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TOLERANCE OF SELECTED CROPS TO GYPSIFEROUS WATER ORIGINATING IN COAL MINES

by

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When I in awesome wonder considered all the plants Thy hands have made…

…my God how great Thou art!

"Through Him all things were made;
without Him nothing was made that has been made"
John 1:3

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To my husband Hannes

who gave of himself in many selfless sacrifices to make this study possible

and

to our children Marié, Jakobie, Hannes and Willem

and their families

whom I love dearly

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ABSTRACT

The disposal of gypsiferous water, generated in coal mining operations, has become a problem in the Mpumalanga Highveld region in South Africa. As part of an investigation into the feasibility of using this water for irrigation, sand and water culture experiments were conducted in a glasshouse and growth chambers to determine growth responses of maize, sorghum, pearl millet, sunflower, soybean, cowpea, dry bean, wheat, rye, triticale, oats, barley, annual ryegrass, and lucerne cultivars to gypsiferous mine water in the germination, seedling and vegetative growth stages. *Germination* %'s were generally not affected. The *seedling growth* of maize, sorghum, pearl millet and lucerne was more sensitive and showed more significant cultivar differences than the seedling growth of soybean and the annual temperate crops. *Seedling growth curves* with increasing concentrations of Ca, Mg and SO_4 followed a similar pattern for most of the crops: where $CaSO_4$ was in solution, growth decreased in a linear manner, but above saturation concentrations with increasing gypsum crystal content, it *increased* despite decreasing osmotic potentials of the treatment solutions. The *vegetative growth* of sunflower, lucerne, dry bean and rye was more tolerant than seedling growth, but was more sensitive for maize and cowpea, and the same as seedling growth for sorghum, pearl millet, wheat, oats, triticale and annual ryegrass. It was concluded that the major property of this water that suppressed growth was the decreased osmotic potential. However, it is the '*effective*' osmotic potential (i.e., the average osmotic potential during the whole growth period) and not that of the treatment solutions, that was mainly responsible for the eventual growth. The 'effective osmotic potential' is determined by evapotranspiration and the *rapidity of gypsum precipitation*, which in turn may be affected by the growth rate, temporal, environmental and soil factors. *Sensitivity* of crops and growth stages is therefore related to its sensitivity to the external osmotic potential, whereas *tolerance* both in the seedling and vegetative growth stages was found in crops primarily affected by the ionic effects of Na and/or Cl. Possible nutrient effects due to the high Ca and SO_4 need further investigation.

Keywords Salt tolerance, gypsiferous water, coal mines, crops, pastures, cultivars, growth stages

CONTENTS

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CHAPTER 1

INTRODUCTION

The disposal of large amounts of gypsiferous water, generated in coal mining operations in the Mpumalanga Highveld region in South Africa, has become a problem of increasing importance. This water is unsuitable for direct uncontrolled discharge into watercourses where it may become a threat to the environment and a problem to potential users. There are several different approaches to this problem. One alternative approach currently being investigated is the use of these waters for the irrigation of agronomic crops and pastures. In this way, large amounts of waste water could become economically useful for irrigation.

This thesis arose out of a screening project to determine the tolerance of crops and pastures to such gypsiferous waters for possible use in irrigation (Barnard, Rethman, Annandale, Mentz & Jovanovic, 1998).

South Africa has a low and variable rainfall with two thirds of the country classified as semi-arid to arid (Department of Water Affairs, 1986). The region in which the coal fields occur has a subtropical summer rainfall climate but is subject to periodic droughts. The area is a major catchment area and rivers originating here supply water to the largest industrial and mining heartland of South Africa, a national power grid and several important irrigation schemes. Due to the increasing use of water by these operations, the disposal of waste water has become a problem that requires constant attention (Kempe, 1983; Van Niekerk, 1992).

These coal fields have been a primary source of energy generation in the country since the latter half of the 19th century. Most South African coal deposits contain pyritic formations (Kempe, 1983). When exposed, iron pyrite is oxidised to sulphuric acid and iron sulphate. This results in the occurrence of large quantities of acid mine drainage water (AMD) being formed, which may be neutralized by other strata present, but where it occurs as a seep, extremely high acidity precludes discharge into natural streams. This is, of course, not only a local problem, but occurs world-wide, where similar deposits are found.

Current measures to prevent pollution of the environment include, inter alia, treatment with calcitic or hydrated lime in order to neutralize the acidity. The major portion of gypsum formed is precipitated in sedimentation basins, but the resulting effluent is a $CaSO_4$-dominated saline water. The estimation that 34 % of the lime mined in South Africa was used for the neutralization of acid mine waters in 1985, is an indication of the volumes of such water being produced (Hart, 1985). There are also other gypsiferous waters emanating from coal mining areas, such as is pumped from old underground workings at the Kleinkopje mine. The volumes of gypsiferous water generated daily on the Mpumalanga Highveld have been estimated at between 14 and 30 ML (P. Tanner, AMCOAL Environmental Services, personal communication, 1999). So far these waters have been used for dust alleviation on dirt roads and irrigation of lawns, but if they can be used for irrigation, large amounts of waste water could become economically useful.

These coal fields underlie one of the most important high potential agricultural areas in South Africa (Schoeman & MacVicar, 1978). This is of particular significance when viewed against the fact that the country has a very low percentage of arable land - some 14 out of 120 million hectares - of which only 4.5 million hectares are regarded as being of high potential. In view of the steady increase in population - 0,2 ha arable land per capita is already being approached whereas 0,4 ha per capita is considered desirable - responsible and effective utilization of the agricultural potential and water resources is very important (Laker, M.C., personal communication, 1996). Moreover, filtering saline water through the soil and precipitating gypsum in the profile could limit environmental pollution hazards. Contamination of water supplies for other potential users could be minimized.

The use of gypsiferous water for irrigation may have several advantages for crop growth:

- Gypsum can be an important S fertilizing agent in this climatic region where excessive summer rainfall has been known to lead to S deficiency in the subsequent seasons; irrigation of the winter crops with this water could replace the leached S. This is of special importance to crops that have a very high demand for S, such as those with a high production of organic material, for example maize. It is also important for protein rich crops such as lucerne and for

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the Cruciferae family (Marschner, 1995).

- Gypsum may have several positive influences when applied to acidified soils:

    - In humid and subhumid climatic conditions acidification and Ca loss by leaching occur simultaneously. The influence of low Ca is furthermore intensified by the inhibition of Ca-uptake from acid soils by the high H^+ concentration and the strongly competing influence of the phytotoxic Al^{3+} ion. Increasing the external Ca concentration, by the application of gypsum, may replenish the Ca of such a soil and also reduce Al-induced inhibition of root elongation (Rengel, 1992a; Rhue & Grogan, 1977). The increased Ca may also stimulate nodulation of legumes which is inhibited by high H^+ together with low Ca^{2+} and high Al^{3+} (Marschner, 1995).

    - An important benefit of gypsum application to growth in acid soils may be the formation of the non-phytotoxic $AlSO_4^+$ ion (Marschner, 1995).

    - With increasing soil acidification and a lower Ca/Al ratio, root penetration into the subsoil can be inhibited. This may lead to a shallow root system and thus a lower utilisation of nutrients and water. Owing to its solubility, gypsum may contribute to the alleviation of subsoil acidity.

- A major concern about the prolonged use of a gypsiferous irrigation water is, however, that the exchange complex may become depleted of Mg and K and dominated by Ca, which may cause nutrient imbalances.

- The most well-known use of gypsum is for the reclamation of sodium-affected soils; the Ca replaces Na adsorbed on the soil colloids, inducing flocculation and thus improving soil structure.

A literature survey on the influence of salinity on plant and crop growth revealed a vast body of literature on plant response to mainly NaCl and other highly soluble salts such as Mg and Na sulphates. Maas and Hoffman (1977) reviewed all available salt tolerance literature and concluded that "in general, yield was not decreased significantly until a threshold salinity level was exceeded, and that yield decreased approximately linearly as salinity increased beyond the threshold". Gypsiferous waters are generally not considered as detrimental to growth as waters with highly soluble salts, because potentially extreme salinity increases are controlled by the precipitation of gypsum. It is thus expected that crop yield would not decrease to the same extent after the solubility product of Ca and SO_4 has been reached in the soil solution. Very little has, however, been reported on growth responses to increasing concentrations of gypsiferous water or the effect that the precipitation of gypsum during evapotranspiration may have on growth responses.

Crop research with $CaSO_4$-dominated water has been very limited and has mainly focussed on yield components and the influence of such an irrigation water on soil chemical properties (du Plessis, 1983; Papadopoulos, 1986; MacAdam, Drost, Dudley and Soltani, 1997; Jovanovic, Barnard, Rethman & Annandale, 1998). The yield and/or quality of moderately sensitive crops such as tomato, bell pepper and eggfruit were decreased (Papadopoulos, 1986), but the moderately tolerant lucerne and tall fescue increased when irrigated with a gypsiferous water (MacAdam et al., 1997). The latter was confirmed by field trials that were conducted simultaneously with the experiments reported in this study (Barnard et al., 1998; Jovanovic et al., 1998). These field trials under irrigation with lime-treated acid mine drainage water, also showed satisfactory yields with soybean, pearl millet, cowpeas and the winter cereals; maize and sorghum, however, suffered from nutrient deficiency which was attributed to shallow rooting depths due to subsoil acidity; lucerne showed K-deficiency symptoms which were corrected by fertilization (Jovanovic et al., 1998).

As salt-tolerance is a multifaceted concept, varying with many environmental and biological factors, the use of such waters for irrigation warranted more information than only the yield response. The influence of biological factors - such as cultivar diversity and growth stage - and the influence of precipitation of gypsum on growth curves, with increasing concentrations of Ca and SO_4 were therefore investigated. Possible nutrient interactions peculiar to this type of saline water were also considered.

The present study focuses purely on the plant and its growth response to $CaSO_4$-dominated growth

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conditions. Most of the experiments conducted were therefore with water and sand cultures. For obvious reasons some extrapolations to soil conditions are considered.

The seedling stage was used for most of the experiments. The tolerance of crops during the seedling stage is important as it is the most sensitive growth stage and effective establishment is necessary for optimum yield. Growth differences are also likely to be clearest at this stage. Some authors have argued that in this growth stage it is the decreased osmotic potential that causes growth decreases (Munns, 1993; Neumann, 1997). This was, however, concluded against the background of osmotic potential versus accumulation of salts being the major suppressing properties of mostly NaCl-dominated saline waters. Other sensitivity mechanisms, such as nutrient imbalances or other ionic effects, were not addressed in these reviews.

The wide range of crops screened afforded an opportunity to investigate whether the physiological salt sensitivity or tolerance mechanisms, which had previously been found for the respective crops, were related to growth responses to this gypsiferous type of water. This may lead to some indication of which properties of such a water are mainly responsible for suppressing growth of crop species.

Seedling growth responses, of a wide variety of crops and cultivars, were firstly investigated in water culture under glasshouse conditions with actual 'worst case' saturated gypsiferous water from the Kleinkopje mine (Chapter 4). The crops and cultivars were selected on the basis of good yields under irrigation and the climatic conditions of the region; they could therefore be expected to possess a measure of tolerance to NaCl saline conditions.

Subsequently a tolerant cultivar of each crop was selected for growth curve investigations with increasing concentrations of Ca, Mg and SO_4 in a simulated $CaSO_4$ mine water; sand culture in growth chambers under controlled environmental conditions was used (Chapter 5). Treatments also included saturated solutions with increasing amounts of undissolved gypsum crystals in order to gain information on growth responses when gypsum had precipitated, and ranges of NaCl, and Na_2SO_4 saturated with $CaSO_4$.

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Comparisons of growth responses to the NaCl, Na_2SO_4 and $CaSO_4$ treatments plotted at similar osmotic potentials could possibly be used to determine sensitivity to ionic effects of Na and Cl.

The tolerance of the different growth stages is compared in Chapter 6. Germination trials in paper rolls were conducted in a growth chamber and the vegetative growth stage was investigated on sand culture in the glasshouse. Nutrient analyses of the top growth were conducted to determine possible nutrient interaction problems (Chapter 6).

The main challenges of these investigations were:

- to compare the tolerances of the different recommended *cultivars* of the crops in the sensitive seedling stage in order to facilitate the choice of suitably tolerant cultivars to ensure agricultural productivity when irrigating with $CaSO_4$-dominated waters

- to determine the seedling growth responses of the crops to increasing Ca, Mg and SO_4 concentrations, with special attention to the question of how increasing amounts of precipitated gypsum may influence growth

- to acquire knowledge of the sensitivities/tolerances of the different *growth stages* of the respective crops to $CaSO_4$-dominated water which may be important for irrigation management

- and finally to gain some insight into which property or properties of a $CaSO_4$- dominated water are mainly responsible for suppressing (or stimulating!) growth.

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CHAPTER 2

LITERATURE SURVEY

This chapter deals with plant response to salinity in the root growth zone. A brief introduction is followed by the general literature which pertains mostly to the influences of NaCl-dominated salinity on plant growth. The influence of salinity on the morphological aspects of growth is first discussed, followed by the physiological responses of the possible sensitivity and tolerance mechanisms.

The general effects of SO_4-salinity are then presented, followed by sections dealing respectively with Na_2SO_4- and $CaSO_4$-salinity.

Apart from salt concentration and composition, the salt tolerance/sensitivity of plants is dependent on many other factors; these are discussed under the section dealing with environmental and plant factors.

Crop salt tolerance has been of commercial importance for many decades, but with the increasing use of marginal soil and poor quality water for agriculture, it has gained importance. The following section thus deals with the evaluation of the salt tolerance of crops, the criteria and parameters used and some yield response functions available to predict growth and yield of crops under saline conditions.

The chapter concludes with the general trends of salt tolerance found for the agronomic groups investigated in this study, namely cereal and forage crops.

2.1 INTRODUCTION

The agricultural productivity of a crop can be limited by excessive concentrations of soluble salts in the growth medium; this is more pronounced in arid and semi-arid regions or where low quality water is used for irrigation. One of the primary options available to ensure agricultural productivity under such conditions is the choice of suitably tolerant crops or cultivars. It must, however, be emphasized that using such crops will have only a temporary beneficial effect, economically, and will not prevent the further degradation of the soil. The only proven way to overcome salinity is by appropriate water management and drainage (Richards, 1995).

'Salt tolerance' has generally been defined as a plant's capacity to endure the effects of excess salts in the root growth medium (Maas, 1990). Agriculturally these 'effects' are caused by some property or properties of a saline soil solution on the physiological processes in the plant which in turn affects growth and yield. A study of the salt tolerance of crops thus requires a knowledge of the composition of a specific saline water as well as the property or properties of this water that are mainly responsible for limiting the growth of a specific crop and/or cultivar.

In most investigations 'salinity' is equated with NaCl, with or without $CaCl_2$, as the sole salinizing agent. Soil and irrigation waters are made up of diverse amounts of various salts. The major ions present are chloride, sulphate and bicarbonate salts of sodium, calcium and magnesium (Bernstein, 1964, 1974). The proportions can vary widely but the concentration of some ions, for example of Na and Cl, can exceed those of essential nutrients by many orders (Epstein & Rains, 1987). The most common type of salinities are nevertheless that of NaCl and Na_2SO_4 sometimes together with Mg salts (Poljakoff-Mayber & Lerner, 1994). As a result studies have tended to concentrate on these types of salinities.

The current investigation is concerned mainly with the use of $CaSO_4$ waters for the irrigation of crops. Salinity studies with sulphates have mostly focussed on the effect of Na_2SO_4 compared to the effect of NaCl. Very little has, however, been published on the tolerance of plants to $CaSO_4$-dominated saline irrigation waters.

The following discussion will first focus on the general literature of salinity effects in plants. This information is based mostly on studies where NaCl was the main salinizing agent. This will be followed by a discussion of sulphate salinity.

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2.2 GENERAL EFFECTS OF SALINITY ON PLANT GROWTH (MOSTLY NACL)

2.2.1 GROWTH RESPONSES

Plants differ in their ability to grow under saline conditions. Greenway & Munns (1980) suggested four groups of plant species according to their growth under saline conditions. They are halophytes, where growth is optimal under sodic and/or saline conditions; a few crop species termed halophylics, where growth is slightly stimulated by low salinity levels and two groups which are non-halophytes (glycophytes) that range from moderately salt-tolerant to salt-sensitive. Most crop species fall under the last two groups which are, however, not clearly defined.

Although salinity affects plants physiologically in many different ways, injury is not readily seen morphologically, except at extreme salt concentrations. The most general effect is a reduction in growth and growth rate. Plants that are salt-sensitive or moderately tolerant show a progressive decline in growth and yield as salinity levels increase (Bernstein, 1964, 1974).

Plant parts are not all equally affected: shoot growth is usually influenced more than root growth with a concomitant decrease in the shoot to root ratio. The leaf to stem ratio is also often affected, which could be important when crops are used for forage (Maas & Hoffman, 1977).

Leaf growth

The initial growth response of a non-halophyte to salinity is that its leaves grow more slowly (Munns & Thermaat, 1986). With low or moderate salinity levels leaves do not necessarily show specific symptoms such as scorching or chlorosis but can be smaller and of a darker green or bluish-green colour when compared to those of plants growing under optimal conditions. Marginal chlorosis, necrosis (leaf burn) and defoliation occur mostly in woody and in some herbaceous species with NaCl salinity; this is mostly due to toxic accumulation of Na and/or Cl (if Na > 0,25 and Cl > 0,5 % dry mass) (Bernstein, 1964). Leaf analyses have shown Cl-toxicity to be the major cause. These effects start at the tips or margins of the leaves due to death of the tissues. The affected parts become brownish and are sharply distinguished from the healthy part of the leaf, which usually retains its normal colour. The more salt accumulated, the bigger the leaf area affected. In citrus and some shrubs a general bronzing of the leaves, followed by leaf drop, may also occur, without leaf burn developing. Leaf burn can, however, also be caused by excess boron which sometimes occurs in saline waters (Bernstein, 1964; Maas, 1986).

Most herbaceous plants do not develop such leaf injury symptoms, even though Na and Cl accumulation can be as high as that which causes injury to woody species. Vegetable, forage and field crops often accumulate these elements up to 5 % and sometimes 10 % of their leaf dry mass without showing leaf injury symptoms (Bernstein, 1974). Leaf injury under saline conditions can also be caused by nutritional imbalances leading to specific nutrient-deficiency symptoms.

In glycophytes *leaf area* is usually decreased by any significant increase in salinity, while for halophytes this will depend on the relationship between the external salinity and the growth optimum; above this optimum halophytes can be expected to respond similarly to glycophytes. In the case of natrophylic species, Na can stimulate growth mainly by its positive effect on cell expansion and water balance. With halophytes the leaf area may be increased, but not necessarily transpiration as the number of stomata per unit also decreases with succulence (Marschner, 1986). In sugar-beet, a tolerant crop, Na increased the leaf area, succulence and the number of stomata per unit leaf area but the chlorophyll content was less (Marschner, 1986).

Changes in leaf area can influence the overall water loss of the plant. The rate of water loss may also be decreased by anatomical and morphological properties or changes in the plant. Leaf surface properties such as hairs (which impede vapour exchange), succulence (which generally reduces the number of stomata per unit area) and the properties of the cuticle, may all contribute in reducing the rate of water movement through the plant and consequently also the accumulation of salts (Ahmad & Wainwright, 1976; Hajibagheri, Hall & Flowers, 1983).

Leaf thickness and succulence (water content per unit leaf area) have been observed as a typical morphological response to high substrate salinity and water stress and is usually observed in salt-tolerant species growing in saline substrates (Jennings, 1968). It is also found in most dicotyledons as an adaptation to high substrate salinity both in salt ‘excluders’ and 'includers' (Longstreth & Nobel, 1979). Succulence can be caused by a decrease in surface area and/or an increase in tissue water content. Apart from salinity, succulence can also be induced by a water deficit and hormone related changes (Marschner, 1986).

Root growth

Generally root growth is affected less by salinity than is shoot growth. At low salinity it may not be influenced or may even show an increase. These observations are, however, mostly based on root dry mass; root length, which is important for nutrient and water uptake, has been shown to be a more sensitive parameter than root dry mass for the influence of salinity on root growth (Shalhevet, Huck & Schroeder, 1995).

At higher concentrations root growth can be inhibited and thus also the capacity for uptake of water and nutrients (Neumann, 1995b). Calcium stimulates and Na inhibits root cell elongation in cotton (Kurth, Cramer, Läuchli & Epstein, 1986). Addition of Ca to the root medium ameliorated salt stress on maize root growth (Cramer, Epstein & Läuchli, 1988), and on peas (Solomon, Gedalovich, Mayer & Poljakoff-Mayber, 1986). The yields of storage roots may, however, be decreased much more than those of fibrous roots (Maas & Hoffman, 1977). The yield and quality of potatoes, however, improved with gypsum amendment under NaCl or Na_2SO_4 saline conditions and the total glycoalkaloids, which are associated with a bitter taste, were decreased (Abdullah & Ahmad, 1982; Bilski, Nelson & Conlon, 1988).

2.2.2 PHYSIOLOGICAL RESPONSES

Physiological responses are twofold: firstly they include mechanisms by which growth is adversely affected (adverse or sensitivity mechanisms); and secondly responses by plants to adapt to saline conditions (mechanisms for salt tolerance).

2.2.2.1 Sensitivity or adverse mechanisms

Sensitivity of plants is due to several properties of salinity that include ionic and osmotic effects as well as nutritional imbalances of which the precise physiological mechanisms are not yet quite clear. The main properties of a saline soil solution that have been found to affect growth adversely are:

- water deficit arising from *the lowered osmotic potential* caused by the high concentration of soluble ions.

- specific ion effects, which include :
  - *toxicity* of mainly Cl and also of Na (especially in Graminaceous species) when taken up in excessive quantities. Sodium toxicity is not as widespread as that of Cl, but unfavourable ratios of Na/Ca, Na/K may disturb inorganic nutrition. High Na can furthermore disturb the Ca-homeostasis of root and leaf cells and therefore the uptake of essential nutrients (Rengel, 1992b). It can also indirectly affect growth by its influence on soil structure and fertility, and the formation of a dense natric B-horizon which can obstruct downward percolation and root growth. A high percentage of exchangeable Mg may also affect soil structure in a similar way as a high exchangeable sodium percentage (ESP) (Driessen & Dudal, 1991); and
  - *nutrient imbalances* where uptake and/or shoot transport is depressed by the lowered activity of nutrient ions, and internal distribution of nutrients and especially Ca is disturbed. This also includes unfavourable ratios of Ca/Mg in the external growth medium.

The earlier belief that it was the actual lack of water that limited growth with a saline root medium, has generally been rejected because plants have been shown to adjust osmotically (Maas & Nieman, 1978). More recent literature suggests that in short-term responses of whole plants to salinity, shoot growth is regulated by the water status of the root, through some "messenger system" to the shoots which could include hormonal substances, for example abscisic acid or other anti-transpirants (Rengel, 1992b).

Osmotic potential or specific ion effects?

From the literature it seems that there are two schools of thought on the relative importance of osmotic potential and/or specific ion effects on growth. Although the toxic influences and nutritional imbalances are recognised, some authors maintain that it is mainly the total salt concentration of the soil solution that causes growth reduction (e.g., Bernstein, 1964, 1974; Maas & Hoffman, 1977; Maas & Nieman, 1978). Evidence connected to the direct toxic influence of some ions or the accumulation of toxic amounts of salts in the leaf tissues, leads others to attach more importance to growth inhibition through ion toxicity or accumulation (e.g., Maas, 1990; Munns, 1993). It is generally recognized that these adverse effects could simultaneously be responsible for growth reduction, but the relative contribution of the three major constraints to growth inhibition at high substrate salinity is difficult to assess (Marschner, 1995; Jacoby, 1994). However, the opinion that growth reduction is primarily due to the osmotic potential is being reviewed as many nutritional and also membrane related studies indicate other possibilities (Reinhold, Braun, Hassidim & Lerner, 1989; Läuchli & Epstein, 1990; Grattan & Grieve, 1992; Rengel, 1992b). Lowered osmotic potential may also influence cell wall hardening and eventually growth (Neumann, 1995a).

Much effort has been made to understand the primary physiological causes of growth reduction in saline environments. These effects are complex and not fully understood (Shannon, 1997). Munns (1993) reviewed work on turgor, photosynthesis and effects on particular metabolites which directly influence growth and concluded:

- “Although turgor is essential for growth...it does not control growth; the rate of cell wall expansion is controlled by the rheological properties of the cell wall and not directly by turgor.” This was confirmed by Neumann (1995a). The decrease in turgor is sensed by a “turgor sensor”, probably in the plasma membrane. The sensor emits an error signal that activates biochemical processes necessary for solute accumulation or synthesis. This results in osmotic adaptation and the recovery of the turgor pressure. Neumann (1995a) however, examined many related studies and found that complete osmotic adjustment and turgor maintenance do not sufficiently prevent stress-induced inhibition of growth.

- “Salinity affects carbon assimilation per plant via a smaller leaf area rather than a reduced rate of photosynthesis. Concentrations of sugars often increase with exposure to salinity indicating a blockage in utilization”.

- Growth reduction and death are mainly due to eventual accumulation of salts in the vacuole above a concentration that the specific specie or cultivar can tolerate and “the cell dies of salt poisoning or dehydration depending on whether salts build up in the cytoplasm or cell wall.”

Furthermore, Neumann (1995a), reviewing the effect of cell wall-hardening on growth, suggests that the long-term growth inhibition of roots, stems and leaves under water stress conditions may involve stress-induced hardening of cell walls which is associated with smaller mature cells. In moderately saline situations cell-wall hardening may negatively affect growth and yield but in terminal survival situations it could be advantageous.

Munns & Termaat (1986) suggested a hypothesis of a biphasic model where the external osmotic potential could be the main growth inhibitory factor for *seedlings* in the first weeks of growth: “This phase of growth reduction is a water stress effect and is regulated by inhibitory signals from the roots.” In *the vegetative growth stage* accumulation and/or specific ion effects are increasingly important in the leaves and can eventually lead to the death of the older leaves when the vacuoles can no longer isolate incoming salts. They suggest that varietal differences would only appear in the more mature growth stage because the growth of these varieties reacted similarly to osmotic effects in the early growth stages. Neumann (1997), however, presents evidence for varietal differences to osmotic stress in early growth stages.

This two-stage process bears similarities to the “short” and “long-term” effects suggested by other authors (e.g., Cramer & Bowman, 1991). The duration of the “short-term” differs for the different authors, but there seems to be agreement that later growth stages are affected more by the specific ion effects of salt accumulation and toxicity than by the osmotic potential of the external solution (Munns, 1993). Plant species and cultivars differ in their ability to compartmentalise salts at the cell, tissue and whole plant level and thus in their salt tolerance or sensitivity to accumulation.

Other reviews stress the nutritional effects of salinity (Grattan & Grieve, 1992, 1994) and the almost immediate effect of excess Na on the Ca-homeostasis of root and leaf cells (Rengel, 1992b). Rengel (1992b p.629) suggested that “the Na-related changes of the normal pattern of Ca fluxes at the plasma membrane is the primary signal of salt stress perceived by roots and translated into almost immediate changes of the leaf cell environment, at least together, if not preceding, the osmotic changes”. With this in mind the hypothesis of Munns & Termaat (1993) of osmotic potential being the main or only growth inhibitor for seedling growth, and of others on “short-term” effects, needs further investigation.

Nutritional disorders

Salinity disrupts nutrition by (i) decreased activity of nutrient ions (decreased availability), due to the ionic strength of the substrate, regardless of its composition (the optimum concentration of most nutrients in a non-saline growth medium could be deficient in saline conditions) (Grattan & Grieve, 1994), and (ii) interactions due to extreme ratios, of for example, Na/Ca, Na/K, Mg/Ca and Cl/NO_3 that can lead to reduced uptake and disrupted translocation of essential nutrients.

As mentioned above, Na-related salinity can also affect the membrane selectivity and efficiency, and the Ca-homeostasis of root and leaf cells (Reinhold, Braun, Hassidim & Lerner, 1989; Rengel, 1992b; Neumann, 1995a; Yermiyahu, Nir, Ben-Hayyim, Kafkafi & Kinraide, 1997).

Nutritional disorders most commonly found with saline soils are reduced uptake or disturbed internal distribution of K and Ca and Mg/Ca interactions (Marschner, 1995).

The influence of salinity on **K** content pertains mainly to the competitive effects of Na on K uptake, regardless of the anion being Cl or SO_4 (Grattan & Grieve, 1994). Cortical root cells have the selective ability to absorb K in preference to Na but the degree of this selectivity varies among species and cultivars (Grattan & Grieve, 1994). Salt tolerance has in some cases been connected to the selective uptake of K over Na by different species. The correlation between the Na/K ratio in plant tissue and salt tolerance has been found significant enough to be used for selection of salt-tolerant wheat cultivars (Suhayda, Redmann, Harvey & Cipywnyk, 1992; Chippa & Lal, 1995).

Ca and **Mg** deficiency can be caused by competition with other cations simultaneously present in excess concentrations, especially by Na. Ca availability can be estimated more accurately as the molar ratio of Ca to the sum of the major cations, rather than the Ca concentration of the soil solution per se. Generally reduced growth is likely to occur when this ratio falls below 0.10-0.15 but this value could be higher, especially if high Na concentrations are present (Grattan & Grieve, 1992).

Salt tolerance of plants is also related to the ability to maintain adequate tissue levels of Ca during salt stress (Suhayda et al., 1992). This was illustrated with kochia and barley where the greater salt tolerance of kochia was related to the degree of selectivity for Ca uptake (Curtin, Steppuhn & Selles, 1993).

Many studies have shown that the addition of Ca (ranging from 5-20 mmol L^{-1}) to NaCl saline growth mediums can ameliorate salt stress and reverse Ca deficiency effects (e.g., Solomon et al., 1986; Fernandez-Ballester, Cerdá & Martinez, 1997). In an investigation of the role of the anion in Ca amelioration of NaCl-stress it was found that $CaSO_4$ was more effective than $CaCl_2$ for *Phaseolus vulgaris* L (Awada, Campbell, Dudley, Jurinak & Khan, 1995). Ca deficiency can also be a result of SO_4 salinity (see 2.3).

A hypothesis has been put forth by Läuchli (1990) that the protective role of Ca against Na-related salt stress operates primarily at the root plasma membrane where Na displaces Ca. Yermiyahu et al. (1997) related quantitative values of the percentage of negative sites occupied by Ca on the plasma membrane, to salt tolerance/toxicity. They found that a salt resistant melon cultivar needed less Ca for protection than the salt sensitive one and that each had a critical value for the fraction of negative sites bound to Ca.

High Mg as part of the Ca/total ions ratio can be partly or largely responsible for a decrease in Ca uptake. If the ratio of Mg to Ca in the growth medium exceeds 1.0, growth can be negatively influenced (Key, Kurtz & Tucker, 1962; Claassens, 1973; Carter, Webster & Cairns, 1979). On the other hand Mg uptake can be depressed by other cations, especially by high levels of K, Ca, Mn and also by H^+ (Claassens, 1973; Heenen & Campbell, 1981; Marschner, 1995).

Salinity can also affect the **N** and **P** content of plants. Nitrate absorption was inhibited to a lesser degree with excess SO_4 than Cl when these were present at equal osmotic potentials (Aslam, Huffaker and Rains, 1984). Although salinity reduces N accumulation in plants, additional N above that considered optimal for normal conditions has generally not proved to increase growth or yield under saline conditions (Grattan & Grieve, 1992).

Crop species also vary in their ability for **P** uptake under saline conditions (Champagnol, 1979). Decreases in **P** uptake have mostly been found with soil studies (probably due to a reduced activity of the $H_2PO_4^{-1}$ ions in the saline solution) and increases in sand or solution studies (Grattan & Grieve, 1992). In one investigation both Cl and SO_4 salts reduced P uptake in barley and sunflower (Zhukovskaya, 1973), but in a more recent study on barley, added P and increased P-uptake increased the salt tolerance (Al-Karaki, 1997). In a review on P nutrition and salt toxicity it was concluded that the influence of added P on the salt tolerance of a variety of crops depended on the severity of the salinity: salt tolerance was increased at low, not affected at moderate and decreased at high salinities (Champagnol, 1979). Grattan & Grieve (1992), found that the most useful conclusion of Champagnol's review was that "P additions to P deficient soils are beneficial provided that the crop is not experiencing severe salt stress." However, in a study by Awad, Edwards & Campbell (1990), it was found that the P requirement of tomato was increased as NaCl salinity intensified from 10 to 100 mmol L^{-1}.

The influence of salinity on **Fe**, **Mn** and **Cu** concentrations in plants is inconsistent; it varies with species, increasing in some crops and decreasing in others (Grattan & Grieve, 1992). High SO_4 can, however, reduce **Mo** and **Se** uptake and thus growth via N and S nutrition respectively (Stout, Meagher, Pearson & Johnson, 1951; Läuchli, 1993).

The above nutritional disorders are dependent on genetic variability, as species and cultivars can vary widely in their nutrient requirements and ability to absorb specific nutrients. There are, however, only a few studies where fertilization with these nutrients increased growth in sodic-saline conditions. Growth is determined by the most limiting factor, in this case salinity stress or nutritional deficiencies (see Bernstein, Francois & Clark, 1974). The amounts needed for corrective fertilization would probably be too large and not economical, especially in the case of K (Grattan & Grieve, 1992, 1994).

2.2.2.2 Tolerance or adaptation mechanisms

Much work has been done to understand the mechanisms by which plants adapt to high concentrations of salt in the root growth medium. The salt tolerance of plants includes complex anatomical and physiological features which makes the breeding of tolerant cultivars very difficult. However, if the property most limited by salinity stress could be identified, salt tolerance could hopefully be improved (Shannon, 1997). Understanding these mechanisms is important for the genetic breeding of salt-tolerant plants, but it will not be discussed here in detail (for reviews see Maas & Nieman, 1978; Greenway & Munns, 1980; Cheeseman, 1988; Jacoby, 1994 and Shannon, 1997).

Mechanisms of salt tolerance have been attributed to:

- selective uptake of ions (salt 'exclusion')

- compartmentation at the cell (vacuoles), tissue or organ levels (ion accumulation - 'includers'), where ions are kept away from the salt sensitive metabolic components of the cytoplasm

- osmotic adjustment (osmoregulation) whereby the osmotic potential in the plant is decreased by an increase of inorganic or organic solutes thus recovering water uptake and turgor; turgor loss, which could lead to stomatal closure, a decrease of gas exchange, photosynthesis and energy for metabolic processes, is therefore prevented.

- morphological characteristics such as a smaller leaf area, fewer stomata and thicker cuticles, but these changes can decrease crop yield and quality. The salt content can also be controlled by excretion and leaf drop.

2.3 SULPHATE SALINITY

Sulphur rich environments cause some plants to die while others survive, but generally plants are tolerant to high sulphate concentrations in the growth medium and are usually only affected when SO_4 is in the order of 50 mmol L^{-1} (4800 mg L^{-1}), with symptoms similar to those of salt affected plants (Mengel & Kirkby, 1987). Toxicity is usually caused by the cation associated with the SO_4 ion, either by ionic effects or disturbed Ca nutrition and root membrane functioning (Tabatabai, 1986).

The effect of excess sulphur on plant growth was reviewed by Rennenberg (1984). He concluded that: "Survival in a sulphur rich environment is seldom achieved through the avoidance of the intake of sulphur. The presence of excess sulphur in the soil or in the air usually results in an intake of excess sulphur into plants. An immediate injury by the excess sulphur taken up, is however, prevented by a series of metabolic processes. Storage of excess sulphur in ... the vacuole, appears to occur in most plants." Sulphate can be translocated in both the xylem and phloem, and can thus be stored in plant parts not directly exposed to the excess. With increasing accumulation of sulphate, an increase of storage glutathione was found, suggesting that with increasing accumulation the reduction of sulphate also increases. The level of glutathione has also been shown to correlate with the adaptation of plants to different stresses (May, Vernoux, Leaver, Van Montagu & Inze, 1998). Sulphate can also be decreased in plants by emission of volatile sulphur compounds. It is thus improbable that excess sulphate per se would influence growth through ion toxicity (Rennenberg, 1984). In this respect citrus is an exception as growth was retarded and interveinal chlorosis occurred in citrus when leaf S-levels exceeded 0.5 % (Haas & Thomas, 1928).

However, in a study with wheat species, it was suggested that the greater negative effect of SO_4 compared to that of Cl salinity could possibly be attributed to the "less effective sequestration or mobility of this ion towards some innocuous centres of plant tissues" (Datta, Kumar, Varma & Angrish, 1995 p.2199). They also observed an interesting phenomenon whereby the presence of high SO_4 in a NaCl growth medium resulted in an increase of the uptake of Na and Cl into the shoot, above that of an equal concentration of only NaCl. Consequently the salt tolerance of the wheat cultivars was also decreased, as salt tolerance in wheat is associated with the exclusion of Cl and to lesser extent of Na from the shoot (Shannon, 1997).

Calcium and Magnesium

Excess SO_4 in the soil solution may, however, have nutritional implications, for example a Ca deficiency where very high SO_4 concentrations are accompanied by low Ca levels (Curtin et al., 1993). On the solonetzic soils of the Canadian prairie nutrient problems arise from high Na and low Ca together with the high SO_4 content (Curtin et al., 1993). Calcium deficiency is also related to the Mg content of the soil; Ca deficiency was found to be severe for barley on the above mentioned soils if the ratio of Mg to Ca exceeded 1.0 or when the Ca to total cations were below 0.15 (Carter et al., 1979).

Ca availability in Na_2SO_4 systems can be influenced by the formation of the $CaSO_4$ ion pair. When the ratio of Ca to other cations is determined in activities (which is a better criterion for plant availability than concentration), the mole fraction of Ca will be less, due to the formation of ion pairs (also applicable for $MgSO_4$). However, in a study where barley and kochia were subjected to high Na_2SO_4 concentrations, the results indicated that at the electrical conductivities (EC_e values) compatible for most glycophytic crops (< 800 mS m^{-1}), Ca-deficiency by SO_4-salinity should not repress growth, except for a limited number of crop species that are inefficient in absorbing or utilizing Ca (Curtin et al., 1993).

Another mechanism of Ca deficiency is by the precipitation of Ca-oxalate in plants. Curtin et al. (1993), observed that with SO_4-salinized plants the oxalate content was higher than with Cl- salinity. This was attributed to the fact that the uptake of SO_4 is slower than that of Cl and that more oxalate was synthesized to compensate for a greater positive charge. Although Ca can become immobilised as Ca-oxalate in the plant, some species (e.g., kochia) has the ability to control the precipitation of Ca-oxalate. Sensitivity to this kind of Ca deficiency may again be species and cultivar dependent.

Molybdenum

High sulphate can also reduce the Mo uptake and/or transport (Stout et al., 1951). Barnard and Fölscher (unpublished data) found that the Mo content of the top growth of wheat doubled in the absence of SO_4; Mo was also diminished by other anions in the order of S >B >P >Cl >NO_3 (Barnard, 1978).

Molybdenum is an essential part of the enzymes nitrogenase and nitrate reductase which are two major enzymes in N metabolism. Nitrogenase catalyzes the fixation of molecular N_2 by bacteria and symbiotic microorganisms, and nitrate reductase catalyzes the biological reduction of nitrate to nitrite. Molybdenum deficiency symptoms can thus be similar to N deficiency, with the exception of necrotic leaf margins, caused by NO_3 accumulation (Maynard, 1979).

Crop species have varying Mo requirements, but generally legumes need two to three times more Mo than non-legumes for the N-fixing nodules (Johnson, 1966), and are thus more prone to Mo deficiency by high levels of SO_4. S-fertilization has depressed growth in legumes growing on soil with low available Mo due to competition of SO_4 with MoO_4 (Reisenauer, 1963).

Although an essential element for livestock, Mo can be toxic at higher concentrations, especially to ruminants. The critical amount of Mo that animals can tolerate depends on the Cu and SO_4 level as the toxicity of Mo is essentially a deficiency of Cu. This Mo/Cu interaction is strongly influenced by the surrounding sulphate level (Albasal & Pratt, 1989).

Plants can tolerate higher levels of Mo in the tissues than the usual 2 mg kg^{-1} dry mass. Forage crops with a high Mo content can therefore be unfit as fodder. Plant Mo availability is low on acid soils and increases to a maximum near neutrality, whereas Cu availability decreases with increasing pH. Sulphate can, however, reduce molybdate uptake by competition. Thus, although Mo would be more available in the pH range of lime treated acid mine drainage water, a high sulphate content should help to prevent excessive levels in forage crops.

To protect animals from toxicity, a guideline of 10 µg L^{-1} exists for irrigation water. In irrigation waters with high SO_4 (such as in the San Joaquin Valley of California), this guideline can be increased to 50 µg L^{-1} due to the effect of SO_4 on Mo absorption (Albasal and Pratt, 1989).

Selenium

Toxic amounts of Se are often present in association with SO_4 salinity in saline soils of semi-arid and arid areas. Selenium is chemically similar to S and in aerated soils is mostly present as the plant available SeO_4. Selenate competes with SO_4, not only in uptake at the SO_4 binding sites, but also by being incorporated into proteins where it can interfere with N and S metabolism (Läuchli, 1993).

Selenium is mainly found in sedimentary and volcanic deposits. Plant availability depends on soil factors such as clay content and pH. Owing to its retention on clay minerals and iron oxides, uptake is more effective from sandy soils. Se is least soluble at slightly acid to neutral pH, and plant availability is low on neutral to acid mineral soils with a high Fe and organic matter content due to fixation.

Selenium is an essential micronutrient for animals and humans but has not been proved essential for higher plants, except for some Se-accumulators. In crop species Se is usually present at concentrations less than 1 mg kg^{-1} dry mass, but as little as 2 mg kg^{-1} has affected growth in sunflower (Shrift, 1969). Selenium can be toxic in animal feeds, causing deformity and death to animals. The desirable level in cereals and forages is 0.05-2 mg kg^{-1} dry mass, the contents differing with species. Selenium is toxic to plants when the content is greater than 50 mg kg^{-1} dry mass.

High concentrations of SO_4 in Se containing soil solutions can reduce the Se content of many plant species by competition and by reduced activity of SeO_4 in the saline water. Growth inhibition and Se uptake in tall fescue at comparable concentrations of Na_2SO_4 and NaCl were reduced with the Na_2SO_4-salinity (Wu & Huang, 1991). In the halophyte, purslane, SO_4-salinity inhibited Se-accumulation to a level where it did not present a dietary hazard to humans, but met the requirement as an essential micronutrient (Grieve & Suarez, 1997). Tall fescue, a moderate Se-accumulator, may also be used as a supplement for livestock feeds with deficient Se content (Wu & Huang, 1991).

Finally, SO_4 in irrigation water could also have a positive effect as a nutrient in areas prone to deficiency, for example in the tropical savannahs, on soils with a low capacity for adsorbing SO_4 where high seasonal rainfall could possibly deplete unbuffered soils of S compounds.

2.3.1 SODIUM-SULPHATE SALINITY

In most studies of SO_4-salinity the associated cation has been Na (Magistad, Ayers, Wadleigh & Gauch, 1943; Curtin et al., 1993; Mayland & Robbins, 1993; Datta et al., 1997; Grieve & Suarez, 1997). Generally the growth-suppressing effect is similar to that of a NaCl salt effect (Mengel & Kirkby, 1986; Curtin et al., 1993). Curtin et al. (1993) compared the effect of a $CaCl_2$/NaCl system with that of $CaSO_4$/Na_2SO_4 in barley and kochia, from which they concluded that “response functions generated by the $CaCl_2$/NaCl salinisation probably provide an acceptable measure of the tolerance of most crops to SO_4-salinity.” Thus, although the response to Na_2SO_4 can be either more or less severe than with NaCl, at isosmotic concentrations, the general linear decrease response is the same as that of Maas & Hoffman (1977). Toxicity and/or nutritional imbalances occurring with Na_2SO_4-salinity are mostly caused by the associated Na ion. As mentioned above, however, the increased uptake of Na and Cl in the presence of SO_4 may possibly be a new adverse mechanism of SO_4-salinity (Datta et al., 1995).

NaCl (or other anions) versus Na_2SO_4 salinity effects have been investigated in a number of nutritional and salinity studies. Some of the effects were mentioned in the above general discussion of SO_4-salinity (2.3). At equal osmotic potentials, SO_4-salinity can often suppress growth more than Cl salinity can (Mengel & Kirkby, 1987); however, even at equal osmotic potentials, plants differ in their responses to the composition of a salinized growth medium. Contrasting results have been reported. Early investigators compared the effect of specific anions on salt tolerance on the basis of equal moles or equivalents with similar cations in the nutrient medium. On this basis SO_4 generally decreased growth to a lesser extent than Cl. Magistad and co-workers (1939-1943) were among the first to compare NaCl and Na_2SO_4 salinity at equal osmotic potentials. They found that “for some crops” (beets, carrots and beans) “chlorides and sulphates at equal osmotic concentrations are equally harmful, while with other crops” (lucerne and peaches) “chlorides are more toxic than sulphate at approximately equal osmotic values.”(Magistad et al., 1943 p.157). This is probably a reflection of either an osmotic potential or of Na/Cl ionic effects respectively. They go on to say that more equivalents of sulphate are needed to produce a given osmotic potential value which explains “why plants can withstand far greater amounts of sulphate than chloride when compared on a parts per million basis” (Magistad et al., 1943).

On the other hand, in the studies of Datta et al. (1997), four genetically diverse wheat cultivars were all more sensitive (differentially) to Na_2SO_4- than to the NaCl salinity at equal osmotic potentials. Furthermore, in a mixed Na/Cl/SO_4 growth medium the presence of SO_4 resulted in increased Na and Cl concentrations in the shoots, the contents of which agreed with the differences in sensitivity of the cultivars. The cereals, barley and sorghum, were also more sensitive to Na_2SO_4 than to NaCl (Curtin et al., 1993; Boursier & Läuchli, 1990; Marschner, 1995).

Reports for halophytic crops also differ : Na_2SO_4 depressed growth more than NaCl in Chenopodium (Warne, Guy, Rollins & Reid, 1990), while kochia grew better with SO_4 salinity (Curtin et al., 1993). Purslane, a common salt-tolerant plant often used as a food source for humans and animals, was evaluated as a prospective salt-tolerant crop for use with a high SO_4 drainage water in the San Joachin valley of California (Grieve &Suarez, 1997). It responded in a similar way as previously found with Cl salinity (Kumamoto, Scora, Clerx, Matsumura, Layfield & Grieve, 1990).

Sodium chloride may also have a greater effect on membrane integrity or leakage than Na_2SO_4, but comparisons are made difficult because different concentrations are needed to acquire treatments with equal osmotic potentials (Jacoby, 1994).

The above mentioned effects would, however, depend on the sensitivity of a specific crop to toxic ions and/or nutrient imbalances.

From the above it can be seen that Na_2SO_4 salinity can affect growth by mechanisms other than, or complemental to, a low osmotic potential.

2.3.2 CALCIUM-SULPHATE SALINITY

Not many studies have investigated the use of irrigation waters with high Ca and SO_4 content. Generally it is considered beneficial to plant growth as salt buildup is restricted by the low solubility and precipitation of gypsum. Growth can however be affected either directly - by decreases of the osmotic potential, nutritional or specific ion effects of the SO_4 or Ca - or indirectly, by influencing soil and soil solution properties.

Papadopoulos (1986) investigated the growth of the moderately sensitive tomato, eggplant and bell pepper with two naturally occurring $CaSO_4$ waters, one with, and the other without Na and Cl in its composition. Both waters - with the same EC's - decreased the yield and/or quality of these crops, the effect of the 'mixed' water being greater. For more tolerant crops, however, soil solutions saturated with $CaSO_4$ may not be limiting. MacAdam et al. (1997) determined the growth of tall fescue and lucerne with ground waters from a plume of high $CaSO_4$ water in Utah near Salt Lake City. The top growth of tall fescue tended to increase and that of lucerne increased significantly at “moderate” SO_4 levels of the soil solution (646 mg L^{-1} SO_4).

The type of soil being irrigated may, however, affect the growth response to a gypsiferous water. Papadopoulos (1984, 1986) stressed the fact that on a *sodic* soil it can actually be harmful by increasing the fraction of Na in the soil solution and consequently also the sodium adsorption rate (SAR); Na is released by cation exchange, and Ca removed by the precipitation of gypsum. It is well known that such an increase can affect the permeability of the soil. [Pore clogging from gypsum precipitation has also been reported (McNeal, 1974; Frankel, Hadas & Jury, 1978)]. Du Plessis (1983), however, found that irrigating with lime-treated acid mine drainage water, did not pose a serious problem to soil physical properties when viewed against “published data on soil hydraulic conductivity as affected by sodium and electrolyte concentration.”

Gypsum is used to ameliorate sodic soils for the correction of imbalances on the exchange complex and to promote good permeability of the soil. Numerous studies have shown that Ca also ameliorates Na stress on plant growth (Rengel, 1992b); in an investigation of the role of the anion of the Ca salt used for this amelioration with *Phaseolus vulgaris* L., it was concluded that $CaSO_4$ treatments ameliorated Na induced salinity stress more than $CaCl_2$ treatments did (Awada et al., 1995). But, when growing *moderately sensitive* crops (2.5.2), it should be taken into account that when leaching with gypsiferous water, the salinity of the soil solution may increase due to an inevitable salt buildup (Papadopoulos, 1986).

When Ca and SO_4 are added to a *calcareous* soil, the Ca can decrease by the precipitation of $CaCO_{3,}$ with a concurrent increase of SO_4 concentration in the soil solution. The presence of Mg will further increase the gypsum solubility by the formation of a moderately strong $MgSO_4$ ion pair (MacAdam et al., 1997). Depending on the Mg content of the soil and irrigation water, this could also have implications for growth via the Ca/Mg ratio.

In soils such as the gypsisols, with a gypsum content higher than 25 %, the nutrient balance can be disturbed by a lower availability of PO_4, K and Mg; the cation exchange capacity also decreases with increasing gypsum (Driessen & Dudal, 1991). Cereal crops and lucerne can be grown where the gypsum content of the upper 30cm is less than 25 %. Yields may, however be depressed due to nutrient imbalances and mechanical hindrances. Soils with more than 25 % gypsum will not be suitable for dry land cultivation, but could possibly be productive with irrigation and effective drainage (Driessen & Dudal, 1991).

In a field trial, conducted simultaneously with the present study, the long-term effects of irrigation with a gypsiferous mine water on inter alia the soil properties were investigated. Using a soil water/salt balance/crop growth model to simulate 30 years of irrigation with gypsiferous mine water, it was concluded that year-round, high frequency irrigation, with a leaching fraction in winter, would not cause irreparable damage to soil resources in this particular summer rainfall climate (Annandale, Jovanovic, Benade & Tanner, 1999).

In conclusion it can be said that the effects of salt stress on growth can be summarised in terms of the energy needed to adapt to saline conditions: "Salt stress essentially increases the energy that must be expended by the plant to extract water from the soil and to make the biochemical adjustments necessary to grow relative to the non-saline condition" (Rhoades & Loveday, 1990 p.1091). Energy is thus diverted from processes needed for normal growth to adaptive mechanisms (Yeo, 1983)

2.4 FACTORS THAT INFLUENCE SALT TOLERANCE

Salt tolerance data in the literature can only be used as a general guideline for crop selection. Such data for a specific crop are mostly average values for different cultivars grown in a variety of environmental conditions (Maas & Hoffman, 1977). Salt tolerance depends not only on salinity but also on many other factors such as edaphic, climatic, plant variety and growth stage, agronomic and irrigation practices. Therefore salt tolerance data in the literature cannot be used for quantitative predictions of crop yield losses from salinity for every situation. It has been found that when the sensitivity is due to some factor, other than the inherent real tolerance of a species or cultivar, the threshold and slope will increase and decrease together, with no change in the salinity of the extrapolated 'zero yield'; but when the salinity at zero yield is also affected, it indicates a difference in real tolerance (Meiri & Plaut, 1985). The complexity of environmental interactions with salinity has been a major obstacle to the breeding of salt-tolerant varieties (Shannon, 1997).

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2.4.1 ENVIRONMENTAL FACTORS

Soil environment

Chemical and *exchange reactions* and *moisture retentivity* can influence growth on saline soils. The physical structure of the soil influences drainage and aeration. Poor soil aeration amplifies the detrimental effects of soil salinity. The application of gypsum under such conditions can increase the salt tolerance by improving soil structure and aeration (Oster & Frenkel, 1980; Frenkel & Meiri, 1985). Salt tolerance in waterlogged conditions can be very different from that in drained conditions. Waterlogged soil conditions increase the uptake of salts from a saline root medium compared to that in aerated conditions (Shannon, 1997; Marschner, 1995). Extraction of water from the underlying water table can also influence the evaluation of salt tolerance of crops in the field, depending on the quality of that water and the rooting pattern of the crops.

The *fertility* and *fertilization* of soil can result in an 'apparent' relative salt tolerance that can be misleading (Bernstein, Francois & Clark, 1974; Grattan & Grieve, 1994). Crops grown at low fertility levels may show an apparently high relative salt tolerance (Feigin, 1985) because yields on non-saline soils can be relatively more affected by infertile conditions than yields on saline soils, resulting in an apparently higher relative salt tolerance. Improving nutrition by fertilization could, on the other hand, improve growth proportionately more under moderate or non-saline conditions than under saline conditions and result in an apparently lower relative salt tolerance. Bernstein et al. (1974) concluded that in the case of cereals at moderate nutrient deficiency and salinity, these effects are independent and additive. At higher stress levels the growth is, however, determined by the more limiting salinity factor. Nutrient/salinity interaction can thus differ substantially as salinity increases from low to high levels (Grattan & Grieve, 1992). This is probably why most plants do not respond positively to N and P fertilization at high salinity. Feigin (1985) reviewed data on fertilization of crops irrigated with saline water and concluded that standard fertilization for non-saline conditions is also suitable for saline conditions.

Salt tolerance also depends on the *combination of specific salts* in the soil solution (the composition). In some regions ions such as Al, B, Mn, Se may be present in toxic or growth limiting concentrations. Different ions have different toxicity levels, and also influence osmotic potential differently. The influence on osmotic potential depends inter alia on the osmotic coefficient of the specific salt of which $NaCl > MgCl_2 / CaCl_2 > Na_2SO_4 > MgSO_4$, depending on the concentration of the particular salt (Robinson & Stokes, 1959). The proportion of $Cl/SO_4/HCO_3$ and Na/Ca/Mg is important for the effect on plant growth. Rana (1985) noted that although crops tolerant to alkali soils are usually also tolerant on non-alkaline saline soils, the opposite is not true. Maas (1990), however, concluded that generally plants respond similarly to salinity over a wide range of salt combinations.

Climate and atmosphere

Climate is probably the factor that has the greatest influence on the salt tolerance of crops. *Temperature, radiation, atmospheric humidity* and *pollution* can all influence salt tolerance. Generally, studies show that crops are more tolerant to salinity under cool, humid conditions than in a hot, dry climate (Magistad et al., 1943). The salt tolerance parameters of threshold and slope can be influenced by hot, dry conditions: the threshold lowered (yield can start decreasing at lower salinities), and the slope increased (a more rapid decrease of yields with increasing salinity). Crops and cultivars can, however, vary in their response. Lucerne and dry bean salt tolerance decreased at higher temperatures (Ahi & Powers, 1938); barley, bean and corn were more sensitive to salinity at low than high air humidity (Maas, 1990), while humidity did not greatly affect the salt tolerance of wheat (Hoffman & Jobes, 1978). High humidity causes greater yield increases in salt-sensitive than in salt-tolerant crops (Maas & Hoffman, 1977).

The *gaseous composition* surrounding the aerial plant parts can also have an effect on the relative salt tolerance. Salinity causes stomatal closure which reduces the CO_2 uptake and consequently the C compounds needed for growth. High CO_2 concentrations can partly reduce this effect. The closing of leaf stomata can also reduce the volume of air pollutants entering the plant, thus possibly reducing the toxic effects on growth. Ozone, a major air pollutant, has a greater effect on the growth of oxidant-sensitive (leafy and forage) crops under non-saline than saline conditions. Such crops may thus seem relatively more tolerant to salinity in such areas (Maas, 1990).

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Agronomic and irrigation practices

Agronomic and irrigation practices can also cause increased injury with saline water. In raised seedbeds with furrow irrigation, for example, seeds should be planted on the shoulders away from the areas of salt accumulation (Ayers & Westcot, 1985). The frequency of irrigation influences sensitivity as plants are exposed to increased salinity with time between applications. Species also differ in their response to sprinkled irrigation. This depends on leaf characteristics and the rate of foliar absorption of salts. The Solonaceae family, for example potato and tomato, is most sensitive to leaf injury by salts. Greenhouse tests indicated sensitivity in the following order: sugarbeet <cotton and sunflower <cauliflower <safflower <barley and sorghum <alfalfa <tomato <potato (Maas, Grattan & Ogata, 1982). Foliar injury depends more on the rate of absorption by leaves than on the salt tolerance of the crop (Ehlig & Bernstein, 1959). The rate of absorption increases not only with concentrations in irrigation water but also with duration of contact.

2.4.2 PLANT FACTORS

Species, cultivars and rootstocks

Plant species and cultivars differ in their ability to grow under saline conditions (Maas & Hoffman, 1977). With the greater emphasis on the genetic breeding of salt-tolerant and other stress-tolerant cultivars, agronomical varieties now originate from a more diverse genetic base than in the past. There is thus a greater possibility of cultivars differing in salt tolerance than in the past and this is an important basis for screening (Francois & Maas, 1994).

Since the 1970's much effort has been put into the development of salt-tolerant crop cultivars but only a few cultivars have been released (Richards, 1995). Breeding salt-tolerant varieties is hampered by the fact that salt tolerance is a multigenetic trait with a variety of different mechanisms by which plants are affected by and can adapt to salinity. The spatial- and time-related heterogeneity of saline soils also make selection for breeding very difficult. Most studies are thus on salinized nutrient solutions, of which the problems of extrapolation to field conditions are well known.

Yield is an important parameter for the selection of agronomic crop cultivars. In practice it has been found that generally the more salt-tolerant varieties are lower yielding while those with higher yields are more salt sensitive. Selecting for salt tolerance can thus develop low yielding cultivars that are not competitive with non-tolerant, high yielding ones (Shannon, 1997). Richards (1995), however, also found that selections made for high yield on naturally saline soils have indirectly developed salt-tolerant cultivars. He concludes that even though the tolerant varieties may survive much better under salt stress, normal high yielding cultivars of for example wheat, barley and sunflower may produce higher yields than their salt-tolerant relatives in saline soils; for breeding it is thus better to select from high yielding rather than from salt-tolerant lines.

Growth stage

The sensitivity of species and cultivars can change during their ontological development. It is important to separate the effects of growth stage from those related to duration of exposure to salinity (Lunin, Gallatin & Batchelder, 1961). Salt tolerance measured at one growth stage does not necessarily correlate well with tolerance at other growth stages. There is, however, little data on specific effects of salinity at the different growth stages of crops. In most studies crops are subjected to salinity either from planting or after the early seedling stage (Francois & Maas, 1994).

The timing of development can also be influenced by salinity. This differs according to crops. In some grains maturity is earlier under saline conditions, for instance in wheat, sorghum and oats; in others it is not affected (rye and barley), while in tomato flowering is delayed (Shannon, 1997).

A major question in the selection or screening of salt-tolerant varieties is whether the tolerance in one growth stage is related to that in other stages. Independent selection at different growth stages and subsequent crossing could possibly then combine salt tolerances at different growth stages into one cultivar (Shannon, 1997). However, where sensitivity is typical for a crop at a specific growth stage, salt tolerance at that stage (e.g., at germination for sugar beet, or early vegetative stages for cereals) could remove a major limitation in its growth.

Germination: Germination can be influenced by salinity through a decreased entry of water (lower osmotic potential) and/or the intake of ions to toxic levels. The percentage of germination is generally not decreased by salinity, but the rate of germination and emergence have been delayed (Francois & Maas, 1994). Exceptions are sugarbeet, lucerne, cotton and sunflower where germination is sensitive to soluble salts (Läuchli & Epstein, 1990). Pearl millet is sensitive to sodicity during germination (Ray, 1988). It is interesting that some halophytes, that grow optimally at relatively high NaCl levels, appear to be salt sensitive during germination (Ungar, 1978). Determining the salt tolerance of a species during germination has generally not been successful for breeding purposes.

Emergence and seedling stages: Plants are usually most sensitive during emergence and early seedling stages and become more tolerant as growth proceeds from the vegetative to the reproductive and grain-filling stages (Francois & Maas, 1994). Leaf and spikelet primordia and tiller buds of cereals are formed during the early vegetative stage. Salinity stress at this stage may significantly affect the eventual seed yield. Sensitivity at these early stages, and thus the crop stand, can be greatly increased because of the exposure of juvenile roots to intensified salt and water stresses by evaporation from the soil surface.

The *vegetative growth stage* of non-halophytes is generally sensitive to salinity.

Anthesis, pollination and fertilization: Although very limited, there are some indications that this could be a sensitive growth stage, for example in the case of rice (Pearson & Bernstein, 1959; Akbar & Yabuno, 1977). In maize, salt sensitivity was found to be particularly high at tasselling (Maas, Hoffman, Chaba, Poss & Shannon, 1983). On the other hand the insensitivity during or just before anthesis in grain crops such as sorghum, wheat and barley has been used successfully as a stage to substitute with more saline waters for irrigation (Maas & Poss, 1988). During *reproductive development* salt tolerance can, however, increase dramatically (e.g., in cotton yields, Rains, 1981).

Comparing the sensitivity of a particular cultivar at different growth stages is complicated by the criteria used at these stages; germination and emergence are usually determined by survival percentage and thereafter salt tolerance is based on relative growth or yield.

When screening for salt tolerance of different cultivars of a species, the most sensitive growth stage would obviously be studied, but within one genotype there could also be shifts in the relative salt tolerance of cultivars at different development stages, for example "Salt resistance of three barley cultivars changed over time, the cultivar most sensitive to early salinisation proved rather resistant at maturity, and the one that had the greatest initial resistance ... was more sensitive at maturity." (Lynch, Epstein & Läuchli, 1982).

2.4.3 MICROBIOLOGICAL FACTORS

Other biological factors are the sensitivities of Rhizobium species, and also possibly those of mycorrhiza. Soil salinities above the threshold values of legume species may severely affect the

survival and N-fixing abilities of Rhizobium species. Chloride salts of Na, K and Mg appear to have specific ion effects on Rhizobium growth and are more toxic than the SO_4 salts. Magnesium inhibits Rhizobium growth at lower concentrations than Na and K (Francois & Maas, 1994).

2.5 EVALUATION OF CROP SALT TOLERANCE

Salt tolerance can be evaluated in several ways: survival under saline conditions; absolute growth or yield reduction for specific salinity levels; or, growth in saline relative to that in non-saline (control) conditions (Maas, 1990). Survival is important for ecological studies and perhaps also for revegetation of problem soils, but not for commercial production. The absolute yield reduction at specific salt concentrations can be useful for farmers, but is complicated by the fact that these yields are influenced by a multitude of other factors pertaining to the climate, environment, soil and the plant itself (2.4). Furthermore, yields of different crop species cannot be compared on an absolute basis. These problems are largely overcome by expressing yield or growth on a relative basis. *Relative growth* or yield was defined by Maas (1990) as the growth or "yield of a crop grown under saline conditions expressed as a fraction of that achieved under nonsaline, but otherwise comparable, conditions". Relative salt tolerances may, however, also be misleading (Bernstein et al., 1974), giving rise to 'apparent' salt tolerances that can be higher or lower depending on the proportionate influence of other factors on the control (2.4.1).

2.5.1 CRITERIA USED FOR SALT TOLERANCE EVALUATION

Many criteria have been used to evaluate the salt tolerance of crops, of which survival, shoot dry mass and seed or fruit yield are the most common. Depending on the criteria, differing salt tolerance responses can be elicited. Seed production can often be less affected than shoot growth. The most recent salt tolerance lists of Francois & Maas (1994) include data on the specific parameters used, such as grain yield, shoot growth and tuber yield. Vegetative shoot growth has been the most widely used parameter with non-halophytic crops. Experience has shown that increased biomass can result in increased economic yields (Arnon, 1977). Because tolerance can differ at different growth stages selection for salt tolerance has often been evaluated over the entire growth cycle. In many cases, however, salinity is imposed from the late seedling stage to maturity. Another approach is to evaluate for salt tolerance at the most sensitive growth stage. This could, however, lead to erroneous deductions for the salt tolerance of the total growth cycle of a species (Ray, 1988; Munns, 1993). Physiological criteria, for example the K/Na ratio and Na and Cl exclusion in wheat, have also been found to be an indication of salt tolerance for some species.

2.5.2 SALT TOLERANCE DATA AND YIELD RESPONSE FUNCTIONS

In earlier salt tolerance data, crops were listed according to their yield under saline conditions (Magistad & Christiansen, 1943) or subsequently more qualitatively by placing crops in groups from sensitive to tolerant. In a later approach, semi-quantitative data were given by listing crops with the salinity values at which different yield percentage decreases could be expected (e.g., Bernstein, 1964, 1974).

In 1977 there was a breakthrough for quantitative evaluation of salt tolerance when Maas and Hoffman reviewed all available salt tolerance information and it became apparent “that, in general, yield was not decreased significantly until a threshold salinity level was exceeded, and that yield decreased approximately linearly as salinity increased beyond the threshold” (Maas & Hoffman, 1977 p.126). Two important parameters emerged from this conclusion: the “*threshold*” that is “the maximum allowable salinity without yield reduction below that of the nonsaline control treatment” and the “*slope*” - “the percent yield decrease per unit salinity increase beyond the threshold” (Maas & Hoffman, 1977 p.121) (Figure 2.1)

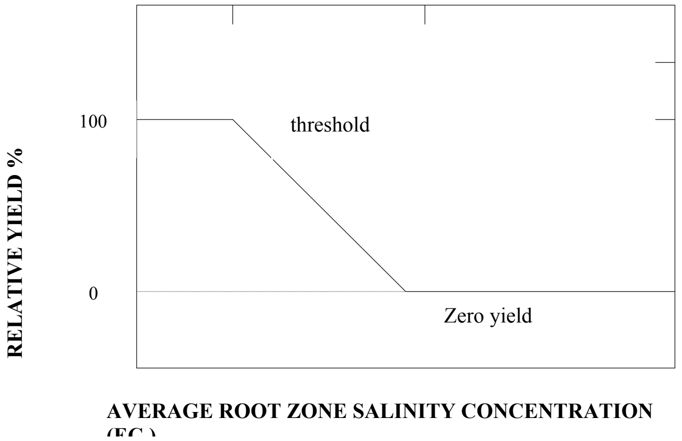

Figure 2.1 Graphic representation of the piecewise linear salt tolerance response function.

The relative yield (Y_r) could now be calculated for any given soil salinity exceeding the threshold, if the threshold and slope values were known, by using the equation

$$Y_r = 100 - B\,(EC_e - A)$$

Where A = the salinity threshold expressed in dS m^{-1} (1 dS m^{-1} = 100 mS m^{-1})

B = the slope expressed in yield decrease % per dS m^{-1} (per 100 mS m^{-1})

EC_e = the mean electrolytical conductivity of the saturated soil extract of the root zone at 25^0C in dS m^{-1} (over the whole growth period)

The threshold hypothesis of the popular two-section linear, yield/salinity response function was confirmed by Feinerman, Yaron and Bielorai (1982), using a switching regression method instead of the least squares approach used by Maas and Hoffman (1977), to estimate the parameters in the two-section linear response curve.

According to Van Genuchten (1983), salinity can also be expressed as concentration (see Hoffman, Rhoades, Letey and Sheng, 1990, for conversion from EC_e), osmotic potential (see Maas, 1990) and the electrolytical conductivity of the soil water per se (EC_{sw}). Most response functions for the effect of salinity on crop growth, however, uses the total salt concentration, measured as the electrolytical conductivity of the growth medium or converted to the osmotic potential. The electrolytical conductivity is, however, not a good representation of the osmotic potential in Ca and Mg sulphate waters as these electrically neutral ion pairs, $CaSO_4$ and $MgSO_4$, are not measured in the electrolytical conductivity, but nevertheless contribute to the osmotic potential (Papadopoulos, 1986).

Because salt tolerance functions are mostly based on the assumption that plants actually respond to the osmotic potential of the soil solution (π_{sw}), EC_e is converted to EC_{sw} at field capacity, or at wilting point and then to osmotic potential of the soil solution. Meiri (1994) points out that this conversion must, however, take into account the structure and chemical characteristics of the specific soil involved. He argues that:

- the calculation of the EC_{sw} is based on the ratio of the saturated water content to that at field capacity (θ_e/θ_{fc}) being 2/1 or the wilting point (θ_e/θ_{wp}) being 4/1. These ratios apply to many soils but depend on the soil's structure and water holding capacity; a range of 2.03 to 8.45 for θ_e/θ_{wp} was reported for very fine and coarse soils respectively *(United States Salinity Laboratory Staff*, 1954 as quoted in Meiri, 1994). This can cause an erroneous calculation of EC_{sw} and the osmotic potential of the soil solution, and consequently of salt tolerance;

- the calculation of EC_{sw} as a simple dilution may be true for NaCl, but with gypsiferous soils and those with a high exchangeable sodium percentage, chemical considerations come into play. The threshold EC_e in gypsiferous soils will be about 200 mS m^{-1} higher than indicated by the EC_e, because of the dissolution of gypsum in the preparation of the saturation extract (Maas and Hoffman, 1977). Furthermore, when gypsum is added to soils with a high exchangeable sodium percentage it will further increase the EC_{sw} by the Na released through Ca exchange, and also by increased dissolution of the gypsum.

A comprehensive list of crop salt tolerances with these 'new' parameters was presented in Maas and Hoffman (1977) and these crop salt tolerances have been updated with ongoing research in expanded lists in Maas (1986, 1990) and Francois and Maas (1994). These lists include results from different countries and should thus be applicable as guidelines anywhere.

For quick qualitative rating, Maas and Hoffman (1977) grouped crops according to the salinities where yield starts to decrease (the threshold EC_e):

Sensitive	$EC_e < 130$ mS m^{-1}
Moderately sensitive	EC_e 130 - 300 mS m^{-1}
Moderately tolerant	EC_e 300 - 600 mS m^{-1}
Tolerant	EC_e 600 - 1000 mS m^{-1}
Unsuitable for most glycophytic crops - unless reduced yield accepted	$EC_e > 1000$ mS m^{-1}

(Ayers & Westcot, 1985)

When using values in these lists for yield prediction, the following must be kept in mind:

- These values are averages - not only from different countries but also with different soil types and for different cultivars.

- The listed values are based on data where salinity treatments were often commenced after seedling establishment, and are not representative of sensitivity during germination and seedling stages (although such information is noted when available).

- Soil salinity was mostly maintained at a relatively uniform value throughout the root zone, by irrigating with a high leaching fraction, thus minimizing salinity variations in concentration over time and space (Hoffman et al., 1990).

- Data in these tables mostly apply to soils where Cl is the main anion. Owing to the dissolution of gypsum when preparing saturated soil extracts, the corresponding EC_e values of gypsiferous soils (non-sodic, low Mg) generally range "from 1 to 3 dS m^{-1} (100 to 300 mS m^{-1}) higher than those of the non-gypsiferous soils having the same conductivity in the soil water at field capacity. Therefore plants grown on gypsiferous soils will tolerate" EC_e values "approximately" 200 mS m^{-1} "higher than those indicated in the tables" (Maas, 1986 p.16).

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- The lists in 1977 only included crop responses to total soluble salts in the root medium. In subsequent reviews salt tolerance data and limits for specific ion effects of for example B, Cl and Na were also included (e.g., Maas, 1986).

Van Genuchten (1983) developed a computer programme, entitled SALT, which facilitates the calculation of the salt tolerance parameters of the piecewise linear and other nonlinear yield-salinity response functions with limited data points.

The threshold and slope parameters were subsequently implemented in a crop-water production function in which three yield relationships were combined, namely yield and evapotranspiration, yield and average root-zone salinity, and average root-zone salinity and leaching (Letey and Dinar, 1986). Existing models for crop growth response with salinity is reviewed in Castrigano, Katerji & Hamdy (1995).

Currently two kinds of salt tolerance tables are used : (i) tables with the threshold and slope values by which relative growth at a specific salinity can be calculated and (ii) tables that show the maximum level of total salinity or the maximum level of specific ion concentrations permitted in irrigation water or soil solutions (Meiri, 1994).

Meiri (1994) however suggests that the existing tables are too conservative. He argues that these values are mostly based on studies with steady-state soil salinities and that a discrepancy arises from the differences with the temporally and spatially changing salinities under field conditions, and also from the interactions of environmental, edaphic and plant factors with crop response. He stresses the need for a salt tolerance data base with a multi-factor expression that takes the above into account. He stresses that the soil salinity parameter that correlates best with crop response should be found (i.e., the "effective salinity"). He suggests the need for possibly a computer programme that will predict the yield quantities and qualities with temporal and spatial changes in salinity, that will also take into account environmental, edaphic and plant growth factors. Product quality is a parameter of increasing importance that has not yet been included into the available salt tolerance tables (Meiri, 1994).

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2.6 SALT TOLERANCE OF AGRONOMIC GROUPS

The general salt tolerance trends for agronomic groups such as cereal, forage, vegetable, fruit and ornamental crops are summarised in Francois and Maas (1994). Most of the crops investigated in the current study fall into the cereal or forage groups.

2.6.1 CEREAL CROPS

With the exception of maize and rice, most cereal crops fall into the moderately salt-tolerant group (2.5.2), for example sorghum, wheat, triticale, rye, oats and barley. Salt tolerance has been indirectly developed in many grain crops by selection for high yield in naturally saline environments (Shannon, 1997).

All cereals seem to follow the same tendencies of sensitivity or tolerance with regard to their growth stage. Seedling and early vegetative stages (‘seedling’ and ‘tillering’ stages of Tottman and Makepeace, 1979) are usually the most sensitive, while subsequent stages are increasingly salt-tolerant. This has been shown to be the case for sorghum, wheat, barley, maize and rice and can also be expected with the other cereal crops (Francois & Maas, 1994).

Developmental events during the life cycle of cereals have been separated into three major phases (Francois & Maas, 1994 p.166):

“In the first phase, which encompasses the early vegetative stage, leaf and spikelet primordia are initiated, leaf growth occurs and tiller buds are produced in the axils of the leaves. High soil salinity at this time reduces the number of leaves per culm, the number of spikelets per spike and the number of tillers per plant.”

During the second growth phase - which includes ‘Stem elongation, booting and inflorescence emergence’ of Tottman & Makepeace (1979) -“the tillers grow, mainstem and tiller culms elongate and the final number of florets is set.” Tiller survival and the number of functional florets per spikelet can be reduced by salinity stress during this phase which ends at anthesis. In the final phase of fertilization and grain filling, seed number and size can be affected by salinity (Francois & Maas, 1994).

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High grain yield of crops such as wheat and sorghum has been found to be a better criterion of salt tolerance than biomass (Shannon, 1997), but Francois & Maas (1994) concluded that through its effect on spikelet and tiller number, salinity has a greater influence on yield in the first phase, than through the yield components of the subsequent two phases.

2.6.2 FORAGE CROPS

Forage crops are mainly from the grass and legume families. Generally the grasses are more tolerant and the legumes more sensitive to saline conditions.

Some grasses are sensitive but there are many salt-tolerant species (e.g., Bermudagrass). As in the case of the cereals, grasses are most sensitive during early seedling growth. Many forage grasses are mostly kept in the vegetative stage by grazing or cutting; when they have passed the early seedling stage and are established, these grasses are less sensitive to soil salinity (Francois & Maas, 1994).

Legumes mostly used for forage or fodder are clovers (*Trifolium* and *Melilotus* species) and lucerne (*Medicago sativa* L.). The salt-tolerance of these crops depends very much on the stage of growth when salinity is imposed. Dark green leaves, decreased leaf area and plant size are typical of the salt effect on these legumes. Owing to the genetic variability of the grass and legume species and cultivars, differences in salt-tolerance do occur (Francois & Maas, 1994).

The salt-tolerance or sensitivity characteristics of the individual crop species will be discussed with the respective results (Chapters 4, 5 and 6).

From this literature survey it should be clear that plant response to salinity must not be over-simplified. Different environmental and plant factors, mechanisms and evaluation methods are involved and should be kept in mind when evaluating and predicting quantitative crop responses to specific saline conditions.

CHAPTER 3

EXPERIMENTAL MATERIALS AND METHODS

In this chapter the plant materials, mine waters, culture methods, statistical analyses, units and terms that were used, are described.

Crops and cultivars to be evaluated were chosen mainly on the basis of irrigation and/or the climate of the eastern Highveld (Mpumalanga) region - a plateau with an elevation of 1500 to 1800 m.a.s.l. and a summer rainfall area.

The chemical composition of the coal mine waters differs with treatment, time and location; the origin and composition of specific waters and treatments used is therefore presented.

The different culture methods for evaluating salt tolerance during the germination, seedling and vegetative growth stages in a glasshouse are described. A sand culture method for determining the effect of increasing concentrations of simulated mine waters on seedling growth in growth chambers is explained.

Finally the statistical analyses are described and the units and special terms used are clarified.

3.1 MATERIALS

3.1.1 PLANT MATERIALS

Crops were selected from those known to be commercially successful for the region in which the coal mines are situated. Seed companies were consulted and asked to recommend the cultivars of these crops that were adapted to the climate of the eastern Highveld (Mpumalanga) region and/or to irrigation. The seed was donated by PANNAR, SENSAKO and CARNIA. The SMALL GRAIN CENTRE of the Agricultural Research Council in Bethlehem S.A., recommended and provided seed for the annual temperate crops and lucerne. More information on the cultivars used is presented in APPENDIX A.

The test crops were:

Annual subtropical	*Zea mays* L. (maize)
	Sorghum bicolor (L.) Moench (sorghum)
	Pennisetum glaucum (L.) R.Br. (pearl millet)
	Glycine max (L.) Merrill (soybean)
	Vigna unguiculata (L.) Walp. (cowpea)
	Phaseolus vulgaris L. (dry bean)
	Helianthus annuus L. (sunflower)
Annual temperate	*Triticum aestivum* L. (wheat)
	Secale cereale L. (rye)
	x *Triticosecale* Wittmack (triticale)
	Avena sativa L. (oats)
	Hordeum vulgare L. (barley)
	Lolium multiflorum Lam. (annual ryegrass)
Perennial temperate	*Medicago sativa* L. (Lucerne)

Other perennial forage crops and four cultivars of *Cynodon dactylon* (L.) Pers. (Bermudagrass) were also investigated. The results can be found in a report published by the sponsor of the project, namely the Water Research Commission of South Africa (Barnard et al., 1998).

3.1.2 MINE WATERS USED

Waters with extreme concentrations of salts were identified from the routine analyses data made available by AMCOAL Environmental Services. Three types of mine waters were used in this evaluation. Initially a SO_4-dominated lime-treated acid mine drainage water (AMD) was used for the evaluations in the vegetative growth stage (Mine A, Kromdraai) (Table 3.1). The particular AMD water was, however, not really a 'problem' water in relation to plant growth. Subsequently seedling growth evaluations were mainly conducted with a more concentrated neutral sulphate water from another location (Mine C, Kleinkopje) (Table 3.1).

A NaCl-dominated water was also included for comparison (Mine B, New Denmark) (Table 3.1).

Mine A water was produced at the Kromdraai coal mine near Witbank by the neutralization of acid mine drainage water with bulk hydrated lime ($Ca(OH)_2$). The water was collected from the irrigation pipe line used for a concomitant field trial (Jovanovic, et al., 1998). The SO_4 and Ca content of this water varied from 998 to 1609, and from 257 to 646 mg L^{-1} respectively (Table 3.1). The Mg content was low, averaging 20,7 mg L^{-1} from 1994 to 1996. Dissolved metals such as Fe and Mn were mostly precipitated by the lime and allowed to settle in sedimentation basins, decreasing the possibility of toxic amounts of these metals in this type of water (see APPENDIX B for an example of trace element analyses). The Mine A water was used to determine its effect on the vegetative growth of crops and for the comparison of the seedling growth of the maize cultivars.

Mine B was a 'worst case' $Na/Cl/SO_4$ NaCl-dominated water of this area with a pH (H_2O) of approximately 8.00. The ratio of $Na:Cl:SO_4$ varied considerably, especially that of Cl to SO_4. This water, however, contains 2 mg L^{-1} F which, although not problematic to plant growth, such crops could eventually be detrimental to animal health. The recommended maximum concentration for irrigation water on acid sandy soils is 1 mg L^{-1} F (Dept of Water Affairs and Forestry, 1993).

Mine C water was a neutral high SO_4 water that was pumped via a borehole directly from old underground workings at the Kleinkopje mine. The sulphate content was higher than that of the lime treated Mine A water, approximately 2500 mg L^{-1} SO_4, with the Ca and Mg content 350 and 200 mg L^{-1} respectively. The Mn content of *ca* 3,5 mg L^{-1} was higher than recommended by water quality guidelines, a maximum of 0,20 mg L^{-1} Mn being suggested (Department of Water Affairs and Forestry, 1993), but it was not anticipated that this would cause any plant nutritional problems in the current trials.

Table 3.1 Chemical composition of mine waters[1] and controls used in the salt tolerance evaluations

Mine/ Controls	pH (H_2O)	EC mS m^{-1}	Σ anions mmol$_c$ L^{-1}	NH_4	NO_3	P	K	Ca	Mg	SO_4	Na	Cl
				mg L^{-1}							mmol L^{-1}	
Control 1	5,6	96	9.93	30	310	10	78	66	28	221	0,7	
A 2/94	6,5	274	38.85	30	310	10	78	646	16	1609		
A 5/94	7,0	274	26.12	30	310	10	78	400	35	998	0,3	
B 3/94	7,8	407	39.26	30	310	10	78	32	30	885	33,5	(15,5)[2]
B 4/94	8,5	407	39.23	30	310	10	70	41	40	802	30,4	(17,2)[2]
Control 2	5,2	92	10.15	31	316	10	78	67	28	227	0,7	
A 7/94[4]	6,5	278	36.38	31	316	10	81	257	40	1371	0,3	2,4
B 7/94	8,4	(405)	45.20	31	316	10	86	41	14	575	40,3	27,8
Control 3	(5,6)	110	8.35	30	207	10	78	67	16	225		
C 10/94	(7,5)	(420)	56.43	30	207	10	80	297	186	2533	2,6	
C 12/94	(7,5)	(370)	52.83	30	207	10	81	419	221	2360	2,3	
B 11/94	8,1	590	62.31	30	207	10	79	110	44	1135	52,3	(35)[2]
B 12/94	(8,1)	(590)	51.07	30	207	10	77	73	21	879	44,8	(29,1)[2]
Control 4	5,6	153	8.97	30	207	10	90	66	30	255	1,1	
C 3/95[5]	7,3	394	50.59	30	207	10	90	425	217	2248	4,6	0,1
B 3/95	7,9	534	44.91	30	207	10	90	67	30	732	39,8	26

Mine A	Lime treated acid mine drainage water
Mine B	NaCl-dominated
Mine C	High sulphate mine water pumped from old underground workings

1. All analyses include the supplemental nutrients; controls are one third strength of a modified Hoagland No 2 (NH_4 + NO_3) solution
2. Calculated
3. Other brackets : estimated
4. Mine A 7/94 Mn 1,84 mg L^{-1}; average values 1994 to 1995: Fe 0,41 Mn 2,85 mg L^{-1}
5. Mine C 3/95 HCO_3 74, Fe 0,44, Mn 3,54, Cu 0,016 & Zn 0,027 mg L^{-1}

A sample of each of the three types of water was taken in October 1996 and analysed by the Institute for Water Quality Studies of the Department of Water Affairs and Forestry, in order to determine the trace metal contents. Be, B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Sr, Zr, Mo, Cd, Ba and Pb contents were determined and were not considered problematic. The results are given in APPENDIX B.

The composition and concentrations of the mine waters varied with time/seasons and especially with the rainfall. The specific water used for each experiment is given, denoting the date on which it was collected from the mine (e.g., Mine A 2/94 collected in February 1994). The chemical composition of the specific waters that were used, together with that of the respective control treatments, is presented in Table 3.1. All values include supplementary nutrients added to approximate that of the controls.

3.2 METHODS

3.2.1 GERMINATION (CHAPTER 6)

Germination percentages were determined by using germination paper rolls (Anchor germination paper and cellulose wadding from MULTASAAD, Kuilsrivier, Cape Town) similar to the method of Covell, Ellis, Roberts and Summerfield (1986). The rolls were prepared by using three paper sheets (28 x 30cm) with absorbent cellulose wadding between two and a third to cover the seeds.

The rolls were first soaked in the respective treatment waters and then wrung by hand until dry enough not to make a shiny liquid film when pressed with a finger. Forty healthy seeds were chosen at random and placed uniformly; the rolls were sealed inside a plastic bag with an elastic band and placed in an upright position in plastic buckets in a growth chamber in total darkness and at a constant temperature of 20°C for both the subtropical and temperate crops.

The rolls were opened on the fourth day to use some of the germinated seedlings for the cultivar comparisons (3.2.2.1). The rolls were returned to the growth chambers and the final number that had not germinated was counted on the twelfth day. Seeds were considered germinated if at least a healthy radicle had formed. In a few cases growth ceased when the radicle was 1 to 2 cm long; these were included in the number not germinated. There were three replicates for each treatment with 40 seeds in each roll.

The treatments were: **Control** deionized water

Mine A a lime treated AMD water (for maize cultivars), or **Mine C**, an untreated neutral high sulphate water with high Ca and Mg

Mine B a NaCl-dominated water with moderate sulphate content.

No supplementary nutrients were added to these treatments.

The specific water used for each crop is given with the respective results, the chemical composition of which is given in Table 3.1.

The germination percentage for each treatment was calculated as a percentage of the total number of seeds ‘planted’, and the relative germination percentage on each treatment as a percentage of the control.

3.2.2 SEEDLING GROWTH

3.2.2.1 Glasshouse studies - growth response and comparison of cultivars in the seedling growth stage (Chapter 4)

The aim of this study was twofold:

- to determine the relative growth of the individual crop cultivars with actual mine waters in relation to a Hoagland control in the seedling growth stage, and

- to compare the relative salt tolerances of cultivars of the selected crops, in the seedling growth stage.

The above was accomplished by a water culture experiment in a glasshouse: germinated seedlings were taken from the paper rolls of the germination trial on the fourth day and 'planted' (secured with foam strips) in seedling trays resting on a 28 L. black plastic container filled with the appropriate treatment solutions. Seedlings damaged in planting were replaced no later than the following morning. The containers were placed on a rotating table. Aeration was given for 3 minutes every 30 minutes through three black plastic pipelets in each container, using an air compressor.

There were two replicates with 10 plants of each cultivar per replicate (except in the case of dry beans where 15 plants and cowpea where 8 plants were used). The cultivars were placed throughout the seedling tray with the help of random numbers.

The treatments were: **Control** - 1/3 strength of modified Hoagland No 2 solution with NO_3 and NH_4(2:1)

Mine A or C - a sulphate-dominated mine water - either Mine A (for maize cultivars), or Mine C (for all the other crops evaluated), with additional nutrients to approximate the control

Mine B - a NaCl-dominated water with moderate sulphate content and additional nutrients to approximate the control

The specific mine waters used are given with the results of each crop, the analyses of which are summarised in Table 3.1. All micronutrients were also given at 1/3 strength Hoagland

No 2 solution; for Mine A or C no Mn was added, as sufficient was present. Nutrients were added weekly: that is on the first and eighth day.

Subtropical crops were evaluated during the summer months from October to February1994/1995 and the temperate crops in winter from March to August 1995. In summer the mean temperatures in the glasshouse were 28°C by day and 14°C by night and in winter 28°C and 6°C. Lighting was the natural sunlight; humidity was not measured, but the glasshouse was cooled by fans, causing a suction of air through a layer of wet coke. In winter the temperature was raised by underfloor heating.

The top and root growth were harvested separately 14 days after ‘planting’ (18 days of growth from seeds), at the three to four leaf stage and the number of plants that survived noted; the material was dried at 65°C for 48 hours and the dry mass of the top and root growth was determined (the mass per ten plants was calculated where necessary). Root masses were, however, not always accurate due to entangling and these results are therefore not given.

3.2.2.2 Growth chamber studies (Chapter 5)

A. Sand culture - relative seedling growth on gradients of simulated artificially mixed mine waters

The objective of these trials was to determine the growth responses of a tolerant cultivar of each crop to increasing concentrations of an artificially mixed high sulphate simulated mine water. A trial with simulated NaCl-dominated mine water was included for comparison to the more common type of salinity. It was also endeavoured to determine threshold and slope values for growth responses to these waters (2.5.2). This was, however, not successful for the SO_4-dominated mine water due to the irregularity of the growth curves and because there were too few data points in the linear sections of the growth curves.

To achieve this objective sand culture experiments were conducted in growth chambers. Dry quartz sand - thoroughly washed with tap and deionized water until free of amorphous material - was weighed (280g) into 250 ml polystyrene vessels with enough holes - covered with a piece of shade netting - to allow free drainage of treatment solutions.. The seeds were planted directly in the sand, then wet with 75 ml of the respective treatment solutions, allowed to drain and the mass determined.

The vessels were placed in growth chambers with day/night light periods of 12/12 hours (except for the subtropical annuals with the simulated NaCl water, where a day/night period of 14/10 hours was followed), and day/night temperatures of 25/15°C for the subtropical, and 23/12°C for the temperate annuals. Until emergence the solutions were replenished daily to the original masses with deionized water. The solutions were replaced at emergence and thereafter on every third day with 120 ml treatment solution and replenished on the other days with deionized water at approximately 08:00. This procedure was followed to minimize daily variations in salinity. The daily mass measurements, however, showed that replenishment did not succeed in maintaining the volume that decreased by up to 40 %. Where evapotranspiration could lead to wilting, especially in the second week, solutions were replenished twice daily at 08:00 and 16:00.

The top growth was clipped at sand level 21 days after planting, at the three to four leaf stage, and the stems rinsed four times with deionized water. The top growth was dried for 48 hours at 65°C and the dry masses per vessel determined. The number of plants per vessel varied for the different crops from 3 plants for dry beans to 20 plants for ryegrass. There were four replicates of each treatment. Results are given as dry mass per 10 plants.

The treatments were:

- A simulated Ca/Mg/SO_4 mine water (Kleinkopje, collected in March 1995)(Table 3.2) at
    a. soluble concentrations (treatments 2 to 5 or 6), and
    b. with increasing undissolved gypsum crystals in suspension (treatments 6 or 7 to10).

- Increasing SO_4 concentrations gained with Na_2SO_4 in a simulated mine water (Kleinkopje) saturated with $CaSO_4$ (treatments 11-14) (Table 3.2).

Table 3.2 Chemical composition of simulated gradients of sulphate salinity

Treatment[1]	Sulphate mg L^{-1} Planned	Sulphate mg L^{-1} Supernatant Analysed	EC mS m^{-1}	pH (H_2O)	Σ anions [1] $mmol_c$ L^{-1}	Ca	Mg	K	Na [3]	NH_4^+ [1]	NO_3 [1]	P
						mg L^{-1}						
1. Control	226	255	97	5.3	8.95/9.63	121	114	81	0/48	31/19	205/247	10
2.	1500	1485	280	6.2	34.57/35.25	345	209	81	0/48	31/19	205/247	10
3.	2000	1866	327	6.3	42.51/43.19	507	304	81	0/48	31/19	205/247	10
4.	2150	2057	349	6.4	46.49/47.17	526	309	81	0/48	31/19	205/247	10
5.	2300	2245	368	6.4	50.41/51.76	599	339	81	0/48	31/19	205/247	10
6.	2500	2428	386	6.4	54.21/54.89	603	411	81	0/48	31/19	205/247	10
7.	3000	2640	403	6.4	58.64/59.31	605	443	81	0/48	31/19	205/247	10
8.	4000	2985	453	6.7	65.82/66.50	589	551	81	0/48	31/19	205/247	10
9.	5000	3300	492	6.8	72.39/73.06	597	678	81	0/48	31/19	205/247	10
10.	6000	3867	525	6.8	84.20/84.88	578	821	81	48	31/19	205/247	10
11.	2500	2474	387	6.4	55.18/55.85	540	328	81	97	31/19	205/247	10
12.	3000	2989	466	6.4	65.91/66.58			81	336	31/19	205/247	10
13.	4000	3896	623	6.5	84.80/85.48	507	303	81	814	31/19	205/247	10
14.	5000	4703	780	6.6	101.6/102.29	526	308	81	1292	31/19	205/247	10
Mine C 3/95		2248	394	7.3	50.48	425	217	90	106	30	207	10

1. Less NH_4 was used for the winter crops and more NO_3; the first value is for summer crops and the second for winter crops.
2. Treatments 2 - 10, salinity increased mainly with $CaSO_4$; 11-14 salinity increased with Na_2SO_4 from 2500 mg L^{-1} SO_4.
3. No Na was added to treatments 1 - 9 for maize CRN 4403, sorghum PAN 888, pearl millet common and sunflower SNK43; for all other crops 48 mg L^{-1} Na was added to treatments 1 - 10.

Table 3.3 Chemical composition of simulated gradients of NaCl-dominated mine water

Treatment	pH (H_2O)	EC [1] mS m^{-1}	Σ Anions [1] $mmol_c$ L^{-1}		Na	Cl	SO_4 [2]	Ca [2]	Mg	K	NH_4[1]	NO_3[1]	P
			Summer crops	Winter crops	mmol L^{-1}		mg L^{-1}						
1. Control	6,2	241/168	27.32	28.00	0,02	0	1137	210	30	90	31/19	205/247	10
2.	5,9	308/286	38.01	38.69	10	10	1170	196	30	90	31/19	205/247	10
3.	5,9	396/372	46.88	47.56	20	16	1308	189	30	90	31/19	205/247	10
4.	5,7	581/565	67.60	68.28	40	29	1440	190	30	90	31/19	205/247	10
5.	5,9	678/664	79.24	79.91	50	35	1949	189	30	90	31/19	205/247	10
6.	5,8	770/756	91.74	92.42	60	42	2213	193	30	90	31/19	205/247	10
7.	5,8	958/934	107.55	108.23	80	54	2396	194	30	90	31/19	205/247	10
Mine B 3/95	7,98	534	44.92		40	26	732	67	30	30	30	207	10

1. Less NH_4 was used for the winter crops and more NO_3; the first value is for summer crops and the second for winter crops.
2. $CaSO_4$ (A.R.) was added to all treatment solutions to prevent a Ca effect on salt tolerance.

- A simulated NaCl-dominated mine water (treatments 2 to 7)(Table 3.3). Gypsum (0,861 g L^{-1} A.R.) was added to all NaCl treatments in order to prevent a Ca deficiency effect on salt tolerance (Rengel, 1992b).

The control was one third strength modified Hoagland No 2 solution with NO_3 and NH_4 in a ratio of 2:1 (treatment 1) except where otherwise indicated (Tables 3.2 and 3.3).

The chemical composition ratios of the Kleinkopje mine were used as a basis for the sulphate salinity. The SO_4, Ca and Mg, were increased to attain a SO_4 gradient, while maintaining the Ca to Mg ratio. The SO_4 concentrations ranged from 226 (control) to 6000 'mg L^{-1} ' sulphate ('mg L^{-1} ' in single quotes denote the total SO_4 present including both the soluble and undissolved or precipitated SO_4). Where the gypsum had not completely dissolved, the solutions were shaken and applied as a suspension. All the chemicals used were analytical reagents.

The limited solubility of gypsum posed a problem in acquiring high sulphate concentrations in solution. In order to obtain such solutions the composition of the 2300 mg L^{-1} sulphate treatment (treatment 5, Table 3.2) was kept constant and the sulphate further increased with Na_2SO_4 (treatments 11 to 14, Table 3.2). The EC, pH (H_2O) and the actual concentrations of Ca, Mg and SO_4 in solution were determined by analyses of microfiltered supernatants of these solutions (Table 3.2).

Nutrient analyses of the top growth with the above treatments were conducted on only one crop, namely maize cv. SNK 2340, to explore possible nutrient effects. The methods employed were similar to those described for the top growth in the vegetative growth stage (3.2.3.4).

B. Soil versus sand culture

A follow-up trial, using the same method and treatments described above (3.2.2.2 A), was conducted to compare the response of maize cv. SNK 2340 on sand with that on acid soil. Two different acid soils were included: a reddish brown sandy loam soil with a high clay content that had been allowed to acidify over a period of years from the Hatfield experimental farm in Pretoria with a pH (H_2O) 4.7; and a ‘virgin’ greyish brown loamy sand from the vicinity of the Kleinkopje coal mine with a pH (H_2O) 4.3, which had not been irrigated or mined.

Day/night temperatures were 25 and 15^0C, and the light *ca* 1400 quantum millivolts. The only differences were that replenishment with either deionized water or nutrient solution was done at strictly the same time of the day and only once daily (08:00), and the quartz sand was thoroughly washed with sulphuric acid, tap water and deionized water respectively. In the previous experiment with maize, replenishment was done twice daily in the second week of growth as the evapotranspiration was feared to cause wilting, which did not however occur during this experiment.

The mass of the vessels was measured daily before and after replenishment to determine the daily water loss and the degree of concentration in the root growth medium by evapotranspiration.

3.2.3 VEGETATIVE GROWTH STAGE (CHAPTER 6)

3.2.3.1 Exploratory trial

An exploratory sand culture experiment in 5 L Mitscherlich vegetation vessels was initially conducted with lime treated acid mine drainage water in December to January 1993/1994, in order to determine the minimum of nutrient concentration that could be used so that the increase of salinity would be minimized; also to obtain an idea of which crops could be used advantageously with high sulphate mine waters and to standardise the sand culture method .

Nutrients were thus added every second day at 1/11 strength of a modified Hoagland No 2 solution with NH_4 and NO_3 (i.e., three weeks’ supply divided by 11), or weekly at one third strength (i.e., three weeks’ supply divided by three). Nutrient deficiency symptoms appeared with the lower concentration; the latter, which increased the electrical conductivity of the mine water by approximately 50 mS m^{-1}, was thus used in subsequent trials in order to eliminate a nutrient factor.

Two trials were subsequently conducted to determine the influence of a lime treated acid mine drainage water, on the vegetative growth stage (26 - 52 days), of subtropical and temperate crops respectively. A NaCl-dominated mine water was also included as an indication of the crop cultivars' sensitivity to NaCl salinity; but when comparing the growth responses to the two types of water, the respective osmotic potentials (represented by the sum of anions), should be taken into account.

3.2.3.2 Glasshouse study with sand culture - subtropical crops

A sand culture experiment was conducted with the following subtropical crops: maize cv. SNK 2340, sorghum hybrid PAN 888, pearl millet (babala) cv. SA standard, soybean cv. Ibis, and cowpea cv. Dr Saunders. The results of the experiment with four Bermudagrass cultivars(Coast cross 2-K11, Primavera, Tierra Verde and Sahara) are not given here and can be found in a report to the sponsors of this project (Barnard et al., 1998).

This trial was conducted on a rotating table in a glasshouse from February to March 1994, using 6 kg of washed quartz sand in 5L Mitscherlich vegetation vessels.. The seeds were germinated in the quartz sand in the vessels with half strength modified Hoagland No 2 (NO_3 / NH_4, 2/1). After thinning to three plants per pot at the three leaf stage, the seedlings were allowed to grow in the same nutrient solution for a further two weeks before the commencement of the comparative study. Prior to full salinisation, which was reached on day 26 and continued to day 52 after planting, the concentration of the mine water treatments was gradually increased as follows in order to avoid salinity shock:

Mine A one week at half strength mine water plus ½ strength of a modified Hoagland No 2 nutrient solution with NH_4 and NO_3

Mine B an incremental concentration increase of mine water over a period of four days.

Solutions were replenished and circulated thoroughly twice daily with deionized water; in this way it was endeavoured to keep concentration fluctuations minimised throughout the experiment. The treatment solutions were replaced weekly to maintain salinity and nutrient levels.

The treatments were: **Control** 1/3 strength of modified Hoagland No 2 solution with NO_3 and NH_4 (2/1)

Mine A Lime treated AMD water from Kromdraai mine near Witbank, with added nutrients to approximate the concentrations of the control (A 2/94 - Table 3.1)

Mine B A NaCl-dominated mine water from New Denmark mine near Standerton, with added nutrients to approximate the concentrations of the control (B 3/94 - Table 3.1)

There were four replicates of each treatment.

The mean temperatures in the glasshouse were 28°C by day and 14°C by night. Lighting was the natural sunlight; humidity was not measured, but the glasshouse was cooled by fans causing a suction of air through a layer of wet coke.

Plants were harvested after 26 days of treatment, on day 52 after planting. The total fresh mass was determined directly after clipping at 'ground' level and the stems rinsed three times in deionized water. Leaf areas were then determined using the LI model 3100 leaf area meter (Li-cor. inc., Lincoln, Nebraska). The dry mass of the top and root components was determined after oven drying at 65°C for 48 hours. The total top growth of the separate replicates was thereafter milled and used for nutrient analyses (3.2.3.4).

The ratios of top growth to roots, and leaves to stems were calculated using the respective dry masses. The water content in the fresh material was calculated from the difference between the fresh and dry mass, and the *succulence* defined as 'mg water per cm^2 leaf area'. The relative growth of both leaf and total top growth with the respective mine waters was calculated as a percentage of the growth with the one-third strength Hoagland control.

3.2.3.3 Glasshouse study with water culture - temperate crops

In May to June 1994, a second trial was conducted, using water culture to evaluate the tolerance of rye cv. SSR 1, oats cv. Overberg, Triticale cv Cloc 1, wheat cv Inia, ryegrass cv. Midmar and lucerne cv. PAN 4860. The perennial forage crops, tall fescue grass (*Festuca elatior* L. cv. A.U. Triumph), crown vetch (*Coronilla varia* L. cv. Penngift), cocksfoot (*Dactylis glomerata* L. cv. Hera) and white clover (*Trifolium repens* L. cv. Dusi) were also investigated. The results of the latter are not given here and can be found in a report published by the sponsors of this project (Barnard et al., 1998).

This experiment was also conducted on rotating tables in a glasshouse. Mitscherlich pots (5 L), lined with black plastic bags, with black plastic covers, were used. The solutions were aerated for three minutes every 30 minutes. Seeds were sown in vermiculite and three seedlings were 'planted' (secured with foam plastic strips) in each pot ten days later. Plants were grown out to the four-leaf stage in a half strength modified Hoagland No 2 nutrient solution with *ca*. 2:1 NO_3 to NH_4. Treatments with the mine waters were started 28 days after planting, after a gradual increase of salinity similar to the previous sand culture experiment. The water level was topped up with deionized water twice daily to maintain the concentrations. Treatment solutions were replaced weekly.

The treatments were the same as for the previous sand culture trial, only now using mine water collected at later dates (Mine A 5/94; Mine B 4/94). The composition is given in Table 3.1. There were again four replicates of each treatment.

The mean glasshouse temperature was 28°C by day and 6°C by night. The temperature was raised by underfloor heating. Lighting was the natural sunlight; humidity was not measured, but as the glasshouse was aerated by fans, causing a suction of air through a layer of wet coke, it was not foreseen to be a limiting factor.

After four weeks of treatment (28 to 56 days after planting) the top and root growth were harvested separately. Fresh and dry mass, leaf areas, chemical analyses and growth ratios were determined as in the sand culture trial for the subtropical crops.

3.2.3.4 Chemical analyses

Nutrient analyses of the *subtropical* crops were conducted on the total top growth, individually for each of the replicates. The leaves and stems of the *temperate crops* were analysed separately. However in this case the replicates were composited for analyses.

For N, P and S analyses the milled material was wet-ashed with the sulphuric acid/selenium method; for K, Ca, Mg, Fe, Zn, Mn, Cu, Mo and Na the nitric/perchloric acid wet-ash method was used (AGRILASA, 1998).

Nutrient content was determined by the following methods: Total N and P were assessed colorometrically with a Technicon Auto Analyzer II. S was determined by the same analyzer using the $BaCl_2$ method and the total S given as SO_4. K, Ca, Mg. Fe, Zn, Mn, Cu and Na were determined by a Perkin-Elmer 272 Atomic-Absorption Spectrophotometer. Chloride was analysed by potensiometric titration with silver-nitrate. Mo was determined spectrophotometrically only for soybean (All methods are described in AGRILASA, 1998).

3.3 STATISTICAL ANALYSES

The statistical analyses for all experiments were conducted with the computer package Statistical Analysis System (SAS) using the General Linear Models (GLM) procedure which fitted linear models to the data. Asterisks(*) indicate differences from the control as indicated in the respective tables.

3.3.1 GERMINATION, SEEDLING GROWTH AND CULTIVAR COMPARISONS

The influence of the mine waters on the germination percentage (Chapter 6), and on seedling growth of individual cultivars (Chapter 4), was determined by comparing the germination percentage or absolute seedling growth of a cultivar on a mine water to that of the control. These differences are indicated by asterisks as given above.

The significance of differences between the relative germination and relative growth percentages of different cultivars, were determined separately for each mine water with Fisher's Least Significant Difference test (LSD_F); these differences are indicated by alphabetical letters.

3.3.2 SEEDLING GROWTH RESPONSE WITH CONCENTRATION GRADIENTS

The influence of increasing concentrations of simulated mine water on the seedling growth of selected crop cultivars (Chapter 5) was assessed by comparing growth on individual concentrations with the control (Treatment 1). Significant differences from the control are indicated by asterisks as shown above.

The computer programme, SALT (Van Genuchten, 1983) was used to fit the unknown coefficients of threshold and slope to the experimental data (2.5.4.2). Where growth decrease was not linear, problems were experienced to acquire a good fit for some regression curves. This programme was successful mainly for the NaCl salinity. It was also used in an attempt to determine the threshold and slope of the linear sections of the $CaSO_4$ growth regressions, but data points in these parts of the growth curves were not sufficient to use this programme successfully. The Statistical Analysis System was, however, used to determine the significance of regression of parts of the $CaSO_4$, and the Na_2SO_4 growth curves.

3.3.3 VEGETATIVE GROWTH

The influence of the mine waters on the vegetative growth (Chapter 6) was determined statistically for each growth parameter of the subtropical crops by comparing values with the mine waters to those of the controls. Each crop was analysed separately. Significant differences from the controls are again shown by asterisks, as indicated above.

3.4 UNITS AND TERMINOLOGY

The salinity of the soil solution and threshold values are usually presented as the electrolytic conductivity of a saturated soil extract (EC_e) in dS m^{-1}, and slope values as a percentage yield decrease per dS m^{-1}. In this study mS m^{-1} is used (1 dS m^{-1} = 100 mS m^{-1}). The electrolytic conductivity of the soil solution is denoted as EC_{sw}, and of irrigation water as EC_{iw}. The EC_e value is about half that of the soil solution (EC_{sw}) at field capacity (Marschner, 1986; *cf.* Meiri, 1994).In this report the electrolytic conductivity of the growth medium is simply referred to as EC, and is comparable to the electrolytic conductivity of the soil water (EC_{sw}). Values in this report are thus about twice the concentration of what they would be if measured as a saturated soil extract (EC_e).

When comparing these results with other investigations, it must be taken into account that in the literature the threshold value is mostly computed from the EC_e of soil samples that have been taken either from the root zone where maximum water is taken up, or from the spatial and temporal mean of the root growth zone (Maas and Hoffman, 1977; see also Meiri, 1994).

The electrolytic conductivity is mostly used as a parameter to indicate the *osmotic potential* of a growth medium. Because of the formation of strong neutral ion pairs of Ca and Mg with SO_4, which are not measured by the electrolytic conductivity, the EC is, however, not a suitable parameter for the osmotic potential of these gypsiferous mine waters. Osmotic potential is determined by both the free ions and ion pairs content of such a water. The sum of the cations or anions would thus be a more correct parameter for osmotic potential than the electrolytical conductivity in $CaSO_4$-dominated mine waters (Papadopoulos, 1986). For this reason the sum of anions ($mmol_c$ L^{-1}) of the treatment solutions was used to illustrate graphically the results of these experiments rather than the electrolytical conductivity. It has the further advantage that the seedling growth on the different types of water can then be compared on a more or less equal basis of osmotic potential. It must, however, be emphasized that the values of the sums of anions (and the EC's) used are those of the nutrient solutions applied and not of the in situ situation in the root growth zone.

Meiri (1994) coined a term, "effective root zone salinity", which he defined as "the soil salinity parameter that correlates best with the crop response". This parameter should, inter alia, incorporate the edafic factors that can influence salinity and crop response. In the current experiments the "effective salinity" should ideally incorporate the daily temporal changes of the osmotic potential in the root zone solutions in the vessels. In the growth curve experiments (Chapter 5) the average osmotic potentials of these solutions in the root zone during the two weeks growth period would thus be a more representative parameter of salinity. These changes could, however, not be measured in situ in the present study. Therefore the term "*effective osmotic potential*" or "*effective salinity*" is used in this study for "*the average osmotic potential (or salinity) in the root zone solutions during the whole growth period* ".

Lastly, 'mg L^{-1} ' SO_4 in single quotes denotes the total SO_4 present in a treatment, including both that in solution and the undissolved or precipitated SO_4, while SO_4 in solution is denoted as mg L^{-1} without inverted commas.

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CHAPTER 4

SEEDLING GROWTH AND CULTIVAR DIFFERENCES

In this chapter the results of the glasshouse experiment to determine the tolerance of crops to actual mine waters in the seedling growth stage, are presented. The aims of these water culture experiments were firstly to determine the seedling growth response of crops and cultivars to two types of actual 'worst case' mine waters relative to a one third Hoagland control; and secondly, to determine whether the recommended cultivars differed in their tolerance to these waters. The study focusses on $CaSO_4$-dominated mine water with a more traditional NaCl-dominated mine water included for comparison. The crops have been subdivided into two groups: the subtropical or summer annual crops and the temperate or winter annual crops and lucerne.

4.1 INTRODUCTION

Numerous studies have investigated why and how species and cultivars differ with NaCl-dominated salinity. Only in a few cases, however, have crops been evaluated for growth with water where $CaSO_4$ is the predominant salt, but to the knowledge of the writer no literature on seedling growth response or cultivar differences with $CaSO_4$ water exist.

Shannon (1997) suggests that cultivar differences should be seen more clearly in the most sensitive growth stage or stages of a particular crop. The seedling stage has been identified as the most sensitive for cereals and grass forages (Francois & Maas, 1994) (2.6.1). This stage is, however, crucial to all crops as the salinity of the top layer of the soil is subject to rapid concentration changes due to evaporation from the soil surface.

It has been concluded that the main adverse mechanism by which salinity retards the growth of *seedlings* is that of a decreased osmotic potential (Munns, Schachtman & Condon, 1995; Neumann, 1997). Munns (1993) suggested that cultivars reacted similarly to salinity in the seedling stage and that selections for salt tolerance based on this growth stage would be inappropriate. By reviewing work done on seedling varietal differences, Neumann (1997) agrees that early growth inhibition has often been due to the decreased osmotic potential (presumably of NaCl-dominated conditions) and not to toxic or osmotic effects of salt accumulation in the plant. However, he disagrees with the conclusion that there is a lack of genotypic diversity in the early growth response (10 days) to osmotic stress and presents clear evidence for such diversity to salt or poly-ethylene-glycol (PEG) induced osmotic stress. He suggests that genotypic differences during seedling growth could be the result of metabolically regulated responses to osmotic potential. Such mechanisms could include the effect on cell wall plasticity and thus growth (Neumann 1995a, 1997), K-deficiency variations associated with NaCl salinity (Wu, Ding & Zhu, 1996) and the ability to compartmentalise Na (Kingsbury, Epstein & Pearcy, 1984).

In the above arguments the possible influence of nutrient interactions in the seedling stage was, however, not really addressed. The biphasic model of growth response to salinity suggested by Munns (1993) is based on the adverse mechanisms either being decreased osmotic potential in the seedling stage, or of the toxic influences of the accumulation of salts in the plants at later growth stages. The above mentioned metabolic effects are not considered. With a $CaSO_4$ water, additional nutrient effects could be due to the interaction between SO_4 and other anions and/or of Ca with other ions. If Mg is also present in appreciable quantities, detrimental ratios of Mg to Ca may develop by the precipitation of $CaSO_4$ while $MgSO_4$ remains soluble; this may be a nutrient effect that must be considered in irrigation studies with this water, especially in arid areas.

The aim of the experiments reported in this chapter is twofold: to determine firstly, the relative salt tolerance of selected subtropical and temperate crops and cultivars in the seedling growth stage on an actual ‘worst case’ $CaSO_4$-dominated water; and secondly, whether cultivars of the respective crops differ in their tolerance to this water in the seedling growth stage (sowing to Day 18). A NaCl-dominated mine water was also included for comparison to a more traditional type of NaCl salinity.

The method used is described in Chapter 3 (3.2.2.1).

4.2 RESULTS AND DISCUSSION

4.2.1 SUBTROPICAL ANNUAL CROPS

The subtropical annual crops evaluated were maize, sorghum, pearl millet (babala), sunflower, soybean, cowpea and dry bean (*Phaseolus vulgaris* L.).

Cereals and pastures

The seedling growth of several hybrids of **maize** and cultivars of **sorghum** and **pearl millet** were suppressed by the high sulphate mine waters: seedling growth of six of the 18 maize hybrids was significantly decreased by *ca* 30 %; four of the 14 sorghum cultivars by 32-42 %, and the growth of pearl millet cv. SA Standard was greatly decreased in contrast to the high forage cultivar, PAN 911, which grew very well on the sulphate water (Tables 4.1 and 4.2).

There were some significant cultivar differences in all three of these crops: With **maize** the relative growth of the two most tolerant hybrids (CRN 4403 and CRN 3631) was significantly higher than that of the four most sensitive ones (SNK 2151, SNK 2665, PAN 6552 and PAN 6549). This was in contrast to the seedling growth on the NaCl-dominated water where no significant cultivar differences occurred and the growth was decreased to a greater extent (Table 4.1). For **sorghum** only the growth of the most tolerant cultivar (CRN 7686) was significantly higher than the least tolerant (SNK 3860) (Table 4.2). The two **pearl millet** cultivars responded very differently to the high $CaSO_4$ waters; PAN 911 grew very well while the seedling growth of SA Standard was decreased by 68 % (Table 4.2).

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TABLE 4.1 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of maize hybrids

Maize Hybrids	Dry mass of top growth per 10 plants (g)			c.v. %	Relative growth %	
	Control	Mine A	Mine B		Mine A	Mine B
SNK 2042	3.99	3.19	3.08 *	13.9	bc	a
SNK 2888	4.26	3.62	2.76 *	16.3	abc	ab
SNK 2266	4.43	3.45 *	2.81 *	10.3	bc	ab
SNK 2151	3.87	2.59 *	2.14 *	20.8	c	ab
SNK 2665	4.32	3.11 *	2.21 *	13.9	c	ab
PAN 6480	3.09	2.48	2.3	12.9	abc	ab
PAN 6364	4.14	3.76	2.37 *	20.1	abc	ab
PAN 6552	3.23	2.25 *	1.84 *	13.0	c	ab
PAN 6363	3.65	3.03	1.78 *	7.1	abc	b
PAN 6549	3.11	2.21 *	1.77 *	13.2	c	ab
PAN 6479	2.98	2.38	1.91 *	11.2	abc	ab
CRN 3816	2.43	2.17	1.57 *	8.8	abc	ab
CRN 3414	2.00	1.71	1.37	12.1	abc	ab
CRN 3818	2.37	2.21	1.55	13.0	abc	ab
CRN 3631	3.25	3.59	1.93 *	18.6	110 ab	ab
CRN 4403	4.16	4.66	2.35 *	14.7	a	ab
CRN 4523	3.91	3.54	2.07 *	7.3	abc	ab
SNK 2340[1.]	2.30	1.73 *	1.33	13.7	-	-
c.v. %	14.6				18.7	20.6
LSD_F					33	26

* Significant difference from control ($P < 0.05$)

Mine A 7/94 Mine B 7/94

[1.] This hybrid was not included with Mine A water, but was evaluated with the sorghums on more concentrated water: Mine C water (10/94) EC 402 mS m^{-1}; 2533 mg L^{-1} sulphate and Mine B (11/94) EC 590 mS m^{-1}, 52 mmol L^{-1} Na, 35 mmol L^{-1} Cl and 1135 mg L^{-1} sulphate (Table 3.1).

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TABLE 4.2 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of sorghum **and** pearl millet **cultivars**

Cultivars	**Dry mass of top growth/10 plants g**			**c.v. %**	**Relative growth %**	
	Control	**Mine C**	**Mine B**		**Mine C**	**Mine B**
SORGHUM						
SNK 3860	1.11	0.64 *	0.37 *	9.5	58 cd	33 cd
SNK 3939	0.79	0.70	0.37 *	14.3	81 abc	43 abc
SENFOR	0.71	0.57	0.33 *	16.2	82 abc	47 abc
SENTOP	0.97	0.66 *	0.47 *	8.8	68 bcd	48 abc
SNK 3000	0.75	0.59	0.36 *	25.6	83 abc	51 abc
PAN 8494	0.67	0.44 *	0.36 *	14.7	66 bcd	55 ab
PAN 8501	0.83	0.55 *	0.32 *	6.8	66 bcd	38 bcd
PAN 8522	0.58	0.56	0.32 *	13.5	97 abc	54 ab
PAN 8564	0.73	0.69	0.36 *	5.0	95 abc	50 abc
PAN 8591	0.89	0.82	0.47 *	6.5	92 abc	52 abc
NK 283	0.90	0.87	0.50 *	12.5	99 abc	55 ab
PAN 888	0.45	0.47	0.23 *	10.7	104 abc	52 abc
CRN 776W	0.73	0.72	0.34 *	12.0	98 abc	47 abc
CRN 7686	0.61	0.62	0.32 *	19.0	105 ab	48 abc
PEARL MILLET						
PAN 911	0.51	0.61	0.11 *	31.5	120 a	22 d
Common	0.82	0.26 *	0.32 *	23.4	32 d	39 abcd
c.v. %	15.7				26.6	19.8
LSD_F					47	19

* Significant difference from control ($P < 0.05$)

Mine B 11/94.

Mine C 10/94.

The decrease of the osmotic potential by salinity has been shown to be the major growth suppressing mechanism for these three crops (Cramer, 1994, maize; Ashraf & Idrees, 1995, pearl millet; Shannon, 1997, sorghum).

Cramer (1994) concluded that the reduction of growth in **maize** by salinity appears to be caused by a reduced leaf area, which seems to be primarily due to an osmotic potential effect. Specific ion effects apparently play a minor role for most saline conditions, but for soil types or irrigation waters with unusual ion ratios it could be a more important growth inhibitory mechanism. Seedling growth up to 21 days was found to be the most sensitive stage for maize (Maas, Hoffman, Chaba, Poss & Shannon, 1983). The decrease of seedling growth for the affected cultivars may therefore be due to the decreased osmotic potential of the lime-treated acid mine drainage water (EC 278 mS m^{-1}). The same could be true for **sorghum** with the Kleinkopje mine water (EC *ca* 400 mS m^{-1}), as osmotic adaptation has also been found to be responsible for differences in the tolerance of sorghum cultivars (Shannon, 1997).

Cultivar differences in **pearl millet** have been attributed to the ability to synthesize organic osmotica and thus also to osmotic adaptation (Ashraf & Idrees, 1995). However, in SA Standard growth decrease could be partly due to the water culture method of screening, as the relative growth in the sand culture experiment (Chapter 5) with water of similar composition was 84 % as opposed to the 32 % in the water culture.The coefficient of variation for the pearl millet cultivars in the water culture was also unacceptably high (Table 4.2).

This phenomenon, that the water culture screening method was more severe than sand culture screening, has also been found with dry bean (Zaiter & Mahfouz, 1993).

The growth of all the **sunflower** cultivars was also severely decreased in the seedling stage with the water culture (Table 4.3). However, SNK 43 sunflower seedlings grew very well on sand culture with a water of similar composition (Chapter 5) – 106% compared to the 58% in the water culture. This could be due to a possible aeration effect, as it was mostly encountered in typical dryland crops.

TABLE 4.3 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of sunflower **cultivars**

Cultivars	**Mass of top growth/10 plants (g)**			**c.v. %**	**Relative growth %**	
	Control	**Mine C**	**Mine B**		**Mine C**	**Mine B**
SNK 43[1]	4.78	2.77 **	2.38 *	9.5	58 abc	50 ab
SNK 34	3.44	2.13 **	1.86 **	2.4	62 ab	54 ab
SNK 37	2.35	1.38 **	1.26 **	12.7	59 abc	54 ab
PAN 7392	3.36	1.82 **	1.52 **	13.5	54 abc	45 ab
PAN 7411	3.94	1.76 **	1.86 **	5.3	45 abc	47 b
PAN 7369	3.39	2.18 **	2.00 **	9.5	64 a	59 a
CRN 1445	3.32	2.03 **	1.56 **	5.2	61 ab	47 b
CRN 543	2.84	1.32 **	1.29 **	17.2	47 abc	46 b
A 1006	4.18	1.81 **	2.08 **	4.6	43 c	50 ab
c.v. %					14.36	9.70
LSD_F	9.2				17	11

* Tendency to differ from control ($P < 0.1$)

** Significant difference from control ($P < 0.05$)

Mine B 7/94

Mine C 10/94

1. SNK 43 seeds were infected with a fungus.

Legumes

Nine recommended **soybean**, one **cowpea** and four **dry bean** cultivars were screened for their tolerance to the actual mine waters in the seedling growth stage. The results are presented in Table 4.4.

In contrast to the cereals discussed above, the $CaSO_4$-dominated water did not significantly affect the seedling growth of the **soybean** cultivars and there were no significant differences between cultivars. The **dry bean** cultivars grew exceptionally well on the high SO_4 water; the seedling growth of three dry bean cultivars, PAN 127, Mkusi and Nandi, were significantly higher than the control with this water while PAN122 was not significantly affected. The relative seedling growth of the most tolerant cultivar (PAN 127) was significantly higher than that of the most sensitive (PAN 122). The seedling growth of **cowpea**, Dr Saunders, seemed to be sensitive and was significantly suppressed by the SO_4-dominated water in these water culture experiments (relative growth 65 %) (Table 4.3).These results are, however, unreliable as the variation was unacceptably high for cowpea. As in the case of pearl millet and sunflower seedlings, growth of cowpea in the sand culture experiment (Chapter 5) with a similar high SO_4 water was not significantly affected, although it did tend to decrease (Figure 5.2).

Soybean has been classified as a moderately tolerant crop with a relatively high threshold value of EC_e 500 mS m^{-1} ($\approx EC_{sw}$ 1000 mS m^{-1}) for yield decrease (Maas & Hoffman, 1977). Sensitivity of soybean is also mainly related to Cl toxicity in the shoots (Abel & McKenzie, 1964; Parker, Gascho & Gaines, 1983). The tolerance of the soybean may therefore be related to these two attributes. It has been possible to breed tolerant cultivars that prevent or restrict the transport of Cl to the shoots; as the seedling growth was not suppressed by the high NaCl-dominated water (52 mmol L^{-1}), these cultivars, that were partly selected because of good performance under irrigation, have probably been bred for tolerance to NaCl salinity.

Meiri & Poljakoff-Mayber (1970) studied the effect of NaCl salinity on the growth, leaf expansion and transpiration of **dry bean**. Retardation of bean growth was found to be dependent on the rate, the ultimate level and the duration of salinity. Growth is mainly suppressed through a smaller leaf area and number of leaves. The adverse effect was due mainly to a reduction in transpiration. However, under constant salinity, beans showed a slight adaptation to saline conditions. Dry bean could also have a low capacity of discrimination of the K-uptake system in the presence of high Na levels, which could account for their sensitivity to NaCl (Benlloch, Ojeda, Ramos & Rodriquez-Navarro, 1994). It has also been found that bean plants adjusted osmotically to salt stress resulting in increased leaf water content and it was suggested that "two major physiological traits enable plants to tolerate salinity: (a) compensatory growth following adjustment to salinity, and (b) the ability to increase both leaf area ratio (LAR) and net assimilation rate (NAR) to achieve this increased growth" (Wignarajah, 1990).

TABLE 4.4 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of soybean, dry bean **and** cowpea **cultivars**

Cultivar	**Dry mass top growth/10 plants (g)**			**c.v.**	**Relative growth %**	
	Control	**Mine C**	**Mine B**	**%**	**Mine C**	**Mine B**
SOYBEAN						
1. Bakgat	3.01	2.40	*	8.3	abc	cd
2. Ibis	3.00	2.47	2.42	10.7	abc	abcd
3. PAN 494	2.79	2.53	*	14.2	ab	d
4. PAN 577 G	2.98	2.62	*	5.1	ab	bcd
5. Prima	2.61	2.29	*	14.2	ab	d
6. Hutcheson[1]	(2.15)	(1.78)	(1.44)	21.4	ab	bcd
7. A2233[1, 2]	(3.24)	(3.05)	(3.31)	10.6	ab	a
8. A5409	3.90	3.21	*	7.2	abc	d
9. A7119	2.73	2.11	*	13.2	bc	cd
COWPEA						
1. Dr Saunders[4]	2.87	*	2.13	26.5	65	80
DRY BEAN[5]						
1. PAN 122	6.16	5.12	*	13.4	c	c
2. PAN 127	7.26	*	6.54	5.3	a	a
3. Mkusi	6.78	*	*	5.3	ab	ab
4. Nandi	7.22	*	*	7.5	ab	b

*Significant difference from control ($P < 0.05$)

Mine C 10/94 (soybean & cowpea); 3/95 (dry bean)
Mine B 11/94 (soybean & cowpea); 3/95 (dry bean)

1. Germination affected in all treatments by infections. The seedlings planted were very weak.
2. Fewer plants survived, especially in the control, probably due to infection; more plants survived with the salt treatments.
3. Brackets indicate that growth could have been influenced by infection of the seeds and young seedlings.
4. The number of surviving plants , as well as growth of individual plants, varied. This is probably an indication of sensitivity in the seedling stage
5. Dry beans were evaluated with 15 plants per replicate.

This osmotic compensation followed by compensatory growth may partly be an explanation for the increased growth of bean seedlings on the $CaSO_4$ water. Furthermore, if bean sensitivity to NaCl is mainly due to “a low capacity of discrimination of the K-uptake system”

(Benlloch et al., 1994) in competition to Na, this could further explain the tolerance to a $CaSO_4$-dominated water where NaCl was present at very low concentrations. Another contributing factor to the increased growth compared to that on the one-third Hoagland of the control, could be a positive nutrient effect, as legumes have a high S-requirement (Mengel & Kirkby, 1987).

The relatively good growth of three dry bean cultivars on the NaCl-dominated water may be an indication that these cultivars also have genetic characteristics for salt tolerance such as an increased K-uptake efficiency and/or an increased ability for osmotic adaptation.There have been indications of cultivar differences in the salt tolerance of seedling growth of dry bean (Zaiter & Mahfouz, 1993).

There has been some indication that Cl content of shoots and not that of Na, may be related to salt sensitivity of **cowpea** (Keating, 1986; West & Francois, 1982); if this is the case, tolerance of cowpea seedlings for a $CaSO_4$ water, as is seen in the sand culture experiment (Table 5.9), may be similar to that of soybean. The results for the NaCl actual mine water (Table 4.4) coincide better with the seedling growth with the simulated NaCl mine water on sand culture (Table 5.10), probably because the Cl was the main limiting factor.

4.2.2 TEMPERATE ANNUAL CROPS

The temperate annual crops evaluated in the seedling growth stage were wheat, triticale, rye,ryegrass, oats and barley. Seven cultivars of wheat, seven triticale, four rye, four ryegrass and six of oats were evaluated. One barley cultivar was included for comparison.

Wheat, triticale and rye

The high sulphate water did not significantly affect the seedling growth in 6 of the 7 **wheat** cultivars evaluated; SST 822 was the most sensitive with the growth suppressed by 31 % (Table 4.5). With **triticale** only Kiewiet was significantly reduced by 14 % (Table 4.6), and none of the **rye** cultivars was influenced (Table 4.7). The NaCl mine water significantly suppressed the seedling growth of all the wheat cultivars significantly, ranging from a relative growth of 42 % for SST 822 to 61 % for Marico. The same was true for triticale cultivars (59 to 85 %) and for rye (43 to 54 %).

There were few significant *cultivar differences* with the sulphate water. In the case of wheat, only SST 822, the most sensitive, differed significantly from Inia, the most tolerant; similarly in the case of triticale, Rex was significantly higher than Kiewiet. With the NaCl salinity, the wheat cultivar SST 822 was also the most sensitive, although not differing significantly from the others, while triticale Rex was again significantly higher than Kiewiet (Tables 4.5 and 4.6). Rye showed no cultivar differences with both waters (Table 4.7). During the selection of these cultivars, a pre-screening for salinity tolerance had, however, already been conducted in a sense, as the cultivars were selected for the geographical area where winter cereals are mostly irrigated, which usually presupposes the possibility of salinization.

TABLE 4.5 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of wheat **cultivars**

Cultivars	Top growth mass/10 plants (g)			c.v. %	Relative growth %	
	Control	Mine C[1]	Mine B[2]		Mine C	Mine B
1. SST 822	1.56	*	*	23.2	69 b	42 b
2. SST 825	1.63	1.68	*	10.20	103 a	48 ab
3. Palmiet	1.58	1.77	*	12.03	113 a	57 ab
4. Marico	1.27	1.20	*	10.9	95 ab	61 ab
5. Kariega	1.53	1.45	*	8.4	94 ab	55 ab
6. Inia	1.56	1.82	*	15.0	115 a	56 ab
7. Nursecrop	1.46	1.56	*	3.11	107 a	70 a
c.v. %					13.7	20.0
LSD_F	11.5				31	25

TABLE 4.6 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of triticale **cultivars**

Cultivars	Top growth mass/10 plants (g)			c.v. %	Relative growth %	
	Control	Mine C	Mine B		Mine C	Mine B
1. Kiewiet	1.66	*	*	9.9	86 a	59 b
2. SShR1	1.43	1.37	*	6.7	97 ab	64 ab
3. Rex	1.40	1.51	*	8.3	108 a	85 a
4. PAN 299	1.33	1.23	*	4.9	92 ab	67 ab
5. SSKR 626	0.97	0.99	*	14.5	103 ab	62 ab
6. SSKR 628	1.15	1.05	*	6.1	91 ab	61 ab
7. Cloc 1	1.10	1.07	*	3.5	97 ab	66 ab
c.v. %	8.2				9.1	14.72
LSD_F					19	23

* Significant difference from control ($P < 0.05$)

Mine C 3/95

Mine B 3/95

TABLE 4.7 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of rye **cultivars**

Cultivars	Top growth mass/10 plants (g)			c.v. %	Relative growth %	
	Control	Mine C	Mine B		Mine C	Mine B
RYE						
1. SSR 727	0.82	0.75	*	4.9	91 a	42 b
2. SSR 729	0.70	0.68	*	14.1	98 a	54 a
3. SSR 1	0.65	0.65	*	9.8	100 a	42 b
4. Henoch	0.61	0.61	*	19.3	104 a	42 b
c.v.%	13.4				19.1	17.2
LSD_F					40	16

* Significant difference from control ($P < 0.5$)

Mine B 3/95 Mine C 3/95

Growth observations of the wheat seedlings, however, indicated a possible toxic NH_4 effect on the control plants (NO_3:NH_4, 2:1). Especially the first emerging leaf of some cultivars was bronze coloured. Growth on the SO_4 water was healthy and showed no signs of bronzing or chlorosis. The 'apparent' salt tolerance (2.4.1) may therefore be higher due to possibly suppressed growth of the controls. The wheat cultivars were subsequently rescreened with half the NH_4 and an equivalent increase in NO_3-N, with the same mine water. In this case the controls were a healthy green and the top growth dry masses generally higher than with the higher NH_4 (except for SST 822) (Table 4.8). It was, however, very notable that with the lower NH_4, the seedlings on the $CaSO_4$-dominated mine water were generally very chlorotic in contrast to the healthy green seedlings of the previous evaluation.

TABLE 4.8 The influence of the $CaSO_4$-dominated mine water with different ratios of NH_4 and NO_3 **on the seedling top growth of** wheat **cultivars**

Cultivars	**First screening** NO_3:NH_4 = 2:1				**Second screening** NO_3:NH_4 = 4:1			
	Top growth mass /10 plants g		**c.v. %**	**Relative growth %**	**Top growth mass /10 plants g**		**c.v. %**	**Relative growth %**
	Control	**Mine C**			**Control**	**Mine C**		
1. SST 822	1.56	1.05	23.2	69	1.48	1.16	1.0	78
2. SST 825	1.63	1.68	10.2	103	1.94	1.23	10.4	63
3. Palmiet	1.58	1.77	12.0	113	1.81	1.21	3.3	67
4. Marico	1.27	1.20	10.9	95	1.65	1.26	0.9	76
5. Kariega	1.53	1.45	8.4	94	1.57	1.21	2.3	77
6. Inia	1.56	1.82	15.0	115	1.78	1.44	3.2	81
7. Nursecrop	1.46	1.56	3.1	107	1.77	1.59	5.0	90
Means	1.56	1.60	LSD_F 0.163		1.71	1.30	LSD_F 0.154	

It thus seems possible that N- uptake/assimilation was inhibited with the high sulphate water, despite the increased NO_3 concentration, which seems to be remedied by the higher NH_4 in the first screening. The effect of higher NH_4 was confirmed by a follow-up nutrient culture solution trial with wheat, where interactive effects of SO_4 salinity at varying levels of N (NO_3 and NH_4), P and K were determined (Ströhmenger et al., 1999). A similar effect of NH_4-nutrition was also previously found for wheat (Shaviv, Hazan, Neumann & Hagin, 1990).

The only cultivar that did not follow this response was SST 822 where the absolute growth of the control was depressed with less NH_4. This cultivar is sensitive to water stress and also responds very well to increasing N applications (P. Van der Merwe, SENSAKO, personal communication, 1996), which may explain the response to the saline waters.

Although N x S interaction has generally been found to be positive or additive (Tandon, 1992), the large difference in SO_4 and NO_3 concentrations (SO_4:NO_3 *ca.* 47: 4 as $mmol_c\ L^{-1}$) may possibly result in a N-deficiency due to competition between these anions. Ammonium could therefore have provided additional N where a ratio of 1:2 was used (*cf.* Ströhmenger, et al., 1999). In practice this could mean that when irrigating wheat with high sulphate water during the seedling growth stage, the inclusion of NH_4 for N-fertilization could be advantageous for most cultivars during establishment. Nitrification would probably cancel such an effect during later growth stages.

Absolute salt tolerance (based on absolute growth in saline conditions) of wheat and triticale was found to be largely dependent on the intrinsic growth rate of cultivars (Rawson, Richards & Munns, 1988). When selecting for salt tolerance this should first be taken into account, together with the physiological tolerance (relative salt tolerance). The main physiological mechanism for tolerance to NaCl salinity for wheat, triticale and rye seems to be the exclusion of mainly Cl, and also of Na (Gorham et al., 1986; Francois et al., 1988; Francois et al., 1989; Maas & Poss, 1989; Shannon, 1997). The influence of the Na ion on nutritional imbalances of the Na/K and Na/Ca ratios and the capacity of cultivars to maintain healthy ratios are major determinants of tolerance and cultivar differences of these crops to salinity (Grattan & Grieve, 1994; Chhipa & Lal, 1995; Ayala, Ashraf & O'Leary, 1997; Shannon, 1997).

Rye may be more tolerant to Cl than wheat and triticale (Francois et al., 1989) and there are indications that rye may be more sensitive to the Na/Ca ratio than wheat (Grattan & Grieve, 1994). Differences for osmoregulation also contribute to the salt tolerance but to a lesser extent (Shannon, 1997).

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These three crops all fall into the moderately tolerant or tolerant category (threshold EC_e 300 - 600 mS m^{-1} or EC_{sw} 600-1000 mS m^{-1}) (Francois & Maas, 1994). This, together with the very low concentrations of Na and Cl in the $CaSO_4$-dominated water, probably explains the general tolerance of these crops to this type of water. The above mentioned mechanisms can also be ample reasons why these crop cultivars were sensitive to the NaCl-dominated water.

The greater sensitivity of these temperate crops to the NaCl-dominated salinity, despite the fact that the osmotic potential of the NaCl-dominated water was *higher* in this instance than that of the $CaSO_4$ water (Table 3.1), suggests that *also in the seedling growth stage* ionic effects are of greater importance than osmotic potential for the sensitivity of these crops and cultivars to salinity (*cf.* Munns, 1993 and Neumann, 1997).

Annual ryegrass

The seedling growth of annual ryegrass was generally not significantly influenced with the $CaSO_4$ water (from 75 % for Midmar to 100 % for Dargle).This was in contrast to the response to the NaCl-dominated water where growth was severely reduced (21 to 52 %) (Table 4.8). In both waters Dargle was the most tolerant and Midmar the most sensitive cultivar (by relative salt tolerance). The absolute tolerance of Midmar was, however, equal to that of Dargle, but the results of Midmar are unreliable due to an unacceptably high variation. There were no significant cultivar differences with the sulphate water, but with the NaCl-dominated water the relative growth of Dargle was significantly higher than the growth of the other three cultivars (Table 4.9).

TABLE 4.9 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of annual ryegrass **cultivars**

Cultivars	**Top growth masses/10 plants (g)**			**c.v. %**	**Relative growth %**	
	Control	**Mine C**	**Mine B**		**Mine C**	**Mine B**
RYEGRASS						
1. Macho	0.55	0.48	*	18.9	88 a	35 bc
2. Dargle	0.24	0.24	*	14.7	100 a	52 a
3. Apollo 64	0.40	0.36	*	9.9	89 a	30 bc
4. Midmar	0.33	0.25	*	33.1	75 a	21 c
c.v.	13.4				19.1	17.2
LSD_F					40	16

* Significant difference from control ($P < 0.5$) Mine C 3/95 Mine B 3/95

Annual ryegrass has been classified as moderately tolerant, the thresholds of which are possibly higher than the EC of the NaCl mine water used (534 mS m^{-1}). For seedlings, however, the threshold for a simulated NaCl mine water was found to be EC 240 mS m^{-1} (≈EC_e 120 mS m^{-1}) (Barnard et al., 1998), which could account for the sensitivity of seedling growth with the NaCl water. Yet on the sulphate water, which had an EC of 394 mS m^{-1}, that was also higher than the calculated threshold value, the growth was not decreased. This may be an indication that osmotic stress plays a lesser role in the salt tolerance of these annual ryegrass cultivars than Na and Cl ionic effects.

In an investigation of the ionic balance and biomass production in annual ryegrass with salinity it was found that the synthesis of organic acids in annual ryegrass was essential for osmoregulation under saline conditions (Sagi, Dovrat, Kipner & Lips, 1997). Tolerance in ryegrass was associated with osmotic adaptation by an increased plant tissue content of both inorganic ions and organic anions; Sagi et al.(1997) furthermore also found that biomass was correlated with the organic anion concentration in the plants, which in turn was in close relationship with the organic N content. The organic N concentrations were again highly correlated with the total inorganic cations in the plants. From this it could be concluded that an increase in cation-uptake could eventually lead to an increase in organic osmoregulation in annual ryegrass.

As osmotic influences are generally seen to be the adverse mechanism restricting growth in seedlings (Neumann, 1997), the high cation content of the sulphate water could be responsible for improved osmoregulation and thus growth of the ryegrass seedlings on this water. In Chapter 5 the influence of increasing gradients of this water (increasing Ca, Mg and SO_4) on seedling growth are reported. It is interesting that with these gradients the ryegrass seedlings showed unusual growth increases (up to 170%!) which could possibly confirm the inorganic cation relationship to osmoregulation. This is a metabolic salt tolerance mechanism related to the external osmotic potential, which manifested in the seedling growth stage and therefore supports the suggestions that cultivar differences may exist in the seedling growth stage due to differences in osmoregulation (Neumann, 1997). In Chapter 5 two ryegrass cultivars were tested with increasing concentrations of $CaSO_4$-dominated water. In both cases the seedling growth was increased but not to the same extent, showing possible cultivar differences at this growth stage.

Furthermore a restriction of Na transport from the roots in ryegrass (Sagi et al., 1997), could point to a possible detrimental ionic effect of high Na in the shoots. The low concentration of Na in the $CaSO_4$ water could therefore also have contributed to the tolerance with this water.

Oats & Barley

The seedling growth of **oats** was not influenced by either water, nor were there any cultivar differences (Table 4.10). Oats is classified as tolerant (threshold EC_e > 600 mS m^{-1}) and is sensitive in the early vegetative growth stage (Francois & Maas, 1994). It has been found to be sensitive to an Na/Ca imbalance (Maas & Grieve, unpublished data, 1984. In: Grattan & Grieve, 1994). These properties are again self explanatory for the tolerance of oats to the $CaSO_4$ water. In South Africa some oats is cultivated in areas adjacent to the sea, and it is also possible that these cultivars (e.g., Overberg) have already been bred for tolerance to NaCl.

Barley was not influenced by the SO_4 water but the NaCl-dominated water significantly suppressed growth by 21 % (Table 4.9). Salt tolerance of barley is related to osmoregulation (by glycine betaine production), the exclusion of Na and Cl and the ability to regulate Cl transport to the shoot (Shannon, 1997). Barley growth has also been found to be stimulated by

SO_4 (Hassan, Drew, Knudsen & Olsen, 1970). These mechanisms could possibly be responsible for the responses to these waters.

TABLE 4.10 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of oats **and** barley **cultivars**

Cultivars	**Top growth/10 plants (g)**			**c.v. %**	**Relative growth %**	
	Control	**Mine C**	**Mine B**		**Mine C**	**Mine B**
OATS						
1. SSH 421	1.12	1.10	1.13	17.6	98 abc	101 a
2. SSH 423	0.99	1.09	0.99	4.8	110 a	99 a
3. Witteberg	0.85	0.76	0.82	18.8	89 ab	96 ab
4. Perdeberg	1.26	1.26	1.16	9.2	100 abc	92 ab
5. Echidna	1.21	1.19	1.08	16.5	98 abc	89 ab
6. Overberg	1.36	1.45	1.28	16.7	107 ab	94 ab
BARLEY						
1. Stirling	2.32	2.04	*	19.0	88 c	79 ab
c.v. %	17.6				7.8	27.0
LSD_F					17	

*Significant difference from control ($P < 0.05$)

Mine B 3/95

Mine C 3/95

The annual temperate cereals all fall into the moderately salt tolerant (threshold EC_e 300 to 600 mS m^{-1}) or tolerant category (600 to 1000 mS m^{-1}) (Francois & Maas, 1994). This is possibly one reason why the growth of the seedlings of these temperate annuals was generally not affected by the $CaSO_4$ water (EC 394 mS $m^{-1} \approx EC_e$197 mS m^{-1}). The main mechanisms by which NaCl salinity suppresses the general (mature) growth of these crops are, however, associated in some way or another with the influence of Na and/or Cl on nutritional imbalances, and is also affected to a lesser or greater extent by osmotic influences. As the above results show a much greater suppression of seedling growth with NaCl salinity compared to that of the $CaSO_4$-dominated salinity, the low concentrations of Na and Cl in this water could once more account for the tolerance of most of these crops to this water.

In the tribe Triticeae salt tolerance has been found to be poligenic (Zong & Dvořák, 1995). Genotypic differences in salt tolerance are mainly associated with the ability for exclusion, the maintenance of a high K/Na ratio, sensitivity to a high Na/Ca ratio and in some species more than in others, to an ability for osmoregulation. Again the very low Na and Cl contents of this water, together with a high Ca content, probably explains the lack of salt tolerance differences of these crop cultivars to the particular $CaSO_4$ mine water evaluated.

Lucerne

Five cultivars of lucerne were compared on two types of mine water in the seedling growth stage. The seedling growth of all the cultivars was significantly reduced by the $CaSO_4$-dominated mine water, ranging from 55 % for PAN 4581 to 76 % for Diamond (Table 4.11). There was a significant difference in seedling growth between these two cultivars. Growth was severely restricted and chlorotic on the NaCl-dominated water and all cultivars responded in a similar way (21-25%).

Salinity affects *seedling* growth of lucerne through osmotic stress, whereas in the more mature stages tolerance is associated with exclusion of Cl or the level of Cl tolerated (Shannon, 1997), but contrasting data indicated that there is a "positive correlation between Na and Cl accumulation and growth" in lucerne (Ashraf & O'Leary, 1994). Salt tolerance differences in the seedling stage were not connected to differences in root and shoot Na, or shoot Cl but K versus Na selectivity was greater in the seedling stage of a more tolerant line (Ashraf & O'Leary, 1994).

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TABLE 4.11 The influence of $CaSO_4$- and NaCl-dominated mine waters on the seedling growth of lucerne **cultivars**

Cultivars	Top growth/10 plants (g)			c.v. %	Relative growth %	
	Control	Mine C	Mine B		Mine C	Mine B
1. PAN 4860	0.46	*	*	8.0	72 ab	22 a
2. PAN 4581	0.36	*	*	15.4	55 b	21 a
3. Baronet	0.48	*	*	2.3	71 ab	21 a
4. Topaz	0.45	*	*	5.7	71 ab	25 a
5. Diamond	0.52	*	*	2.6	76 a	22 a
c.v. %	6.9				10.9	15.1
LSD_F					19	8

* Significant difference from control ($P < 0.5$)

Mine C 3/95

Mine B 3/95

Lucerne is classified as moderately sensitive to salinity (threshold EC_e 200 mS m^{-1} ≈ EC_{sw} 400 mS m^{-1}) (Maas & Hoffman, 1977). Growth decreases with these two waters (EC 396 mS m^{-1} and 534 mS m^{-1}) are thus not unexpected. Salt tolerance in legumes has been associated with osmoregulators (Tramontana & Jouve, 1997), and in lucerne with an increase in proline content of the roots, where it may serve a protective function (Petrusa & Wincov, 1997). This mechanism may also be operative in the seedling growth stage and could possibly be responsible for cultivar differences.

The growth decrease with the $CaSO_4$ water found in this study could be due to sensitivity to a decreased osmotic potential. The threshold for shoot growth of lucerne has been determined at EC_e 200 mS m^{-1} (≈EC_{sw} 400 mS m^{-1}) (Bernstein, 1974; Maas & Hoffman, 1977) and as the seedling growth is more sensitive (Forsberg, 1953 as quoted in: Noble, Halloran & West, 1984; see also Figure 5.2), it can be deduced that the decrease in seedling growth was probably due to the decreased osmotic potential (EC 394 mS m^{-1}) and the cultivar differences were probably due to osmotic adaptive abilities (Neumann, 1997).

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In the experiment with increasing concentrations of simulated NaCl mine water (Chapter 5), a threshold of EC 170 mS m^{-1} ($\approx EC_e$ 85 mS m^{-1}) was determined for the seedling growth of PAN 4860 (Barnard et al., 1998). A decrease at EC 534 mS m^{-1} is thus inevitable on the NaCl mine water for these lucerne cultivars.

4.3 CONCLUSION

Seedling growth on the actual 'worst case' mine water showed that the *annual subtropical cereal* crops exhibited a greater sensitivity and more cultivar differences with the $CaSO_4$-dominated water than did the legumes. Although the seedling growth decreases of some crop cultivars were quite severe, there remains a relatively wide choice of cultivars that could be used for irrigation with $CaSO_4$ saline water in the sensitive seedling stage. Soybean and dry bean grew exceptionally well on the sulphate water, while cowpea seemed to be sensitive with the water culture in contrast to the response with sand culture where the growth was not severely affected.

Generally the seedling growth of the *annual temperate crops* was more tolerant to the sulphate water than that of the subtropicals, except for one sensitive wheat and one triticale cultivar. Wheat seedling growth was less sensitive to the sulphate water when N was partly supplied as NH_4. Lucerne cultivars were generally sensitive to the $CaSO_4$ mine water. With the NaCl-dominated water the seedling growth of all the temperate crops, with the exception of oats, was severely suppressed.

The presence and concentration of Na, Cl and Mg in $CaSO_4$-dominated waters could however influence the seedling growth, depending on the adverse and tolerance mechanisms operative in specific crops and cultivars. The general sensitivity/tolerance mechanisms that are known for specific crops may be an indication of the tolerance of the respective crops to this type of water. For instance, cultivars of crops sensitive to a decreasing osmotic potential - such as maize, sorghum and pearl millet - would be more sensitive to this water in the seedling growth stage; where tolerance is mainly connected to ionic effects of Na and Cl the crops may probably be more tolerant to this water.

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The seedling growth of both the subtropical and temperate annual cereals was generally suppressed with the *NaCl-dominated* 'worst case', actual mine water. Again the subtropical annuals were influenced to a greater extent than the temperate annuals. The relative seedling growth of the *subtropical legumes* (soybean, dry bean and cowpea) was generally less suppressed than that of the *subtropical cereals* on the NaCl-dominated water. Oats seedling growth was exceptional in that none of the cultivars was sensitive to this particular concentration of NaCl-dominated water. As tolerance of soybean and oats is generally associated with the exclusion of Na and/or Cl, these cultivars may already have genetic properties for this purpose. All lucerne cultivars were very sensitive. Generally the choice of cultivars to be grown under irrigation with the NaCl-dominated mine waters is limited. There are, however, some cultivars that should be tolerant enough to bridge the sensitive seedling growth stage successfully.

There were significant *cultivar differences* in the seedling growth of maize, sorghum, pearl millet and dry bean with the $CaSO_4$-dominated water, whereas very few differences were found with the temperate cereals and lucerne. With the NaCl-dominated water some differences were manifested for wheat, triticale and ryegrass where significant differences mainly occurred between the most sensitive and tolerant cultivars.

In conclusion it can be said that although the seedling growth of some cultivars, especially of the subtropical cereals, was decreased by a saturated $CaSO_4$ water, there remains a wide choice of high yielding cultivars that can be successfully utilised for irrigation with this water.

Cultivar differences, especially among the cereals, should be considered when irrigating with these mine waters, as yield may be influenced by the effect of salinity on the primordial development of spikelets in the seedling growth stage.

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CHAPTER 5

SEEDLING GROWTH WITH INCREASING CONCENTRATIONS OF MINE WATERS

In this chapter seedling growth with increasing concentrations of Ca, Mg and SO_4, and of increasing amounts of gypsum crystals in the growth medium is explored.

Firstly, the influence of increasing concentrations of three types of SO_4-dominated waters on the seedling growth in sand culture, is discussed. The three types of gradients were: where $CaSO_4$ was soluble; where $CaSO_4$ crystals were increasingly present; and where increasing SO_4 concentrations were gained with Na_2SO_4 in a simulated mine water saturated with $CaSO_4$. A gradient of simulated NaCl mine water was included for comparative purposes.

Secondly, the seedling growth on sand is compared to the growth on two acid soils with the same $CaSO_4$-dominated waters as in the above treatments.

The chapter is concluded with a general discussion of the results and a comprehensive conclusion.

5.1 INTRODUCTION

The piecewise linear response function of Maas & Hoffman (1977) for crop growth with increasing salinity has generally served as the guideline for salt tolerance of crops. The composition of the salinity has, however, mostly consisted of highly soluble salts such as NaCl, Na_2SO_4 and $MgSO_4$.

In a saline water with predominantly $CaSO_4$, the precipitation of $CaSO_4$ with increasing concentrations could affect growth responses differently from those instances when salinity is increased with highly soluble salts. It remains to be seen whether the above linear response function is applicable to this type of water. Some differences between these two saline compositions are the following :

- the precipitation of $CaSO_4$ can increase the osmotic potential

- the higher equivalents of SO_4 needed to gain a similar osmotic potential to that of NaCl could possibly bring nutrient imbalances into operation differing from those of NaCl

- Ca, Mg and SO_4 ions form strong ion pairs that are not reflected in the measurement of the electrical conductivity (EC), which may result in underestimating the effects of such waters on growth and yield (Papadopoulos, 1986).

"Effective osmotic potential"

Meiri (1994) stresses the need for salinity parameters that incorporate, inter alia, the temporal changes in root zone salinity during the growth of a crop. The replenishment of water on a daily basis - and sometimes twice daily - in these experiments, did not succeed in keeping the soil solution at a steady-state field capacity. The result was that the solutions in the vessels daily concentrated up to as much as 60 % of the original volumes of solutions used. The concentrations could thus generally be higher than those of the nutrient solutions and the osmotic potentials could be lower. The average osmotic potential in situ over the whole growth period is therefore referred to as the 'effective osmotic potential' (see 3.4). This could be lower or higher than that in the original treatment solutions, depending on the daily withdrawal of water by evapotranspiration and the precipitation of $CaSO_4$ respectively.

This chapter reports on sand culture experiments conducted in a growth chamber which investigated the seedling growth responses to increasing salt concentrations of three types of simulated $CaSO_4$ mine waters (Table 3.2). A Na/Cl/SO_4 simulated NaCl-dominated mine water was also included for comparative purposes (Table 3.3).

As the precipitation of $CaSO_4$, and hence also the osmotic potential of a soil solution, may be influenced by soil crystallizing nuclei (Papadopoulos, 1986), an additional experiment was conducted to compare the seedling growth of one crop - maize cv. SNK 2340 - on quartz sand with the seedling growth on two acid soils using the same method and treatments as for the above sand culture experiments.

5.2 SAND CULTURE EXPERIMENTS

Seedling growth response to increasing concentrations of simulated mine waters was investigated in a sand culture experiment in growth chambers with controlled environmental conditions. The annual crops included seven subtropical and six temperate species; lucerne was the only perennial evaluated in this investigation. Seeds were planted in washed quartz sand in 250 ml polystyrene vessels, allowing free drainage of treatment solutions. The method is discussed in Chapter 3 (3.2.2.2A).

The crop cultivars selected for this investigation were:

Annual subtropical	maize hybrids SNK 2340 and CRN 4403,
	sorghum hybrid PAN 888,
	pearl millet cv. SA Standard
	sunflower cv. PAN 43
	soybean cv. Ibis
	cowpea cv. Dr Saunders
	dry bean cv's PAN 122 and 127
Annual temperate	wheat cv. Inia
	rye cv. SSR 1
	triticale cv.'s Rex and Cloc 1
	oats cv. Overberg
	barley cv. Stirling

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annual ryegrass cv.'s Dargle and Midmar

Perennial temperate lucerne cv. PAN 4860.

The simulated mine waters investigated were:

1. Simulated Ca/Mg/SO_4 mine water (Kleinkopje, mine C 3/95) at
    a. soluble concentrations (treatments 2 to 5 or 6) (Table 3.2), and
    b. with increasing undissolved $CaSO_4$ crystals in suspension (treatments 6 or 7 to 10) (Table 3.2);

2. Increasing SO_4 concentrations gained with Na_2SO_4 in a simulated mine water (mine C 3/95) saturated with $CaSO_4$ (treatments 11 to 14) (Table 3.2);

3. Simulated NaCl-dominated mine water (New Denmark, mine B 3/95) (Table 3.3).

5.2.1 RESULTS AND DISCUSSIONS

The relative seedling growth on gradients of these four groups of simulated mine waters was plotted against the sum of anions as a parameter representing the osmotic potential (Papadopoulos, 1986) (3.4). The results are presented in tabulated form in APPENDIX C. Although responses were not always significant and differed in intensity, the seedling growth curves of the different crops generally followed similar patterns with each of the above mentioned types of water.

5.2.1.1 Simulated $CaSO_4$-dominated mine water (Kleinkopje, mine C 3/95)

With increasing $CaSO_4$ gradients the seedling growth of most of the crops followed a similar, irregular three-piece or four-piece growth curve to a greater or lesser extent. There was an initial gradual decrease, increase or no effect from the control to *ca* 2000 mg L^{-1} SO_4, followed by a

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sharper decrease to 2300 or 2500 mg L^{-1} SO_4 (more or less where saturation with $CaSO_4$ was expected). Then an unexpected tendency to *increased* growth followed where gypsum crystals were increasingly present, from 3000 to 5000 'mg L^{-1}' SO_4 (treatments 7 to 9) ('mg L^{-1} ' includes the undissolved gypsum - see 3.4), and a decrease at 5000 or 6000 'mg L^{-1}' SO_4 (treatment 9 or 10) where the ratio of Mg to Ca in solution was >1 (Table 3.2) (Figures 5.1 to 5.4).

A. Soluble $CaSO_4$ gradients (1500 to 2300 or 2500 mg L^{-1} SO_4)(Treatments 2 to 5 or 6, Table 3.2)

Where $CaSO_4$ was totally soluble in the treatment solutions, the seedling growth generally decreased in a linear manner with increasing concentrations, above a threshold value. Linear regression for decreases below 100 % in this part of the curve was significant for maize SNK 2340, sorghum, pearl millet, dry bean, wheat, rye, triticale cv. Cloc1, barley, oats and lucerne.

Up to *ca* 2000 mg L^{-1} SO_4 the effects were very gradual and generally not significantly different from the control. At 1500 mgl L^{-1} SO_4 maize SNK 2340, sorghum, pearl millet and lucerne already showed decreases in seedling growth, while wheat, triticale and oats were not affected and the seedling growth of maize CRN 4403, dry bean, cowpea, rye, barley, ryegrass Dargle and sunflower was increased at this SO_4 concentration (Figures 5.1 to 5.4). These groupings may be an early indication of the tolerance of these crops.

From 2150 to *ca* 2500 mg L^{-1} SO_4 (EC 300 to 400 mS m^{-1}) there were sharper decreases, with the seedling growth generally reaching a minimum in this concentration range. These decreases were significantly different from the controls for maize SNK 2340, sorghum, pearl millet, lucerne and oats, but not for soybean, cowpea and dry bean, or for the annual temperates, wheat, rye, triticale and barley (Figures 5.1 to 5.3). In contrast to the above mentioned crops the seedling growth of both ryegrass cultivars *increased* significantly in this part of the growth curve (Figure 5.4).

Maize, sorghum, **pearl millet** and **lucerne** responded in a similar way, the decreases becoming significant at *ca* 2300 mg L^{-1} SO_4 with the exception of pearl millet. Maize cv. SNK 2340 responded with a lower absolute and relative seedling growth than that of cv. CRN 4403, and was therefore more sensitive to increasing $CaSO_4$ concentrations (Figures 5.1 and 5.2) (Tables 5.1, maize; 5.3, sorghum; 5.5, pearl millet and 5.27 for lucerne).

The seedling growth of the subtropical legumes **soybean**, **cowpea** and **dry bean** followed a similar pattern to the subtropical cereals, but the growth was influenced to a lesser extent. The minimum growth was again in the vicinity of 2150 to 2500 mg L^{-1} SO_4 (treatments 4 to 6) (EC 349 to 386 mS m^{-1}), but was not significantly less than the seedling growth of the control (Figure 5.2) (Tables 5.7, soybean; 5.9, cowpea and 5.11 for dry bean).

The seedling growth of the annual temperate cereals **wheat**, **rye**, **triticale**, **barley** and **oats** also decreased with increasing concentrations but not as sharply as that of the subtropical annuals (Figures 5.3 and 5.4). These decreases were not significant with the exception of **oats** at 2500 mg L^{-1} SO_4 (EC 349 mS m^{-1}) (Tables 5.15, wheat; 5.17, rye; 5.19, triticale; 5.21, barley and 5.23 for oats)[1] .

In contrast to the above crops, both **ryegrass** cultivars showed an almost inverse response to the $CaSO_4$ gradient compared to the other temperate annuals. There was a dramatic and significant increase of 69 % in seedling growth at 3000 ‘mg L^{-1}’ SO_4 for Dargle and 31 % at 4000 ‘mg L^{-1}’ SO_4 for Midmar (Figure 5.4)(Table 5.25)[1].

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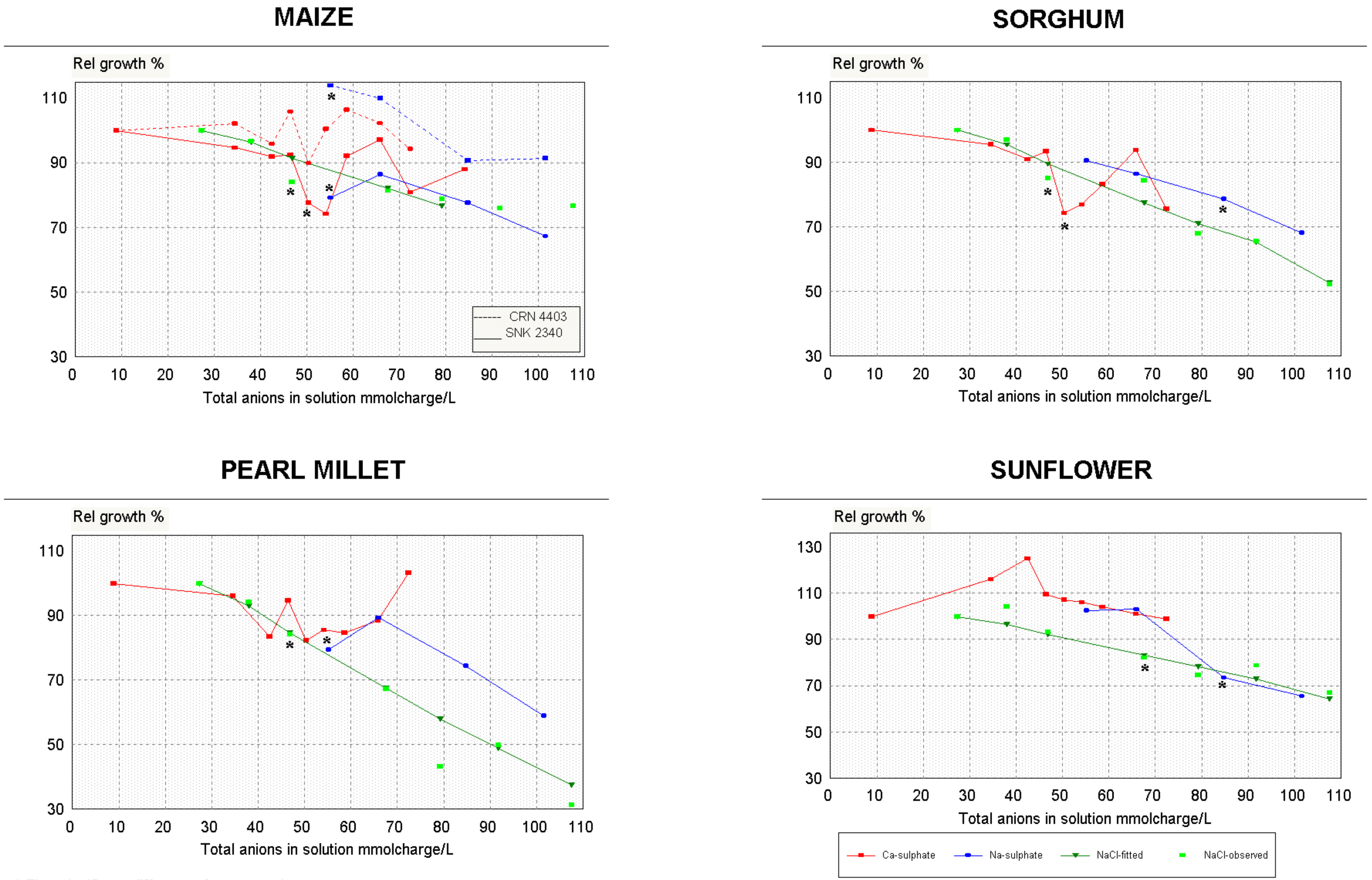
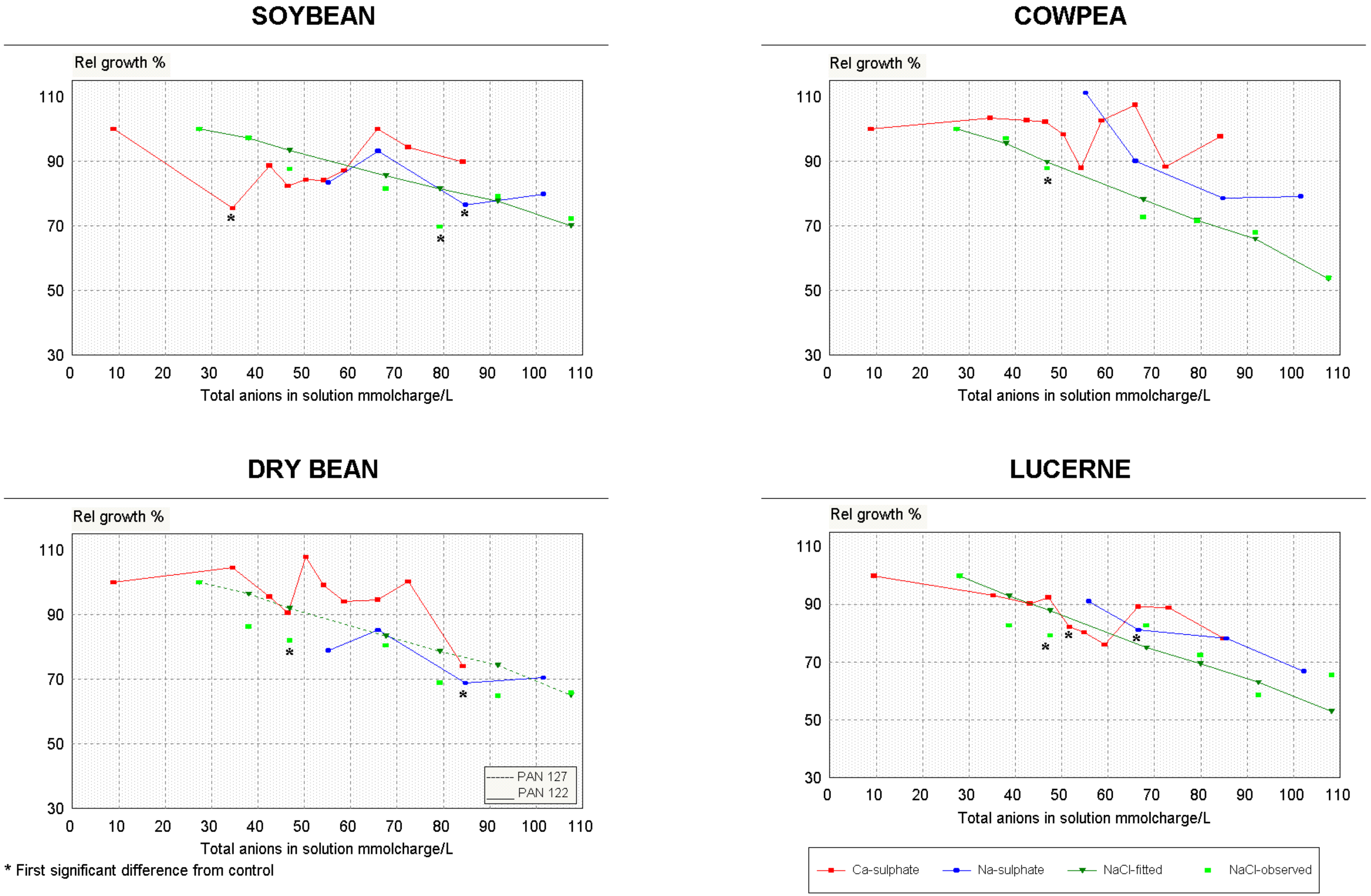
1 A higher ratio of NO_3 to NH_4 (4:1) was used for the temperate crops; if a 2:1 ratio had been used as in the case of the subtropical crops, the absolute tolerance would possibly have been greater (see wheat in the discussion).



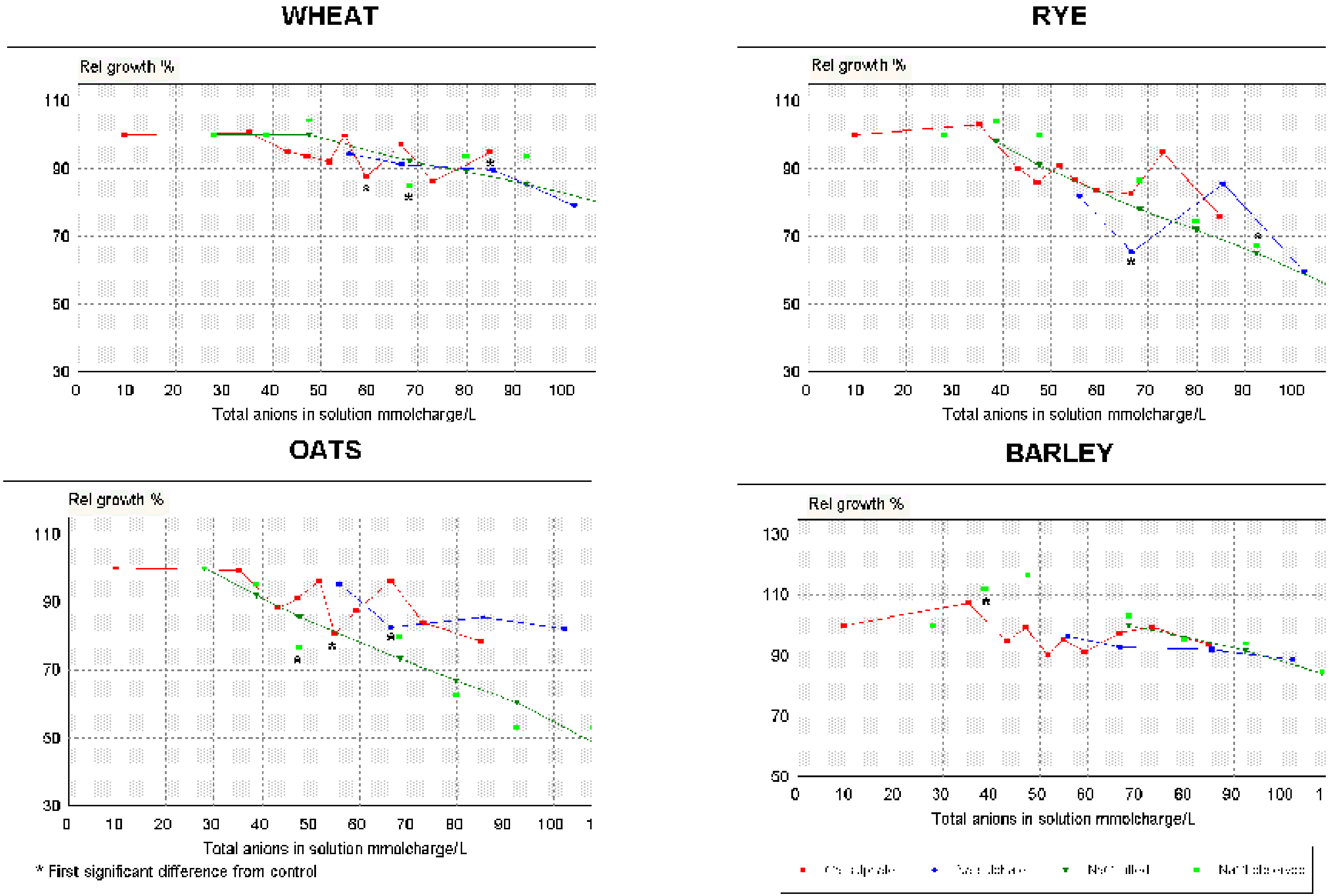
* First significant difference from control

FIGURE 5.1 **The influence of gradients of simulated mine waters on the seedling growth of maize SNK 2340 and CRN 4403, sorghum PAN 888, pearl millet SA Standard and sunflower SNK 43.**



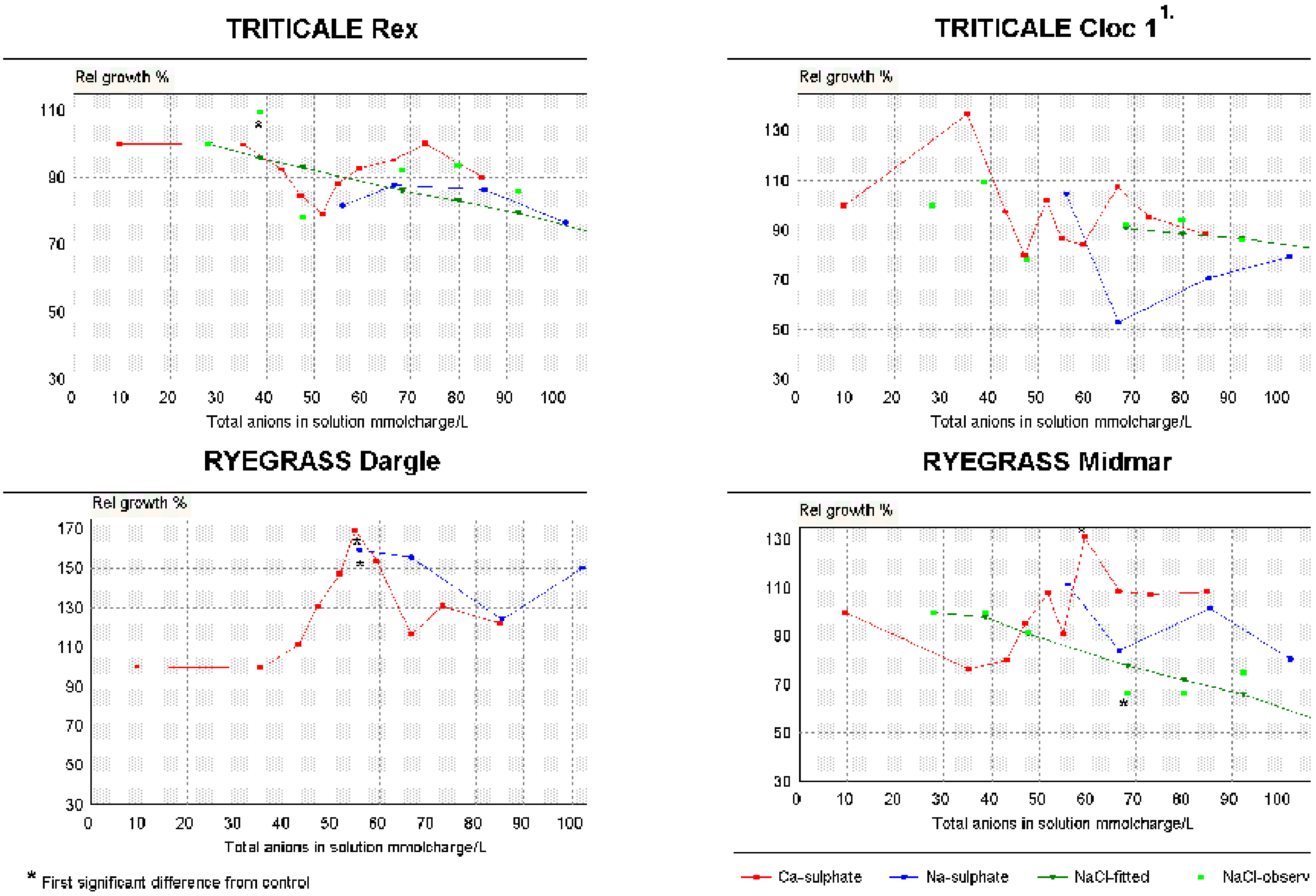
* First significant difference from control

FIGURE 5.2 **The influence of gradients of simulated mine waters on the seedling growth of soybean Ibis, cowpea Dr. Saunders, dry bean PAN 122 and 127 and lucerne PAN 4860.**



* First significant difference from control

FIGURE 5.3 **The influence of gradients of simulated mine waters on the seedling growth of wheat Inia, rye SSR1, oats Overberg and barley Stirling**



* First significant difference from control
1 c.v. was high 36,8%

FIGURE 5.4 The influence of gradients of simulated mine waters on the seedling growth of triticale Rex and Cloc 1 and annual ryegrass Dargle and Midmar

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Sunflower, a dryland crop, responded with increased seedling growth to all these $CaSO_4$ treatments, which could be due to its high tolerance to osmotic potential decreases (Chimenti & Hall, 1993, 1994) and a possible nutrient effect by increased Ca, Mg and S in comparison to the one third strength Hoagland in the control (Figure 5.1) (Table 5.13).

Cultivar differences were evident in the growth curves of the crops where two cultivars were evaluated, that is between the two maize (Figure 5.1), triticale and ryegrass cultivars (Figure 5.4)[1].

Discussion

Where $CaSO_4$ was soluble, with increasing concentrations of Ca, Mg and SO_4, seedling growth generally followed the expected linear decrease above a threshold value as expounded by Maas and Hoffman (1977).

Although the solutions in the vessels were regularly replenished in an effort to maintain field capacity and concentrations, this was not successful. Daily mass decreases of 17 to 40 % were recorded. The actual salinities in the root growth media over the two weeks growth period were therefore probably higher than those of the treatment solutions applied.

It was expected that when the growth medium reached the saturation point of $CaSO_4$, the increase of salinity would be slower (and thus decreases of seedling growth less) in comparison to the stage before saturation. Generally, however, such a second stage was not clearly evident in these curves. This could probably be explained by a too slow rate of precipitation of $CaSO_4$ which could probably be due to the short time intervals and the absence of crystallizing nuclei in the washed quartz sand.

It is suggested that the responses of the different crops were probably connected to the major mechanisms by which NaCl salinity affects seedling or general growth of a specific crop, and to the sensitivity of the particular cultivar used. In **maize** (Cramer, 1994), **sorghum** (Shannon, 1997)

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[1] Statistical comparison was not possible with maize as the two cultivars were not evaluated in the same experiment; and it was not viable with the other two crops as in each case one of the cultivars showed high variation.

and **pearl millet** (Ashraf & Idrees, 1995) it is mainly the decreased osmotic potential that suppresses growth generally, and seedling growth in the case of **lucerne** (Ashraf & O'Leary, 1994). With **soybean** (Abel & McKenzie, 1964; Parker, Gascho & Gaines, 1983), **cowpea** (Keating, 1986), **wheat** (Gorham et al., 1986; Maas & Poss, 1989), **rye** (Francois et al., 1989) and **triticale** (Francois et al., 1988) an ionic effect of Cl is the main adverse mechanism, with decreased osmotic potential exerting a lesser influence (Shannon, 1997) (see Chapter 4).

The sensitivities of **oats** (Maas & Grieve, unpublished data, 1984 in: Grattan & Grieve, 1994) and **barley** (Shannon, 1997) are mainly connected to the presence of Na and/or Cl, and to the degree in which adaptative mechanisms such as osmoregulation are present in a cultivar (see Chapter 4 p.78).

These respective mechanisms together with the low concentration of Na and Cl explain the greater effect of these treatments on the seedling growth of maize, sorghum, pearl millet and lucerne in contrast to the crops where the suppression of growth is by Cl and other ionic effects.

The main mechanism for the salt tolerance of **annual ryegrass** has been found to be an ability for osmoregulation, which is indirectly enhanced by an increase of the cation content of the growth medium (Sagi et al,1997) (see Chapter 4 p.76). The increased seedling growth could thus be explained by the increased Ca and Mg contents of these treatments. Furthermore, as these two cultivars were probably bred for a good yield on acid soils, which usually has a fairly low cation content due to leaching, it may grow even better in the presence of increased cations. In **dry bean** and **sunflower** positive nutrient effects were also possibly operative as the seedling growth increased with some of these Ca, Mg and SO_4 increases compared to the control.

With Na and Cl being absent in these treatments it is suggested that the degree in which seedling growth decreased with increasing concentrations of $CaSO_4$ is probably an indication of its sensitivity to decreasing osmotic potential. This is undergirded by the observation that the crops most sensitive to these simulated waters were those where osmotic potential was the main mechanism by which salinity suppresses growth.

Negative nutrient interactions such as competition of SO_4 with MoO_4 or other anions, and of Ca with other cations, may have been an additional factor in suppressing growth in this part of the growth curve (*cf.* Ströhmenger et al., 1999). Chemical analyses of the seedling top growth of maize cv. SNK 2340 on the SO_4-dominated water, did not, however support possible competition effects: the dry matter contents of N, P, K, Mg and most of the micro-nutrients were higher than those of the control (Table 5.29) (5.2.1.4). The only exception was a decrease of Mn-uptake which may, however, be beneficial with mine waters where the Mn-uptake was increased as in the case of the vegetative growth trials with Kromdraai lime-treated acid mine drainage water (Mine A 2/94) (Table 6.14).

Conclusion

In the first part of the growth curve, where $CaSO_4$ was soluble, it is suggested that the tolerance of seedling growth was mainly connected to

- the mechanism by which salinity generally suppresses growth of specific crops and the absence of Na and especially Cl from the $CaSO_4$ water,

- the sensitivity of a crop or cultivar to decreasing osmotic potential, and

- the presence and nature of adaptive mechanisms of specific crops and cultivars.

B. **Simulated $CaSO_4$ mine water** (Kleinkopje, mine C 3/95) **with increasing $CaSO_4$ crystals in suspension** (Treatments 6 or 7 to 10) (Table 3.2)

From treatments of *ca* 3000 to 5000 'mg L^{-1}' SO_4, where undissolved gypsum crystals were increasingly present, there was a general tendency of unexpected increases of seedling growth, despite an increasing EC of the treatment solutions applied (Figures 5.1 to 5.4).

Seedling growth increased significantly from 2500 to 4000 'mg L^{-1}' SO_4 for **maize** cv. SNK 2340 and **sorghum**, and without statistical significance for **pearl millet** and **maize** cv. CRN 4403 (Figure 5.1) (Tables 5.1, maize; 5.3, sorghum and 5.6 for pearl millet).With **lucerne** the seedling growth increased at 4000 and 5000 'mgL^{-1}' SO_4 ($P< 0.10$) (Figure 5.2) (Table 5.27). Similar increases of **soybean**, **cowpea** and **dry bean** were not significant. These tendencies were more pronounced with soybean than cowpea, with only slight increases with dry bean which was probably overshadowed with positive nutrient effects (Figure 5.2) (Table 5.7, soybean; 5.9, cowpea and 5.11 for dry bean cv. Pan 122).

There was no apparent trend for **wheat** and **rye**, while **triticale**, **barley** and **oats** tended to increase from 2300 or 2500 to 5000 'mg L^{-1}' SO_4, triticale cv. Rex and oats being the most prominent (Figure 5.3) (Table 5.15, wheat; 5.17, rye; 5.19, triticale; 5.21, barley; and 5.23 for oats).

Both **ryegrass** cultivars showed an almost inverse response to the $CaSO_4$ gradient compared to the other temperate annuals: In contrast to the increased growth of the above crops there was a significant *decrease* above 2500 up to 4000 'mg L^{-1}' SO_4 for Dargle (and a tendency for Midmar, $P = 0.09$); the seedling growth levelled off to 120 % for Dargle and 110 % for Midmar at 4000, 5000 and 6000 'mg L^{-1}' SO_4 (Figure 5.4) (Table 5.25).

The seedling growth of **sunflower** decreased very gradually with these treatments but was still higher than that of the control (Figure 5.1) (Table 5.13).

With increasing gypsum crystal content the increased seedling growth was significant for maize, sorghum and lucerne ($P<0.05$ or $P<0.10$), while for pearl millet, soybean, cowpea, triticale, oats and barley it was evident but not significant. Still another group (wheat and rye) showed no such increases, while the seedling growth of ryegrass and sunflower was decreased with increasing crystal content, but was still greater than the control.

For most of the crops evaluated there was a second growth decrease at 5000 and/or 6000 'mg L^{-1}' SO_4 (treatments 9 and/or 10). This was probably due to an unfavourable Mg to Ca ratio in solution of these treatments because of the lower solubility of $CaSO_4$ (Table 3.2); maize (both cultivars), pearl millet, soybean, cowpea and triticale Rex were the least affected (seedling growth *ca* 90 %) (Figures 5.1 to 5.4) (Tables 5.1, maize; 5.6, pearl millet; 5.7, soybean; 5.9, cowpea; and 5.19, triticale). Sorghum, dry bean cv. PAN 122, lucerne, rye and oats were decreased to a greater extent (72 to 79 %) (Figures 5.1 to 5.4) (Tables 5.3, sorghum; 5.11, dry bean ; 5.27, lucerne; 5.17, rye and 5.23 for oats). No further suppression of ryegrass was perceptible (Figures 5.4) (Table 5.25).These decreases at 5000 and/or 6000 'mg L^{-1}' SO_4 might have been aggravated by a pore clogging effect caused by the repeated replacement of the treatment suspensions.

Discussion

It is suggested that the increased seedling growth with the suspension treatments was due to an *increase* of the effective osmotic potential; this was probably caused by rapid daily precipitation of gypsum, which was accelerated by the presence of the undissolved gypsum crystal nuclei, during the daily withdrawal of water from the root growth medium. The increasing crystal contents from treatment 7 to 9 (thus an increase of crystal surface) may also have increased the rate of precipitation, and thus the effective osmotic potential. This could account for increased growth with increasing crystal content.

It is furthermore suggested that the degree in which seedling growth increased is coupled to the sensitivity of specific crops to changes in osmotic potential in the seedling growth stage. This is indicated by the observation that the crops where this response was most perceptible, were once again those where osmotic potential has been found to be the major suppressing mechanism of salinity. This data confirms those of the soluble $CaSO_4$ treatments (5.2.1.1 A).

Conclusion

It is concluded that the responses of seedling growth to these treatments are linked firstly to the influence of the undissolved $CaSO_4$ crystals on the effective osmotic potential via accelerated precipitation of $CaSO_4$, and secondly to the major salt tolerance or sensitivity mechanisms of particular crops and cultivars. It is suggested that the degree of the response is probably an indication of the particular crop and cultivars' sensitivity to changes in the effective osmotic potential.

5.2.1.2 A simulated mine water (Kleinkopje 3/95) saturated with $CaSO_4$ with increasing

concentrations of Na_2SO_4 (Treatments 11 to14) (Table 3.2)

Where increasing SO_4 concentrations were obtained with Na_2SO_4 in simulated mine water solutions saturated with $CaSO_4$, seedling growth generally decreased in a linear manner. This linear regression was significant for maize cv.'s SNK 2340 and CRN 4403, sorghum, pearl millet, cowpea, lucerne and wheat, but not for soybean, dry bean PAN 122 and the other temperate cereals. The seedling growth of the last mentioned crops was therefore not significantly suppressed by osmotic potential and/or Na ionic effects.

Comparison of the seedling growth on $CaSO_4$, Na_2SO_4 and NaCl at similar osmotic potentials may give an indication of possible ionic effects of Na and/or Cl. This comparison of the growth curves on the different types of simulated mine waters was, however, complicated by the influence of evapotranspiration and precipitation of gypsum (especially by the effect of the speed of crystallization) on the effective osmotic potential. As these factors could affect the in situ osmotic potentials in the root zone, a comparison of the growth curves with $CaSO_4$, Na_2SO_4 and NaCl at the osmotic potentials of the applied treatment solutions would therefore not be valid for the actual in situ situation.

However, when comparing these growth curves there are indications that the presence of Na and Cl may have had ionic effects on the seedling growth of some of the crops (Figures 5.1 to 5.4). This may be in contrast to the findings of some authors that the adverse mechanism of salinity on seedling growth is mainly due to osmotic potential effects (Munns et al., 1995; Neumann, 1997). It is suggested that these growth curves may indicate that ionic effects in the seedling growth stage need further investigation.

There is a strong indication of a Na and/or Cl ionic effect in **dry bean** cv. PAN 122 (Figure 5.2). This is similar to findings that Na ionic effects suppressed growth more than decreasing osmotic potential in snapbean cv. Contender (Awada et al., 1995). Tolerance in soybean and dry bean has also been associated with Na accumulation in the roots (Cordovilla , Ocaña, Ligero & Lluch, 1995), which could be a protective mechanism against the effect of Na in the shoots. The fact that the regression with increasing Na_2SO_4 was not significant for soybean and dry bean may indicate the presence of such a mechanism.

A negative Na effect was also indicated in maize cv. SNK 2340 by the chemical analyses of the top growth, where the uptake of Na was accompanied by a decrease of nutrient cations (Table 5.29). In contrast a positive effect of Na, possibly by inorganic osmotic adaptation, is strongly apparent with the seedling growth of the maize cv. CRN 4403 (Figure 5.1).

The absence of high Na concentrations from a $CaSO_4$ water can thus be advantageous to the seedling growth of some crop cultivars. This once again emphasizes the importance of the nature of the suppressing mechanism and the composition of specific waters in the tolerance to a $CaSO_4$-dominated water.

5.2.1.3 NaCl gradients (Mine B 3/95)(Table 3.3)

With increasing Na/Cl/SO_4 concentrations, simulating a NaCl-dominated mine water, seedling growth generally decreased in a linear fashion according to the Maas & Hoffman (1977) theory. The threshold and slope values for these growth curves were computed with the SALT programme (Van Genuchten, 1983) (3.3.2), and are given in Barnard et al. (1998).

5.2.1.4 Chemical analyses

The top growth of maize SNK 2340 with selected treatments of the three SO_4 waters was analysed to investigate whether the growth differences with soluble $CaSO_4$, suspensions and Na_2SO_4 were possibly the result of nutrient effects. No apparent nutrient interactions were evident for either of the $CaSO_4$ treatments. The dry matter contents of the shoots were: N in the optimum to high range; P, K and Mg very high; Ca high and the micronutrients in the optimum range for maize (Loué, 1987)(Table 5.29). These high values were probably due to the plants being in the seedling stage. The total amounts of N, P and K taken up for each treatment were in proportion to the growth. The Ca and Mg uptake was not markedly different from that on the control. The SO_4 uptake was increased on both SO_4 waters and the Mn uptake was decreased (Table 5.29).

With increasing Na_2SO_4 treatments the relative growth was decreased, with a concurrent increase of dry matter Na and a decrease of K, Ca, Mg, Fe, Mn and Cu (Table 5.29). Maize cv. SNK 2340 is therefore not an excluder of Na which seems to have caused some nutrient imbalances that affected the growth.

From these analyses it is concluded that seedling growth decreases and increases in maize cv. SNK 2340 with increasing Ca, Mg and SO_4 were probably not the result of nutrient imbalances caused by the high SO_4 or Ca. It does seem, however, that an increase of Na led to increased uptake that suppressed seedling growth via interactions with K, Ca, Mg, Fe, Mn and Cu. In contrast the seedling growth of the maize cv. CRN 4403 with Na_2SO_4 treatments saturated with $CaSO_4$, was generally higher than that of the $CaSO_4$ treatments at similar osmotic potentials of the treatment solutions (Figure 5.1). In this case Na was either excluded or compartmentalized, or the Na may have contributed to inorganic osmotic adaptation.

TABLE 5.29 Concentration of nutrient elements in the seedling top growth of maize SNK 2340 with a gradient of simulated $CaSO_4$ and Na_2SO_4 mine water (Figure 5.1)

Treatment	SO_4 in solution	Ca in solution	Mg in solution	N	P	K	Ca	Mg	Na	Sulphate	Fe	Mn	Cu	Zn	Total mass	Relative growth
	mg L^{-1}	mg L^{-1}	mg L^{-1}	%							mg kg^{-1}				g/10 plants	%
$CaSO_4$:																
Control 1	255	121	114	3.3	0.5	2.9	0.6	0.4	0	2.11	146	165	9	23	2.05	100
2	1485	345	209	3.45	0.6	3	0.6	0.7	0	3.43	138	77	14	30	1.94	95
6	2428	603	411	3.72	0.6	3.4	0.7	0.7	0	3.88	182	69	14	29	1.59	78
8	2985	589	551	3.57	0.6	3.1	0.6	0.8	0	4.03	155	65	12	29	1.99	97
9	3300	597	678	4.94	0.7	3.8	0.6	0.7	0	3.76	189	63	12	38	1.48	72
Na_2SO_4 :																
12	2989	526	313	3.7	0.7	3.9	0.7	0.6	0.3	3.94	198	62	14	27	1.77	86
14	4703	526	308	3.5	0.7	2.8	0.5	0.4	1.4	3.4	171	50	9	27	1.38	

5.2.2 CONCLUSION FOR SAND CULTURE EXPERIMENTS

It is suggested that the seedling growth responses with the simulated $CaSO_4$ mine waters were mainly related to the effective osmotic potentials of the solutions in the vessels, which was in turn dependent on the rapidity of the precipitation of gypsum during evapotranspiration.

In the treatments where $CaSO_4$ was soluble, seedling growth decreased in a linear manner above a threshold value with increasing concentrations of Ca, Mg and SO_4, which was similar to the growth response function expounded by Maas and Hoffman (1977). Precipitation of gypsum in these treatments was probably retarded by the absence of nuclei for crystallization in the washed quartz sand, and by the short time intervals between applications, which probably resulted in decreases of the effective osmotic potential. The presence of undissolved gypsum crystals, however, probably caused an *increase* of the effective osmotic potential in the vessels by acting as crystallizing nuclei

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and therefore accelerating precipitation during the withdrawal of water from the root growth medium by evapotranspiration.

At high concentrations of the $CaSO_4$ - dominated water the relative seedling growth decreased. This was probably because the Mg to Ca ratio in solution reached values detrimental to growth, caused by the withdrawal of Ca from solution by precipitation.

A comparison of the seedling growth curves with $CaSO_4$ waters of varying compositions, and Na and Cl contents, indicates that - in addition to osmotic potential - other mechanisms, such as Na and/or Cl ionic effects, could also determine the response of seedling growth of some crops and cultivars to these waters. Although the trends seen in these growth curves generally agree with the findings of Munns et al. (1995) and Neumann (1997) that the effect of salinity on *seedling* growth was mainly due to the decreased osmotic potential of salinity, the above ionic effects may need further consideration.

Generally the sand culture experiments indicate that the tolerance of seedling growth to $CaSO_4$ dominated waters was mainly related to

- the sensitivity of a crop or cultivar to decreasing osmotic potential,

- the mechanism by which salinity generally suppresses growth of specific crops and the absence of Na and especially Cl from the $CaSO_4$ water, and

- the presence and nature of adaptive mechanisms of specific crops and cultivars and the influence of $CaSO_4$- dominated water on these mechanisms.

5.3 SOIL versus SAND EXPERIMENT

Soil contains abundant nuclei which could accelerate the crystallization of $CaSO_4$; this in turn would increase the effective osmotic potential of the soil solution during evapotranspiration and probably also the seedling growth. An experiment similar to the above sand cultures was thus conducted with maize SNK 2340, to compare the seedling growth on quartz sand with that on two acid soils.

The method is given in Chapter 3 (3.2.2.2 B).

The acid soils were chosen because the soils in the vicinity of the coal mines, where this water can be utilised for irrigation, are generally acidic. Seedling growth in quartz sand was compared to that in two acid soils with a gradient of simulated $CaSO_4$ - dominated mine water.

5.3.1 RESULTS AND DISCUSSION

The growth curves found with these soils were similar to the responses of most of the crops in the previous sand culture experiments. With increasing concentrations of Ca, Mg and SO_4 the seedling growth with both the soils and the sand followed the now familiar three-piece or four-piece irregular growth curve found in the above sand culture experiments (Figure 5.5).The growth curves on the soils as well as the sand were however ‘flatter’, with less significance between treatments than in the previous sand culture trial (Figures 5.5 vs. 5.1).

The *relative* salt tolerance was less on the soils than on the sand (Figure 5.6) (Table 5.30). This is, however, a typical example of an “apparent salt tolerance” (2.4.1), which is probably caused here by the differences of fertility of the soils and sand. The growth of the three controls differ significantly, which clearly indicates fertility differences. It was thus considered more correct to evaluate the tolerance with the absolute growth curves (Figure 5.5).

There was a gradual decreasing tendency up to 2000 mg L^{-1} SO_4, followed by a sharper decline from 2150 to 2300 or 2500 mg L^{-1} SO_4 (EC 350 to 400 mS m^{-1}) (Figure 5.5). These decreases were highly significant for the reddish-brown soil (pH 4.7), but not for the greyish-brown mine soil and the sand.

With the presence of undissolved $CaSO_4$ crystals the growth again *increased* at 3000 'mg L^{-1}' SO_4 (treatment 7), though not significantly. With a further increase of concentration and gypsum crystal content at 4000 'mg L^{-1}'SO_4 (treatment 8), the seedling growth on the soils tended to decrease, in contrast to growth on the sand (Figure 5.5); these trends at treatments 7 and 8 were, however, not significant. This indicates that added undissolved $CaSO_4$ crystals could have had a greater effect on osmotic potential and growth in sand than in the acid soils. As soil nuclei were present in all the treatments of the soils, it is understandable that additional nuclei in the form of $CaSO_4$ crystals would have a less marked effect on growth than in the acid-washed sand where no other nuclei were present.

In these acid soils crystallization of gypsum may have been inhibited by adsorptive coatings of aluminium phosphates or humic substances on the soil crystal nuclei faces (as quoted in Van Den Ende, 1991); the consequence would be a slower precipitation of $CaSO_4$ that would not increase the effective osmotic potential as much as in sand where these inhibiting soil properties were absent.

The absolute seedling growth on the reddish-brown soil (pH 4.7) was > mine soil (pH 4.3) > sand; differences between the first mentioned soil and sand were highly significant for all treatments, but seedling growth on the mine soil was not significantly greater than that on the sand. The seedling growth on the two soils did not differ significantly. These growth differences could be due to the greater fertility of the two soils compared to the sand, as indicated by better growth on the control treatments.

The weaker growth at 6000 'mg L^{-1} ' (treatment 10) for sand and the mine soil, though not significant, is again probably due to the unfavourable ratio of Mg to Ca in solution (Table 3.2). This was not evident with the reddish-brown soil, probably because of the fertility and higher cation exchange (CEC) properties of this particular soil.

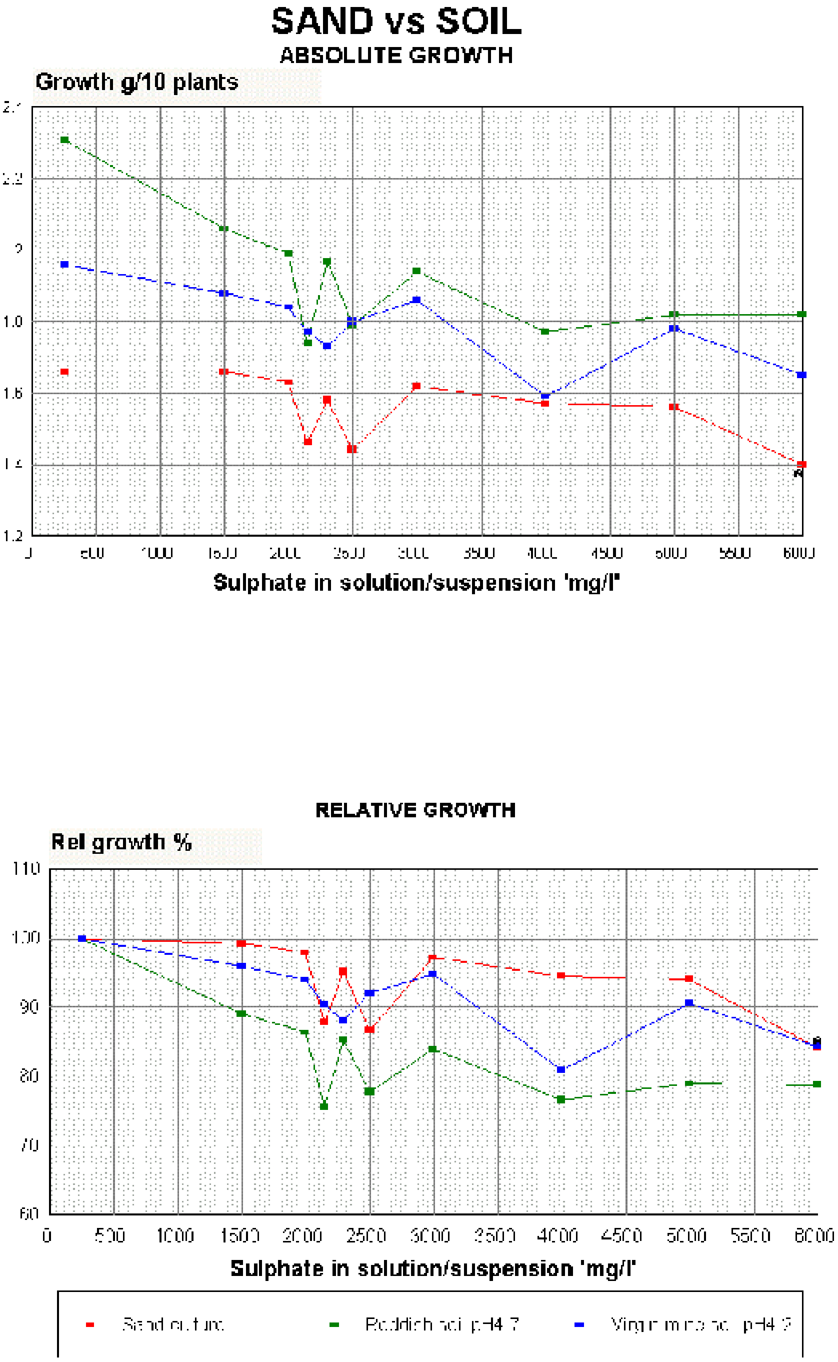

FIGURE 5.5 The influence of a gradient of simulated CaSO$_4$ mine water on the absolute and relative seedling growth of maize SNK 2340, comparing the response on sand with that on two acid soils.

TABLE 5.30 The influence of a gradient of a simulated $CaSO_4$ mine water on the seedling top growth of maize SNK 2340 with quartz sand compared to that with two acid soils (Figure 5.5)

Treatment	SO_4	EC	Sand		Reddish-brown soil (pH 4.7)		Virgin mine soil (pH 4.2)	
	(mg $^{L-1}$)	mS m^{-1}	g/10 plants	Rel. growth %	g/10 plants	Rel. growth %	g/10 plants	Rel. growth %
1	255	97	1,66	100	2,31**	100	1,96**	100
2	1500	280	1,66	100	2,06 **	89**	1,88	96
3	2000	327	1,63	98	1,99 **	86**	1,84	94
4	2150	349	1,46	88	1,74 **	76**	1,77	90
5	2300	368	1,58	95	1,97 **	85**	1,73	88
6	2500	386	1,44	87	1,79 **	78**	1,80	92
7	3000	403	1,62	97	1,94 **	84**	1,86	95
8	4000	453	1,57	95	1,77	77	1,59	81
9	5000	492	1,56	94	1,82 **	79 *	1,78	91
10	6000	525	1,40	84	1,82 **	79**	1,65 *	84

* Tendency of soils to differ from sand ($P < 0,1$)

** Significant difference of soils to sand ($P < 0,05$)

5.3.2 DISCUSSION FOR SOIL EXPERIMENT

The decrease of osmotic potential by salinity has generally been found to be the major growth suppressing mechanism for seedling growth (Neuman, 1997) and for the general growth of maize (Cramer, 1994). The effective osmotic potential is determined firstly by the degree of daily concentration of the root growth medium, and secondly by the precipitation of gypsum.

Withdrawal of water from the root zone is mainly related to the rate of growth and evapotranspiration. As the root growth medium concentrates, crystallization of $CaSO_4$ may take place. The rate of crystallization may be stimulated by many types of nuclei in soils, or inhibited by various adsorptive coatings such as aluminium phosphates in acid soils, $CaCO_3$ and humic substances (as quoted by Van den Ende, 1997).

Soluble $CaSO_4$ gradients (Treatments 2 to 5 or 6) (1500 to 2300 or 2500 mg L^{-1} SO_4) (Table 3.2)

It was expected that the abundant nuclei in soil would accelerate precipitation of gypsum with the soluble treatments in the same way as the undissolved crystals in the suspension treatment. The seedling growth, however, still decreased with these treatments. It is possible that the presence of aluminium phosphates in these acid soils and - to a lesser extent - humic substances in the virgin mine soil may have contributed to a possible diminished precipitation of gypsum which could result in the effective osmotic potential and seedling growth being decreased.

The time interval between replenishing is another important factor in precipitation, especially in situations with no nuclei present. In this trial the time interval was about twice that of the time interval in the previous experiments with maize, where water was replenished twice daily in the second week to prevent wilting. As the growth curves on both the soils, as well as the sand, are ‘flatter’, with less significance between treatments than in the previous sand culture trial, it is probably the longer time interval and not the presence of soil nuclei that ameliorated the seedling growth in this trial, compared to the previous trial (Figure 5.1 vs. Figure 5.5).

It is interesting to compare the relative growth percentages of maize SNK 2340 on the sand in this evaluation (where the time interval was about twice that of the previous experiment) to those of the first sand culture trials (Table 5.30 vs. Table 5.1): with the longer interval the minimum relative growth on sand was 87 % at 2500 mg L^{-1} SO_4 compared to the 78 % with the previous trial with shorter time intervals. It is possible that with the longer interval more gypsum precipitated with a simultaneous greater increase of the effective osmotic potential.

The decreases of seedling growth with the soluble $CaSO_4$ solutions (treatments 2 to 6) were not only due to diminishing osmotic potentials (increasing concentrations) of the treatments but also to

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additional increases in concentration by evapotranspiration, even possibly to short periods of super-saturation (see Van den Ende, 1991). Greater concentration effects can be expected in these treatments, due to a slower precipitation of gypsum; precipitation would probably be slower due to the absence of added gypsum crystals, together with a possible inhibiting influence of aluminium phosphate and humic substance adsorption on the soil nuclei surfaces.

Simulated $CaSO_4$ mine water (Kleinkopje, mine C 3/95) **with increasing crystals in suspension** (Treatments 6 or 7 to10) (Table 3.2)

The increases of growth in treatments with added $CaSO_4$ crystals, can again be attributed to accelerated precipitation in the soil solutions between replenishments; this was probably caused by the presence of the added undissolved crystals (possibly strengthened by the soil nuclei) in the treatments. These increases were not significant in this trial in contrast to significance in the previous trial where the time intervals between replenishments were shorter (Figure5.1) (5.2.2). With shorter intervals the effect of the added gypsum crystals would be expected to be more manifest compared to precipitating crystals in the soluble treatments. With increased time intervals precipitation would take place more readily in both these solutions and there should be a smaller difference between them.

Generally the results may also be related to the *hydraulic conductances* of the growth media which decreased in the following order: sand > virgin mine soil > reddish brown soil. When nutrient solutions were added to the red soil, there was a greater draining time lag which could lead to greater salinisation of the soil, and thus a greater growth suppression, compared to the control (Figure 5.5).

Another contributing factor could be that owing to better growth on the soils, the average *water loss* by evapotranspiration was generally higher on the soils than with the sand: mine soil > red soil>sand (*ca* 33>31>22 ml/vessel/day).Concentration of the soil solutions would thus be more on the soils than on the sand. This could subsequently lead to a greater decrease of the osmotic potential in the root zone with the soils, which may however be counteracted by a speedier

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precipitation of gypsum in a non-acidic soil. It did not seem to have an effect in the acid soils.

To summarise, the *final seedling growth response* would thus depend on the *effective osmotic potential* which in turn would be determined by:

- The balance between the inhibition and stimulation of the precipitation of $CaSO_4$, together with

- the daily concentration by evapotranspiration; and

- the time interval between replenishing.

5.3.3 CONCLUSION FOR SOIL EXPERIMENT

The expected ameliorating influence by soil crystal nuclei on the effective osmotic potential - and thus on seedling growth - was not evident with these acid soils. This is probably due to the inhibiting effect of aluminium phosphates - present in the acid soils - on the rapidity of precipitation, and the concomitant influence on the effective osmotic potential which, in turn, could affect the seedling growth. The seedling growth on the acid soils followed similar growth decreases and increases to that on the quartz sand. It is suggested that the similarity of the growth curves is mostly due to the absence, or presence, of the added undissolved gypsum crystals in the respective treatments.

The *absolute* seedling growth was generally higher with the soils, which was probably due to the higher fertility of the soils compared to that of the one third strength Hoagland nutrition. The *relative* growth decreases on the soils were, however, greater on these acid soils than on the sand. This probably was mainly due to the superior growth of the respective controls. The greater decreases in relative growth on the soils may be a warning that this water can be relatively more harmful to seedling growth on acid soils, than to seedling growth as was found on the sand. This would depend on the chemical and physical properties of individual soils which are illustrated by the different growth responses on the two soils.

5.4 DISCUSSION

Gypsum precipitation and 'effective osmotic potential'

The precipitation of gypsum from the root growth solution results in a decrease of the electrolytical conductivity, thereby increasing the osmotic potential and probably also the 'effective osmotic potential' (3.4).

It is well known that the presence of crystals accelerates crystallization; Van den Ende (1991) also found that the addition of gypsum crystals to soil press extracts decreased the Ca and SO_4 contents as well as the electrolytical conductivity values much *sooner* than in an identical extract where no gypsum crystals had been added . The washed quartz sand used in the current experiments was virtually free of amorphous material and was probably depleted of possible nuclei which could stimulate the crystallization of gypsum. It could therefore be expected that the *rate* of precipitation and the simultaneous increase of osmotic potential would be much slower in treatment solutions devoid of crystallization nuclei (treatments 1 to 5 or 6) than in those where undissolved gypsum crystals were present (treatments 7 to 10). Precipitation with increasing concentration by evapotranspiration would thus be slower, and more dependent on time, in treatment solutions 1 to 5 or 6, whereas in the treatments with undissolved gypsum, crystallisation would take place almost immediately when saturation point was reached. The resultant effective osmotic potential would thus be increased.

The *time interval between replenishing* is another important factor in precipitation. Given enough time, gypsum will eventually precipitate but without crystallizing nuclei it may be retarded. It is probable that - with longer intervals - more gypsum would precipitate, leading to a greater increase of the effective osmotic potential and a higher relative seedling growth. This was illustrated in the case of maize seedling growth on sand with different time intervals in the first sand culture experiments compared to sand in the 'soil experiment'; with the longer interval the minimum relative growth was 87 % (Table 5.30) compared to 74 % (Table 5.1) in the previous trial with shorter time intervals.

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Precipitation of gypsum could also possibly have affected the osmotic potential in the Na_2SO_4 solutions. Although the Na salt is soluble, these solutions were saturated with $CaSO_4$ that could have precipitated when water was withdrawn by evapotranspiration. In the current trials such an effect was, however, improbable as the rate of such precipitation would be slow, due to the absence of nuclei in the washed quartz sand and the short time intervals between replenishing.

The expected response with a gradient of $CaSO_4$-dominated water is that below the solubility product of Ca and SO_4 growth will decrease with increasing salinity. When gypsum starts precipitating, it is expected that salinity - and thus growth - would either not decrease any further, or at a much slower rate. The trend, however, was that although the electrolytical conductivity of the treatment solutions still *increased* (due to increasing $MgSO_4$), instead of a further decrease, the seedling growth of most of the crops in these trials now tended to *increase*. This could be explained by the presence of undissolved gypsum crystals in the suspensions applied. These crystals probably increased the rapidity of crystallization as evapotranspiration daily depleted the water in the vessels, resulting in a higher effective osmotic potential. The increasing tendency of seedling growth with the suspensions (treatments 7 to 8 or 9) corresponds with an increasing content of undissolved gypsum crystals.

The decrease of seedling growth up to 2500 'mg L^{-1} ' SO_4 was probably more severe in the washed quartz sand than it would be in soil in field conditions where abundant nuclei are usually present. The salinity in the sand culture was probably much higher than that of the initial treatment solutions, even possibly to the point of super-saturation for short periods (see Van den Ende, 1991). In contrast the increase of salinity in soil may be ameliorated by accelerated precipitation of gypsum by soil nuclei. It can thus be expected that in a field situation the seedling growth would be higher than in this sand culture.

However, when extrapolating these results to a soil environment, the rapidity of the gypsum precipitation - and thus the effective osmotic potential - may be influenced not only by the abundance of the soil nuclei but also by the mineral and organic composition of the soils involved.

The formation of gypsum crystals in situ in soil can be affected by a number of soil factors. Van den Ende (1991) refers to the findings of various researchers who observed that organic anionic poly-electrolytes can inhibit the formation of gypsum crystals and that humic substances in the soil solution occur as such electrolytes. Adsorption of these substances on crystal and other nuclei surfaces was considered the cause of this inhibition. He also draws on research that other substances, such as polyphosphates (especially in the presence of abundant orthophosphates), and various mineral coatings such as $CaCO_3$ in calcareous, and aluminium phosphates in acid soils, could contribute to inhibiting the precipitation of gypsum. In the current soil experiment this seems to be confirmed for the two acid soils evaluated.

Salt sensitivity/tolerance mechanisms of crops and tolerance to $CaSO_4$-dominated water

The degree in which a specific crop or cultivar showed the above tendencies seemed to be connected to the mechanism by which NaCl-dominated salinity generally influences the growth of a particular crop or cultivar. In both parts of the growth curve it was mostly those crops where osmotic potential effects have been found to be the major adverse mechanism that showed the most evident response to these waters e.g., maize (Cramer, 1994), and sorghum (Shannon,1997). Where Na and/or Cl ionic effects have been found to be the main property of salinity that suppresses growth - as with for example soybean (Abel & McKenzie, 1964; Parker, Gascho & Gaines, 1983), cowpea (Keating, 1986) and wheat (Shannon, 1997) - the seedling growth was suppressed to a lesser extent by increasing concentrations of the $CaSO_4$-dominated waters. This was probably due to the very low Na and Cl content of these waters and/or to the genetic osmotic adaptation properties of the chosen cultivars.

The above observations, together with the very low Na and Cl concentrations in these treatments, suggest that the degree to which a specific crop or cultivar responded to the increasing $CaSO_4$ concentrations in these growth curves may be an indication of its sensitivity to osmotic potential changes; it could also be an indication of how this type of water influences the metabolic processes of osmoregulation as is probably the case with ryegrass (Sagi et al., 1997) (4.2.2.1); and of possibly being nutritionally beneficial to the growth of crops that have a high Ca and S requirement such as legumes.

No conclusions on possible suppression of growth by the high Ca and SO_4 concentrations could be obtained from these experiments, except that nutrient analyses of the top growth of maize did not indicate any such negative interactions. Possible Ca and S interactions are discussed in greater detail in Chapter 6 (6.4.1 and 6.4.2).

5.5 SUMMARY AND CONCLUSIONS

1. *The seedling growth of most of the crops followed a similar irregular three-piece or four-piece growth curve with increasing Ca, Mg and* SO_4 *concentrations in the simulated* $CaSO_4$*-dominated mine waters*. This growth curve included treatments where $CaSO_4$ was soluble, as well as suspensions where gypsum crystals were increasingly present.

Initially there was a gradual decrease, increase or no effect; this was followed by a sharper decrease up to concentrations of Ca and SO_4 where the solubility product was more or less reached in the treatment solutions, then a tendency to *increased* growth where undissolved $CaSO_4$ crystals were increasingly present. Where the Mg to Ca ratio in solution was >1 seedling growth of most of the crops finally decreased again.

In the first and second parts of the growth curve where $CaSO_4$ was soluble, the seedling growth generally followed the expected linear decrease above a threshold value as expounded by Maas and Hoffman (1977). The significance of the decreases differed and may be seen as an indication of the sensitivity or tolerance of specific crops and cultivars. The actual concentrations in the root zones were considerably increased by daily evapotranspiration , but the continued linear decreases up to saturation concentrations of the applied treatment solutions, were an indication that salinity was not noticeably limited by precipitation of gypsum during evapotranspiration in this part of the curve.

The increases of seedling growth in the third part of the growth curve, despite decreasing

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osmotic potential in these treatment solutions, were unexpected. It was anticipated that beyond saturation concentrations salinity would be limited by the precipitation of gypsum. Only small decreases in the osmotic potential and seedling growth were expected.

It is suggested that the differences in seedling growth response between the second and third part of the curve could probably be attributed to the rate of precipitation of gypsum when saturation concentrations were reached through the withdrawal of water by evapotranspiration. In the first and second part of the curve crystallization is probably retarded by the absence of any nuclei in the washed quartz sand (it is possible that supersaturation occurred for a part of the day in some of the treatments where the salts were soluble - seeVan den Ende, 1991), whereas the presence of undissolved gypsum crystals in the suspension treatments used in the third part of the growth curve, probably accelerated precipitation. The result would be a decrease in the effective osmotic potential in the first two parts of the curve and an increase in the third part, with corresponding decreases or increases of the seedling growth respectively.

The *minimum seedling growth* generally occurred with the treatments where concentrations were in the vicinity of saturation but where undissolved crystals were still apparently absent. In a soil environment where nuclei are usually abundantly present, it can therefore be expected that precipitation would be much quicker than in the sand and that the resulting effective osmotic potential and seedling growth would be higher than indicated by the results on the sand culture. Drying of the soil in field conditions should thus not lead to the same rate of concentration and a concomitant lower osmotic potential, than in the sand culture. Seedling growth in soil would probably be similar to that in the treatments where the seedling growth was increased by the presence of gypsum crystals stimulating the precipitation rate.

The precipitation of gypsum could, however, also be retarded by several soil factors. The acceleration of crystallization by soil nuclei can be reduced by coatings of - for example - aluminium phosphates, $CaCO_3$ and humic substances (as quoted by Van den Ende, 1991). If the soil water is kept at field capacity by frequent irrigation, precipitation can also be slower than with longer time intervals and subsequent drying of the soil (Van den Ende, 1991).

Overall the seedling growth of the crops and cultivars evaluated did not, however, decrease beyond 70 %. Although it should be sufficient growth to bridge the sensitive seedling stage to the more tolerant vegetative growth stage (see Chapter 6), primordial spikelet development of especially cereal crops may be impaired, which may eventually affect the yield (Francois & Maas, 1994) (2.6.1).

The differences in seedling growth curves between the two ryegrass, maize and triticale cultivars with these treatments also confirm the conclusion of Chapter 4, that *cultivars could differ* in their response to a $CaSO_4$-dominated water.

2. It is suggested that *the main property of the $CaSO_4$-dominated mine water that caused suppression of seedling growth was the decreased osmotic potential*. It was observed that the above mentioned trends were most evident with crops where the major adverse mechanism is related to osmotic potential effects. It is therefore suggested that the extent to which a crop or cultivar manifested decreases (and increases) of seedling growth with increasing concentrations of simulated $CaSO_4$-dominated mine waters, may therefore be an indication of its sensitivity to osmotic potential changes of the external solution.

The most sensitive crops were maize cv. SNK 2340, sorghum cv. PAN 888, pearl millet cv. SA Standard and lucerne cv. PAN 4860. Although following the same trend, the subtropical legumes soybean cv. Ibis and cowpea cv. Dr Saunders were less sensitive, indicating either a lesser influence of osmotic potential, a greater inbred tolerance to osmotic potential decreases or positive nutritional effects. However, salt tolerance of these last mentioned crops is mainly related to the absence of Cl which has been found to be the main growth inhibiting effect of saline water for these legumes. Generally the annual temperate crops were less sensitive to osmotic potential than the subtropical annuals.

When selecting crops and cultivars for use with this type of water, a knowledge of the adverse and adaptation mechanisms may be a useful indicator of tolerance to a $CaSO_4$-dominated water.

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Crops where an ionic effect is the main growth suppressing mechanism with NaCl-dominated salinity may be relatively tolerant to this type of water, depending on the degree to which osmotic potential plays a part in the growth suppression of such a crop.

It is therefore concluded that *the seedling growth of crops that are mainly sensitive to osmotic potential effects would be more sensitive to* $CaSO_4$*-dominated mine water than those where the adverse mechanisms are mainly related to ionic effects of Na and Cl.*

3. This water may also be *nutritionally beneficial* to crops. It could benefit crops such as legumes and the Cruciferae family that have a high S requirement. The exceptionally good growth of dry bean with this water illustrates the possibility of such an effect. In ryegrass the increased Ca and Mg content probably stimulated the metabolic process of osmoregulation and thus the seedling growth. In leached acid and irrigated soils, the high Ca and Mg could also be valuable to replenish the depleted Ca and Mg content of these soil types.

4. The *chemical composition of a specific* $CaSO_4$*-dominated water must be carefully considered* as the presence of ions such as Mg, Na, Cl and certain trace elements could have distinctive effects on plant growth and animal or human health.

Where Mg is present in appreciable amounts, the precipitation of gypsum over long periods without leaching by rain or a good quality water, could lead to a growth suppressing ratio of Mg to Ca. The general trend of suppressed seedling growth with the highest $Ca/Mg/SO_4$ treatment is a *warning against prolonged irrigation with such a gypsiferous water during periods of drought.*

The presence and concentration of Na and/or Cl in a $CaSO_4$-dominated water can affect tolerance, depending on the adverse and adaptation mechanisms of specific crops and cultivars. In a saturated $CaSO_4$ water *with increasing* Na_2SO_4 *concentrations,* seedling growth generally decreased in a linear manner above 20 mmol L^{-1} Na; and in a NaCl-dominated mine water the seedling growth of most crops also decreased in a linear manner with significance mostly reached at 20 or 40 mmol L^{-1} Na and 16 or 29mmol L^{-1} Cl respectively.

5. It is suggested that when using this type of water for irrigation, soil can be expected to have an ameliorating effect as the presence of crystallizing nuclei may positively affect the osmotic potential by accelerating the precipitation of gypsum. This would, however, depend on the chemical properties of individual soils which, together with the physical properties of individual soils, could affect the overall response of seedling growth to a $CaSO_4$-dominated irrigation water.

The data suggest that the tolerance to a $CaSO_4$-dominated water in the seedling growth stage depends mainly on the sensitivity of a crop or cultivar to external osmotic potential changes and on the chemical composition of specific irrigation waters. The severity of suppression would furthermore depend on the influence of the rapidity of gypsum precipitation on the effective osmotic potential, which in turn can be influenced by the rate of growth, evapotranspiration, soil properties and time.

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CHAPTER 6

TOLERANCE TO GYPSIFEROUS MINE WATERS AT THE GERMINATION, SEEDLING AND VEGETATIVE GROWTH STAGES

In this chapter the influence of actual $CaSO_4$-dominated mine waters on germination, seedling and vegetative growth of selected agronomic and forage crops are considered.

Firstly, the germination percentages on a Kleinkopje $CaSO_4$-dominated mine water are reported. This is followed by investigations into the influence of a lime-treated acid mine drainage water on the vegetative growth of, firstly, maize, sorghum, pearl millet, soybean, cowpea and sunflower in sand culture; and, secondly, of wheat, rye, oats, triticale, annual ryegrass and lucerne in water culture.

The tolerances of the crops in the seedling and vegetative growth stages are subsequently compared and discussed.

Finally the chapter is completed with a conclusion on the tolerance of these crops in the germination, seedling and vegetative growth stages, to $CaSO_4$-dominated mine water.

6.1 INTRODUCTION

It is well known that the sensitivity of crops to the traditional NaCl-dominated salinity can vary during the different ontological growth stages (Bernstein, 1964). Tolerance and sensitivity are, however, also intimately related to the chemical composition of the saline medium (Francois & Maas, 1994; Shannon, 1997). Research with $CaSO_4$-dominated water has been limited and has mainly been conducted on the yield components with no reference to differences in tolerance of the growth stages (Papadopoulos, 1986; MacAdam et al., 1997; Jovanovic et al., 1998).

It is important for irrigation management to determine whether and to what extent the various growth stages were influenced by $CaSO_4$-dominated water. The tolerance of the germination and generally vulnerable vegetative growth stages was thus investigated and compared with the seedling stage.

The purpose of the trials reported on in this chapter was to investigate

- the influence of actual $CaSO_4$ Kleinkopje mine waters on the *germination percentage* of selected agronomic and forage crop cultivars;
- the *vegetative growth* response and *nutrient uptake* with an actual lime-treated acid mine drainage water from the Kromdraai mine complemented with nutrients; a NaCl-dominated type of salinity from the New Denmark mine was included for comparative purposes; and
- possible differences in the tolerance in the *seedling* and *vegetative growth stages*.

6.2 GERMINATION

Germination can be influenced by salinity through a decreased entry of water due to lower osmotic potential, and/or the intake of ions to toxic levels. The percentage of germination is generally not decreased by salinity, but the rate of germination and emergence has been delayed by NaCl-type of waters (Francois & Maas, 1994).

6.2.1 METHOD AND MATERIALS

The germination percentages of the respective crops and cultivars with two types of actual mine waters, compared to deionized water, were determined in paper rolls; the method is described in Chapter 3 (3.2.1). A lime-treated AMD mine-water was initially used for the maize hybrids (Mine A 7/94), but maize SNK 2340 and all the other crops were evaluated with mine water from the Kleinkopje mine with higher Ca and SO_4 concentrations (Mine C) (3.1.2).

Several cultivars of the following crops were screened for their tolerance during the germination growth stage: annual subtropical - maize, sorghum, pearl millet, sunflower, soybean, cowpea, and dry bean; temperate - wheat, rye, triticale, oats, barley, annual ryegrass, and lucerne.

The mine waters used were:

1. a $CaSO_4$-dominated mine water from Kleinkopje, Mine C 10/94, for the subtropical crops, and Mine C 3/95 for the temperate crops (Table 3.1).

2. NaCl-dominated mine water from New Denmark, mine B 11/94, for the subtropical, and Mine B 3/95, for the temperate crops (Table 3.1).

6.2.2 RESULTS AND DISCUSSION

Generally the germination percentages of most of the cultivars of the above crops were not influenced by either the $CaSO_4$- or the NaCl-dominated waters. The results of the crops where germination was not influenced, are not given, but can be found in a previous report (Barnard et al., 1998).

The germination percentages of the 18 recommended **maize** hybrids were not significantly influenced by either the lime-treated acid mine drainage water or the sodic-saline mine water (Barnard et al., 1998). One high forage producing **sorghum** cv. SENTOP tended ($P< 0.1$) to be sensitive during very early seedling growth with the sulphate water, but the seedlings where the radicles had died when less than two centimetres long, are included (Table 6.1). With **pearl millet** the sulphate water significantly reduced the germination percentage of the high forage cultivar PAN 911, by eleven percent (Table 6.1).

TABLE 6.1 The influence of two mine waters on the germination percentage[1] **of** sorghum

and pearl millet **cultivars**

Cultivars	**Germination %**			**c.v. %**	**Relative germination %**	
	Deionised water	**Mine C**	**Mine B**		**Mine C**	**Mine B**
SORGHUM						
1. SNK 3860	93	93	93	2.2	100	100
2. SNK 3939	97	97	94	2.1	100	97
3. SENFOR[1]	80	84	92**	5.8	105	115**
4. SENTOP[1]	97	91*	92	3.1	94*	95
5. SNK 3000	88	91	95	6.6	103	108
6. PAN 8494	88	85	83	4.4	97	94
7. PAN 8501	93	90	91	4.4	97	98
8. PAN 8522	89	91	88	3.5	102	99
9. PAN 8564	99	98	98	1.9	99	99
10. PAN 8591	98	98	98	1.9	100	100
11. NK 283	96	92	94	5.1	96	98
12. PAN 888	99	98	98	1.9	99	99
13. CRN 776W	95	98	92	2.3	103	97
14. CRN 7686[1]	82	80	74**	10.7	98	90**
PEARL MILLET						
PAN 911	95	85**	98	3.8	89**	103
SA Standard	91	92	92	4.6	101	101

c.v. 4.3%

* Tending to significant difference from control ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

Mine C 10/94.

Mine B 11/94.

1. This includes seedlings that died after the radicle grew to 1-2 cm; more apparent with control for SENFOR and NaCl-dominated water for CRN 7686.

The **soybean** cultivars were not significantly influenced by the high sulphate water; the cultivar

A5409 showed a tendency to a germination decrease of six percent. The germination percentage of **cowpea** cv. Dr Saunders was increased significantly by five percent, while **dry bean** germination was not affected significantly (Table 6.2). On the NaCl-dominated water germination of four **soybean** cultivars was suppressed and that of **dry bean** and **cowpea** not affected (Table 6.2).

TABLE 6.2 The influence of two mine waters on the germination percentage of soybean, dry bean **and** cow pea **cultivars**

Cultivar	Germination %			c.v. %	Relative germination %	
	Deionized water	Mine C	Mine B		Mine C	Mine B
SOYBEAN						
1. Bakgat	82	79	75**	7.3	96a	92a
2. Ibis	88	90	92	5.6	104a	106a
3. PAN 494	100	98	99	1.7	98a	99a
4. PAN 577G	99	98	99	2.1	99a	100a
5. Prima	98	99	100	1.9	101a	102a
6. Hutcheson[1.]	(38)	(25)**	(28)**	25.8	(68) b	(74) b
7. A2233[1.]	(88)	(86)	(80)**	9.0	(98) a	(89) ab
8. A5409	95	89*	86**	2.1	94a	90ab
9. A7119	85	83	86	3.6	97a	101a
COWPEA						
1. Dr Saunders	95	100**	97	1.7	105a	102a
c.v. %	4.4				11.1	10.7

[1.] Brackets indicate that values were probably influenced by an infection.

DRY BEAN						
1. PAN 122	93	94	96	1.5	101 a	103 a
2. PAN 127	99	98	100	2.1	99 a	101 a
3. Mkusi	98	99	100	1.9	101 a	102 a
4. Nandi	98	99	95	2.4	101 a	97 a
c.v. %	4.4				11.1	10.7

* Tendency to differ from control ($P < 0.1$)
** Significant difference from control ($P < 0.05$)
Mine C 10/94 (soybean & cowpea); 3/95 (dry bean)
Mine B 11/94 (soybean & cowpea); 3/95 (dry bean)

The temperate annuals were generally more tolerant to both types of mine water than the subtropical annuals. Germination percentages were generally not influenced by either the sulphate or sodic-saline mine water. Exceptions were an **oats** cultivar, Overberg, with sulphate salinity where the germination was possibly affected by infection (Table 6.4), and **rye** SSR1 with the NaCl-dominated water (Table 6.5).

TABLE 6.3 The influence of two mine waters on the germination percentage of oats **cultivars**

Cultivars	Germination %			Relative germination %		c.v. for cultivars %
	Deionized water	Mine C	Mine B	Mine C	Mine B	
OATS						
1. SSH 421	99	99	99	100	100	1.5
2. SSH 423	98	99	98	101	100	2.2
3. Witteberg	100	100	100	100	100	0.0
4. Perdeberg	98	99	99	101	101	2.8
5. Echidna	100	99	100	99	100	0.8
6. Overberg[1]	93	86 **	88	92 **	95	2.7

c.v. 2.6%

1. All treatments had a black powdery infection.

TABLE 6.4 The influence of two mine waters on the germination percentage of rye cultivars

Cultivars	Germination %			Relative germination %		c.v. %
	Deionized water	Mine C	Mine B	Mine C	Mine B	
RYE						
1. SSR 727	88	93	92	106	105	6.4
2. SSR 729	95	98	95	103	100	2.7
3. SSR 1[1.]	63	60	53 **	95	84 **	16.6
4. Henoch	98	96	94	98	96	2.46

c.v. 6.06%

1. The low germination of SSR 1 could be possibly be due to the seed having aged.

* Tendency to differ from control ($P < 0.1$)
** Significant difference from control ($P < 0.05$)

The germination percentages of the **lucerne** cultivars were not significantly suppressed on the high sulphate mine water. Topaz, however, tended to decrease by eleven percent. On the NaCl-dominated water the germination of only Diamond was significantly depressed by twelve percent (Table 6.5). As lucerne has been found to be sensitive during germination (Läuchli and Epstein, 1990), this is an indication of the tolerance of the remaining lucerne cultivars.

TABLE 6.5 The influence of two mine waters on the germination percentage of lucerne **cultivars**

Cultivars	Germination %			c.v. %	Relative germination %	
	Deionized water	Mine C	Mine B		Mine C	Mine B
1. PAN 4860	91	90	90	7.4	99	99
2. PAN 4581	93	96	94	3.9	104	102
3. Baronet	93	95	93	4.5	103	101
4. Topaz	71	63 *	75	9.8	89 *	106
5. Diamond	98	93	87 **	4.0	94	88 **

c.v. 5.9%

* Tendency to differ from control (P<0.10)

** Significant difference from control (P < 0.05)

Mine C 3/95 (2248 mgl^{-1} SO_4, EC 394 mSm^{-1})

Mine B 3/95 (40 mmoll^{-1} Na, 26mmoll^{-1} Cl, EC 534 mSm^{-1})

The germination of some crop cultivars seemed to be stimulated by the $CaSO_4$ water. The germination percentage was increased significantly for **cowpea** cv. Dr Saunders (Table 6.2) and **triticale** cv. PAN 299 (Table 6.6), and tended (P< 0.1) to increase in maize cv. PAN 6564 and sunflower cv. SNK 34 (Barnard et al., 1998).

TABLE 6.6 The influence of two mine waters on the germination percentage of triticale **cultivars**

Cultivars	Germination %			Relative germination %		c.v. %
	Deionized water	Mine C	Mine B	Mine C	Mine B	
1. Kiewiet	100	99	99	99	99	1.2
2. SShRI	88	88	89	100	101	3.5
3. Rex	98	98	98	100	100	2.3
4. PAN 299[1]	57	63 **	58	110 **	102	11.1
5. SSKR 626	98	96	96	98	98	1.9
6. SSKR 628	98	98	94	100	96	3.5
7. Cloc 1	91	91	92	100	101	3.9

c.v. 3.4%

1.These percentages included a marked number that had died when the radicle was *ca* 1 cm: more with the control than with the mine waters (Control 7.5-10 %. Mine C 2.5-5 % and Mine B 2.5 %) giving an apparent increase in germination.

Germination was influenced more where there was a black powdery infection, for instance with some soybean and oats cultivars (Tables 6.2 and 6.4) . With some cultivars of triticale and sorghum there seemed to be a sensitivity of very early seedling growth with the radicle dying after about one centimetre's growth (Tables 6.6 and 6.1).

Although the rate of germination was not measured no obvious indications of such a delay were observed except where infections had occurred.

6.2.3 CONCLUSION FOR GERMINATION

The germination percentages of most cultivars of both the subtropical and temperate annual crops were not influenced by either the $CaSO_4$- or NaCl-dominated mine waters used. There were, however, exceptions where germination percentages of some cultivars of sorghum, pearl millet and soybean were slightly suppressed with sulphate salinity, and also of soybean and lucerne with the NaCl-dominated water.

Germination should, however, not be a problem if these crops are irrigated with these waters; where it was suppressed, it ranged from 5 to 12 %, which could be compensated for by sowing more densely.

Generally it is thus not expected that the decreased osmotic potential of $CaSO_4$-dominated mine waters, will affect the germination percentage of the majority of cultivars of the crops that were evaluated. This is in agreement with findings for NaCl salinity that germination percentages of most crops are generally not affected at osmotic potentials below *ca* 700 mS m^{-1} (Francois & Maas, 1994).

6.3 VEGETATIVE GROWTH

6.3.1 ANNUAL SUBTROPICAL CROPS

6.3.1.1 Method and materials

Selected subtropical crops and cultivars were evaluated in the vegetative growth stage with a lime-treated acid mine drainage and a NaCl-dominated mine water in a sand culture experiment conducted in the glasshouse. Full strength salinization was imposed from the Day 26 after planting, when the plants had approximately four leaves. Plants were harvested on Day 52 after planting at the beginning of the tasselling stage, that is 26 days after full strength treatment had begun. The method is described in chapter 3 (3.2.3.2).

Chemical analyses were conducted on the composited material of the stems, leaves and spikes separately for each replicate (3.2.3.2). The individual mine waters used are indicated with the respective tables of which the analyses are given in Table 3.1. The following crops were evaluated: maize cv. SNK 2340, sorghum cv. PAN 888, pearl millet (babala) cv. SA Standard, soybean cv. Ibis, and cowpea cv. Dr Saunders.

6.3.1.2 Results for subtropical crops

Maize, sorghum and pearl millet

The total top growth of maize, sorghum and pearl millet was not depressed significantly in the vegetative growth stage by either the $CaSO_4$- or NaCl-dominated water but seemed to be less on the $CaSO_4$ water than with the controls for all three these crops (Table 6.7 and 6.8). There was a significant decrease in stem mass in the case of maize and sorghum with the $CaSO_4$-dominated water (Figure 6.1).

The vegetative growth of these three species generally seemed to be influenced to a lesser extent by the NaCl mine water than by the $CaSO_4$ water despite an apparently higher EC of the NaCl water (but, Σ anions 39 *vs* 39 $mmol_c\ L^{-1}$) (Figure 6.1) (Table 6.7). The significant increases of

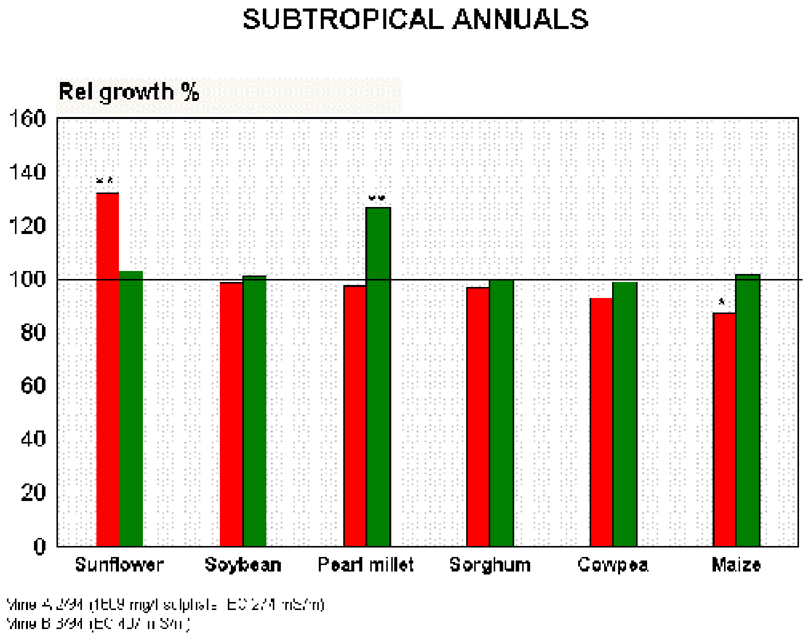
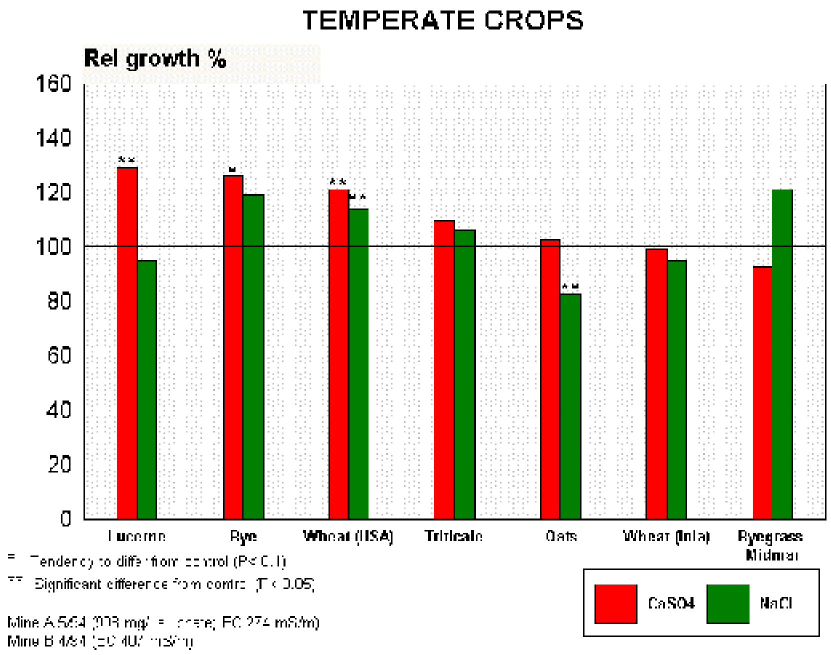


FIGURE 6.1 The influence of $CaSO_4$ and NaCl mine water on the vegetative growth of subtropical and temperate crops.



leaf area and succulence of maize on the NaCl water may indicate an osmotic adaptation to salinity (Table 6.7 and 6.8). This together with the increased tissue concentrations of Na, Cl and K in the maize hybrid (and possibly also in sorghum) with the NaCl-dominated mine water (Table 6.13), may indicate an osmotic adaptation by the uptake of these inorganic ions. The apparently higher growth masses may, however, also be due to earlier maturity with the NaCl water (Shannon, 1997). No symptoms indicating accumulation of toxic amounts of Na and Cl were observed and accumulation was therefore apparently not a major growth depressing mechanism in this more mature stage with the particular water and cultivars used.

Soybean, cowpea and dry bean

The relative top growth of soybean and cowpea was not significantly different from that of the control (Figure 6.2) (Table 6.7 and 6.8).

Soybean, however, developed a white marginal chlorosis on a few of the younger mature leaves after two to three weeks of salinisation (Figure 6.1). This could possibly be due to a Mo deficiency by sulphate competition; in the field trial where this cultivar was irrigated with a similar water, these symptoms did not occur (N.Z.Jovanovic, personal communication, 2000).The leaf area of **cowpea** decreased significantly together with a significant increase in succulence without the leaf mass being depressed; this could be an indication of a possible osmotic adaptation (2.2.2.2) (Table 6.7 and 6.8). Satisfactory yields were obtained with a similar water for soybean and cowpea in the field trial (Jovanovic et al., 1998).

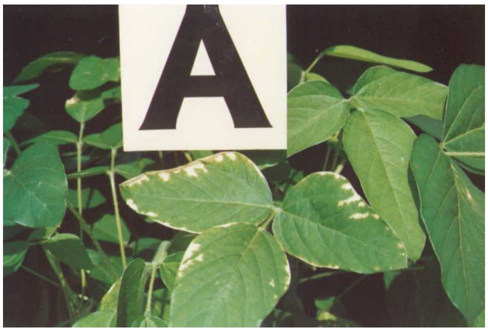


Figure 6.2 White marginal chlorosis on a few of the younger mature leaves of soybean on $CaSO_4$ water

Table 6.7 Growth parameters for subtropical annuals in the vegetative growth stage

Crop	Treat-Ment	Dry mass top growth g				Dry mass roots g	Leaf Area cm^2
		Stems	Leaves	Pods/ spikes	Total		
Maize SNK 2340	Control	47.64	41.26	3.92	92.81	26.45	8764
	Mine A	38.17**	39.00	3.51	80.68*	26.16	8571
	Mine B	46.29	43.70	4.45	94.43	29.77	9811**
c.v. %		13.08	7.13	25.72	9.54	13.62	5.75
Sorghum Hybrid PAN 888	Control	55.18	18.92	6.53	80.63	23.68	4872
	Mine A	51.84**	19.34	6.85	78.03	23.80	4980
	Mine B	54.34	19.91	6.31	80.55	21.87	5345
c.v. %		3.72	6.83	17.69	3.19	7.53	8.50
Soybean Ibis	Control	16.40	17.15	4.89	38.43	8.66	6158
	Mine A	16.36	16.75	4.67	37.78	7.11 *	6318
	Mine B	15.05	16.61	7.09***	38.75	6.72 **	5523*
c.v. %		15.60	9.33	16.24	9.09	15.68	7.33
Pearl millet ommon (babala)	Control	33.67	17.55	5.58	56.79	16.75	4229
	Mine A	31.27	17.32	6.50	55.09	15.96	3881
	Mine B	44.11 **	18.23	9.48	72.05***[1.]	14.00	4281
c.v. %		12.85	16.82	59.15	9.61	15.02	12.47
Cowpea Dr Saunders	Control	38.14	20.40	3.81	62.35	6.09	7606
	Mine A	34.33	19.58	4.14	58.04	6.89	6331***
	Mine B	35.73	21.34	4.50	61.56	6.70	6238***
c.v. %		10.84	9.95	16.93	9.38	17.64	5.39

* Tends to significant difference from control ($P<0.10$)
** Significant difference from control ($P<0.05$)
*** Highly significant difference from control ($P<0.01$)
Mine A 2/94
Mine B 3/94
1. This increase was due to earlier maturity of pearl millet on this water.

TABLE 6.8 Growth ratios of subtropical annuals in the vegetative growth stage

Species	**Treat-Ment**	**Water % In top Growth**	**Succulence mg H_2O/cm^2 leaves**	**Leaves/ Stems**	**Top growth/ Roots**	**Relative growth %**	
						Leaves	**Top growth**
Maize	Control	83.91	16.69	0.87	3.59	100	100
SNK 2340	Mine A	84.08	17.36	1.03**	3.09	94.52	86.93*
	Mine B	85.77***	18.75***	0.96	3.18	105.91	101.43
Sorghum Hybrid PAN 888	Control	79.70	13.44	0.34	3.41	100	100
	Mine A	79.32	12.83	0.37	3.28	102.22	96.78
	Mine B	80.73	13.54	0.37	3.71	105.23	99.90
Soybean Ibis	Control	79.40	9.90	1.06	4.52	100	100
	Mine A	80.13	10.28	1.03	5.35	97.67	98.33
	Mine B	79.34	10.39	1.11	5.82**	96.85	100.83
Pearl millet Common (babala)	Control	81.36	22.27	0.53	3.40	100	100
	Mine A	82.71	21.88	0.56	3.49	98.69	97.01
	Mine B	81.05	21.15*	0.41	5.23***	103.87	126.87***
Cowpea Dr Saunders	Control	83.46	17.96	0.53	10.34	100	100
	Mine A	83.44	19.17**	0.57	8.50***	95.98	93.09
	Mine B	84.11	20.22***	0.61	9.53	104.60	98.73

Mine A 2/94 Mine B 3/94

TABLE 6.9 The influence of two mine waters on the vegetative top growth of sunflower cultivar SNK 43

Cultivar	**Dry mass of topgrowth g/pot**			**Relative growth %**		**c.v. %**
	Control	**Mine C**	**Mine B**	**Mine C**	**Mine B**[1.]	
SNK 43	41.85	55.38**	43.50	132**	103	5.2

Mine C 10/94; Mine B 7/94 & 11/94

TABLE 6.10 The influence of two mine waters on the yield of dry bean PAN 122

Cultivar	**Yield (65°C) g/pot**		**Relative yield %**	**c.v.**
	Control	**Mine C**	**Mine C**	**%**
PAN 122	34.17	45.71**	134*	26.7

Mine C 12/94

* Tends to significant difference from control ($P<0.10$)
** Significant difference from control ($P<0.05$)
*** Highly significant difference from control ($P<0.01$)

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[1] This particular sodic-saline water (Mine B 12/94), however seemed to 'improve' with time (2 months) probably due to the unusual presence of a black substance that settled, leaving a supernatant solution that was not at all saline with very low Na, Cl and SO_4 contents.

Dry bean cv. PAN 122 was evaluated separately on sand culture with a high sulphate Kleinkopje mine water (Mine C 12/94) but only dry seed yield was measured. The seed yield was increased by 34 % ($P<0.1$) on this water (Table 6.10).

As a relationship also exists between salt tolerance and the macro-nutrient accumulation in the vegetative organs of legumes (Cordovilla et al., 1995), the increased nutrients in the $CaSO_4$ water may have given rise to increased vegetative growth. Nutrient analyses of the top growth of **soybean** and **cowpea** confirmed an increased nutritional status with the $CaSO_4$ water: The N, P, K and total S tended to be increased above that of the control (Table 6.13 and 6.14). The dry matter content of all the nutrients except N was in the optimum range for soybean (Small & Ohlrogge, 1973).

Sunflower

The effect of the Kleinkopje mine water on sunflower growth from planting to 52 days (before flower buds appeared), was evaluated on sand culture in the glasshouse. The relative top growth was increased significantly by 32 % in the vegetative growth stage with the $CaSO_4$-dominated water (Table 6.9).

6.3.2 TEMPERATE CROPS

6.3.2.1 Method and materials

The vegetative growth of the following temperate crops was evaluated in water culture in a glasshouse. The method is described in Chapter 3 (3.2.3.3). The crops evaluated were: wheat cv. Inia and USA cultivar used on mine spoils, rye cv. SSR 1, oats cv. Overberg, triticale cv. Cloc 1, annual ryegrass cv. Midmar, and lucerne cv. PAN 4860.

6.3.2.2 Results for temperate crops

The annual temperate crops produced very few significant growth responses. The notable exceptions were rye, the wheat cultivar bred as a nurse-crop for use on mine spoils, and lucerne. With **rye** lime-treated acid mine drainage water had a significant beneficial effect on total top growth, the mass of roots and leaves produced, and the top growth to root ratio. Lime-treated acid mine drainage water produced 24 % more leaf material and 26 % more top growth than the control nutrient solution. The nurse-crop responded with significant increases in most of the growth parameters except for root dry mass (Tables 6.11 and 6.12) (Figure 6.1).

Lucerne grew exceptionally well in the vegetative growth stage with this lime-treated acid mine drainage water. Lucerne leaf, stem and root masses increased significantly (there were, however, a few 'cupped' leaves with a thin marginal necrosis) (Table 6.11). The yield of lucerne in the field trial confirmed the tolerance in the vegetative growth stage (Jovanovic et al., 1998).

The $CaSO_4$-dominated water also improved the leaf yield of **oats** and the leaf to stem ratio of **ryegrass** Midmar, both aspects of importance where these crops are used as forages. **Triticale** was not significantly influenced in any way although most growth parameters seemed to improve on this lime-treated acid mine drainage water (Tables 6.11 and 6.12).

6.4 CHEMICAL ANALYSES OF TOP GROWTH

Chemical analyses were conducted on the top growth to establish whether the high Ca and SO_4 concentrations in the lime-treated acid mine drainage mine waters showed cation or anion antagonistic effects on nutrient uptake.

Nutrient analyses were conducted on the composited material of the total top growth separately for each replica for the subtropical crops; in the case of the temperate crops the replicates of the leaf and stem material respectively were combined (3.2.3.4). The concentration and total uptake of nutrient elements in the subtropical crops are given in Tables 6.13 and 6.14 respectively, and those of the temperate crops are given in Tables 6.15 and 6.16. Statistical analyses were conducted for the subtropical crops to determine the effect of these waters on the nutrient uptake (3.1.2), but was not possible for the temperate crops as replicates were combined.

6.4.1 CALCIUM INTERACTIONS

There were increased dry mass concentrations and total uptake of **Ca** in the top growth of all the crops (Tables 6.13 to 6.16), that compared well with the average Ca content of plants (0.5 to 3.0 %) (Marschner, 1986).

Although **K** uptake has been found to be competitively affected by Ca in maize roots (Elzam & Hodges, 1967), it was not significantly diminished in the shoots of maize or the other subtropical crops with the lime-treated acid mine drainage water. K uptake even seemed generally higher, with a significant increase in sorghum (Tables 6.13 and 6.14). In the seedling growth stage of maize cv. SNK 2340 nutrient analyses also revealed that the high Ca and Mg content of the Kleinkopje water did not significantly affect the K uptake (Table 5.29).

Antagonistic Ca effects were not evident in a decrease of **Mg** in the top growth of the subtropical crops with the lime treated acid mine drainage water; on the contrary Mg concentrations in the top growth of the subtropical crops generally *increased* significantly (Table 6.13). In contrast the Mg uptake in the temperate crops was *decreased* to about half that of the control in rye, oats, triticale and wheat Inia but was not influenced in ryegrass and the wheat nurse-crop (Table 6.15). The concentrations were low compared to the average 0,5 % of the dry mass of the vegetative parts for optimal plant growth (Marschner, 1986) (Table 6.15). Slight Mg deficiency in the vegetative growth stage of cereals does not, however, always result in a decreased yield (Mengel & Kirkby, 1987).

The Mg content in the lime treated acid mine drainage water was very low probably resulting in active uptake of Mg. The higher uptake of Mg in the summer crops compared to the suppressed uptake in the winter crops may possibly be due to the effect of the temperatures on the active uptake of Mg.

The greater uptake of Mg in ryegrass and the wheat nursecrop compared to the other crops, may be due to genetic characteristics such as osmotic adaptation, which may be partly responsible for the good growth of these crop cultivars on this water (Sagi, et al., 1997) (Table 6.15 and 6.16).

A high external **Ca:Mg** ratio has been found to decrease the photosynthetic rate and water use efficiency for maize (Plaut and Grieve, 1988). Although there were such indications in maize and sorghum, the high Ca:Mg ratio in the lime treated acid mine drainage water was, however, not accompanied by significantly suppressed growth in most of these crops (Table 6.7). The significant increase in Mg uptake with the NaCl water could be due to a higher external Mg:Ca ratio in this water (Mengel & Kirkby, 1987) (Table 6.13 and 6.15).

There was a significant increase in the **Mn** uptake with the lime-treated acid mine drainage water (Table6.13), but it was still well below toxicity levels (Chapman, 1966); however, for soybean, toxicity could be induced at only 160 mg kg^{-1} (Mengel & Kirkby, 1987). This increase was probably due to the higher Mn content of the water used in comparison to the control. Additional manganese was also given with the nutrients without which the uptake would probably have been less. With a water with a higher Mg content (as was the case with the Kleinkopje water used in the seedling experiments), Mn uptake would probably not be increased as Mg has an antagonistic effect on Mn uptake and can prevent Mn toxicity (Mengel & Kirkby, 1987). This was confirmed by the analyses of the seedling top growth of maize cv. SNK 2340 on the Kleinkopje water, where Mn uptake was significantly decreased (Table 5.29).

With the **NaCl**-dominated mine water (Mine B 4/94), the total **Ca**-uptake per pot was increased significantly for sorghum, cowpea and the wheat nursecrop indicating an efficient Ca uptake of these crop cultivars. The **Na** and **Cl** content of maize was the highest of the subtropical crops and together with the significantly *increased* leaf area and succulence it may be an indication of an inorganic osmotic adaptation of this maize cultivar via the uptake of Na and/or Cl (Cramer,

1994) (Table 6.13). The Na and Cl uptake was least for wheat cv. Inia and high for oats cv. Overberg. This corresponds with a tolerance of Inia and a sensitivity of oats for NaCl water (Table 6.11). The Cl content of the two wheat cultivars was furthermore the least of all the crops, which could be due to exclusion from the shoots. The vegetative growth of annual ryegrass seemed to be unaffected by the high concentrations of Na and Cl in the top growth (Tables 6.11), but ryegrass is generally tolerant to NaCl-dominated salinity at these concentrations (Marschner, 1995).

6.4.2 SULPHATE INTERACTIONS

The **total S** (given as SO_4) in the top growth of all crops evaluated was increased significantly with the high sulphate water (Tables 6.13 to 6.16). S concentration in the total top growth of the subtropical crops varied between 1.08 % of the dry mass for maize and 2.56 % for soybean. In the temperate crops S uptake was increased in both the leaves and stems; the S (given as SO_4) content in the leaves ranged from 1,92 % in ryegrass to 2,24 % in rye; this is higher than the critical requirement of cool season grasses of 0,2 to 0,26 % (Martin and Walker, 1966).These levels should, however, not be detrimental to plant growth (2.5.2.2).

Table 6.11 The influence of two types of mine water on the vegetative growth parameters of annual temperate crops

Crops		Treat-Ment	Leaves Wet mass g/pot$^{1.}$	Leaves dry mass g/pot	Tillers/stems		Total dry mass top growth g/pot	Rel. Growth %	Roots dry mass g/pot	Leaf area cm^2/pot
					No.	Dry mass g/pot				
1	Rye	Control	185.91	20.99	75	13.03	34.01		3.41	7182.06
	SSR1	Mine A	187.27	26.01**	88	17.00	43.01*	126 *	5.38**	7544.38
		Mine B	195.81	25.50*	74	15.13	40.63	119	3.53	8966.92**
	c.v. %		8.93	12.56	19.34	21.33	14.51		27.22	6871.79
2	Oats	Control	188.56	24.85	23	30.81	55.66		5.05	6871.79
	Overberg	Mine A	189.35	27.18*	25	30.15	57.33	103	4.64	6935.84
		Mine B	180.98	22.09**	28 **	23.92*	46.01**	83 **	3.42***	6389.17
	c.v. %		6.21	6.50	11.51	15.63	10.91		15.90	7.16
3	Triticale	Control	172.38	19.28	102	6.47	25.75		4.17	6536.34
	Cloc 1	Mine A	177.01	21.14	111	7.18	28.32	110	3.99	6588.43
		Mine B	167.63	20.02	110	7.24	27.26	106	3.82	5880.44***
	c.v. %		5.47	8.11	8.63	13.42	9.29		19.57	6.63
4	Wheat	Control	43.24	8.81	26	14.80	28.23		3.26	2349.61
	Inia	Mine A	46.14	9.03	29	14.37	28.06	99	2.76*	2414.01
		Mine B	48.67	9.95*	34 ***	13.39	26.68	95	2.76*	2527.73
	c.v. %		10.23	9.09	5.53	10.7	10.48		10.99	10.51
5.	Ryegrass	Control	137.50	15.63	173	7.50	23.13		4.31	4985.77
	Midmar	Mine A	140.48	15.18	167	6.22	21.40	93	4.04	5756.82
		Mine B	173.59**	18.47	173	9.57*	28.04	121	4.97	7256.26
	c.v. %		13.15	18.39	26.17	22.65	19.16		37.61	26.69
6	Wheat (USA)	Control	127.81	17.50	45	8.05	25.55		3.55	4178.19
	Nursecrop for mine	Mine A	163.16***	20.58**	51	10.37**	30.95***	121 ***	3.98	5724.85 **
	Spoils	Mine B	143.53*	19.49*	72 ***	9.68*	29.17 **	114 **	3.61	4918.81
	c.v %		7.58	7.22	10.41	11.42	7.41		12.58	14.46

* Tendency to differ from control ($P < 0.1$) ** Significant difference from control ($P < 0.05$) *** Highly significant difference from control ($P < 0.01$)

Mine A 5/94 Mine B 4/94 (Both waters diluted by rain)

1. 3 Plants per pot

Lucerne PAN 4860	Control	51.73	8.65	30	7.68	16.33	100	3.97	2969.67
	Mine A	60.06 *	11.55 **	31	9.32*	20.87**	129 **	5.85**	3364.25
	Mine B	42.80	7.66	24	6.49	14.15	95	2.91**	2412.36
c.v. %		14.64	14.79		16.02	13.77		12.47	24.1

* Tendency to differ from control ($P < 0.1$)   ** Significant difference from control ($P < 0.05$)   *** Highly significant difference from control ($P < 0.01$)

Mine A 5/94 Mine B 4/94 (Both waters diluted by rain)
1. 3 Plants per pot

Table 6.12 Growth ratios for temperate annuals

Crops	Treat-Ment	Leaves/ Stems	Tops/ Roots	leaf area mg H_2O/cm^2	Relative top growth %
1. Rye SSR1	Control	1.62	10.59	23.00	100
	Mine A	1.60	8.23**	21.48	126
	Mine B	1.69	11.97	19.51*	119
2. Oats Overberg	Control	0.81	11.19	23.81	
	Mine A	0.91	12.40	23.46	103
	Mine B	0.95	13.58**	24.87	83 *
3. Triticale Cloc 1	Control	3.00	6.24	23.51	100
	Mine A	2.99	7.42	23.65	110
	Mine B	2.77	7.35	30.25***	106
4. Wheat Inia	Control	0.60	8.70	14.66	100
	Mine A	0.63	10.31	15.17	99
	Mine B	0.74	9.71	15.33	95
5. Ryegrass Midmar	Control	2.11	5.92	25.96	100
	Mine A	2.49*	5.61	23.20	93
	Mine B	1.95	5.74	22.14*	121
6. Wheat (USA) Nursecrop on mine spoils	Control	2.18	7.20	26.46	100
	Mine A	1.99	7.84	25.04	121 **
	Mine B	2.04	8.21	25.60	114 *
c.v. %		11.55	16.24	12.55	

Lucerne PAN 4860	Control	0.95	7.63	12.49	100
	Mine A	1.06	7.90	13.96	129 *
	Mine B	0.89	9.87***	14.78**	95
c.v. %		20.26	15.59	12.89	12.89

* Tends to significant difference from control(P<0.10)
** Significant difference from control (P<0.05)
*** Highly significant difference from control(P<0.01)

Table 6.13 Concentration of nutrient elements in the top growth of subtropical annuals with two mine waters in the vegetative growth stage

Species	Treatment	N	P	K	Ca	Mg	Na	Sulphate	Cl	Fe	Mn	Cu	Zn
		%								mg kg^{-1}			
Maize SNK 2340	Control	0.955	0.145	0.983	0.173	0.138	0.00	0.813	0.072	18	45	3	6
	Mine A	0.820*	0.140	1.183	0.337*	0.163	0.00	1.080*	0.158	21	131 *	2	12 *
	Mine B	0.688*	0.118*	1.178	0.213	0.323*	0.63*	0.210*	2.752*	35 *	30 *	3	3
	c.v. %	7.01	6.92	12.27	12.59	12.51	30.00	14.45	20.0	50.78	9.17	42.42	34.62
Sorghum Hybrid PAN 888	Control	1.088	0.145	0.855	0.213	0.165	0.00	0.965	0.079	30	89	6	13
	Mine A	0.943	0.140	1.123*	0.370*	0.250*	0.00	1.428*	0.144	51	221 *	6	25 *
	Mine B	1.100	0.155	1.033*	0.283*	0.443*	0.058*	0.240*	2.634*	59 *	33 *	5	11
	c.v. %	13.09	9.64	7.64	9.21	10.67	45.18	12.27	4.41	48	8.03	25.53	17.89
Soybean Ibis	Control	2.23	0.263	1.648	0.703	0.330	0.00	1.410	0.077	68	188	8	29
	Mine A	2.46	0.293	1.868	1.493*	0.408*	0.00	2.565*	0.122	77	316 *	8	52 *
	Mine B	2.29	0.308	1.995*	1.263*	0.625*	0.058*	0.518*	0.800*	75	99 *	6	26
	c.v. %	14.01	9.15	11.58	4.31	6.47	66.79	4.99	10.96	12.81	6.64	17.00	10.85
Pearl millet SA Standard Common (Babala)	Control	1.298	0.208	1.525	0.243	0.268	0.00	1.405	0.088	47	128	4	17
	Mine A	1.258	0.215	1.763	0.383*	0.435*	0.10*	1.755*	0.180	41	350 *	4	33 *
	Mine B	1.178	0.193	1.595	0.218	0.423*	0.24*	0.385*	2.12*	31 *	55 *	4	11
	c.v. %	20.65	14.03	17.24	22.34	16.45	27.84	11.22	16.64	31.70	20.96	19.76	36.69
Cowpea Dr Saunders	Control	2.523	0.198	1.425	0.685	0.235	0.00	1.613	0.077	71	302	5	21
	Mine A	3.235*	0.215	1.428	1.143*	0.308*	0.00	2.205*	0.134*	72	486 *	2*	37 *
	Mine B	3.060	0.193	1.490	1.048*	0.465*	0.255*	0.973*	0.866*	67	132 *	4	12 *
	c.v. %	12.40	9.71	9.59	18.01	9.76	19.61	7.87	9.52	18.65	16.22	15.38	18.96

*Significant difference from control ($P < 0.05$)

TABLE 6.14 Total uptake of nutrients in the top growth of subtropical annuals with two mine waters in the vegetative growth stage

Crop	Treatment	N	P	K	Ca	Mg	Na	Sulphate	Chloride	Fe	Mn	Cu	Zn
		g/pot								mg/pot			
Maize SNK 2340	Control	0.884	0.135	0.909	0.159	0.127	0.00	0.748	0.067	1.63	4.17	0.24	0.58
	Mine A	0.662*	0.113*	0.940	0.270*	0.132	0.00	0.869*	0.127	1.68	10.57*	0.18	0.97
	Mine B	0.648*	0.111*	1.105*	0.199	0.303*	0.591*	0.196*	2.580*	3.18*	2.78	0.24	0.24
Sorghum Hybrid PAN 888	Control	0.877	0.117	0.689	0.171	0.133	0.00	0.777	0.063	2.39	7.12	0.51	1.07
	Mine A	0.733	0.109	0.875	0.289*	0.195*	0.00	1.113*	0.112	3.38	17.25*	0.44	1.96*
	Mine B	0.889	0.125	0.831	0.228*	0.356*	0.046*	0.193*	2.121*	4.78*	2.69*	0.39*	0.85
Soybean Ibis	Control	0.861	0.101	0.624	0.269	0.126	0.00	0.543	0.031	2.61	7.26	0.32	1.08
	Mine A	0.928	0.110	0.705	0.564*	0.154	0.00	0.969*	0.046	2.90	11.94*	0.30	1.97*
	Mine B	0.882	0.118	0.768	0.488*	0.241*	0.022	0.198*	0.308*	2.92	3.85*	0.25	1.01
Pearl millet common (Babala)	Control	0.742	0.117	0.857	0.135	0.150	0.00	0.786	0.049	2.56	7.19	0.23	0.94
	Mine A	0.699	0.119	0.978	0.210*	0.241*	0.056*	0.969*	0.100	2.26	19.40*	0.21	0.84
	Mine B	0.843	0.138*	1.144*	0.157	0.305*	0.170*	0.277*	1.525*	2.22	3.96*	0.30	0.82
Cowpea Dr Saunders	Control	1.577	0.123	0.883	0.423	0.146	0.00	1.002	0.048	4.40	18.62	0.28	1.33
	Mine A	1.875	0.125	0.828	0.663*	0.178	0.00	1.280*	0.077	4.18	28.21*	0.09*	2.15*
	Mine B	1.868	0.117	0.913	0.633*	0.283*	0.159*	0.598*	0.531*	4.12	8.12*	0.23	0.75

*Significant difference from control ($P < 0.05$).

Table 6.15 Concentration of some nutrient elements in the top growth of annual temperate crops with two types of mine water

Crops	Treat-ment	Ca		Mg		SO_4		Na		Cl	
		%									
		Lea ves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
1. Rye SSR 1	Control	1.43	0.92	0.62	0.44	1.51	0.9	0.03	0.03	0.05	0.05
	Mine A	1.99	1.37	0.35	0.27	2.24	1.68			0.20	0.19
	Mine B	1.05	0.77	0.13	0.36	1.27	0.69	1.10	0.72	2.60	3.20
2. Oats Overberg	Control	0.70	0.40	0.42	0.26	1.47	0.66	0.14	0.44	0.02	0.04
	Mine A	1.28	0.85	0.20	0.16	2.12	1.40			0.10	0.17
	Mine B	0.63	0.51	0.38	0.26	1.62	0.69	1.94	3.34	2.88	4.44
3. Triticale Cloc 1	Control	0.85	0.69	0.40	0.38	1.59	1.03	0.02	0.04	0.06	0.07
	Mine A	1.51	1.27	0.20	0.25	2.20	1.80			0.20	0.20
	Mine B	0.73	0.50	0.33	0.28	1.48	0.79	1.12	1.06	2.09	2.73
4. Wheat Inia	Control	1.31	0.37	0.45	0.14	1.57	0.87	0.01	0.02	0.03	0.06
	Mine A	2.03	0.67	0.22	0.09	2.00	1.52			0.14	0.23
	Mine B	0.99	0.37	0.65	0.26	1.65	0.96	0.39	0.33	0.99	1.27
5. Ryegrass Midmar	Control	0.81	0.62	0.41	0.27	1.39	0.76	0.12	0.38	0.06	0.07
	Mine A	1.35	1.06	0.32	0.31	1.92	1.48			0.19	0.19
	Mine B	0.87	0.51	0.39	0.24	1.24	0.63	1.96	2.27	3.08	3.84
6. Wheat (USA) Nursecrop for mine spoils	Control	2.19	1.20	0.28	0.13	1.49	0.60	0.06	0.06	0.05	0.04
	Mine A	3.17	1.86	0.22	0.25	2.12	1.52			0.26	0.21
	Mine B	3.29	1.37	0.38	0.27	1.06	0.90	1.95	1.93	0.59	0.62

Lucerne PAN 4860	Control	0.83	0.49	0.53	0.44	1.03	0.56	0.04	0.1	0.04	0.05
	Mine A	1.10	0.63	0.42	0.11	1.68	1.40	0.25		0.25	-
	Mine B	0.77	0.57	0.44	0.40	0.98	0.79		1.67	2.79	2.53

Mine A 5/94
Mine B 4/94

1. Both these waters were diluted by heavy rain.

Table 6.16 Total uptake of nutrient elements by annual winter crops

Crops	**Treat-ment**	**Ca**		**Mg**		**SO_4**		**Na**		**Cl**	
		g/pot[1.]									
		Leaves	**Stems**	**Leaves**	**Stems**	**Leaves**	**Stems**	**Leaves**	**Stems**	**Leaves**	**Stems**
1. Rye SSR 1	Control	0.30	0.12	0.13	0.06	0.32	0.12	0.006	0.004	0.01	0.007
	Mine A	0.52	0.23	0.09	0.05	0.58	0.28			0.05	0.03
	Mine B	0.27	0.12	0.03	0.05	0.32	0.10	0.28	0.11	0.66	0.48
2. Oats Overberg	Control	0.17	0.12	0.10	0.08	0.37	0.20	0.03	0.14	0.005	0.01
	Mine A	0.35	0.26	0.05	0.05	0.58	0.42			0.027	0.05
	Mine B	0.14	0.12	0.08	0.06	0.36	0.17	0.43	0.80	0.64	1.06
3. Triticale Cloc 1	Control	0.16	0.04	0.08	0.02	0.31	0.07	0.004	0.002	0.01	0.005
	Mine A	0.32	0.09	0.04	0.02	0.47	0.13			0.04	0.01
	Mine B	0.15	0.04	0.07	0.02	0.30	0.06	0.22	0.08	0.42	0.20
4. Wheat Inia	Control	0.12	0.05	0.04	0.02	0.14	0.13	0.001	0.003	0.003	0.009
	Mine A	0.18	0.10	0.02	0.01	0.18	0.22			0.013	0.03
	Mine B	0.10	0.05	0.06	0.03	0.16	0.13	0.04	0.04	0.10	0.17
5. Ryegrass Midmar	Control	0.13	0.05	0.06	0.02	0.22	0.06	0.02	0.03	0.009	0.005
	Mine A	0.20	0.07	0.05	0.02	0.29	0.09			0.03	0.01
	Mine B	0.16	0.05	0.07	0.02	0.23	0.06	0.36	0.23	0.57	0.37
6. Wheat (USA)	Control	0.38	0.10	0.05	0.10	0.26	0.05	0.01	0.005	0.009	0.003
Nursecrop for mine spoils	Mine A	0.65	0.19	0.05	0.03	0.44	0.16			0.05	0.02
	Mine B	0.64	0.13	0.07	0.03	0.21	0.09	0.38	0.19	0.11	0.06

Lucerne PAN 4860	Control	0.12	0.08	0.08	0.07	0.15	0.09	0.01	0.02	0.007	0.01
	Mine A	0.22	0.12	0.08	0.02	0.33	0.27			0.02	0.04
	Mine B	0.10	0.09	0.06	0.06	0.13	0.12	0.27	0.25	0.11	0.17

Mine A 5/94

Mine B 4/94

[1.] 3 plants per pot

Anion antagonistic effects have been evident where Cl, SO_4 and H_2PO_4 uptake were stimulated when the NO_3 uptake was strongly depressed (Kirkby & Knight, 1977). Although the most common anion antagonism is between NO_3 and Cl, such an effect of high SO_4 concentrations on the uptake of H_2PO_4 and NO_3 is not excluded.

N nutrition

The **N** uptake of **maize** cv. SNK 2340 was significantly decreased by 0,135 % which may indicate a possible competition of SO_4 more probably with MoO_4 than with NO_3 . The **N** content was in the low range for maize (Chapman, 1966), but even though there were significant decreases of N on both waters, there were no obvious symptoms of deficiency except in the decreased stem masses.

Although N x S interaction has generally been found to be positive or additive (Tandon, 1992), a large difference in SO_4 and NO_3 concentrations may possibly result in a N-deficiency due to competition between these anions. In the seedling growth trials (4.2.2.1) wheat seedlings growing on an actual $CaSO_4$-dominated mine water with an NH_4:NO_3 ratio of 1:2 showed no deficiency symptoms (4.2.2). This was in contrast to severe chlorosis when less NH_4 was given but with similar solution concentrations of N (46,8 $mmol_c\ L^{-1}$ SO_4 , 4.1 $mmol_c\ L^{-1}$ NO_3 and *ca.* 1 $mmol_c\ L^{-1}$ NH_4) (Table 4.8). Ammonium could therefore have provided additional N where a ratio of 1:2 was used.

The influence of NH_4 was confirmed by a subsequent study where the effect of differential levels of K, NO_3 and NH_4 on the top growth of wheat with Ca/Mg/SO_4 salinity was investigated (Ströhmenger, et al., 1999). Top dry matter was improved with K and NH_4 treatments. The data suggest enhanced NH_4 nutrition under SO_4 salinity. The following possible reasons for this are suggested: an antagonistic effect of NH_4 on plant Mg concentration, a synergistic effect on K uptake and/or to NH_4 being a supplementary N source when large SO_4 concentrations suppressed NO_3 uptake. N utilization efficiency was also higher with NH_4 than with NO_3 at similar solution concentrations of N (Ströhmengher, P. H. F., personal communication, 2000).

There were indications that the N uptake in cowpea may have been favourably influenced by the $CaSO_4$ water (Tables 6.13 and 6.14). In the exploratory trial (3.2.3.1), the growth of root nodules with a similar lime-treated acid mine drainage water (but with a lesser nutrient content) was exceptional. The $CaSO_4$ water may thus possibly have influenced N uptake of legumes via a positive effect on nodule growth.

The greater decrease of N content generally found with the NaCl water could be due to competition between chloride and nitrate ions (Table 6.13 and 6.15).

Sulphate can also compete with **molybdate** for uptake (Stout et al., 1951). As molybdate is necessary for protein synthesis this could reduce growth (Barnard, 1978; Albasel & Pratt, 1989). Growth reduction was, however, not evident in the top growth of most of the crops evaluated with the $CaSO_4$ water (Tables 6.7 and 6.11). Analysis for Mo was conducted only in the case of soybean, where a few leaves showed possible deficiency symptoms (Figure 6.1). The Mo content of the total top growth was, however, 2 mg kg^{-1} which is generally sufficient (Chapman, 1966).

P uptake

The high SO_4 concentrations in the lime-treated acid mine drainage waters were not generally accompanied by P decreases in the top growth concentration of the subtropical crops. The significant decrease in the total uptake of P in maize was an exception, but may be due to the decreased growth (Table 6.14).The P concentration was not significantly affected (Table 6.13).

Studies with maize in solution cultures have shown that P concentrations that are optimal under non-saline conditions, could be toxic to and adversely affect the growth of maize when grown under saline conditions (Nieman & Clark, 1976). This was not the case in the current investigation for the vegetative growth stage of most crops on either the high sulphate lime-treated acid mine drainage or NaCl water. The P uptake was less for maize with the NaCl water relative to the control, indicating competitive effects with the Cl (Table 6.13).

In conclusion it can be said that the high Ca and SO_4 concentrations and uptake with the lime-

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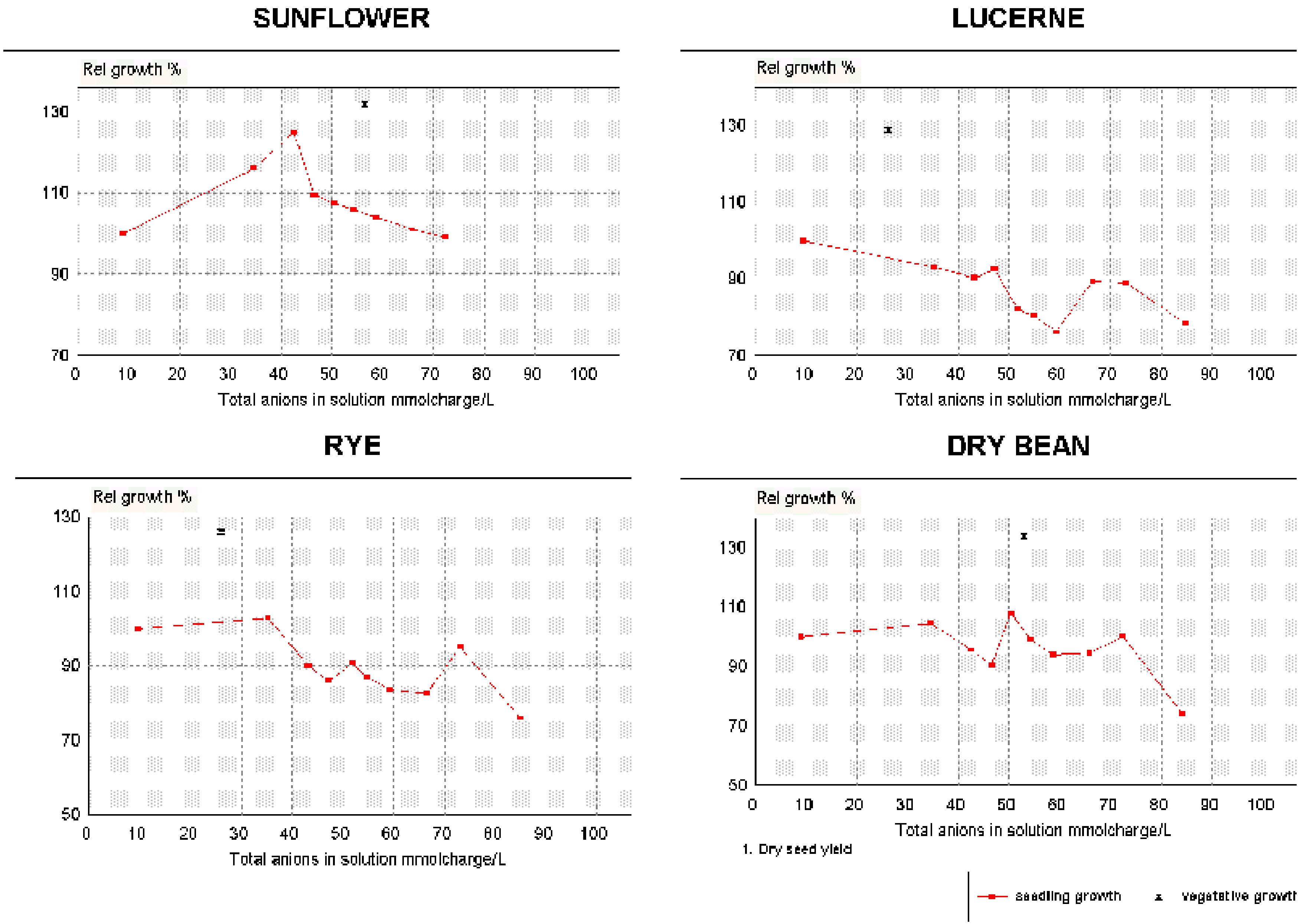
treated acid mine drainage water did not generally affect the uptake of other nutrients. There were two exceptions: Mg plant concentrations were increased in summer crops and decreased in the winter crops, and the N concentration in maize top growth was significantly decreased.

This research was not designed to investigate nutrient interactions. Further research into the possible interactions of especially Ca, Mg and SO_4 with other macro- and micronutrients is therefore recommended with soil chemistry also taken into account.

6.5 COMPARISON OF THE SEEDLING AND VEGETATIVE GROWTH STAGE WITH GYPSIFEROUS MINE WATER

The vegetative growth stage in this investigation cannot be compared per se to that of the germination and seedling stages as the lime-treated acid mine drainage waters available at the time of the vegetative experiments were diluted by heavy rain. The seedling and germination growth stages, and also the vegetative growth of sunflower and the yield of dry bean, were subsequently investigated on Kleinkopje mine water with higher Ca, Mg and SO_4 concentrations than those initially used for the vegetative growth (3.1.2).

The vegetative growth may, however, be compared to the seedling growth in the gradient trials plotted at the appropriate total anion concentration values (indicative of osmotic potential) of the respective mine waters used (Figures 6.3 to 6.5).



SUNFLOWER

LUCERNE

RYE

DRY BEAN

FIGURE 6.3 Crops where the vegetative growth was greater than the seedling growth at similar osmotic potentials.

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These comparisons revealed that crops could be divided into three groups, namely:

1. Crops where the relative *vegetative growth* and thus the tolerance was very much *greater than* that of the *seedling growth* at similar osmotic potentials; this was the case for **sunflower**, **lucerne** and **rye**, and the relative *yield* of **dry bean** (Figure 6.3). It is probable that the major mechanism by which salinity affects seedling and vegetative growth of these crops differs, and that decreased osmotic potential is less suppressing in the vegetative than in the seedling growth stage.

   Sunflower possesses the capacity for osmotic adjustment (Chimenti & Hall, 1993, 1994), which moderates stomatal closure, thus allowing continued photosynthetic activity under drought conditions (Conroy, Virgona, Smillie & Barlow, 1997). This characteristic would be advantageous for tolerance to the $CaSO_4$ water where it is increasingly evident from the present study that osmotic potential is the major suppressing quality of this type of saline water. The ability for osmotic adjustment together with its high stomatal conductance, even under drying conditions (Robinson, 1978), probably explains why both seedling and vegetative growth were increased on this water. The high stomatal conductances of sunflower lead to a lavish water use (Rawson & Constable, 1980), which together with the tolerance shown for the $CaSO_4$ saline water both during seedling and vegetative growth stages, make it a prime candidate for water disposal via irrigation.

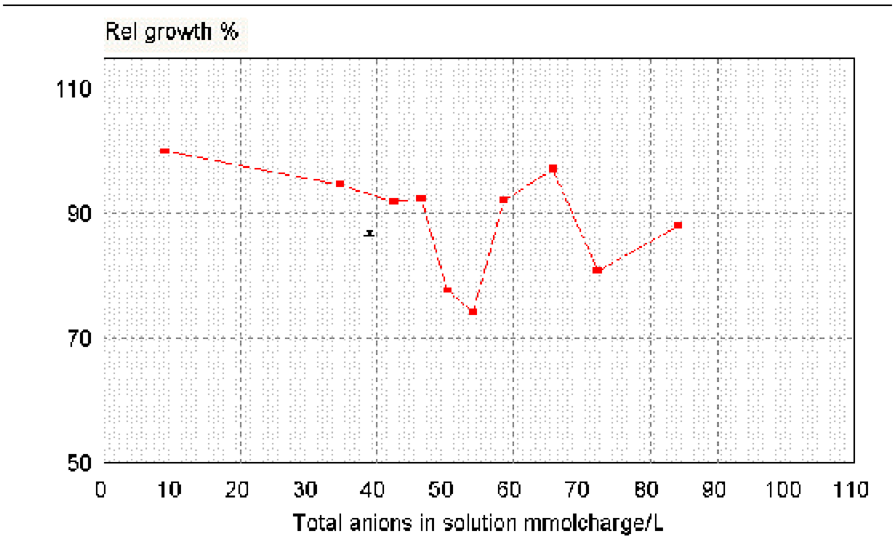
   In the case of **lucerne** the seedling growth is mainly affected by osmotic potential and the mature growth by Cl toxicity; tolerance is associated with the exclusion of Cl or the level of Cl tolerated (Noble, Halloran & West, 1984). Salt tolerance of lucerne is also associated with rapid increases of proline - especially in roots - which is an adaptation to a decrease in external osmotic potential (Petrusa & Wincov, 1997). The increased vegetative growth on this water may also be due to increased nutrient levels above those of the one-third Hoagland control, as legumes have a high Ca and S requirement (Cordovilla et al., 1995). A $CaSO_4$-dominated water may thus lead to decreased seedling growth but increased vegetative production of lucerne, due to the absence of Cl, osmoregulation and nutrient effects.

The tolerance of **rye** has been mainly connected to the Na/Ca ratio in the growth medium (Grattan & Grieve, 1994), and the exclusion of Na from the leaves (Francois et al., 1989). It is thus understandable why rye grew so well on this water where Na and Cl were virtually absent and Ca abundantly present; it can be expected that vegetative growth and yield should not decrease to the same extent on a $CaSO_4$ water as on a NaCl water with low Ca content. The good vegetative growth with the NaCl water (Table 6.11), may possibly be ascribed to an ability of this particular cultivar to restrict Na and/or Cl from the leaves.

In **dry bean** growth suppression is mainly connected to high Na concentrations (Benlloch, Ojeda, Ramos & Rodriguez-Navarro, 1994), and Na toxicity has been found to be a greater growth suppressing factor than osmotic potential (Awada et al., 1995). Under constant salinity dry bean also showed a slight adaptation to saline conditions (Meiri & Poljakoff-Mayber, 1970). Wignarajah (1990) found that dry bean plants adjusted osmotically to salt stress and suggested that "two major physiological traits enable plants to tolerate salinity: (a) compensatory growth following adjustment to salinity, and (b) ability to increase both leaf area ratio (LAR) and net assimilation rate (NAR) to achieve this increased growth". As mentioned previously, a relationship has also been suggested to exist in legumes between NaCl-salt tolerance and the macro-nutrient accumulation in the vegetative organs (Cordovilla et al., 1995).The low Na content, as well as osmotic adaptation and the increased nutrients in the Kleinkopje $CaSO_4$-dominated water could thus have given rise to the increased yield of dry bean and possibly also to better vegetative growth (Figure 6.2, Table 6.10).

2. Crops where the relative *vegetative* growth and thus the tolerance was apparently *less* than that of the *seedling* growth at similar osmotic potentials, namely with **maize** cv. SNK 2340 and **cowpea** (Figure 6.4).

MAIZE



COWPEA

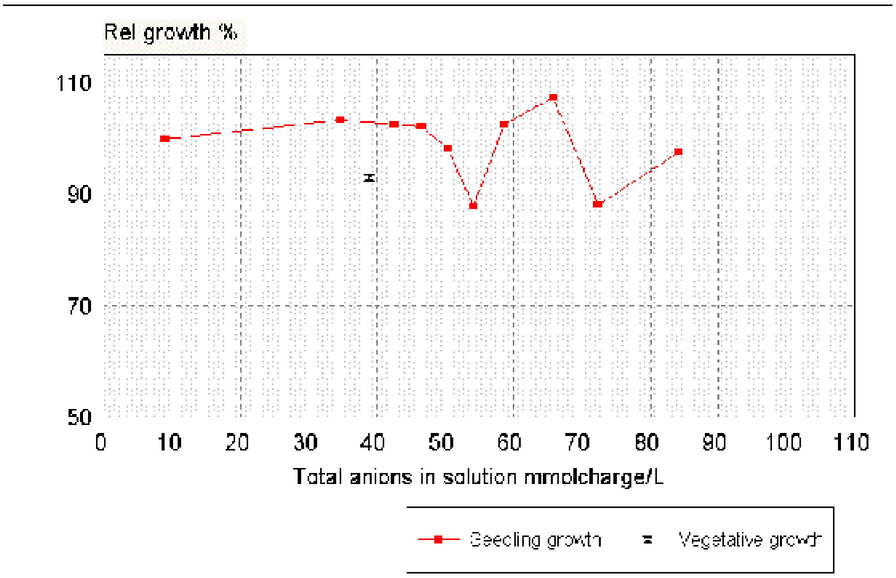


FIGURE 6.4 **Crops where the vegetative growth was less than that of the seedling growth at similar potentials.**

This is in contrast to the studies of Maas et al. (1983) on the sensitivities of the growth stages of **maize** with NaCl salinity where the seedling growth stage up to 21 days was the most sensitive. The salt tolerance thresholds for growth after 21 days, and for the ear and grain yields were, however, higher than for seedling growth.

Cramer (1994) concluded that the reduction of growth in maize by salinity appears to be caused by a reduced leaf area, primarily caused by an osmotic potential effect. The greater suppression of vegetative growth compared to the seedling growth (Figure 6.5), can possibly be attributed to a cumulative effect of the osmotic potential on the leaf area as the number of leaves increased, leading to a reduction of total photosynthesis and growth. Although the leaf area of maize was not significantly decreased, it did tend to be less (Table 6.7). Alternatively the growth decrease may also be due to antagonistic cation and anion effects (6.4.2). A further possibility is that the difference between the seedling and the vegetative growth of maize may also be due to the difference in composition, especially the Ca and Mg contents and ratios, of the two waters used (Mine C 3/95 vs. Mine A 2/94, Table 3.1).

Although the tolerance of **cowpea** has been connected to Cl exclusion (Keating, 1986), decreased osmotic potential may be an additional growth-retarding factor. As the lime-treated acid mine drainage water resulted in a significant reduction of the leaf area in the vegetative growth stage (Table 6.7), it is possible that the vegetative growth of cowpea was affected in a similar manner to that attributed to maize by Cramer (1994).

It is therefore possible that if osmotic potential suppresses the vegetative growth of a particular crop or cultivar, mainly via decreasing the leaf area, this could result in greater sensitivity of such crops in the vegetative growth stage compared to the seedling stage.

3. Crops where the relative *vegetative growth* and thus the tolerance was *similar to* the seedling growth. This seemed the case with **sorghum**, **pearl millet** and **wheat**, and probably also **oats**, **triticale** and **ryegrass**, although there were indications of better growth in the

vegetative stage of these last mentioned crops, but to a much lesser extent than with lucerne, rye and sunflower (Figure 6.5). With these crops osmotic potential seems to have a similar effect in both the seedling and vegetative stage.

The decrease of the osmotic potential has been shown to be the major property of salinity by which the growth of **maize**, **sorghum** and **pearl millet** is suppressed (Cramer, 1994, maize; Ashraf & Idrees, 1995, pearl millet; and Shannon, 1997, sorghum). The relative leaf growth of sorghum in the vegetative stage tended to be affected less than that of maize (Table 6.7). This is possibly one reason why the growth of sorghum was affected to a lesser extent than that of maize in the vegetative growth stage. The seedling growth of sorghum and maize did not differ markedly.

The seedling and vegetative growth stages of wheat and other cereals are the most sensitive in the case of NaCl salinity (Maas & Poss, 1989). Generally the sensitivity of wheat, as of other cereals, decreases with age (Francois & Maas, 1994; Maas & Poss, 1989). This sensitivity is mainly connected to Cl toxicity with osmotic potential exerting a lesser influence, which may be why seedling and vegetative growth are affected in a similar way by the $CaSO_4$ water.

Vegetative growth at higher concentrations of SO_4 water?

When interpreting the results of the vegetative growth stage, it must be taken into account that it was evaluated at much lower concentrations than that used for the seedling growth stage (Mine A *ca* 1000 or 1600 vs. Mine C *ca* 2500 mg L^{-1} SO_4). These evaluations may thus not be representative of vegetative growth in the higher concentration range especially between 2000 and 2500 mg L^{-1} SO_4.

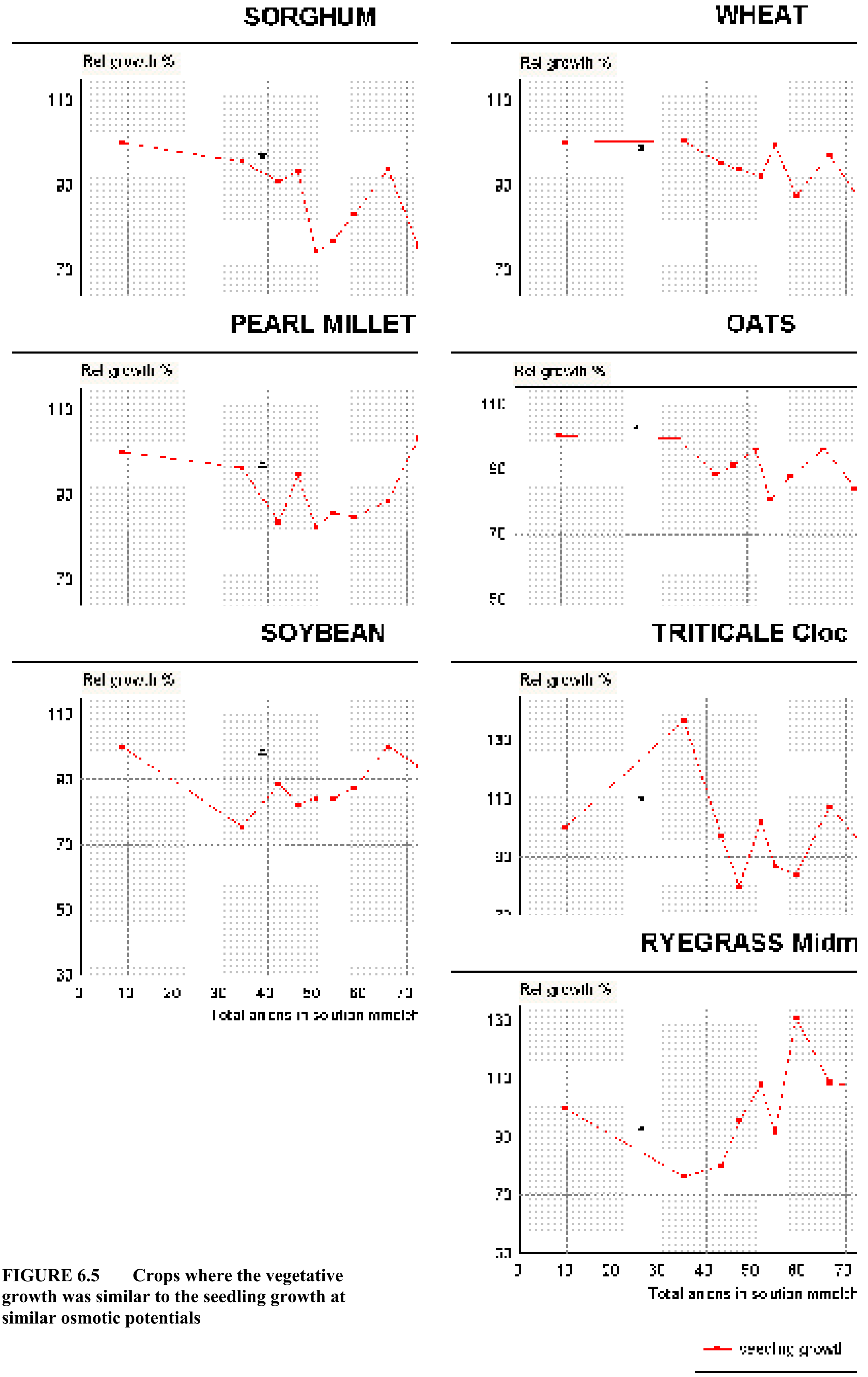


FIGURE 6.5 Crops where the vegetative growth was similar to the seedling growth at similar osmotic potentials

These responses together with that of the *seedling* growth at the higher concentrations may, however, serve as indications of what might be expected in the vegetative growth stage at higher concentrations; this would, however, depend greatly on whether the mechanisms by which growth is affected or adapted to salinity are similar in these two growth stages. The tolerance of cereals to NaCl-salinity has generally been found to increase with ontological development (Francois & Maas, 1994). This too would depend on the mechanism by which growth is suppressed or adapted in a high SO_4 water compared to that of NaCl water in the two growth stages.

If the mechanisms of the adverse effects of salinity, and of adaptation are known, it may serve as indications by which the vegetative growth may be predicted:

In **maize** where the decreased osmotic potential has been found to be the major suppressing quality of salinity in both the seedling and vegetative growth stages (Cramer, 1994), the vegetative growth can also be expected to decrease further with increasing concentrations of $CaSO_4$ mine water. If the particular cultivar possesses an osmotic adaptation mechanism, this may be less marked.

In contrast the seedling growth of **lucerne** is mainly affected by osmotic potential and the mature growth by Cl toxicity (Noble et al., 1984), Na content (Ashraf & O'Leary, 1995) and osmoregulation (Petrusa & Wincov, 1997). A $CaSO_4$-dominated water at the higher concentrations may thus result in decreased seedling growth but increased vegetative production due to the absence of Cl, and the presence of osmoregulation and nutrient effects.

In the case of **annual ryegrass** osmoregulation is stimulated by an increase of cations in the external medium (Sagi et al., 1997); it is therefore possible that at higher concentrations of the Ca/Mg/SO_4 water (Kleinkopje) the vegetative growth will also increase, as was the case with the seedling growth on simulated Kleinkopje water (Figure 6.5).

Although salt tolerance of the Tritiaceae tribe has been found to be poligenic (Zong & Dvorak, 1995), **temperate cereals** generally tolerate saline waters by Na and/or Cl exclusion or are affected by a Na/Ca imbalance (Francois et al., 1989). The tolerance for $CaSO_4$ water would thus depend on the Na/Cl content of such a water and also, but to a lesser extent, on the sensitivity of a particular crop cultivar for osmotic potential decreases.

These examples suffice to illustrate that the tolerance of the vegetative stage to $CaSO_4$-dominated water will mainly depend on the adverse and adaptive mechanisms in this growth stage. As Na and Cl are virtually absent in this water, the vegetative growth of the crops where ionic effects are the main cause of sensitivity, should be more tolerant to these $CaSO_4$-dominated waters. Where osmotic potential is the main suppressive mechanism, the vegetative growth is also expected to be sensitive. The response to this water will also depend on the ability and degree of osmotic adaptation in the particular crop or cultivar.

6.6 CONCLUSION

The relative germination percentage of most of the cultivars of both the subtropical and temperate annual crops was not influenced by either the $CaSO_4$- or NaCl-dominated mine waters used. Some cultivars of sorghum, pearl millet and soybean were moderately sensitive to the $CaSO_4$ water, but significant decreases for these did not exceed 12 % which could be easily compensated for by sowing more densely.

A comparison of seedling and vegetative growth at similar osmotic potentials of $CaSO_4$-dominated waters revealed that:

- the vegetative growth of sunflower cv. SNK 43, rye cv. SSR 1, lucerne cv. PAN 4860 and the yield of dry bean cv. PAN 122, was increased on this water, and that the tolerances of the vegetative growth stage were much *greater* than that of the seedling growth stage;

- maize cv. SNK 2340 and cowpea cv. Dr Saunders were possibly *more sensitive* in the

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vegetative than in the seedling growth stage; and

- the tolerance of sorghum cv. PAN 888, pearl millet cv.SA Standard, soybean cv. Ibis, wheat cv. Inia, oats cv. Overberg, triticale cv. Cloc 1 and annual ryegrass cv. Midmar *did not differ* markedly in these two growth stages.

It is suggested that the vegetative growth may be

- *more tolerant than seedling* growth in crops where tolerance in the vegetative stage is generally correlated to ionic effects and/or osmotic adaptation, rather than to a sensitivity to osmotic potential effects as is probably the case in the seedling stage. Nutrient effects may be responsible for stimulation of growth or of osmotic adaptation.

- *more sensitive than seedling growth* in crops where a reduction of growth appears to be due to the decreased osmotic potential reducing the leaf area. Where osmotic potential is nevertheless the growth reducing mechanism in the vegetative stage, but without affecting leaf area, vegetative growth may be influenced in a similar way to the seedling growth.

It is concluded that if the concentration of Na, Cl, or other possibly toxic ions, are negligible in a $CaSO_4$-dominated water, the tolerance of crops is mainly connected to the degree in which growth in the respective growth stages is affected by external osmotic potential decreases.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

This research originated from the need to dispose of large volumes of $CaSO_4$-dominated water generated in the neutralization of acid mine drainage water with lime in coal mining operations in the eastern highveld of Mpumalanga, South Africa. One possible means of disposal is the use of such waters for irrigation of agricultural crops.

Previous research on the influence of $CaSO_4$-dominated waters on plant and crop growth has, however, been extremely limited. Where researched, it mainly pertained to the influence on yield, quality of crops and the influence on soil chemistry (du Plessis, 1983; Papadopoulos, 1986; MacAdam et al., 1995).

The current studies were initiated to screen crops and cultivars already cultivated in the geographical area of the coal mines, for tolerance to $CaSO_4$-dominated mine waters, as part of a South African Water Research Commission funded project. As salt tolerance is a multifaceted concept, varying with many environmental and biological factors, the use of such waters for irrigation warranted more information than simply the yield response.

The main focus of this study was to investigate **seedling growth responses to increasing concentrations of Ca, Mg and SO_4 and the influence of the precipitation of gypsum on growth** response curves. It is generally expected that the relatively low solubility and precipitation of gypsum would limit extreme increases of salinity which could be beneficial for crop growth. In order to gain some insight into the role of gypsum precipitation on growth, suspensions where $CaSO_4$ crystals were increasingly present, were included in the growth response treatments.

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The seedling growth stage is generally regarded as the most sensitive of growth stages and was therefore considered to be the best developmental stage to demonstrate growth response differences.

The seedling growth of most of the crops followed an irregular three-piece or four-piece pattern in the growth curves:

> In the *first* and *second* parts of the growth response curve, where the concentrations of Ca and SO_4 in the treatment solutions were below the solubility product for $CaSO_4$, seedling growth generally decreased in a linear manner above a threshold salinity, which was similar to the growth response curves expounded by Maas & Hoffman (1977). The continued linear decreases up to the saturation concentrations indicated that in this part of the growth curve salinity increases were not limited by precipitation during evapotranspiration. It is suggested that the rate of precipitation was probably too slow, due to the absence of crystallizing nuclei in the quartz sand and to the short time intervals between replenishing. Seedling growth generally reached a minimum where saturation concentrations of Ca and SO_4 were present in the original treatment solutions.
>
> In the third part of the growth curve, where undissolved $CaSO_4$ crystals were increasingly present, seedling growth increased unexpectedly, despite further increasing conductivities of the treatment solutions. These growth curves suggest that the seedling growth was improved when crystallizing nuclei were present in the growth medium.
>
> In the treatment with the highest concentration, where the Mg to Ca ratio in solution was ≥1, seedling growth finally decreased again. In practice such a situation could develop in an arid area with prolonged irrigation with a $CaSO_4$ water with relatively high Mg content, where leaching with good quality water is not possible.
>
> When increasing SO_4 concentrations were obtained with Na_2SO_4 in a simulated mine water saturated with $CaSO_4$, seedling growth generally tended to decrease in a linear manner.

It is suggested that when the solubility product is reached, whether by evapo-transpiration or in treatments with higher concentrations, the effective osmotic potential - that is the average osmotic potential in the root growth zone over the whole growth period - is determined by the rapidity of the precipitation of gypsum. In a *soil* environment this rate of precipitation may be stimulated by the abundantly present soil nuclei which would have an eventual effect of *increasing* the effective osmotic potential and probably also the seedling growth. Drying of the soil in a field situation should thus not lead to the same rate of concentration by evapotranspiration, with a concomitantly lower osmotic potential, as in the sand culture. If the soil water is kept at field capacity by frequent irrigation, precipitation may, however, also be slower than with longer time intervals and subsequent drying of the soil. The rate of precipitation may, however, also be *inhibited* by coatings of different humic and mineral substances present in soils, for instance aluminium phosphates on nuclei in acid soils (as quoted by Van den Ende, 1991). The extent of such an influence would depend on the amount of coating.

It has furthermore been found for annual ryegrass that osmoregulation is influenced by the cation concentration in the plant tissue and therefore indirectly by that of the soil solution (Sagi et al., 1997). The decrease of the Ca content of the soil solution by rapid precipitation may therefore also diminish the osmotic adaptation and growth of ryegrass and possibly of other crop species with a similar osmoregulation metabolism.

For practical agronomic application it was also important to establish **whether cultivars** of crops likely to be utilized in this area, **differed in their tolerance** to this type of water.

Cultivar differences on a $CaSO_4$-dominated water were investigated in water culture in a glasshouse, where the seedling growth of several cultivars of each crop species was compared on actual $CaSO_4$-dominated mine waters from the Kleinkopje mine. There were significant cultivar differences in the seedling growth of maize, sorghum, pearl millet, dry bean, wheat and lucerne with the $CaSO_4$ water. Soybean cultivars did not differ in their response to this water. The subtropical cereal crops exhibited more cultivar differences with the high sulphate water than

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did the subtropical legumes and the annual temperate cereals.

These cultivar differences found in the seedling growth stage may not necessarily be as prominent in the mature stages. When considering irrigation with these mine waters, it should nevertheless be taken into consideration that the yield of especially the cereals can be influenced by the effect of salinity on the primordial development of spikelets in the seedling growth stage (Francois & Maas, 1994).

It must be emphasized that the crops and cultivars used in these trials were selected for their tolerance under irrigation or drought conditions, and were therefore probably genetically developed for salinity. Other cultivars not suitable for irrigation may be more sensitive to $CaSO_4$-dominated water, especially if the tolerance of a specific crop is mainly related to the osmotic potential effect of salinity.

It can therefore be said that although the seedling growth of some cultivars, especially of the subtropical cereals, was decreased by a saturated $CaSO_4$ water, the seedling growth of several high yielding cultivars was tolerant enough to be successfully utilised for irrigation with these waters.

Tolerance to NaCl-dominated water has been found to differ during *ontological development*. The findings with seedling growth may therefore not be applicable to further growth stages. This would depend on the property/ies of this type of water that are responsible for limiting growth of specific crops/cultivars in the respective growth stages. **Possible differences in the tolerance of the germination, seedling and vegetative growth stages** with actual $CaSO_4$-dominated mine waters were therefore investigated.

- The relative *germination percentages* of most cultivars of both the subtropical and temperate annual crops were not influenced by the $CaSO_4$-dominated mine waters used. This is in agreement with observations that germination percentages of most crops are generally not affected at osmotic potentials below *ca* 700 mS m^{-1} for NaCl salinity (Francois and Maas,

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1994). There were exceptions where germination percentages of some cultivars of sorghum, pearl millet and soybean were slightly suppressed with sulphate salinity. Germination should, however, not be a problem if these crops are irrigated with these waters. Where it was suppressed, it ranged from 5 to 12 %, which could be compensated for by sowing more densely.

- The relative *seedling growth* on both the actual 'worst case' and the simulated $CaSO_4$ mine waters showed that the *subtropical cereal* crops exhibited greater sensitivity with the high sulphate water than the subtropical legumes or the annual temperate crop species. The soybean cultivars were not significantly affected while most of the dry bean cultivars showed significant *increases*. The seedling growth of the annual temperate crops did not generally show significant sensitivity.The seedling growth of rye, oats, ryegrass and barley cultivars was not significantly decreased on the actual mine water, but with simulated $CaSO_4$ mine water in the growth response curves, the seedling growth of wheat, rye and oats was significantly decreased, although at higher salinities than were present in the actual mine water. The concentrations where growth started to decrease (the threshold) were lower with the subtropical cereals than those of the temperates. The relative seedling growth of wheat was more tolerant to the sulphate water when N was partly supplied as NH_4 in the ratio of NO_3 to NH_4 of 2:1 rather than 4:1. All the selected lucerne cultivars were significantly sensitive to the sulphate mine water in the seedling growth stage.

The tolerance to the $CaSO_4$-dominated mine water was generally greater than with the NaCl-dominated mine water where the seedling growth of the majority of cultivars, with the exception of oats and cowpea, was significantly and severely suppressed.

The data suggest that crops and cultivars which are generally sensitive to the decreased osmotic potential of salinity such as maize, sorghum and pearl millet - and lucerne in the seedling stage - are more sensitive to this $CaSO_4$water in the seedling growth stage than crops where tolerance is mainly connected to ionic effects of Na and Cl.

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The known mechanisms by which salinity generally affects the salt tolerance of specific crops, may therefore be an indication of how the seedling growth of the respective crops will respond to this type of water. As salt tolerance is mostly poligenic, the tolerance to $CaSO_4$ water of the crop species which are subject to ionic effects, would also depend on the degree to which the decreased osmotic potential of salinity contributes to sensitivity (Shannon, 1997).

- A comparison of the tolerance in the *seedling* versus the **vegetative growth stages** for $CaSO_4$-dominated waters at similar osmotic potentials revealed that tolerance in the vegetative growth stage could be much *greater*, *less* or *similar* in a specific crop cultivar :

  - The vegetative growth of sunflower cv. SNK 43, rye cv. SSR 1, lucerne cv. PAN 4860 and the yield of dry bean cv. PAN 122 was increased on this water, with the tolerance being *much greater than in that of the seedling growth stage*.

    It is suggested that the vegetative growth may be more tolerant than seedling growth in crops where sensitivity in the vegetative stage is generally correlated to ion imbalances - such as Na/Ca (rye) and Na/K ratios (dry bean) - accumulation effects of Na and/or Cl (lucerne), and/or osmotic adaptation (sunflower).

    Stimulation of the relative growth may also be due to a nutrient effect of for instance Ca and S in legumes or to the improvement of osmotic adaptation caused by the high Ca and Mg content as seemed to be the case with annual ryegrass (Sagi et al., 1997).

  - The tolerance of maize cv. SNK 2340 and cowpea cv. Dr Saunders *was less in the vegetative growth stage than in the seedling stage.*

  - The tolerance of sorghum cv. PAN 888, pearl millet cv. SA Standard, soybean cv. Ibis, wheat cv. Inia, oats cv. Overberg, triticale cv. Cloc 1 and annual ryegrass cv. Midmar did *not differ markedly in these two growth stages*. Whether this would be the case at

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higher concentrations remains unanswered, but it would probably depend on the degree of sensitivity to osmotic potential of the specific crop cultivar in the vegetative growth stage. In the case of ryegrass, tolerance can be expected to increase with increasing concentrations as osmoregulation is indirectly related to the cation content of the growth medium (Sagi et al., 1997).

It is suggested that vegetative growth may be affected more than seedling growth in crops where a reduction of growth appears to be caused by a reduced leaf area which is primarily due to an osmotic potential effect, as is the case with maize (Cramer, 1994). With cowpea, the SO_4 water also resulted in a significantly reduced leaf area and may therefore have had an effect similar to that of maize on the vegetative growth.

In the case of sorghum and pearl millet, where osmotic potential is nevertheless an accepted growth reducing mechanism in the vegetative stage, the leaf area was not affected; vegetative growth may therefore tend to be influenced in a similar way to the seedling growth.

It is therefore clear that tolerance to a $CaSO_4$-dominated water may be influenced by the growth stage during which crops are irrigated.

These observations, together with the above conclusions on growth suppressing mechanisms in the seedling growth stage, explain why the presence of high concentrations of Na, Cl, Mg and other possibly toxic ions in $CaSO_4$ waters should also be taken into account when evaluating such waters for irrigation in the seedling growth stage.

It is concluded that the tolerance to a $CaSO_4$-dominated water depends mainly on the sensitivity of a crop or cultivar to external osmotic potential and on the chemical composition of specific irrigation waters. The severity of suppression would furthermore depend on the rapidity of $CaSO_4$ precipitation, which influences the effective osmotic potential; the rate of precipitation may in turn be influenced by soil properties, the rate of evapo-transpiration and the time interval between irrigations. It is suggested that a knowledge of the major tolerance/sensitivity mechanisms generally operative in a specific crop species and in the different growth stages, would be helpful in indicating tolerance to this type of water.

Probably the most pressing question on $CaSO_4$ salinity for future use is **which property/ies of such a water are the major growth suppressing (or stimulating) properties of $CaSO_4$-dominated waters?** There are two possibilities that warrant consideration, namely *osmotic potential* and negative or positive *nutrient effects*.

When evaluating the various investigations in this study, it was noticed that it was mostly the same crops that were sensitive to $CaSO_4$-dominated waters. These crops were maize and sorghum in both the seedling and vegetative stage and lucerne in the seedling growth stage. The common characteristic shared by these crops is that the property of NaCl-salinity that mainly suppresses their growth has been found to be the lower osmotic potential of the external saline growth medium.Other crops such as soybean, wheat, triticale and rye, where ionic effects such as accumulation or nutrient imbalances by Na and/or Cl were the main growth suppressing properties of salinity, were generally more tolerant to the $CaSO_4$ waters.

Salt tolerance of plants may, however, be poligenic with, for instance, both osmotic potential and ionic properties affecting growth in saline conditions (Shannon, 1997). It is therefore possible that osmotic potential effects can also contribute to the salt tolerance, even though ionic effects have been found to be the major suppressing property for a specific crop. As Na and Cl were virtually absent in the $CaSO_4$ waters used it is suggested that the degree of suppression on thése crops could be an indication of the sensitivity to osmotic potential effects.

Another possibility is that *interactions* of the high Ca and SO_4 with other nutrients may have affected growth. The current research was mainly designed to investigate growth responses. Some indications could, however, be elicited from firstly comparing seedling growth responses with increasing concentrations of $CaSO_4$, Na_2SO_4 and NaCl respectively at similar osmotic potentials of the applied treatment solutions, and secondly from the nutrient analyses of the top growth.

Generally $CaSO_4$ treatments decreased seedling growth *less* than NaCl or Na_2SO_4 at similar osmotic potentials of the treatment solutions. Because the actual effective osmotic potentials in the root zones were influenced by evapo-transpiration and the precipitation of $CaSO_4$, a comparison with the in situ osmotic potentials of the root growth zones was, however, not possible. No conclusive deductions on Ca and SO_4 interactions with other nutrients in the seedling stage could therefore be drawn from these comparisons. Nutrient analyses of the top growth of the maize seedlings did not, however, reveal any antagonistic cationic or anionic effects in the seedling growth stage. On the other hand severe chlorosis did develop in wheat seedlings with the $CaSO_4$ water when less NH_4 was applied (Ströhmenger et al., 1999).

In the vegetative growth stage nutrient analyses of the top growth showed that the high concentrations and uptake of Ca and SO_4 with the lime-treated acid mine drainage water did not generally affect the uptake of other nutrients. There were however, two exceptions:

> The N concentration in maize top growth was decreased significantly. The significant decreases in stem growth of maize and sorghum may be related to either less growth due to an osmotic potential effect, or the high concentrations of Ca and SO_4 could have influenced growth via nutrient effects. A white marginal chlorosis on a few of the younger mature leaves of soybean could also have been an indication of Mo deficiency (Bennett, 1993), although differential plant analyses were not carried out to confirm this.
>
> The second exception was decreases of Mg concentrations in the temperate crops, and increases in the summer crops, which were not reflected in visual symptoms of the top growth.

This water may also be *nutritionally beneficial* to crops. It may benefit crops such as legumes that have a high Ca and S requirement. The exceptionally good yield of dry bean and lucerne with this water illustrates the possibility of such an effect. In ryegrass the increased Ca and Mg content probably stimulated the metabolic process of osmoregulation (Sagi et al., 1997).

Excessive rainfall in the area of these coal mines has also been known to result in S deficiencies in crops in subsequent seasons which could be alleviated when irrigating with this water.

It is concluded that the tolerance to a $CaSO_4$-dominated water where the concentrations of Na, Cl or other possibly toxic ions are negligible, is mainly related to the degree in which growth of a specific crop cultivar is affected by the external osmotic potential in the different growth stages. Possible nutrient interactions, especially between SO_4 and N nutrition should, however, be investigated in greater depth.

When considering the tolerance of a crop species or cultivar for irrigation with a $CaSO_4$-dominated water it is important that the following should be taken into account :

Climate - if leaching with adequate rainfall or good quality water is not possible it must be kept in mind that leaching with this type of water is ineffective in controlling the electrical conductivity derived from the $CaSO_4$; the latter can lead to salinity with a higher conductivity than that of the irrigation water (Papadopoulos, 1984).These mines are, however, situated in a summer rainfall area, which should prevent a serious salt build-up. In an arid climate - besides salt build-up - the precipitation of gypsum from waters with appreciable amounts of Mg may also lead to ratios of Mg to Ca that could be detrimental to growth.

The *composition of the water* - as the tolerance to this type of water was found to be closely related to the salt tolerance/sensitivity mechanisms operative in specific crops, the presence of high concentrations of Na and Cl may affect crops where tolerance is related to ionic effects. As mentioned above, a too high Mg content may also influence growth negatively.

Soil types to be irrigated can influence the usefulness of this water. It is a well known practice to improve sodic soils with the application of gypsum. Precipitation or absorption of Ca could, however, influence growth via an increase in the fraction of Na in the soil solution and therefore also the soil solution sodium adsorption ratio (SAR), and the permeability of the soil. In an evaluation of a lime-treated acid mine drainage water du Plessis (1983), however, concluded that

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no serious Na related soil physical problems were expected with this type of water. On a calcareous soil the precipitation of $CaCO_3$ and the formation of $MgSO_4$ ion pairs can enhance gypsum solubility and SO_4 accumulation in the soil solution (MacAdam et al., 1997).

The major **conclusion** of the current research is therefore that the *sensitivity* to $CaSO_4$-dominated water is mainly related to osmotic potential effects, whereas *tolerance* is found in crops that are generally sensitive to ionic effects and in crops that possess the ability of osmotic adaptation.

It is furthermore suggested that the effective osmotic potential of the soil solution (i.e. the average osmotic potential throughout the whole growth period) is determined by the rapidity of the precipitation of gypsum which in turn can be influenced by growth rate (evapotranspiration), temporal, environmental and soil factors. A decrease of Ca in the soil solution by rapid precipitation may also suppress the growth of crop species such as annual ryegrass where the cation content of the soil solution may indirectly influence organic syntheses related to osmoregulation.

In summary it can be said that the major growth suppressing property of a $CaSO_4$-dominated water is the decreased osmotic potential. Nutrient effects were less prominent, but there were indications of a possible effect on N nutrition and Mg uptake.

These conclusions have the practical advantage of facilitating the choice of suitable crops and cultivars for irrigation with $CaSO_4$-dominated water, which may be very different from those hitherto recommended for NaCl-dominated waters. They also give some insight into environmental conditions where the use of this water would be advantageous or harmful to crop growth and soil properties in the long-term. These conclusions may also be useful in crop growth models which incorporate a $CaSO_4$-dominated type of salinity.

The following areas of future research into the tolerance of agronomic crops and pastures to

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$CaSO_4$-dominated water are recommended:

- The dynamics of gypsum precipitation during evapo-transpiration in different soil types and its influence on the effective osmotic potential.

- The influence of high Ca and SO_4 content on N nutrition and other nutrient interactions at concentrations encountered in saturated gypsum waters, together with the fertilization needed to ensure balanced nutrition with this type of water.

- The effect on the tolerance in the reproductive stage, yield and especially on the quality of fodder and grains for animal and human consumption.

- The tolerance should be further tested under practical field conditions on the different soil types found in the agricultural areas within reach of these waters. Careful monitoring of plant growth, soil conditions - both chemical and plant nutritional - and drainage water would be required.

- The long term effect of a saturated $CaSO_4$ water on the physical, exchange and soil solution properties of the local soil types and its influence on crop growth.

- Other aspects relating to gypsiferous water, such as wetland dynamics, other possible usages as for instance for hydroponic culture or the cultivation of plant species used for purposes other than human or animal nutrition, could also be followed up.

It is clear that a large spectrum of agronomic and pasture species have the growth potential with gypsiferous water to make irrigation with this type of water viable. With this knowledge of plant tolerance and the necessary irrigation and fertilization management, $CaSO_4$-dominated water could play an important role in at least augmenting irrigation water, of which both the supply and quality are steadily decreasing in the Mpumalanga region of the Republic of South Africa. Whether this can be done in an environmentally acceptable manner is currently being further

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investigated in a comprehensive multifaceted project co-sponsored by the Environmental Services of the AMCOAL mining group and the South African Water Research Commission.

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APPENDIX A

PLANT MATERIALS USED IN GLASSHOUSE AND LABORATORY SCREENING

SUBTROPICAL ANNUAL CROPS

Maize

1.	SNK 2042	Yellow; excellent performance with stress: drought resistant; also used with irrigation; medium growth length; planting early to medium.
2.	SNK 2888	Yellow; good performance with stress; good drought resistance; good with irrigation; good acid tolerance (Al); medium growth length.
3.	SNK 2266	Yellow; performs well on acid soil.
4.	SNK 2151	White; performs well over a wide range of environments - dryland and irrigation; very good acid tolerance; medium growth length.
5.	SNK 2665	White; performs with stress (dryland) and irrigation; very good acid tolerance (also Al); medium-tall growth length; suitable for most planting times.
6.	PAN 6480	Yellow; outstanding agronomic balance; very good resistance to grey leaf spot; medium growing season.
7.	PAN 6364	Yellow; exceptionally high yield potential; proved under drought stress; medium-short growing season.
8.	PAN 6552	Yellow; high potential; quick grain fill with particularly good standability; medium growing season.

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9.	PAN 6363	White; quick, recommended for late plantings.
10.	PAN 6549	White; outstanding performance under widely varying conditions; known for good standability and grain quality.
11.	PAN 6479	White; good performance under widely varying conditions including stress; outstanding resistance to grey leaf spot.
12.	CRN 3816	Yellow
13.	CRN 3414	Yellow
14.	CRN 3818	Yellow
15.	CRN 3631	White
16.	CRN 4403	White
17.	CRN 4523	White
18.	SNK 2340	Yellow; performs particularly well in eastern Highveld; good with centre pivot irrigation and dryland conditions; for early planting; medium growth length. SNK 2340 was also used in the vegetative evaluation and in the field trials.

Sorghum

1.	SNK 3860	Grain; birdproof; very high hay production; used in Middelburg/Stofberg area.
2.	SNK 3939	Grain; sweet malt (GM); excellent (outstanding) production; any planting date; medium growth length.
3.	SENFOR	Forage; very high forage production; regrowth very fast; high protein; very palatable.

4.	SENTOP	Forage; very high forage production; regrowth very good; high protein; low hydrocyanic acid.
5.	SNK 3000	Grain for ensiling; high biomass and grain; medium growth length; good drought resistance.
6.	PAN 8494	
7.	PAN 8501	Grain; good livestock feed (sweet type); medium to long growing season; stands exceptionally until harvest; strong "stay-green" characteristic; short even plant with thick stalk.
8.	PAN 8522	
9.	PAN 8564	Grain; reliable medium to long growing season; good yield potential; good malting and feed characteristics.
10.	PAN 8591	Grain; medium to long growing season; good yield potential; medium plant height; wide area adaptability; GM malt class.
11.	NK 283	Industrial standard (PANNAR); most popular sorghum hybrid; high yield potential, long growing period.
12.	PAN 888	Leafy forage hybrid; performs well on marginal soils; also used in the vegetative evaluation and field trials.
13.	CRN 766W	
14.	CRN 7686	

Pearl Millet (Babala)

1.	PAN 911	Hybrid forage millet; outstanding summer grazing; recovers quickly after drought; can be planted as soon as soil temperatures are suitable

		(early October); also for haymaking and ensiling. Seed variable.
2.	SA Standard	The same seed that was used in the vegetative evaluation and field trials. Seed variable.

Soybean

1.	Bakgat (Sensako)	Short growing season; planting time 15 November to 15 December; short growth length; used for irrigation.
2.	Ibis (Sensako)	Strongly recommended for warmer areas. Also used in sand culture pot trials and in the field trial.
3.	PAN 494	Top performance; excellent standability; intermediate growth habit; good protein and oil content.
4.	PAN 577G	Short to medium growing season; recommended for coal production areas; recommended for later plantings in warm areas; very good standability; stable above average yield potential; fairly branched upright determinate growth habit.
5.	PRIMA (Pannar)	Most widely planted in Highveld; medium-short growing period; excellent yield potential; widely recommended particularly for temperate regions.
6.	HUTCHESON (Pannar)	
7.	A 2233 (Carnia)	
8.	A 5409 (Carnia)	
9.	A 7119 (Carnia)	

Dry bean (for furrow irrigation)

1.	PAN 122	small white canning bean

2.	PAN 127	speckled sugar bean
3.	MKUSI	very aluminium tolerant; does well in marginal conditions; responds very well to fertiliser; seed type carioca not popular; soil temperature critical - must be at least 11-12°.
4.	NANDI	genetically similar to MKUSI with the same characteristics.

Cowpea

1.	Dr Saunders	used in field trial - generally produces better under hot, dry conditions; generally not well adapted to cooler areas.

Sunflower

1.	SNK 43	Medium-long growing period; increased resistance to disease.
2.	SNK 34	Short growing period; early-late and late planting; drought resistance good; short growth length.
3.	SNK 37	Medium-long growing period; early and first in later planting, drought resistance good; used with irrigation on Highveld.
4.	PAN 7392	Medium growing period; top performer in National trials.
5.	PAN 7411	
6.	PAN 7369	Medium growing period; high potential; very adaptable; best yield reliability of all cultivars in the one to two ton category.
7.	CRN 1445	
8.	CRN 543	
9.	A 1006 9 (CARNIA)	

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TEMPERATE ANNUAL CROPS

Oats

1.	SSH 421 (SENSAKO)	Plant height - tall; medium growing season; fast grower can be cut every 3 weeks.
2.	SSH 423 (SENSAKO)	Plant height - tall; medium/late growing season
3.	Witteberg (Small Grain Centre)	
4.	Perdeberg (Small Grain Centre)	
5.	Echidna (Small Grain Centre)	
6.	Overberg	Probably developed for winter rainfall area; the same seed that was used in the vegetative evaluation and in the field trial

Barley

1. Stirling (Small Grain Centre)

Triticale

1.	Kiewiet (Small Grain Centre)	
2.	SShR1 (Small Grain Centre)	
3.	Rex (Small Grain Centre)	
4.	PAN 299	
5.	SSKR 626 (SENSAKO)	Tall; fast grower; very late
6.	SSKR 628 (SENSAKO)	Tall; slow grower; very late; used for winter pasture

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7.	Cloc 1	Same seed as used in the vegetative evaluation and field trial

Wheat (all cultivars recommended for irrigation as in cooler eastern Highveld areas)

1.	SST 822 (replaces SST 86)	Short growth period; best response to increasing N-fertilisation; sensitive to drought stess; needs efficient irrigation management; good resistance to sprouting; good Al tolerance.
2.	SST 825	Medium growth period.
3.	Palmiet	Medium growth period; poor Al tolerance (?); good resistance to sprouting.
4.	Marico	Longer growth period; poor Al tolerance.
5.	Kariega	Longer growth period; poor Al tolerance.
6.	Inia	For later planting; also popular for warmer Transvaal irrigation regions, e.g. Springbok flats; poor Al tolerance; used in vegetative evaluation and field trial.
7.	Wheat cultivar bred for use as a nursecrop on mine spoils from USA.	

Rye

1.	SSR 727	Same qualities as SSR 1 but resistant to aphids.
2.	SSR 729	Drought resistant
3.	SSR 1	Uses moisture efficiently; also used in vegetative evaluation and field trial
4.	Henoch	

Ryegrass

1.	Macho	
2.	Dargle	
3.	Apollo 64	
4.	Midmar	Used in vegetative evaluation and field trial

TEMPERATE PERENNIAL

Lucerne (used for seedling trials)

1.	PAN 4860	Good for Highveld; Feb/March planting; synthetic composite - some genetic variation; also used for vegetative evaluation and in field trial.
2.	PAN 4581	Good for Highveld; Feb/March planting. Synthetic composite - some genetic variation.
3.	Baronet	
4.	Topaz	Used with irrigation; high biomass.
5.	Diamond	

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APPENDIX B

ANALYSES OF FINAL SAMPLES OF MINES A, B & C WATER

MAINLY FOR TRACE METALS

MINE A - lime-treated acid mine drainage water Kromdraai		
DETERMINAND	**UNIT**	**RESULT**
Major inorganic determinands		
PH		6.6
NH_4-N	mg/l	3.10
$NO_3 + NO_2$-N	mg/l	1.42
F	mg/l	0.5
TAL AS $CaCo_3$	mg/l	10
Na	mg/l	4
Mg	mg/l	20
Si	mg/l	< 0.4
PO_4-P	mg/l	0.028
SO_4	mg/l	1386
Cl	mg/l	4
K	mg/l	2.7
Ca	mg/l	552
EC	mS/m	219.0
TDS	mg/l	1991
Trace metals		
Be	mg/l	< 0.001
Be-ACID SOL	mg/l	< 0.001
B	mg/l	< 0.002
B-ACID SOL	mg/l	< 0.002
Al	mg/l	0.673
Al-ACID SOL	mg/l	0.741
Ti	mg/l	< 0,001

DETERMINAND	UNIT	RESULT
Ti-ACID SOL	mg/l	< 0.001
V	mg/l	0.028
V-ACID SOL	mg/l	0.052
Cr	mg/l	0.063
Cr-ACID SOL	mg/l	0.068
Mn	mg/l	2.159
Mn-ACID SOL	mg/l	2.128
Fe	mg/l	< 0.003
Fe-ACID SOL	mg/l	0.114
Co	mg/l	< 0.005
Co-ACID SOL	mg/l	0.012
Ni	mg/l	0.068
Ni-ACID SOL	mg/l	0.075
Cu	mg/l	< 0.004
Cu-ACID SOL	mg/l	0.103
Zn	mg/l	< 0.003
Zn-ACID SOL	mg/l	< 0.003
Sr	mg/l	0.312
Sr-ACID SOL	mg/l	0.256
Zr	mg/l	< 0.001
Zr-ACID SOL	mg/l	< 0.001
Mo	mg/l	< 0.006
Mo-ACID SOL	mg/l	< 0.006
Cd	mg/l	< 0.001
Cd-ACID SOL	mg/l	< 0.001
Ba	mg/l	0.015
Ba-ACID SOL	mg/l	0.018
Pb	mg/l	< 0.020
Pb-ACID SOL	mg/l	< 0.020

MINE B - $CaSO_4$-dominated mine water from the Kleinkopje mining area

DETERMINAND	UNIT	RESULT
Major inorganic determinands		
PH		8.5
NH_4-N	mg/l	1.18
$NO_3 + NO_2$-N	mg/l	< 0.04
F	mg/l	3.7
TAL AS $CaCo_3$	mg/l	299
Na	mg/l	1252
Mg	mg/l	48
Si	mg/l	4.1
PO_4-P	mg/l	0.029
SO_4	mg/l	1384
Cl	mg/l	871
K	mg/l	10.3
Ca	mg/l	49
EC	mS/m	570.0
TDS	mg/l	3984
Trace metals		
Be	mg/l	< 0.001
Be-ACID SOL	mg/l	< 0.001
B	mg/l	< 0.002
B-ACID SOL	mg/l	< 0.002
Al	mg/l	0.269
Al-ACID SOL	mg/l	0,406
Ti	mg/l	< 0.001
Ti-ACID SOL	mg/l	< 0.001
V	mg/l	0.050
V-ACID SOL	mg/l	0.053
Cr	mg/l	< 0.003
Cr-ACID SOL	mg/l	0.014
Mn	mg/l	< 0.001
DETERMINAND	UNIT	RESULT

Mn-ACID SOL	mg/l	< 0.001
Fe	mg/l	< 0.003
Fe-ACID SOL	mg/l	< 0.003
Co	mg/l	0.023
Co-ACID SOL	mg/l	0.030
Ni	mg/l	0.147
Ni-ACID SOL	mg/l	0.152
Cu	mg/l	< 0.004
Cu-ACID SOL	mg/l	0.039
Zn	mg/l	< 0.003
Zn-ACID SOL	mg/l	< 0.003
Sr	mg/l	4.006
Sr-ACID SOL	mg/l	3.547
Zr	mg/l	< 0.001
Zr-ACID SOL	mg/l	< 0.001
Mo	mg/l	< 0.006
Mo-ACID SOL	mg/l	< 0.006
Cd	mg/l	< 0.001
Cd-ACID SOL	mg/l	< 0.001
Ba	mg/l	0.077
Ba-ACID SOL	mg/l	0.073
Pb	mg/l	< 0.020
Pb-ACID SOL	mg/l	< 0.020

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M I N E C - NaCl-dominated mine water the New Denmark mine, Standerton		
DETERMINAND	**UNIT**	**RESULT**
Major inorganic determinands		
PH		8.3
NH_4-N	mg/l	0.18
$NO_3 + NO_2$-N	mg/l	0.29
F	mg/l	0.4
TAL AS $CaCo_3$	mg/l	94
Na	mg/l	56
Mg	mg/l	191
Si	mg/l	6.4
PO_4-P	mg/l	0.029
SO_4	mg/l	2065
Cl	mg/l	19
K	mg/l	10.9
Ca	mg/l	537
EC	mS/m	318.0
TDS	mg/l	2996
Trace metals		
Be	mg/l	< 0.001
Be-ACID SOL	mg/l	< 0.001
B	mg/l	< 0.002
B-ACID SOL	mg/l	< 0.002
Al	mg/l	0.615
Al-ACID SOL	mg/l	0.647
Ti	mg/l	< 0.001
Ti-ACID SOL	mg/l	< 0.001
V	mg/l	0.050

DETERMINAND	UNIT	RESULT
V-ACID SOL	mg/l	0.059
Cr	mg/l	< 0.003
Cr-ACID SOL	mg/l	< 0.003
Mn	mg/l	5.508
Mn-ACID SOL	mg/l	6.920
Fe	mg/l	< 0.003
Fe-ACID SOL	mg/l	< 0.003
Co	mg/l	0.047
Co-ACID SOL	mg/l	0.051
Ni	mg/l	0.131
Ni-ACID SOL	mg/l	0.148
Cu	mg/l	0.039
Cu-ACID SOL	mg/l	0.086
Zn	mg/l	< 0.003
Zn-ACID SOL	mg/l	< 0.003
Sr	mg/l	2.745
Sr-ACID SOL	mg/l	2.602
Zr	mg/l	< 0.001
Zr-ACID SOL	mg/l	< 0.001
Mo	mg/l	< 0.006
Mo-ACID SOL	mg/l	< 0.006
Cd	mg/l	< 0.001
Cd-ACID SOL	mg/l	< 0.001
Ba	mg/l	0.027
Ba-ACID SOL	mg/l	0.027
Pb	mg/l	< 0.020
Pb-ACID SOL	mg/l	< 0.020

Hg, As, Se: There were traces of Hg in all three mine waters but no As or Se.

APPENDIX C

TABLE 5.1 The influence of a gradient of a simulated sulphate saline mine water on the seedling top growth of maize (Figure 5.1)

Maize Hybrid	Treatment[1.]			Dry mass of top growth/ 10 plants	Relative Growth
		EC_{iw}[2.] mS m^{-1}	Sulphate[3.] mg L^{-1}	g	%
Maize	1.	97	226	2.05	100
SNK 2340	2.	280	1500	1.94	95
	3.	327	2000	1.88	92
	4.	349	2150	1.90	93
	5.	368	2300	1.59**	78**
	6.	386	2500	1.52**	74**
	7.	403	3000	1.89	92
	8.	453	4000	1.99	97
	9.	492	5000	1.66**	81**
	10.	525	6000	1.80	88
	11.	387	2500	1.62**	79**
	12.	466	3000	1.77	87
	13.	623	4000	1.59**	78**
	14.	780	5000	1.38**	67**

c.v. 13.3%

Maize	1.	97	226	2.29	100
CRN 4403	2.	280	1500	2.34	102
	3.	327	2000	2.19	96
	4.	349	2150	2.42	106
	5.	368	2300	2.06*	90*
	6.	386	2500	2.30	101
	7.	403	3000	2.44	107
	8.	453	4000	2.34	102
	9.	492	5000	2.16	94
	11.	387	2500	2.61 **	114 **
	12.	466	3000	2.52 *	110 *
	13.	623	4000	2.08 *	91 *
	14.	780	5000	2.09	92

c.v. 7.6%				LSD_F 0.24	12

* Tendency to differ from control (Treatment 1) ($P < 0.1$).
** Significant difference from control ($P < 0.05$).

[1.] Treatment 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.
[2.] EC measured in micro-filtered supernatant of treatment solutions.
[3.] Total sulphate in suspension.

TABLE 5.2 **The influence of a gradient of a simulated NaCl-dominated mine water on the top growth of maize seedlings (Figure 5.1)**

Hybrid	Treatment					Dry mass of top growth/ 10 plants g	Relative Growth %
		EC mS m^{-1}	Na	Cl	SO_4		
			mmol L^{-1}				
Maize SNK 2340	1.	241	0	0	12	3.39	100
	2.	308	10	10	12	3.28	97
	3.	396	20	16	13.8	2.85**	84**
	4.	581	40	29	17.5	2.76**	81**
	5.	678	50	35	19.3	2.60**	77**
	6.	770	60	42	21.1	2.58**	76**
	7.	958	80	54	24.8	2.60	77**

c.v. 8.10% LSD$_F$ 0.34

** Significant difference from control ($P < 0.05$).

TABLE 5.3 **The influence of a gradient of a simulated $CaSO_4$-dominated mine water on the seedling top growth of** sorghum **seedlings (Figure 5.1)**

Cultivars	Treatment[1.]		Dry mass of top growth/ 10 plants	Relative Growth
	EC[2.] mS m^{-1}	Sulphate[3.] mg L^{-1}	g	%
Sorghum PAN 888	1. 97	226	1.39	100
	2. 280	1500	1.33	96
	3. 327	2000	1.26	91
	4. 349	2150	1.29	93
	5. 368	2300	1.03**	74**
	6. 386	2500	1.07**	77**
	7. 403	3000	1.15**	83**
	8. 453	4000	1.30	94
	9. 492	5000	1.05**	76**
	11. 387	2500	1.26	91
	12. 466	3000	1.20	86
	13. 623	4000	1.09**	79 **
	14. 780	5000	0.95**	68 **

c.v. 15.2% LSD$_F$ 0.22 15.8

TABLE 5.4 **The influence of a gradient of a simulated NaCl-dominated mine water on the seedling top growth of** sorghum **PAN 888 (Figure 5.1)**

Cultivar	Treatment				Dry mass of top growth/ 10 plants g	Relative Growth %
	EC mS m^{-1}	Na	Cl	SO_4		
		mmol L^{-1}				
Sorghum PAN 888	1. 241	0	0	12	1.34	100
	2. 308	10	10	12	1.30	97
	3. 396	20	16	13.8	1.14**	85**
	4. 581	40	29	17.5	1.13**	84**
	5. 678	50	35	19.3	0.91**	68**
	6. 770	60	42	21.1	0.88**	66**
	7. 958	80	54	24.8	0.70**	52**

c.v. 12.4 % LSD$_F$ 0.19 14

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1.Treatment 1-9 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.

2. EC measured in micro-filtered supernatant of treatment solutions.

3. Total sulphate in suspension.

TABLE 5.5 The influence of a gradient of a simulated CaSO$_4$-dominated mine water on the seedling top growth of pearl millet **(Figure 5.1)**

Crop Cultivar	Treatment[1.]		Dry mass of top growth/ 10 plants g	Relative Growth %
	EC[2.] mS m^{-1}	Sulphate[3.] mg L^{-1}		
Pearl millet SA Standard (babala)	1. 97	226	1.09	100
	2. 280	1500	1.05	96
	3. 327	2000	0.91	83
	4. 349	2150	1.03	95
	5. 368	2300	0.90	82
	6. 386	2500	0.93	85
	7. 403	3000	0.92	85
	8. 453	4000	0.96	88
	9. 492	5000	1.13	103
	11. 387	2500	0.87**	80**
	12. 466	3000	0.98	89
	13. 623	4000	0.81**	75**
	14. 780	5000	0.64**	59**
c.v. 12.6 %			LSD$_F$ 0.21	15.8

TABLE 5.6 The influence of a gradient of simulated NaCl-domionated mine water on the seedling top growth of pearl millet **(Figure 5.1)**

Cultivar	Simulated sodic-saline mine water				Dry mass of top growth/ 10 plants g	Relative Growth %
	EC mS m^{-1}	Na	Cl	SO$_4$		
		mmol L^{-1}				
Pearl millet SA Standard	1. 241	0	0	12	1.04	100
	2. 308	10	10	12	0.98	94
	3. 396	20	16	13.8	0.88**	85**
	4. 581	40	29	17.5	0.70**	67**
	5. 678	50	35	19.3	0.45**	44**
	6. 770	60	42	21.1	0.52**	50**
	7. 958	80	54	24.8	0.39**	38**
c.v. 10.3 %					LSD$_F$ 0.10	9.6

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatment 1-9 salinity with mainly CaSO$_4$; 11-14 with added Na$_2$SO$_4$.

2. EC measured in micro-filtered supernatant of treatment solutions.

3.Total sulphate in suspension.

TABLE 5.7 **The influence of a gradient of a simulated $CaSO_4$-dominated mine water on the top growth of** soybean **seedlings (Figure 5.2)**

Cultivar	Treatment$^{1.}$		Dry mass of top growth/ 10 plants g	Relative Growth %
	EC$^{2.}$ mS m^{-1}	Sulphate$^{3.}$ mg L^{-1}		
Soybean Ibis	1. 97	226	2.17	100
	2. 280	1500	1.64 **	75**
	3. 327	2000	1.93	89
	4. 349	2150	1.79 *	82*
	5. 368	2300	1.83	84
	6. 386	2500	1.83	84
	7. 403	3000	1.89	87
	8. 453	4000	2.17	100
	9. 492	5000	2.05	94
	10. 525	6000	1.95	90
	11. 387	2500	1.81	83
	12. 466	3000	2.02	93
	13. 623	4000	1.66 **	77**
	14. 780	5000	1.73 *	80*

c.v. 17.0 %

TABLE 5.8 **The influence of a gradient of a simulated NaCl-dominated mine water on the top growth of** soybean **seedlings (Figure 5.2)**

Cultivar	Treatment				Dry mass of top growth/ 10 plants g	Relative Growth %
	EC mS m^{-1}	Na	Cl	SO_4		
		mmol L^{-1}				
Soybean Ibis	1. 241	0	0	12	3.53	100
	2. 308	10	10	12	3.43	97
	3. 396	20	16	13.8	3.09	88
	4. 581	40	29	17.5	2.88 *	82*
	5. 678	50	35	19.3	2.46 **	70**
	6. 770	60	42	21.1	2.79 *	79*
	7. 958	80	54	24.8	2.55 **	72**

c.v. 19.3 %

LDS_F 0.75 21

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatment 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.
2. EC measured in micro-filtered supernatant of treatment solutions.
3. Total sulphate in suspension.

TABLE 5.9 **The influence of a gradient of a simulated sulphate mine water on the top growth of** cowpea **Dr Saunders seedlings (Figure 4.7, p. 117)**

Crop Cultivar	Treatment[1.]			Dry mass of top growth/ 10 plants g	Relative Growth %
		EC[2.] mS m^{-1}	Sulphate[3.] mg L^{-1}		
Cowpea Dr Saunders	1.	97	226	2.31	100
	2.	280	1500	2.38	103
	3.	327	2000	2.37	103
	4.	349	2150	2.36	102
	5.	368	2300	2.27	98
	6.	386	2500	2.03	88
	7.	403	3000	2.36	103
	8.	453	4000	2.48	107
	9.	492	5000	2.04	88
	10.	525	6000	2.25	98
	11.	387	2500	2.56	111
	12.	466	3000	2.08	90
	13.	623	4000	1.81	79
	14.	780	5000	1.83	79

c.v. 18.9 %

TABLE 5.10 **The influence of a gradient of simulated NaCl-dominated mine water on the top growth of** cowpea **seedlings (Figure 5.2)**

Crop Cultivar	Treatment					Dry mass of top growth/ 10 plants g	Relative Growth %
		EC mS m^{-1}	Na	Cl	SO_4		
			mmol L^{-1}				
Cowpea Dr Saunders	1.	241	0.02	0.02	12	3.62	100
	2.	308	10	10	12	3.53	97
	3.	396	20	16	13.8	3.18**	88**
	4.	581	40	29	17.5	2.63**	76**
	5.	678	50	35	19.3	2.59**	72**
	6.	770	60	42	21.1	2.46**	68**
	7.	958	80	54	24.8	1.95**	54**

c.v. 7.8 % LSD_F 0.32

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatment 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.

2. EC measured in micro-filtered supernatant of treatment solutions.

3.Total sulphate in suspension.

TABLE 5.11 The influence of a gradient of a simulated $CaSO_4$-dominated mine water on the top growth of drybean **seedlings (Figure 5.2)**

Crop Cultivar	Treatment[1.]			Dry mass of top growth/ 10 plants g	Relative Growth %
		EC[2.] mS m^{-1}	Sulphate[3.] mg L^{-1}		
Drybean PAN 122	1.	97	226	1.86	100
	2.	280	1500	1.94	104
	3.	327	2000	1.78	96
	4.	349	2150	1.68	91
	5.	368	2300	2.00	108
	6.	386	2500	1.84	99
	7.	403	3000	1.75	94
	8.	453	4000	1.76	95
	9.	492	5000	1.86	100
	1 0.	525	6000	1.38 *	74*
	11.	387	2500	1.46	79
	12.	466	3000	1.58	85
	13.	623	4000	1.28 **	69**
	14.	780	5000	1.31 **	70**

c.v. 21.1 %

TABLE 5.12 The influence of a gradient of a simulated NaCl-dominated mine water on the top growth of drybean **seedlings (Figure 5.2)**

Crop Cultivar	Treatment					Dry mass of top growth/ 10 plants g	Relative Growth %
		EC mS m^{-1}	Na	Cl	SO_4		
			mmol L^{-1}				
Drybean PAN 127	1.	241	0.02	0.02	12	3.98	100
	2.	308	10	10	12	3.43	86
	3.	396	20	16	13.8	3.26*	82*
	4.	581	40	29	17.5	3.20**	80**
	5.	678	50	35	19.3	2.74**	69**
	6.	770	60	42	21.1	2.58**	65**
	7.	958	80	54	24.8	2.62**	66**

c.v. 15.9 % LSD$_F$ 0.72 18

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatment 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.

2. EC measured in micro-filtered supernatant of treatment solutions.

3. Total sulphate in suspension.

TABLE 5.13 **The influence of a gradient of a simulated $CaSO_4$-dominated mine water on the top growth of** sunflower **seedlings (Figure 5.1)**

Crop Cultivar	**Treatment[1.]**		**Dry mass of top growth/ 10 plants g**	**Relative Growth %**
	EC[2.] mS m^{-1}	**Sulphate[3.] mg L^{-1}**		
Sunflower SNK 43	1. 97	226	2.50	100
	2. 280	1500	2.91 *	116*
	3. 327	2000	3.12	125
	4. 349	2150	2.74	106
	5. 368	2300	2.68	107
	6. 386	2500	2.66	106
	7. 403	3000	2.61	104
	8. 453	4000	2.52	101
	9. 492	5000	2.47	99
	11. 387	2500	2.57	1.03
	12. 466	3000	2.58	1.03
	13. 623	4000	1.84**	74**
	14. 780	5000	1.64*	66*

c.v. 13.1%

TABLE 5.14 **The influence of a gradient of simulated NaCl-dominated mine water on the top growth of** sunflower **seedlings (Figure 5.1)**

Crop Cultivar	**Treatment**				**Dry mass of top growth/ 10 plants g**	**Relative Growth %**
	EC mS m^{-1}	**Na**	**Cl**	**SO_4**		
		mmol L^{-1}				
Sunflower SNK 43	1. 241	0.02	0.02	12	2.45	100
	2. 308	10	10	12	2.55	104
	3. 396	20	16	13.8	2.28	93
	4. 581	40	29	17.5	2.01**	82**
	5. 678	50	35	19.3	1.83**	75**
	6. 770	60	42	21.1	1.93**	79**
	7. 958	80	54	24.8	1.64**	67**

c.v. 7.2%

LSD_F 0.22

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatment 1-9 salinity with mainly $CaSO_4$; 10-13 with added Na_2SO_4.
2. EC measured in micro-filtered supernatant of treatment solutions.
3. Total sulphate in suspension.

TABLE 5.15 **The influence of a gradient of a simulated $CaSO_4$-dominated mine water on the seedling growth of** wheat **(Figure 5.3)**

Cultivars	**Treatment**[1.]		**Dry mass of top growth/ 10 plants g**	**Relative Growth %**
	EC[2.] **mS m^{-1}**	**Sulphate**[3.] **mg L^{-1}**		
Wheat Inia	1. 97	226	0.55	100
	2. 263	1500	0.55	100
	3. 330	2000	0.52	95
	4. 332	2150	0.51	94
	5. 338	2300	0.50	92*
	6. 349	2500	0.55	100
	7. 364	3000	0.48	88**
	8. 398	4000	0.53	97
	9. 473	5000	0.47	86**
	10. 507	6000	0.52	95
	11. 352	2500	0.52	94
	12. 424	3000	0.50	91*
	13. 572	4000	0.49	90**
	14. 782	5000	0.43	79**

c.v. 6.8%

TABLE 5.16 **The influence of a gradient of simulated NaCl-dominated mine water on the seedling top growth of** wheat **(Figure 5.3)**

Crop Cultivar	**Treatment**				**Dry mass of top growth/ 10 plants g**	**Relative Growth %**
	EC mS m	**Na**	**Cl**	**SO_4**[3.]		
		mmol L^{-1}				
Wheat Inia	1. 168	0.02	0.02	12	0.47	100
	2. 286	10	10	12	0.47	99
	3. 382	20	16	13.8	0.49	104
	4. 565	40	29	17.5	0.40**	85**
	5. 664	50	35	19.3	0.44	94
	6. 756	60	42	21.1	0.44	94
	7. 934	80	54	24.8	0.35**	75**

c.v. 10.3%

LSD_F 0.06 12.7

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatment 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.

2. EC measured in micro-filtered supernatant of treatment solutions.

3. Total sulphate in suspension.

TABLE 5.17 The influence of a gradient of a simulated $CaSO_4$-dominated mine water on the top growth of rye seedlings· (Figure 5.3)

Crop Cultivar	Treatment[1.]			Dry mass of top growth/ 10 plants g	Relative Growth %
		EC[2.] mS m^{-1}	Sulphate[3.] mg L^{-1}		
Rye SSR 1	1.	97	226	0.63	100
	2.	280	1500	0.65	103
	3.	327	2000	0.57	90
	4.	349	2150	0.54	86
	5.	368	2300	0.57	91
	6.	386	2500	0.55	87
	7.	403	3000	0.53	84
	8.	453	4000	0.52	83
	9.	492	5000	0.60	95
	10.	525	6000	0.48 *	76*
	11.	387	2500	0.52	82
	12.	466	3000	0.41**	65**
	13.	623	4000	0.54	85
	14.	780	5000	0.38**	60**

c.v. 23.5 %

TABLE 5.18 The influence of a gradient of simulated NaCl-dominated mine water on the seedling top growth of rye (Figure 5.3)

Crop Cultivar	Treatment					Dry mass of top growth/ 10 plants g	Relative Growth %
		EC mS m^{-1}	Na	Cl	SO_4		
			mmol L^{-1}				
Rye SSR 1	1.	228	0.02	0.02	12	0.67	100
	2.	336	10	10	12	0.70	105
	3.	434	20	16	13.8	0.67	100
	4.	610	40	29	17.5	0.58 *	88 *
	5.	694	50	35	19.3	0.50 **	75 **
	6.	780	60	42	21.1	0.45 **	68 **
	7.	946	80	54	24.8	0.47 **	71 **

c.v. 9.9% LSD$_F$ 0.08 12

* Tendency to differ from control (Treatment 1) ($P < 0.1$).
** Significant difference from control ($P < 0.05$).

1. Treatments 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.
2. EC measured in micro-filtered supernatant of treatment solutions.
3. Total sulphate in suspension.
4. Did not germinate very well. possibly due to the age of the seed - high variation.

TABLE 5.19 The influence of a gradient of a simulated $CaSO_4$-dominated saline mine water on the seedling top growth of triticale **(Figure 5.4)**

Crop Cultivar	**Treatment**[1]			**Dry mass of top growth/ 10 plants g**	**Relative Growth %**
		EC[2] **mS m^{-1}**	**Sulphate**[3] **mg L^{-1}**		
Triticale Cloc 1[4]	1.	97	226	0.34	100
	2.	280	1500	0.46	136
	3.	327	2000	0.33	97
	4.	349	2150	0.27	80
	5.	368	2300	0.35	102
	6.	386	2500	0.29	87
	7.	403	3000	0.29	84
	8.	453	4000	0.36	107
	9.	492	5000	0.32	95
	10.	525	6000	0.30	88
	11.	387	2500	0.36	105
	12.	466	3000	0.18*	53*
	13.	623	4000	0.24	71
	14.	780	5000	0.27	79
c.v. 36.8 %					
Triticale Rex	1.	97	226	0.42	100
	2.	280	1500	0.41	100
	3.	327	2000	0.38	92
	4.	349	2150	0.35	85
	5.	368	2300	0.33	79
	6.	386	2500	0.37	88
	7.	403	3000	0.39	93
	8.	453	4000	0.40	95
	9.	492	5000	0.42	100
	10.	525	6000	0.37	90
	11.	387	2500	0.34	82
	12.	466	3000	0.36	88
	13.	623	4000	0.36	86
	14.	780	5000	0.32 *	77*
c.v. 20.0 %					

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatment 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.
2. EC measured in micro-filtered supernatant of treatment solutions.
3. Total sulphate in suspension.
4. High variation probably due to age of seed. Numbers of plants per container varied.

TABLE 5.20 **The influence of a gradient of a simulated NaCl-dominated mine water on the seedling top growth of** triticale **(Figure 5.4)**

Crop Cultivar	Treatment					Dry mass of top growth/ 10 plants g	Relative Growth %
		EC mS m^{-1}	Na	Cl	SO_4		
			mmol L^{-1}				
Triticale Cloc 1	1.	168	0.02	0.02	12	0.41	100
	2.	286	10	10	12	0.35	86
	3.	382	20	16	13.8	0.40	96
	4.	565	40	29	17.5	0.37	90
	5.	664	50	35	19.3	0.39	94
	6.	756	60	42	21.1	0.34	83
	7.	934	80	54	24.8	0.34	83
c.v. 16.1%							
Triticale Rex	1.	168	0.02	0.02	12	0.64	100
	2.	286	10	10	12	0.70	110
	3.	382	20	16	13.8	0.50	78**
	4.	565	40	29	17.5	0.59	92
	5.	664	50	35	19.3	0.60	93
	6.	756	60	42	21.1	0.55	85**
	7.	934	80	54	24.8	0.43	68**
c.v. 9.9%						LSD_F 0.08	12.5

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. EC electrical conductance measured in supernatant of treatment solutions.

TABLE 5.21 The influence of a gradient of a simulated $CaSO_4$-dominated mine water on the seedling top growth of barley **(Figure 5.3)**

Crop Cultivar	Treatment[1.]			Dry mass of top growth/ 10 plants g	Relative Growth %
		EC[2.] $mS\ m^{-1}$	Sulphate[3.] $mg\ L^{-1}$		
Barley Stirling	1.	97	226	0.79	100
	2.	280	1500	0.85	107
	3.	327	2000	0.75	95
	4.	349	2150	0.79	99
	5.	368	2300	0.71 *	90*
	6.	386	2500	0.76	95
	7.	403	3000	0.72	91
	8.	453	4000	0.77	97
	9.	492	5000	0.79	99
	10.	525	6000	0.74	94
	11.	387	2500	0.76	96
	12.	466	3000	0.73	93
	13.	623	4000	0.73	92
	14.	780	5000	0.70 *	89*

c.v. 8.7%

TABLE 5.22 The influence of a gradient of a simulated NaCl-dominated mine water on the seedling top growth of barley **(Figure 5.3)**

Crop Cultivar	Treatment					Dry mass of top growth/ 10 plants g	Relative Growth %
		EC $mS\ m^{-1}$	Na	Cl	SO_4		
			$mmol\ L^{-1}$				
Barley Stirling	1.	168	0.02	0.02	12	0.66	100
	2.	286	10	10	12	0.74**	112**
	3.	382	20	16	13.8	0.77	116
	4.	565	40	29	17.5	0.68	103
	5.	664	50	35	19.3	0.63	96
	6.	756	60	42	21.1	0.62	94
	7.	934	80	54	24.8	0.56**	85**

c.v. 10.6% LSD$_F$ 0.10 15

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatment 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.
2. EC measured in micro-filtered supernatant of treatment solutions.
3. Total sulphate in suspension.

TABLE 5.23 The influence of a gradient of a simulated $CaSO_4$-dominated saline mine water on the seedling top growth of oats **(Figure 5.3)**

Cultivars	**Treatment**[1]			**Dry mass of top growth/ 10 plants g**	**Relative Growth %**
		EC[2] **mS m^{-1}**	**Sulphate**[3] **mg L^{-1}**		
Oats Overberg	1.	97	226	0.47	100
	2.	280	1500	0.46	99
	3.	327	2000	0.41 *	88 *
	4.	349	2150	0.43	91
	5.	368	2300	0.45	96
	6.	386	2500	0.38 **	88 **
	7.	403	3000	0.41 *	87 **
	8.	453	4000	0.45 *	96 *
	9.	492	5000	0.39 **	84 **
	10.	525	6000	0.37 **	78 **
	11.	387	2500	0.45	95
	12.	466	3000	0.39 **	82**
	13.	623	4000	0.40 **	86**
	14.	780	5000	0.38 **	82**

c.v. 10.4 %

TABLE 5.24 The influence of a gradient of a simulated NaCl-dominated mine water on the seedling top growth of oats **(Figure 5.3)**

Crop Cultivar	**Treatment**					**Dry mass of top growth/ 10 plants g**	**Relative Growth %**
		EC mS m^{-1}	**Na**	**Cl**	**SO_4**[3]		
			mmol L^{-1}				
Oats Overberg	1.	168	0.02	0.02	12	0.64	100
	2.	286	10	10	12	0.61	95
	3.	382	20	16	13.8	0.49**	76**
	4.	565	40	29	17.5	0.51**	80**
	5.	664	50	35	19.3	0.40**	63**
	6.	756	60	42	21.1	0.34**	53**
	7.	934	80	54	24.8	0.34**	53**

c.v. 13.2 % LSD_F 0.09 14

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatments 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.
2. EC electrical conductance measured in micro-filtered supernatant of treatment solutions.
3. Total sulphate in suspension.

TABLE 5.25 **The influence of a gradient of a simulated $CaSO_4$-dominated mine water on the seedling top growth of** annual ryegrass **cultivars (Figure 5.4)**

Crop Cultivar	**Treatment**[1.]			**Dry mass of top growth/ 10 plants g**	**Relative Growth %**
		EC[2.] **mS m^{-1}**	**Sulphate**[3.] **mg L^{-1}**		
Annual Ryegrass Midmar	1.	97	226	0.06	100
	2.	280	1500	0.04*	76*
	3.	327	2000	0.04	80
	4.	349	2150	0.05	95
	5.	368	2300	0.06	108
	6.	386	2500	0.05	91
	7.	403	3000	0.07**	131**
	8.	453	4000	0.06	109
	9.	492	5000	0.06	107
	10.	525	6000	0.06	109
	11.	387	2500	0.06	111
	12.	466	3000	0.05	84
	13.	623	4000	0.06	102
	14.	780	5000	0.04	81
c.v. 18.5%					
Annual Ryegrass Dargle	1.	97	226	0.04	100
	2.	280	1500	0.04	99
	3.	327	2000	0.05	111
	4.	349	2150	0.06	131
	5.	368	2300	0.06*	147*
	6.	386	2500	0.07**	169**
	7.	403	3000	0.07**	154**
	8.	453	4000	0.05	116
	9.	492	5000	0.06	131
	10.	525	6000	0.05	122
	11.	387	2500	0.07**	159**
	12.	466	3000	0.07**	156**
	13.	623	4000	0.05	124
	14.	780	5000	0.06**	150*
c.v. 23.8 %					

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatment 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.
2. EC electrical conductance measured in micro-filtered supernatant of treatment solutions.
3. Total sulphate in suspension.

TABLE 5.26 **The influence of a gradient of a simulated NaCl-dominated mine water on the seedling top growth of** annual ryegrass **(Figure 5.4)**

Crop Cultivar	Treatment					Dry mass of top growth/ 10 plants g	Relative Growth %
		EC mS m^{-1}	Na	Cl	SO_4		
			mmol L^{-1}				
Annual Ryegrass Midmar	1.	168	0.02	0.02	12	0.13	100
	2.	286	10	10	12	0.12	97
	3.	382	20	16	13.8	0.12	93
	4.	565	40	29	17.5	0.09**	70**
	5.	664	50	35	19.3	0.09**	70**
	6.	756	60	42	21.1	0.10**	77**
	7.	934	80	54	24.8	0.06**	50**

c.v. 15.2 %

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

TABLE 5.27 **The influence of a gradient of a simulated $CaSO_4$-dominated mine water on the seedling top growth of** lucerne **(Figure 5.2)**

Crop Cultivar	Treatment[1]			Dry mass of top growth/ 10 plants g	Relative Growth %
		EC[2] mS m^{-1}	Sulphate[3] mg L^{-1}		
Lucerne PAN 4860	1.	97	226	0.23	100
	2.	280	1500	0.22	93
	3.	327	2000	0.21	90
	4.	349	2150	0.22	93
	5.	368	2300	0.19**	82**
	6.	386	2500	0.19**	80**
	7.	403	3000	0.18**	76**
	8.	453	4000	0.21	89
	9.	492	5000	0.21	89
	10.	525	6000	0.18**	78**
	11.	387	2500	0.21	91
	12.	466	3000	0.19**	81**
	13.	623	4000	0.18**	78**
	14.	780	5000	0.16**	67**

c.v. 11.8 %

TABLE 5.28 **The influence of a gradient of simulated NaCl-dominated mine water on the seedling top growth of** lucerne **(Figure 5.2)**

Crop Cultivar	Treatment					Dry mass of top growth/ 10 plants g	Relative Growth %
		EC mS m^{-1}	Na	Cl	SO_4		
			mmol L^{-1}				
Lucerne PAN 4860	1.	168	0.02	0.02	12	0.29	100
	2.	286	10	10	12	0.24**	82**
	3.	372	20	16	13.8	0.23**	79**
	4.	565	40	29	17.5	0.24**	84**
	5.	664	50	35	19.3	0.21**	72**
	6.	756	60	42	21.1	0.17**	59**
	7.	934	80	54	24.8	0.19**	66**
c.v. 9.8%						LSD_F 0.03	10

* Tendency to differ from control (Treatment 1) ($P < 0.1$).

** Significant difference from control ($P < 0.05$).

1. Treatment 1-10 salinity with mainly $CaSO_4$; 11-14 with added Na_2SO_4.
2. EC electrical conductance measured in micro-filtered supernatant of treatment solutions.
3. Total sulphate in suspension.