

## 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<sub>4</sub>-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 ( $\text{Ca}(\text{OH})_2$ ). The water was collected from the irrigation pipe line used for a concomitant field trial (Jovanovic, et al., 1998). The  $\text{SO}_4$  and Ca content of this water varied from 998 to 1609, and from 257 to 646  $\text{mg L}^{-1}$  respectively (Table 3.1). The Mg content was low, averaging 20,7  $\text{mg L}^{-1}$  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/ $\text{SO}_4$  NaCl-dominated water of this area with a pH ( $\text{H}_2\text{O}$ ) of approximately 8.00. The ratio of Na:Cl: $\text{SO}_4$  varied considerably, especially that of Cl to  $\text{SO}_4$ . This water, however, contains 2  $\text{mg L}^{-1}$  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  $\text{mg L}^{-1}$  F (Dept of Water Affairs and Forestry, 1993).

**Mine C** water was a neutral high  $\text{SO}_4$  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  $\text{mg L}^{-1}$   $\text{SO}_4$ , with the Ca and Mg content 350 and 200  $\text{mg L}^{-1}$  respectively. The Mn content of *ca* 3,5  $\text{mg L}^{-1}$  was higher than recommended by water quality guidelines, a maximum of 0,20  $\text{mg L}^{-1}$  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<sup>1</sup> and controls used in the salt tolerance evaluations**

Mine/ Controls	pH (H <sub>2</sub> O)	EC mS m <sup>-1</sup>	Σ anions mmol <sub>c</sub> L <sup>-1</sup>	NH <sub>4</sub>	NO <sub>3</sub>	P	K	Ca	Mg	SO <sub>4</sub>	Na	Cl
				mg L <sup>-1</sup>								mmol L <sup>-1</sup>
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) <sup>2</sup>
B 4/94	8,5	407	39.23	30	310	10	70	41	40	802	30,4	(17,2) <sup>2</sup>
Control 2	5,2	92	10.15	31	316	10	78	67	28	227	0,7	
A 7/94 <sup>4</sup>	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) <sup>2</sup>
B 12/94	(8,1)	(590)	51.07	30	207	10	77	73	21	879	44,8	(29,1) <sup>2</sup>
Control 4	5,6	153	8.97	30	207	10	90	66	30	255	1,1	
C 3/95 <sup>5</sup>	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<sub>4</sub> + NO<sub>3</sub>) solution
2. Calculated
3. Other brackets : estimated
4. Mine A 7/94 Mn 1,84 mg L<sup>-1</sup>; average values 1994 to 1995: Fe 0,41 Mn 2,85 mg L<sup>-1</sup>
5. Mine C 3/95 HCO<sub>3</sub> 74, Fe 0,44, Mn 3,54, Cu 0,016 & Zn 0,027 mg L<sup>-1</sup>

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:

<b>Control</b>	deionized water
<b>Mine A</b>	a lime treated AMD water (for maize cultivars), or <b>Mine C</b> , an untreated neutral high sulphate water with high Ca and Mg
<b>Mine B</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<sub>3</sub> and NH<sub>4</sub>(2:1)

**Mine A** - a sulphate-dominated mine water - either Mine A (for maize  
**or C**        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 February 1994/1995 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<sub>4</sub>-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<sub>4</sub> 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 to 10).
  
- Increasing SO<sub>4</sub> concentrations gained with Na<sub>2</sub>SO<sub>4</sub> in a simulated mine water (Kleinkopje) saturated with CaSO<sub>4</sub> (treatments 11-14) (Table 3.2).

**Table 3.2 Chemical composition of simulated gradients of sulphate salinity**

Treatment <sup>1</sup>	Sulphate mg L <sup>-1</sup>		EC mS m <sup>-1</sup>	pH (H <sub>2</sub> O)	Σ anions <sup>1</sup> mmol <sub>c</sub> L <sup>-1</sup>	Ca	Mg	K	Na <sup>3</sup>	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>1</sup>	P
	Planned	Supernatant Analysed										
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	101.6/102.2 9	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<sub>4</sub> was used for the winter crops and more NO<sub>3</sub>; the first value is for summer crops and the second for winter crops.

2. Treatments 2 - 10, salinity increased mainly with CaSO<sub>4</sub>; 11-14 salinity increased with Na<sub>2</sub>SO<sub>4</sub> from 2500 mg L<sup>-1</sup> SO<sub>4</sub>.

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<sup>-1</sup> Na was added to treatments 1 - 10.

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

Treatment	pH (H <sub>2</sub> O)	EC <sup>1</sup> mS m <sup>-1</sup>	Σ Anions <sup>1</sup> mmol <sub>c</sub> L <sup>-1</sup>		Na	Cl	SO <sub>4</sub> <sup>2-</sup>	Ca <sup>2+</sup>	Mg	K	NH <sub>4</sub> <sup>1</sup>	NO <sub>3</sub> <sup>1</sup>	P
			Summer crops	Winter crops	mmol L <sup>-1</sup>		mg L <sup>-1</sup>						
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<sub>4</sub> was used for the winter crops and more NO<sub>3</sub>; the first value is for summer crops and the second for winter crops.

2. CaSO<sub>4</sub> (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 ( $0,861 \text{ g L}^{-1}$  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  $\text{NO}_3$  and  $\text{NH}_4$  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  $\text{SO}_4$ , Ca and Mg, were increased to attain a  $\text{SO}_4$  gradient, while maintaining the Ca to Mg ratio. The  $\text{SO}_4$  concentrations ranged from 226 (control) to 6000 ' $\text{mg L}^{-1}$ ' sulphate (' $\text{mg L}^{-1}$ ' in single quotes denote the total  $\text{SO}_4$  present including both the soluble and undissolved or precipitated  $\text{SO}_4$ ). 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  $\text{mg L}^{-1}$  sulphate treatment (treatment 5, Table 3.2) was kept constant and the sulphate further increased with  $\text{Na}_2\text{SO}_4$  (treatments 11 to 14, Table 3.2). The EC, pH ( $\text{H}_2\text{O}$ ) and the actual concentrations of Ca, Mg and  $\text{SO}_4$  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<sub>2</sub>O) 4.7; and a ‘virgin’ greyish brown loamy sand from the vicinity of the Kleinkopje coal mine with a pH (H<sub>2</sub>O) 4.3, which had not been irrigated or mined.

Day/night temperatures were 25 and 15<sup>0</sup>C, 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<sub>4</sub> and NO<sub>3</sub> (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<sup>-1</sup>, 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 ( $\text{NO}_3 / \text{NH}_4$ , 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  $\frac{1}{2}$  strength of a modified Hoagland No 2 nutrient solution with  $\text{NH}_4$  and  $\text{NO}_3$

**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  $\text{NO}_3$  and  $\text{NH}_4$  (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<sup>2</sup> 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<sub>3</sub> to NH<sub>4</sub>. 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<sub>2</sub> method and the total S given as SO<sub>4</sub>. K, Ca, Mg, Fe, Zn, Mn, Cu and Na were determined by a Perkin-Elmer 272 Atomic-Absorption Spectrophotometer. Chloride was analysed by potentiometric 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<sup>-1</sup>' SO<sub>4</sub> in single quotes denotes the total SO<sub>4</sub> present in a treatment, including both that in solution and the undissolved or precipitated SO<sub>4</sub>, while SO<sub>4</sub> in solution is denoted as mg L<sup>-1</sup> without inverted commas.