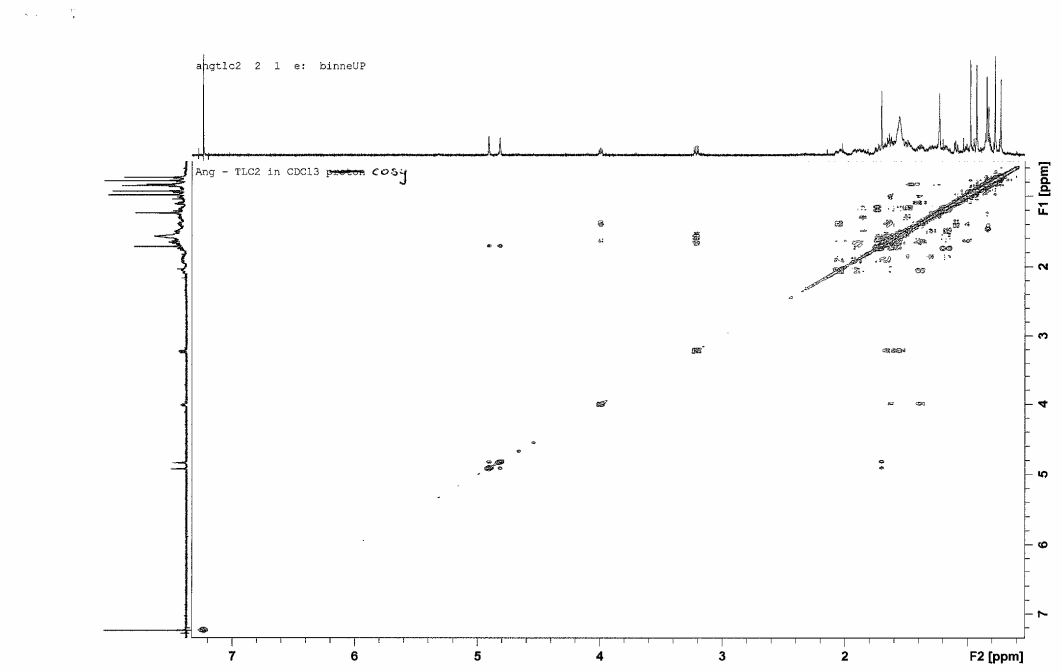

Figure B.3. Cosy spectrum.

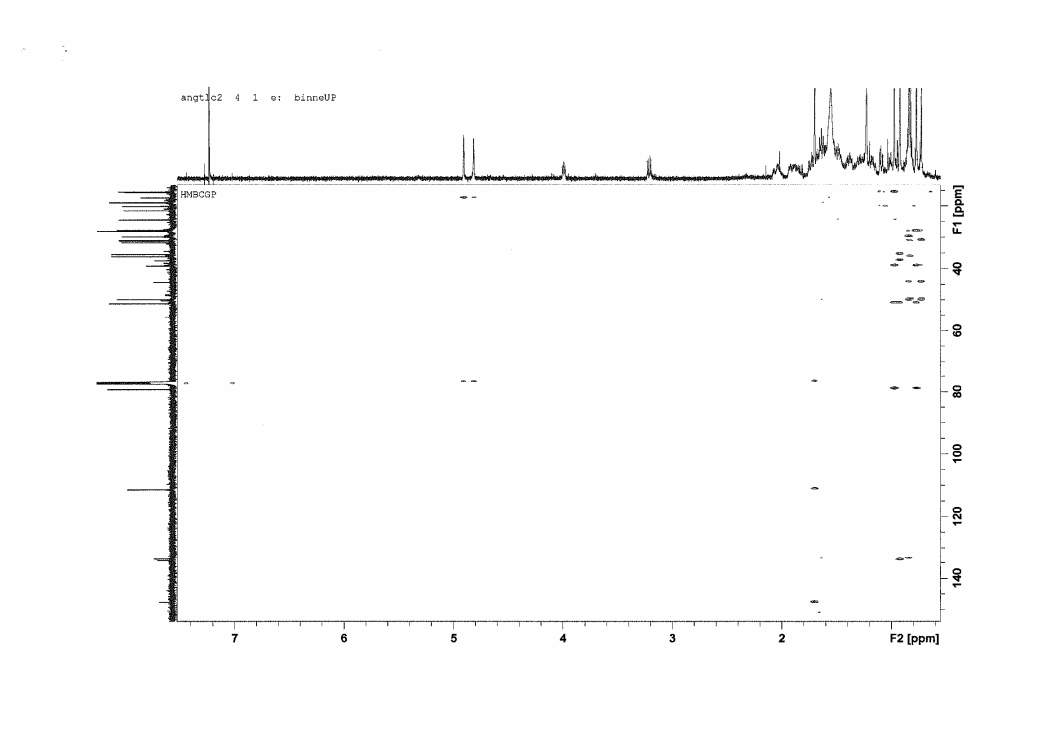

Figure B.4 HMBCGP Spectrum.

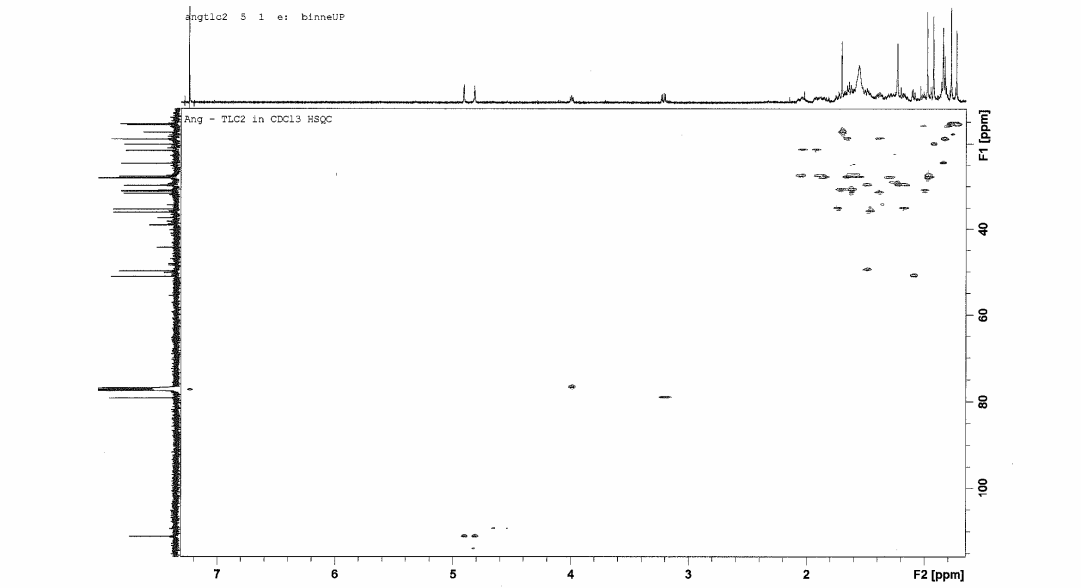

Figure B.5. HSQC spectrum.

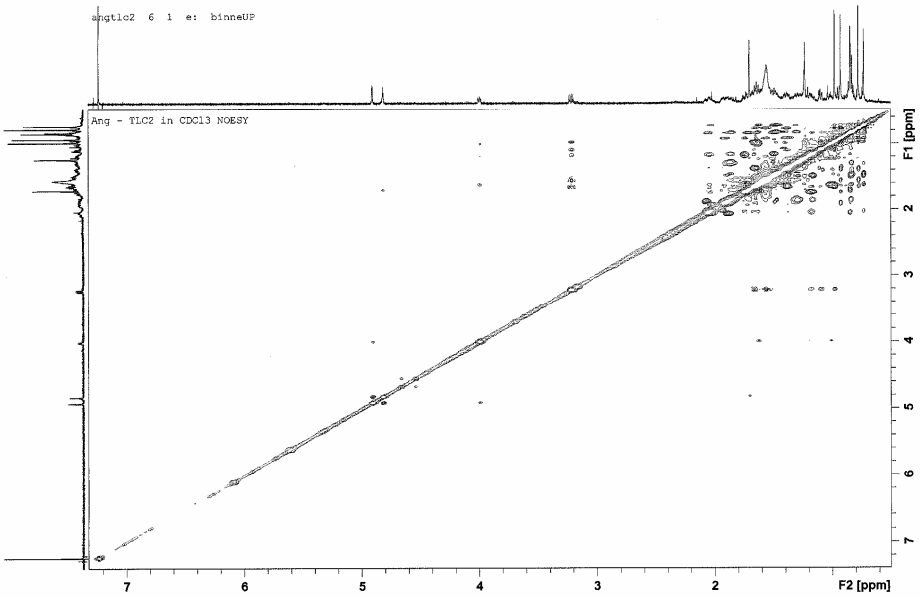

Figure B.6. NOESY spectrum.

APPENDIX C

ICP-MS DATA FROM SOIL ANALYSES

Sample number	Location	Region of circle	Depth in m	Li	Be	B	Na	Mg	Al	Si	P	K	Ca	Sc	Ti	V	Cr
1	Bushmanland	Inside	0	0	10	87	4788	23192	30190	1170	3142	13167	18849	13	1611	1030	321
3	Bushmanland	Inside	0	1	8	97	4879	21177	29417	8071	4277	13059	17163	14	1612	1023	315
5	Bushmanland	Inside	0	0	9	119	4801	18830	26956	1070	3211	11278	18488	12	1473	1077	314
7	Bushmanland	Inside	0	0	6	129	5154	17711	27687	1190	5501	11120	24209	11	1361	888	257
8	Bushmanland	Inside	0	0	8	80	5134	19057	28490	1247	5861	10480	23429	14	1457	904	274
10	Bushmanland	Inside	0	0	8	92	5032	19104	30003	4458	5725	11312	24156	14	1538	1004	273
11	Bushmanland	Inside	0	0	13	100	5772	24494	31948	1627	5425	10751	36190	12	1547	1144	297
14	Bushmanland	Inside	0	0	6	84	5118	18652	27071	1894	6340	10067	24087	11	1491	985	283
15	Bushmanland	Inside	0	0	7	89	5685	17545	24817	1813	5452	11314	24089	11	1469	987	266
2	Bushmanland	Matrix	0	3	7	109	4819	20089	27102	1546	4350	10937	17276	12	1563	1070	311
4	Bushmanland	Matrix	0	2	9	94	4933	18486	24288	2854	3873	10031	15682	11	1490	996	295
6	Bushmanland	Matrix	0	3	5	104	5745	22458	29942	2733	4427	12552	19719	13	1601	1088	326
9	Bushmanland	Matrix	0	1	10	67	5500	22074	32970	4326	6461	13420	29050	14	1577	1026	303
12	Bushmanland	Matrix	0	0	7	86	5441	18448	28451	1175	6189	11984	25007	11	1453	932	291
13	Bushmanland	Matrix	0	1	10	108	6318	24968	31050	1484	5775	11826	50773	11	1533	1097	300
16	Bushmanland	Matrix	0	0	7	88	5311	23254	34263	1237	7281	14639	41262	13	1708	1078	323
29	Bushmanland	Edge	0	388	21	319	5087	244368	317164	4985	36411	149256	158853	77	21473	2663	1430
30	Giribes	Edge	0.5	359	16	296	8140	210567	279024	4000	26359	112567	674896	66	15683	2720	1334
31	Giribes	Edge	1	380	15	232	14712	214717	275053	3190	43360	102154	1529424	61	14606	2622	1232
36	Giribes	Edge	0	198	15	385	4680	133112	180127	6788	27696	88594	117646	80	8575	1941	1472
39	Giribes	Edge	0	140	10	432	3675	107088	144985	5555	20595	74121	105258	68	7497	1675	1076
20	Giribes	Inside	0	212	9	219	3917	146801	198093	4220	23585	85003	78651	49	12596	1706	1069
21	Giribes	Inside	0.5	340	17	355	29132	239398	315406	4648	30537	124284	350254	74	17649	2981	1439
22	Giribes	Inside	1	263	14	246	3869	180048	232991	3537	32776	91976	440012	59	13153	2445	1169
23	Giribes	Inside	0	370	16	269	5721	266722	343333	5884	38059	146977	142606	92	20944	3007	1599
24	Giribes	Inside	0.5	416	19	248	5206	264694	346995	4260	37370	130687	379332	79	19388	3205	1591
25	Giribes	Inside	1	439	26	272	6120	282561	384599	30306	45359	148829	974911	101	22009	3504	1825
26	Giribes	Inside	0	357	9	309	4593	241203	324697	28868	40127	136801	145039	96	21866	2971	1554
27	Giribes	Inside	0.5	344	14	311	6200	217258	286916	3436	30566	112422	445352	68	16738	2895	1374
28	Giribes	Inside	1	312	15	290	4646	198887	242825	2470	37302	88282	1759145	52	13078	2486	1172
37	Giribes	Inside	0	169	21	427	3626	118664	159944	5780	25954	86100	109639	76	7798	1842	1128
40	Giribes	Inside	0	108	9	405	3058	76511	108022	6188	14366	53892	81295	65	5079	1503	955
32	Giribes	Matrix	0	263	5	273	2602	166446	218905	3275	25171	100933	99946	57	13651	1915	1034
33	Giribes	Matrix	0.5	302	23	448	8482	210356	239234	3650	13460	79353	478401	82	13847	2587	1444
34	Giribes	Matrix	1	340	20	575	22753	212986	260966	5101	30070	102333	1286703	89	13269	2574	1517
35	Giribes	Matrix	0	150	10	449	3229	92269	130498	5797	18779	65147	91607	67	5650	1517	1009
38	Giribes	Matrix	0	132	6	417	2806	89734	121055	7490	16795	64705	83800	64	5951	1413	992
42	Keerweder	Edge	0	34	10	157	3320	23776	43744	4911	3432	15101	39771	44	6007	1913	1217
45	Keerweder	Edge	0	35	12	152	3772	26698	51624	4533	3583	16453	36709	52	6613	2073	1408
47	Keerweder	Edge	0	33	14	100	3043	35135	64361	4347	4194	18054	41234	64	7180	2465	1723
50	Keerweder	Edge	0	31	10	101	2071	26892	51421	3911	4185	17913	40117	46	7453	2252	1447
41	Keerweder	Inside	0	32	11	198	2028	26531	49606	11029	3400	14067	33823	54	6065	1931	1365
44	Keerweder	Inside	0	40	14	139	2923	33243	62671	4028	4133	18095	39668	62	7295	2376	1635
48	Keerweder	Inside	0	37	12	122	9638	27168	50889	4245	5050	15083	36122	53	7030	2284	1402
49	Keerweder	Inside	0.5	25	14	83	3586	24606	42671	4099	2826	11787	32828	43	6323	2149	1341
43	Keerweder	Matrix	0	39	12	153	3459	29326	55119	4115	3679	18184	55933	56	7054	2210	1493
46	Keerweder	Matrix	0	40	14	87	5529	28572	53524	4379	4009	16592	37483	53	6461	2068	1415
51	Keerweder	Matrix	0	37	11	134	2444	30346	57970	4591	4245	18467	37534	59	7376	2359	1558
52	Keerweder	Matrix	0.5	31	13	125	6673	25519	44335	3596	2717	11985	32570	44	6237	2037	1320
60	Marienfluss	Edge	0	37	14	93	2083	29452	67865	3901	4970	38622	25037	52	11785	1557	477
64	Marienfluss	Edge	0	29	10	60	1742	30822	66019	3007	6071	38503	28496	48	10668	1295	432
69	Marienfluss	Edge	0	23	8	41	4787	24597	53277	3166	5667	29539	24727	35	9222	1160	415
55	Marienfluss	Inside	0	126	61	145	2516	91333	173139	4027	27038	92091	85454	94	15883	2067	703

Sample number	Location	Region of circle	Depth in m	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Rb	Sr	Y	Zr	Nb	Mo
1	Bushmanland	Inside	0	1899	203601	57	109	156	520	33	2	247	22	2383	53	155	290	36	10	10
3	Bushmanland	Inside	0	1791	209379	50	116	136	530	33	2	246	36	1909	58	134	301	32	9	14
5	Bushmanland	Inside	0	1762	201276	48	189	190	534	33	1	260	21	2116	49	141	300	30	11	13
7	Bushmanland	Inside	0	2198	134121	46	104	130	541	25	1	250	27	1906	51	149	248	25	10	5
8	Bushmanland	Inside	0	2230	137758	50	317	133	644	25	1	251	23	1125	58	130	264	38	10	6
10	Bushmanland	Inside	0	2283	143456	49	99	151	497	28	2	287	27	1340	58	146	279	29	11	6
11	Bushmanland	Inside	0	2392	152439	62	301	855	831	31	2	299	36	4043	56	175	274	22	11	7
14	Bushmanland	Inside	0	1948	135092	45	103	135	477	25	2	284	37	2569	52	141	256	31	10	6
15	Bushmanland	Inside	0	1927	131884	41	288	339	815	24	1	305	32	5119	50	143	234	25	11	7
2	Bushmanland	Matrix	0	1696	219159	49	149	142	622	32	2	265	24	1572	51	139	288	33	13	10
4	Bushmanland	Matrix	0	1948	177542	45	165	129	467	28	1	268	32	2481	49	121	260	28	11	11
6	Bushmanland	Matrix	0	1887	199905	54	114	146	499	32	2	262	32	1913	55	144	284	35	11	9
9	Bushmanland	Matrix	0	2690	150812	57	110	170	674	30	1	274	31	2205	62	163	287	31	12	6
12	Bushmanland	Matrix	0	2186	140502	45	164	145	495	26	1	272	26	2202	51	148	242	26	11	7
13	Bushmanland	Matrix	0	2241	147462	59	194	220	599	29	1	298	26	3094	55	209	281	22	12	6
16	Bushmanland	Matrix	0	2492	167026	55	124	161	665	32	2	288	29	2652	60	193	294	27	13	6
29	Bushmanland	Edge	0	12335	724407	361	675	1200	2030	151	4	291	35	2932	924	906	292	108	27	26
30	Giribes	Edge	0.5	9036	585707	375	716	999	1361	137	3	324	21	3166	804	1870	294	35	20	25
31	Giribes	Edge	1	8035	537545	325	478	933	1389	126	3	333	66	4445	742	3187	340	34	22	16
36	Giribes	Edge	0	9986	446723	260	585	666	1810	82	4	222	78	9040	436	668	272	70	13	46
39	Giribes	Edge	0	7409	327975	182	547	467	1138	61	3	248	69	11266	352	636	216	51	11	38
20	Giribes	Inside	0	8578	476238	289	728	898	1006	97	3	200	30	3231	559	486	199	130	20	25
21	Giribes	Inside	0.5	9619	663376	395	708	1044	1217	149	4	343	66	5239	861	1042	312	75	26	10
22	Giribes	Inside	1	7960	494987	338	524	827	954	117	4	264	26	1859	630	1152	289	53	19	11
23	Giribes	Inside	0	13371	815866	448	587	1263	1871	171	5	328	33	766	944	892	382	214	29	31
24	Giribes	Inside	0.5	10154	733608	453	714	1216	1402	165	4	363	35	2901	936	1236	360	100	23	32
25	Giribes	Inside	1	11809	784141	498	1498	1541	2207	176	5	431	62	5195	1021	2347	413	67	22	29
26	Giribes	Inside	0	12261	756589	482	563	1148	1486	170	4	340	48	4541	892	869	376	191	22	25
27	Giribes	Inside	0.5	9063	619236	378	873	1239	1474	140	3	347	40	3700	790	1327	308	45	23	28
28	Giribes	Inside	1	7507	519580	370	723	1144	2286	119	3	334	57	3311	640	2959	274	31	27	16
37	Giribes	Inside	0	9715	389688	266	515	563	1409	74	3	217	68	8979	406	557	245	78	10	39
40	Giribes	Inside	0	6472	287559	181	512	482	1162	53	3	224	75	9731	260	427	191	59	11	37
32	Giribes	Matrix	0	8611	509850	293	378	819	998	105	2	215	18	1636	631	572	233	101	18	18
33	Giribes	Matrix	0.5	6530	430227	220	620	580	829	64	2	201	60	4972	462	1058	179	48	15	15
34	Giribes	Matrix	1	7413	556097	464	815	1616	1533	108	4	302	93	7665	620	2759	300	33	20	37
35	Giribes	Matrix	0	7568	316610	198	477	536	1098	59	3	190	53	8133	305	482	197	49	8	38
38	Giribes	Matrix	0	6740	293149	180	462	392	954	55	2	176	69	8663	308	469	164	50	9	37
42	Keerweder	Edge	0	2910	321817	71	0	178	942	35	2	211	24	3278	76	320	174	28	23	6
45	Keerweder	Edge	0	3562	367499	87	0	185	742	42	2	234	27	5353	89	367	190	70	24	12
47	Keerweder	Edge	0	4454	467999	118	0	229	984	50	3	228	25	3385	104	422	209	126	25	15
50	Keerweder	Edge	0	3291	409390	84	0	802	1916	42	2	220	40	5064	92	388	188	80	39	11
41	Keerweder	Inside	0	3251	362674	85	0	154	839	39	2	176	30	4104	83	307	175	114	21	12
44	Keerweder	Inside	0	4350	450585	108	0	198	772	48	2	211	37	5303	103	425	208	108	26	13
48	Keerweder	Inside	0	3505	419021	87	0	201	1216	44	3	227	25	3188	90	331	206	127	31	11
49	Keerweder	Inside	0.5	2921	361231	81	0	176	1293	35	2	228	16	2817	80	266	177	102	24	12
43	Keerweder	Matrix	0	4075	413859	102	0	190	1399	45	2	209	40	5022	92	438	204	68	25	13
46	Keerweder	Matrix	0	3791	391299	95	0	198	1027	43	2	208	23	5229	89	393	185	77	35	9
51	Keerweder	Matrix	0	4165	458350	103	0	222	845	47	2	215	38	5150	103	411	206	95	24	11
52	Keerweder	Matrix	0.5	2943	362295	80	0	155	638	36	2	196	35	4574	80	275	172	64	22	12
60	Marienfluss	Edge	0	4578	654491	62	0	179	1685	95	5	450	22	1246	384	152	236	51	68	18
64	Marienfluss	Edge	0	6119	660571	62	0	185	931	94	4	191	22	907	399	190	244	71	56	20
69	Marienfluss	Edge	0	3546	524213	46	0	169	1551	76	4	164	23	868	323	160	199	55	53	13
55	Marienfluss	Inside	0	18460	1072737	211	0	460	2936	232	11	199	51	1059	1215	679	1261	308	178	48

Sample number	Location	Region of circle	Depth in m	Ru	Rh	Pd	Ag	Cd	Sn	Sb	Te	I	Cs	Ba	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho
1	Bushmanland	Inside	0	1	0	0	2	1	50	2	0	3	3	855	829	1442	199	737	125	12	105	14	76	13
3	Bushmanland	Inside	0	0	0	0	1	0	19	1	0	3	3	764	846	1485	207	753	130	13	114	15	78	13
5	Bushmanland	Inside	0	0	4	0	1	4	13	1	0	3	3	754	845	1454	205	747	128	12	124	15	74	14
7	Bushmanland	Inside	0	0	1	0	1	0	15	1	0	3	3	843	718	1195	172	606	97	11	95	12	61	11
8	Bushmanland	Inside	0	0	0	0	1	0	11	1	1	3	4	891	772	1288	184	662	104	12	100	13	67	11
10	Bushmanland	Inside	0	0	0	0	1	0	12	2	1	3	4	929	841	1422	195	696	113	12	102	14	72	13
11	Bushmanland	Inside	0	0	3	0	2	0	23	1	1	9	4	994	798	1333	192	644	112	13	98	13	68	13
14	Bushmanland	Inside	0	0	0	0	2	0	16	1	0	4	3	881	740	1207	177	630	101	12	94	12	63	11
15	Bushmanland	Inside	0	0	0	3	1	0	30	1	1	3	3	845	711	1184	169	591	101	11	88	12	59	10
2	Bushmanland	Matrix	0	0	0	0	2	0	20	1	0	3	3	728	839	1471	203	729	121	12	113	14	76	13
4	Bushmanland	Matrix	0	0	0	0	2	0	15	1	0	2	3	722	762	1280	184	654	107	11	98	14	66	12
6	Bushmanland	Matrix	0	0	0	0	2	0	45	1	0	4	3	763	867	1489	212	738	120	13	112	14	74	13
9	Bushmanland	Matrix	0	0	0	0	2	0	14	1	0	3	4	1026	856	1402	199	722	116	13	108	14	76	14
12	Bushmanland	Matrix	0	0	6	0	2	0	18	1	0	3	3	886	733	1213	170	627	102	11	91	12	60	11
13	Bushmanland	Matrix	0	0	0	0	1	0	14	1	0	11	4	975	838	1382	196	701	112	13	104	14	72	12
16	Bushmanland	Matrix	0	0	0	0	1	0	26	1	0	4	4	1033	832	1405	199	688	113	12	111	14	72	13
29	Bushmanland	Edge	0	0	0	4	0	0	48	3	0	17	37	3826	1029	1982	201	723	113	17	107	12	57	9
30	Giribes	Edge	0.5	0	0	3	0	7	354	3	0	40	39	3275	999	1837	191	681	101	17	109	12	53	9
31	Giribes	Edge	1	0	0	2	0	7	231	2	0	94	37	3356	857	1374	167	619	91	17	107	12	60	10
36	Giribes	Edge	0	0	14	4	2	10	45	2	0	15	18	3029	958	1770	187	703	101	17	110	12	48	9
39	Giribes	Edge	0	0	0	5	2	9	25	2	3	11	16	2334	717	1245	142	496	74	12	65	9	41	7
20	Giribes	Inside	0	0	0	4	0	0	35	2	3	3	26	2408	612	1183	123	433	68	11	70	9	37	6
21	Giribes	Inside	0.5	0	0	2	0	5	46	3	0	20	44	3604	850	1622	173	616	99	17	105	12	53	10
22	Giribes	Inside	1	0	0	2	0	5	41	3	1	24	34	2837	1157	2273	226	779	117	17	111	13	56	9
23	Giribes	Inside	0	0	0	6	0	8	50	3	0	8	43	4045	1653	3250	306	1044	144	22	155	17	76	11
24	Giribes	Inside	0.5	0	0	2	0	0	56	3	0	20	49	3893	1089	1995	211	736	116	20	126	14	66	11
25	Giribes	Inside	1	0	0	3	0	0	80	4	0	60	52	4588	1248	2314	244	858	131	23	141	15	73	12
26	Giribes	Inside	0	0	0	6	0	7	42	3	2	10	37	3761	1824	3568	328	1104	153	23	153	17	74	11
27	Giribes	Inside	0.5	0	0	2	0	6	100	2	0	24	40	3144	908	1623	179	638	94	16	103	12	52	9
28	Giribes	Inside	1	1	0	3	0	0	87	2	0	120	33	3324	853	1557	166	581	88	16	92	11	46	8
37	Giribes	Inside	0	0	1	6	2	9	23	1	0	11	20	2670	781	1378	153	560	90	15	93	10	46	7
40	Giribes	Inside	0	0	1	5	2	7	21	1	0	10	12	1840	801	1504	156	531	76	12	76	8	38	6
32	Giribes	Matrix	0	0	0	3	0	0	39	2	0	13	29	2657	768	1451	152	532	80	13	83	10	43	7
33	Giribes	Matrix	0.5	0	5	3	1	2	28	1	0	13	17	1166	310	624	69	250	40	8	48	5	26	4
34	Giribes	Matrix	1	0	1	18	3	352	55	3	2	72	36	3327	1078	1918	203	710	101	18	116	12	59	9
35	Giribes	Matrix	0	0	0	4	3	8	23	2	0	13	15	2263	659	1248	137	475	71	12	73	8	38	6
38	Giribes	Matrix	0	0	0	4	2	7	19	2	2	10	13	1987	550	1006	113	396	58	10	62	7	33	5
42	Keerweder	Edge	0	0	0	1	2	0	113	4	0	4	5	741	384	466	93	354	69	7	71	8	38	6
45	Keerweder	Edge	0	0	0	1	2	4	37	4	0	10	5	775	407	500	98	361	71	7	72	9	39	6
47	Keerweder	Edge	0	0	0	2	2	4	16	3	0	10	6	854	444	572	107	380	69	7	78	10	43	7
50	Keerweder	Edge	0	0	0	1	1	2	25	4	0	13	5	686	410	496	98	351	63	7	68	8	40	6
41	Keerweder	Inside	0	0	0	2	3	1	19	3	0	5	5	620	382	478	89	334	59	7	66	8	35	6
44	Keerweder	Inside	0	0	0	2	2	4	22	4	0	8	6	853	444	588	103	378	71	8	78	10	43	7
48	Keerweder	Inside	0	0	0	2	2	4	17	3	0	8	5	770	456	559	110	404	78	8	78	9	44	6
49	Keerweder	Inside	0.5	0	0	2	2	3	13	2	0	14	5	639	358	470	86	320	63	6	65	7	37	6
43	Keerweder	Matrix	0	0	0	2	2	4	26	4	0	6	6	833	426	540	105	370	70	8	78	9	44	7
46	Keerweder	Matrix	0	0	0	1	2	5	21	3	0	6	5	1006	385	497	90	332	60	8	68	8	39	6
51	Keerweder	Matrix	0	0	0	1	1	4	130	5	0	11	5	806	440	542	103	383	72	8	75	9	42	7
52	Keerweder	Matrix	0.5	0	0	1	1	2	22	22	0	19	5	656	359	431	88	316	55	6	61	7	36	6
60	Marienfluss	Edge	0	0	0	1	1	3	22	2	0	13	10	795	825	1281	178	633	96	11	93	11	45	7
64	Marienfluss	Edge	0	0	0	1	1	3	20	1	0	11	9	968	782	1176	167	584	84	11	87	10	40	6
69	Marienfluss	Edge	0	0	0	1	1	2	59	2	0	8	8	810	683	997	148	512	79	9	75	8	37	6
55	Marienfluss	Inside	0	0	0	5	4	6	91	2	0	15	28	2963	3070	5894	682	2444	425	23	430	54	243	41

Sample number	Location	Region of circle	Depth in m	Er	Tm	Yb	Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Th	U
1	Bushmanland	Inside	0	34	4	27	4	1	1	9	0	0	0	0	0	1	0	168	4	9184	3293
3	Bushmanland	Inside	0	35	5	28	4	1	1	9	0	0	0	0	0	0	0	155	4	9432	3451
5	Bushmanland	Inside	0	33	5	26	4	1	1	10	0	0	0	0	0	0	0	198	4	9482	3196
7	Bushmanland	Inside	0	27	4	23	3	1	1	10	0	0	0	0	0	0	0	136	3	4778	0
8	Bushmanland	Inside	0	30	4	25	3	1	1	9	0	0	0	0	0	2	0	171	3	5404	0
10	Bushmanland	Inside	0	34	4	26	4	1	1	9	0	0	0	1	0	0	0	203	3	6913	0
11	Bushmanland	Inside	0	31	4	26	4	1	1	11	0	0	0	1	0	1	0	204	3	6418	0
14	Bushmanland	Inside	0	30	4	24	3	1	1	9	0	0	0	1	0	0	0	167	3	6201	0
15	Bushmanland	Inside	0	26	4	22	3	1	1	8	0	0	0	0	0	0	0	181	3	4901	0
2	Bushmanland	Matrix	0	33	4	26	4	1	1	10	0	0	0	1	0	0	0	192	4	10317	4001
4	Bushmanland	Matrix	0	31	4	24	3	1	1	10	0	0	0	0	0	3	0	187	3	8187	0
6	Bushmanland	Matrix	0	32	4	26	4	1	1	10	0	0	0	0	0	0	0	199	4	8898	0
9	Bushmanland	Matrix	0	32	4	27	4	1	1	10	0	0	0	1	0	1	0	158	4	6448	0
12	Bushmanland	Matrix	0	27	4	20	3	0	1	10	0	0	0	0	0	0	0	178	3	4193	0
13	Bushmanland	Matrix	0	32	4	25	3	1	1	10	0	0	0	0	0	1	0	189	3	4858	0
16	Bushmanland	Matrix	0	33	5	27	4	1	1	12	0	0	0	0	0	0	1	191	4	6898	0
29	Bushmanland	Edge	0	21	3	18	3	1	0	7	0	0	1	2	0	0	6	323	9	232	22
30	Giribes	Edge	0.5	22	3	17	2	1	0	6	0	0	1	1	0	0	6	282	5	216	24
31	Giribes	Edge	1	25	3	19	3	0	0	6	0	0	1	1	0	0	5	242	6	142	26
36	Giribes	Edge	0	20	3	16	2	1	0	6	0	0	1	3	0	9	2	268	7214	171	15
39	Giribes	Edge	0	17	2	11	2	1	0	5	0	0	1	3	0	9	2	183	6	127	11
20	Giribes	Inside	0	15	2	11	2	2	0	6	0	0	6	2	0	0	4	201	6	157	15
21	Giribes	Inside	0.5	20	3	18	3	1	0	8	0	0	5	2	0	0	6	271	5	213	24
22	Giribes	Inside	1	21	3	17	2	1	0	6	0	0	3	1	0	0	4	252	8	272	21
23	Giribes	Inside	0	27	4	22	3	3	0	8	0	0	3	2	0	0	7	364	6	435	27
24	Giribes	Inside	0.5	28	3	20	3	1	0	8	0	0	2	1	0	0	6	298	6	233	25
25	Giribes	Inside	1	32	4	22	4	1	0	7	0	0	3	2	0	0	7	370	9	263	30
26	Giribes	Inside	0	27	3	21	3	3	0	7	0	0	2	1	0	0	6	331	5	463	28
27	Giribes	Inside	0.5	23	3	17	3	1	0	6	0	0	2	1	0	0	6	272	7	172	22
28	Giribes	Inside	1	20	3	15	2	1	0	6	0	0	1	2	0	0	4	246	4	156	24
37	Giribes	Inside	0	17	2	14	2	1	0	6	0	0	1	2	0	9	3	219	7	145	14
40	Giribes	Inside	0	16	2	11	2	1	0	5	0	0	1	3	0	9	2	182	8	173	10
32	Giribes	Matrix	0	18	2	13	2	1	0	4	0	0	1	1	0	0	4	224	13	185	16
33	Giribes	Matrix	0.5	12	1	10	1	0	0	5	0	0	1	2	0	9	2	108	4	222	32
34	Giribes	Matrix	1	23	3	18	3	1	0	7	0	0	1	3	0	15	5	266	4	185	22
35	Giribes	Matrix	0	16	2	11	2	1	0	5	0	0	1	3	0	56	2	177	25	117	10
38	Giribes	Matrix	0	13	2	10	2	0	0	5	0	0	1	2	0	8	2	167	7	98	9
42	Keerweder	Edge	0	16	2	12	1	0	0	5	0	0	0	0	6	180	1	461	5	115	10
45	Keerweder	Edge	0	17	2	12	2	1	0	5	0	0	0	1	18	199	1	244	10	145	11
47	Keerweder	Edge	0	17	2	13	2	1	0	4	0	0	0	1	35	110	1	330	9	154	12
50	Keerweder	Edge	0	15	2	11	2	1	2	3	0	0	0	1	9	20	1	424	34	154	12
41	Keerweder	Inside	0	14	2	12	2	2	0	6	0	0	1	0	0	87	2	231	5	148	11
44	Keerweder	Inside	0	18	2	14	2	1	0	6	0	0	0	1	1	122	1	229	12	179	12
48	Keerweder	Inside	0	16	2	13	2	2	0	4	0	0	0	0	18	116	1	326	8	171	12
49	Keerweder	Inside	0.5	14	2	11	1	1	0	4	0	0	0	1	1	149	1	316	9	124	10
43	Keerweder	Matrix	0	17	2	13	2	1	0	6	0	0	0	1	49	119	1	259	7	149	11
46	Keerweder	Matrix	0	16	2	12	2	1	8	4	0	0	0	1	52	41	1	992	7	146	17
51	Keerweder	Matrix	0	15	2	13	2	1	0	4	0	0	0	1	1	26	1	461	13	158	12
52	Keerweder	Matrix	0.5	14	2	11	1	1	0	4	0	0	0	0	1	109	1	225	13	113	10
60	Marienfluss	Edge	0	17	2	12	2	1	0	2	0	0	0	0	1	38	2	282	3	157	10
64	Marienfluss	Edge	0	16	2	12	2	1	0	2	0	0	0	0	10	50	2	134	2	141	10
69	Marienfluss	Edge	0	13	2	10	2	1	0	2	0	0	0	0	0	75	1	234	2	114	6
55	Marienfluss	Inside	0	107	14	86	11	5	1	16	0	0	0	1	41	53	8	524	8	800	64

Sample number	Location	Region of circle	Depth in m	GPS co-ordinates
1	Bushmanland	Inside	0	S29º10.960' / E 18º26.103
3	Bushmanland	Inside	0	S29º10.960' / E 18º26.103
5	Bushmanland	Inside	0	S29º10.960' / E 18º26.103
7	Bushmanland	Inside	0	S29º08.784' / E 18º23.057
8	Bushmanland	Inside	0	S29º08.784' / E 18º23.057
10	Bushmanland	Inside	0	S29º08.784' / E 18º23.057
11	Bushmanland	Inside	0	S29º08.784' / E 18º23.057
14	Bushmanland	Inside	0	S29º08.784' / E 18º23.057
15	Bushmanland	Inside	0	S29º08.784' / E 18º23.057
2	Bushmanland	Matrix	0	S29º10.960' / E 18º26.103
4	Bushmanland	Matrix	0	S29º10.960' / E 18º26.103
6	Bushmanland	Matrix	0	S29º10.960' / E 18º26.103
9	Bushmanland	Matrix	0	S29º08.784' / E 18º23.057
12	Bushmanland	Matrix	0	S29º08.784' / E 18º23.057
13	Bushmanland	Matrix	0	S29º08.784' / E 18º23.057
16	Bushmanland	Matrix	0	S29º08.784' / E 18º23.057
29	Bushmanland	Edge	0	S19º07.801' / E 13º18.466
30	Giribes	Edge	0.5	S19º07.801' / E 13º18.466
31	Giribes	Edge	1	S19º07.801' / E 13º18.466
36	Giribes	Edge	0	S19º07.812' / E 13º18.456
39	Giribes	Edge	0	S19º07.822' / E 13º18.462
20	Giribes	Inside	0	S19º07.801' / E 13º18.466
21	Giribes	Inside	0.5	S19º07.801' / E 13º18.466
22	Giribes	Inside	1	S19º07.801' / E 13º18.466
23	Giribes	Inside	0	S19º07.801' / E 13º18.466
24	Giribes	Inside	0.5	S19º07.801' / E 13º18.466
25	Giribes	Inside	1	S19º07.801' / E 13º18.466
26	Giribes	Inside	0	S19º07.801' / E 13º18.466
27	Giribes	Inside	0.5	S19º07.801' / E 13º18.466
28	Giribes	Inside	1	S19º07.801' / E 13º18.466
37	Giribes	Inside	0	S19º07.812' / E 13º18.456
40	Giribes	Inside	0	S19º07.822' / E 13º18.462
32	Giribes	Matrix	0	S19º07.801' / E 13º18.466
33	Giribes	Matrix	0.5	S19º07.801' / E 13º18.466
34	Giribes	Matrix	1	S19º07.801' / E 13º18.466
35	Giribes	Matrix	0	S19º07.812' / E 13º18.456
38	Giribes	Matrix	0	S19º07.822' / E 13º18.462
42	Keerweder	Edge	0	S24º58.343 / E 15º55.963
45	Keerweder	Edge	0	S24º58.373' / E15º55.971'
47	Keerweder	Edge	0	S24º58.373' / E15º55.971'
50	Keerweder	Edge	0	S24º58.331' / E15º55.988'
41	Keerweder	Inside	0	S24º58.343 / E 15º55.963
44	Keerweder	Inside	0	S24º58.373' / E15º55.971'
48	Keerweder	Inside	0	S24º58.331' / E15º55.988'
49	Keerweder	Inside	0.5	S24º58.331' / E15º55.988'
43	Keerweder	Matrix	0	S24º58.343 / E 15º55.963
46	Keerweder	Matrix	0	S24º58.373' / E15º55.971'
51	Keerweder	Matrix	0	S24º58.331' / E15º55.988'
52	Keerweder	Matrix	0.5	S24º58.331' / E15º55.988'
60	Marienfluss	Edge	0	S17º35.915' /E12º35.919'
64	Marienfluss	Edge	0	S17º35.915' /E12º35.919'
69	Marienfluss	Edge	0	S17º35.9355' /E12º35.917
55	Marienfluss	Inside	0	S17º35.904' /E12º35.923'

Sample number	Location	Region of circle	Depth in m	Li	Be	B	Na	Mg	Al	Si	P	K	Ca	Sc	Ti	V	Cr
56	Marienfluss	Inside	0.5	99	53	101	2155	76407	141649	3069	10060	71988	66189	73	13405	2020	598
57	Marienfluss	Inside	1	81	72	114	2736	88344	153047	4498	11202	71559	222481	79	13762	2236	696
58	Marienfluss	Inside	1.5	110	58	176	2046	79114	147923	3234	9924	70395	131993	76	14022	2155	600
59	Marienfluss	Inside	0	52	23	152	1706	37576	90417	3514	6514	45609	25451	72	14333	1661	463
62	Marienfluss	Inside	0	35	17	83	1466	33882	74222	3327	5389	41731	24663	60	12028	1439	474
63	Marienfluss	Inside	0.5	36	14	96	2623	35292	80275	3335	4102	39877	25931	69	12670	1500	513
67	Marienfluss	Inside	0	32	14	50	2614	33977	73101	2893	6722	39165	26671	54	11918	1397	403
61	Marienfluss	Matrix	0	40	11	46	1536	33908	80143	3448	5650	41425	29290	59	12482	1516	467
65	Marienfluss	Matrix	0	39	11	48	2557	37131	81328	3323	5770	45435	28609	60	13107	1523	452
66	Marienfluss	Matrix	0.5	35	14	54	2470	37454	78140	2909	4315	39130	27208	62	13359	1494	498
68	Marienfluss	Matrix	0	30	8	41	2303	25188	56978	3366	4921	34525	23855	42	9604	1084	384

Sample number	Location	Region of circle	Depth in m	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Rb	Sr	Y	Zr	Nb	Mo
56	Marienfluss	Inside	0.5	14424	866128	185	0	356	1645	190	10	429	57	741	1071	444	1161	213	186	43
57	Marienfluss	Inside	1	16421	1057127	197	0	414	2228	221	11	236	80	917	1104	905	1324	177	188	49
58	Marienfluss	Inside	1.5	23849	936862	189	0	363	2133	209	11	437	59	815	1110	682	1185	134	167	47
59	Marienfluss	Inside	0	7684	794795	85	0	654	1417	115	5	416	12	594	467	172	285	281	71	19
62	Marienfluss	Inside	0	6889	757327	74	0	163	734	108	5	192	22	805	423	169	279	95	70	26
63	Marienfluss	Inside	0.5	7291	788200	78	0	202	871	112	4	199	25	869	488	167	292	126	67	24
67	Marienfluss	Inside	0	6769	768871	74	0	196	876	113	4	183	25	783	450	183	283	127	68	23
61	Marienfluss	Matrix	0	6449	717570	75	0	188	1196	103	4	214	19	875	431	189	265	76	63	19
65	Marienfluss	Matrix	0	7884	858386	88	0	186	1004	130	4	203	34	758	507	203	300	100	72	21
66	Marienfluss	Matrix	0.5	6986	783897	82	0	193	1300	119	5	212	19	825	502	189	291	166	76	23
68	Marienfluss	Matrix	0	5558	598846	63	0	173	4822	88	3	138	9	561	356	186	223	66	82	13

Sample number	Location	Region of circle	Depth in m	Ru	Rh	Pd	Ag	Cd	Sn	Sb	Te	I	Cs	Ba	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho
56	Marienfluss	Inside	0.5	0	0	2	3	6	74	1	0	54	28	2141	2687	5332	576	2046	338	19	362	44	214	37
57	Marienfluss	Inside	1	0	0	3	3	4	75	2	0	97	30	2352	3156	5995	690	2458	395	24	436	53	250	41
58	Marienfluss	Inside	1.5	0	0	1	2	5	76	9	0	74	30	2506	2700	5180	597	2136	339	19	374	45	220	37
59	Marienfluss	Inside	0	0	0	3	2	3	29	5	0	25	11	1039	907	1440	197	717	111	14	120	13	53	9
62	Marienfluss	Inside	0	0	2	1	1	3	23	1	0	16	10	989	929	1405	196	690	105	12	106	12	50	8
63	Marienfluss	Inside	0.5	0	0	1	1	2	22	2	0	35	12	873	913	1414	198	712	107	13	106	12	51	8
67	Marienfluss	Inside	0	0	0	1	1	1	53	3	0	11	10	1014	900	1392	188	677	98	12	98	11	47	8
61	Marienfluss	Matrix	0	0	0	1	1	2	18	2	0	14	10	952	839	1265	182	653	93	13	104	11	52	8
65	Marienfluss	Matrix	0	0	0	2	1	2	21	3	0	15	11	1061	910	1425	198	697	103	13	102	12	52	8
66	Marienfluss	Matrix	0.5	0	0	2	1	3	20	1	1	26	12	974	899	1391	195	692	103	12	105	12	49	8
68	Marienfluss	Matrix	0	0	0	0	1	12	69	1	0	10	8	1702	722	1059	155	538	79	10	79	9	41	6

Sample number	Location	Region of circle	Depth in m	Er	Tm	Yb	Lu	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Th	U
56	Marienfluss	Inside	0.5	95	12	75	10	3	0	12	0	0	0	1	4	65	7	333	8	691	61
57	Marienfluss	Inside	1	104	14	83	12	3	1	20	0	0	0	0	39	40	7	480	8	696	56
58	Marienfluss	Inside	1.5	97	13	78	11	2	0	11	0	0	0	0	4	102	7	300	7	628	55
59	Marienfluss	Inside	0	22	3	18	3	3	0	3	0	0	0	0	1	82	2	222	3	200	12
62	Marienfluss	Inside	0	19	2	15	2	1	0	2	0	0	0	0	0	183	2	131	3	191	11
63	Marienfluss	Inside	0.5	20	3	15	2	1	0	3	0	0	0	0	0	99	2	125	3	172	9
67	Marienfluss	Inside	0	18	2	15	2	1	0	2	0	0	0	0	0	19	2	158	2	179	10
61	Marienfluss	Matrix	0	19	3	15	2	1	0	3	0	0	0	0	1	49	2	250	3	185	16
65	Marienfluss	Matrix	0	20	3	15	2	1	0	3	0	0	0	0	1	82	2	122	2	165	11
66	Marienfluss	Matrix	0.5	19	3	16	2	1	0	2	0	0	0	0	3	25	2	149	2	148	10
68	Marienfluss	Matrix	0	14	2	12	2	2	11	1	0	0	0	0	0	8	1	1091	4	143	18

Sample number	Location	Region of circle	Depth in m	GPS co-ordinates
56	Marienfluss	Inside	0.5	S17º35.904' /E12º35.923'
57	Marienfluss	Inside	1	S17º35.904' /E12º35.923'
58	Marienfluss	Inside	1.5	S17º35.904' /E12º35.923'
59	Marienfluss	Inside	0	S17º35.915' /E12º35.919'
62	Marienfluss	Inside	0	S17º35.915' /E12º35.919'
63	Marienfluss	Inside	0.5	S17º35.915' /E12º35.919'
67	Marienfluss	Inside	0	S17º35.9355' /E12º35.917
61	Marienfluss	Matrix	0	S17º35.915' /E12º35.919'
65	Marienfluss	Matrix	0	S17º35.915' /E12º35.919'
66	Marienfluss	Matrix	0.5	S17º35.915' /E12º35.919'
68	Marienfluss	Matrix	0	S17º35.9355' /E12º35.917