## THE EFFECT OF COPPER ON THE GROWTH, DEVELOPMENT AND CHEMICAL COMPOSITION OF SOME DRYLAND WHEAT CULTIVARS

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#### SUMMARY

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## M.Sc.thesis, Department of biodiversity and Conservation Biology, University of the Western Cape

Heavy metal accumulation in arable land as a result of mining activities, pesticides and fertilisers has become a global concern. Steinkopf and Concordia in the Northern Cape are well-known for subsistence farming, but just as well-known for the nearby copper mining industry. Very little research has been done on heavy metal toxicity in these areas, thus it was of importance to assess the wheat cultivars (*Triticum aestivum*) historically used in the study areas, to ensure the viability of wheat farming. The nine wheat cultivars screened were Flameks, Knoppies, Rooiwol, Rooigys, Yecoro Royo, Charchia, Witwol, Kariega and Losper. A comparative study was done by determining the concentration levels of Cu, Fe, Zn, Mn, K, Mg, Ca, Na, N and P in the roots and shoots of sensitive and tolerant wheat cultivars. It was established that Witwol and Rooigys were the most tolerant to these adverse conditions. Kariega and Rooiwol were most sensitive. Their tolerance was achieved by excluding copper from the roots and limiting the translocation of copper to the shoots. This trend to exclude copper uptake in Witwol and Rooigys, warrants further investigation on a molecular level to explain these adaptive mechanisms.

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### DECLARATION

I declare that *The effect of copper on growth, development and chemical composition of some dryland wheat cultivars* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Julie Gordon

October 2004

Signed: \_\_\_\_\_

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## **<u>Chapter 1:</u>** Literature Review

### **1.1 General Information:**

Wheat is a very nutritious, convenient and economical source of food. Twenty percent of the world's food supplies are provided by wheat and it is a staple food for nearly 40% of the world's population. Only rice, corn and perhaps potatoes are as important. The per capita consumption of wheat in many countries is greater than for any other food source. Wheat provides the body with proteins, carbohydrates, minerals and vitamins. Wheat is consumed principally as bread, but is also a basic ingredient for numerous food products. Wheat is an important subsistence and staple diet crop in South Africa, and a large proportion of small scale farmers grow wheat as a subsistence crop (Wiese, 1977).

#### **1.2 Origin of this Study:**

On the mountain slopes and valleys of the winter rainfall region of Steinkopf, arable farming involves mainly grains such as wheat and oats. Chapter 2 will deal with the climate, topography, pollution and socio-economic problems of the area from which the wheat used in this study was obtained. Due to inability to afford the necessary equipment and lack of knowledge of methods to sustain more profitable crops, most of these farmers use traditional wheat cultivars (commonly known as 'Witwol', 'Rooiwol' and 'Rooigys'), that have been passed on through many generations within the area (Steinkopf and Concordia), and this has led to the use of old cultivars of lesser quality. 'Rooiwol' and 'Witwol' already went out of general use in the 1950's. Historically, subsistence farmers did and do not make a valuable contribution to the country's wheat production and consequently they were overlooked by agricultural companies that advise and provide new, more productive wheat cultivars. As a result, the outdated cultivars were retained and re-used year-after-year till the present. This situation is worsened by copper leaching from nearby copper mines resulting in low wheat production. Another factor that may affect wheat farming is copper containing dust from copper ore that can also be carried by wind for considerable distances. Very little research has been done to alleviate the dilemma facing the subsistence farmers (Thomas, 1998). The study area is also well-known for its copper mines and migratory labour.

### **1.3 Copper in the soil, Health and Environmental Effects:**

Copper is a reddish metal that occurs naturally in rock, soil, water, sediment, and air. Its average concentration in the earth's crust is about 50 mg.kg<sup>-1</sup> soil (Anonymous a, 2002). The total amount of native copper in the soil depends on the amount of copper in the parent material. The soil copper concentration is usually greater than in the parent rock because of weathering of the parent rock and its accumulation in the upper soil horizons by growing and dying plants. The most abundant copper mineral in the soil is chalcopyrite (CuFeS<sub>2</sub>), which is common in both igneous and sedimentary rocks (Green, 1990).

Copper is a trace element and its absorption is essential for human health. Large amounts of copper are absorbed each day and can be found in many kinds of food, drinking water and even in the air. Water-soluble compounds occur in the environment after release through agricultural application and then form the largest threat to human health. Copper levels in the air are usually quite low, so that exposure through breathing is negligible. Houses with copper plumbing expose people to higher levels of copper because copper is released into drinking water through corrosion of pipes. Long-term exposure to copper can cause irritation of the nose, mouth and eyes and causes headaches, stomach aches, dizziness, vomiting and diarrhea. High uptake of copper may cause liver, kidney damage and even death (Anonymous b, 2002).

An ever increasing amount of copper enters the environment as the world's copper production rises. The disposal of copper-containing wastewater results in sludge contaminated with copper being deposited on riverbanks. The combustion of fossil fuels releases copper into the air where it remains until it starts to rain and it finally ends up in the soil. Copper pollution is widespread since it can be released into the environment by both natural processes and human activities. Natural sources include wind-blown dust, decaying vegetation, forest fires and sea spray. Human activities contributing to copper release include mining, metal production, wood production and phosphate fertiliser production. A limited number of plants are able to survive on copper-rich soils since copper does not break down in the environment and is continually accumulated by plants and animals. Thus copper can influence plant diversity depending on the acidity of the soil and the presence of organic matter (Anonymous b, 2002).

#### **<u>1.4 Copper's role in Plant Growth and Development:</u>**

All living organisms need specific elements for their growth, reproduction and survival. The most commonly needed elements are boron, calcium, carbon, chlorine, chromium, cobalt, copper, hydrogen, iron, magnesium, molybdenum, nitrogen, oxygen, phosphorus, potassium, selenium, strontium, sulphur, vanadium and zinc. Elements are considered essential for plant life when:

- i) a plant can not complete its life cycle in the absence of that element.
- ii) if the element forms part of any molecule or constituent of the plant, that is itself essential to the plant.

Essential elements are classified as micronutrients and macronutrients.

Macronutrients are elements needed in large quantities in plants (1 000 mg.kg<sup>-1</sup> dry mass and more), while micronutrients are elements needed in small quantities in plants (100 mg.kg<sup>-1</sup> dry mass) (Marschner, 1995; Salisbury and Ross, 1992).

The Serbo-Macedonian massif of Northern Greece is notable for varying degrees of porphyry copper mineralisation associated with post Miocene volcanic rocks in agricultural wheat fields (Cook *et al*, 1997). Although Cu is a trace element essential to plant nutrition, in excess it is phytotoxic causing stunte d growth, chlorosis and root malformation. (Cook *et al*, 1997) investigated the relationship between the copper concentration of the soil and plant tissue copper concentration. Copper levels encountered in the field were phytotoxic to wheat. Copper levels in plant tissues increased with increasing soil copper levels. Copper was primarily accumulated in the roots and excluded from the shoots, indicating restricted transport of Cu across the endodermis. This suggests that a primary exclusion mechanism is involved in the restriction of the internal metal transport from root to shoot. The relationship between

plant tissue copper levels and soil copper levels is linear, thus the copper concentration in plant tissue is a function of the copper concentration in the soil. However, these patterns differ amongst plant species and plant parts. Uncontaminated soils contain from 6-60 mg Cu.kg<sup>-1</sup> DM while phytotoxic soils contain between 60-125 mg Cu.kg<sup>-1</sup> DM. Normal copper levels in various plants are 1-10 mg Cu.kg<sup>-1</sup> DM while toxic levels contains from 20-100 mg Cu.kg<sup>-1</sup> DM. The copper content of the tested mature leaves contained from 5-30 mg Cu.kg<sup>-1</sup> DM, levels that are potentially phytotoxic (Cook *et al*, 1997).

Organic chelators are said to protect plants from phytotoxic effects of metals such as Al, Cd, Co, Cr, Cu, Mn, Ni and Zn. Divalent cations were supplied to plants as chelates to reduce metal uptake, accumulation and phytotoxicity compared to an equal supply of the same ionic metal. Wheat plants were grown in 0-50  $\mu$ M Cu as CuSO<sub>4</sub> (ionic form) and 0-800  $\mu$ M Cu as CuEDTA. Plants exposed to 50  $\mu$ M CuSO<sub>4</sub> accumulated 43  $\pm$  6  $\mu$ g.g<sup>1</sup> Cu in leaf tissue and 2300  $\pm$  130  $\mu$ g.g<sup>1</sup> Cu in root tissue. These wheat plants showed acute signs of copper toxicity. Concentrations of Fe, Mn and Mg in leaves injured by CuSO<sub>4</sub> were low, possibly leading to deficiencies (Taylor and Foy, 1985).

However, plants exposed to 800  $\mu$ M CuEDTA accumulated 260  $\pm$ 70  $\mu$ g.g<sup>-1</sup> Cu in leaf tissue and 6 600 +1 200  $\mu$ g.g<sup>-1</sup> Cu in root tissue. Plants injured by CuEDTA showed systematic toxic symptoms. The concentrations of Fe, Mn and Mg in leaves of CuEDTA injured plants were higher in comparison with plants grown on CuSO<sub>4</sub>. Chelators are said to be able to protect plants from metal stress as a result of

competition between chelating ligands and root absorption sites for free metal ions (Taylor and Foy, 1985).

## **1.5 Effect of Selenium on Copper and Cadmium:**

Landberg and Greger (1994) investigated heavy metal and selenium uptake and distribution in plants as well as their effect on growth. Results showed that selenium does not reduce heavy metal toxicity to plants but instead enhanced heavy metal uptake and toxicity. Selenate increased the Cd content of wheat shoots by up to 50%. Cadmium is toxic at even low levels while copper is essential at low levels and only toxic at elevated levels. Plants have several tolerance mechanisms to avoid metal toxicity, for cadmium and copper may be trapped by intercellular metal binding compounds, phytochelatines or metallothionein-like cysteine-rich polypeptides. Organic acids exudated by plants may also trap copper outside the plant (Landberg and Greger, 1994).

## **<u>1.6 Alternative versus Conventional Agriculture:</u>**

The practice of agriculture branches into alternative and conventional agriculture. Farmers practising in the study area fall under alternative agriculture, because they are self-sufficient, rather than being dependent on the market and consumerism like conventional farmers. Alternative farmers rely on personal knowledge, skills and local wisdom rather than on science, specialists and experts. Alternative farmers preserve rural culture and farm traditions unlike conventional farmers. Agriculture is not just a business but also a way of life to alternative farmers. They value nature for its own sake and not just as a valuable resource to be used. They also maintain production by the development and maintenance of healthy soil rather than by the use of agricultural chemicals. In alternative agriculture most plants are grown in polycultures rather than monocultures as in conventional farming. Alternative farmers consider all external costs, whereas conventional farmers ignore them. Finally, in alternative farming, production is used to benefit future generations, whereas in conventional farming high production is used to maintain economic growth (Beus and Dunlap, 1990).

## **References:**

Anonymous a (2003) Habek M http://www.eco-usa.net/toxics/copper.shtml

Anonymous b (2004): http://www.lenntech.com/Periodic-chart-elements/Cu-en.htm

Beus CE, Dunlap RE (1990) Conventional *versus* alternative agriculture. The paradigmatic roots of the debate. Rural Society **55**: 590-616

Cook CM, Vardaka E, Lanaras T (1997) Concentration of copper, growth and chlorophyll content of field-cultivated wheat growing in natural enriched copper soil. Bull.Environ.Contam.Toxicol. **58**: 248-253

Green DH (1990) A comparative study of the influence of different copper concentrations on the growth, chemical composition and activities of certain coppercontaining enzymes in *Atriplex nummularia* Lindl. and A. vestita (Thunb) Aell. Unpublished M.Sc.Thesis, University of the Western Cape, Bellville

Landberg T, Greger M (1994) Influence of selenium uptake and toxicity of copper and cadmium in pea (*Pisum sativum*) and wheat (*Triticum aestivum*). Physiol. Plant. **90**: 637-644

Marschner H (1995) Mineral Nutrition of higher plants ( 2<sup>nd</sup> edition). Academic Press, London

Salisbury FB, Ross CW (1992) Plant Physiology (4<sup>th</sup> Edition). Wadsworth Publishing Company, Belmont, California

Taylor GJ, Foy CD (1985) Differential uptake and toxicity of ionic and chelated copper in *Triticum aestivum*. Can.J.Bot. **63**: 1271-1275

Thomas G (1998) The effect of fungicides, including biological control, on germination and development of five wheat cultivars (*Triticum aestivum*). Unpublished M.Sc.Thesis, University of the Western Cape, Bellville

Wiese MV (1977) Compendium of Wheat Diseases. The American Phytopathological Society, Minnesota

#### ABSTRACT

#### SELECTING COPPER TOLERANT WHEAT CULTIVARS

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Wheat is important in subsistence agriculture in the copper mining impacted areas of Concordia and Steinkopf in the Northern Cape. Thus local dryland wheat cultivars were evaluated for their copper tolerance. The effect of copper on percentage germination, coleoptile length, root length, shoot mass and root mass were determined. The initial assessment of cultivars (Flameks, Knoppies, Rooiwol, Rooigys, Yecoro Royo and Charchia) was carried out over a range up to 32 mg Cu.L<sup>-1</sup>. From this Rooigys emerged as the most tolerant and Rooiwol as most sensitive. A second assessment was carried out over a much higher copper range of up to 125 mg Cu.L<sup>-1</sup>. The cultivars used for this included Witwol, Kariega and Losper, and Flameks. Witwol and Rooigys were the most tolerant, and Kariega and Rooiwol the most sensitive to copper

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#### **Chapter 2: Selecting copper tolerant wheat cultivars**

#### 2.1 Introduction:

Namaqualand can be described as 'semi-desert' with weakly developed soils. The region generally receives summer rainfall and the mean is less than 200 mm annually and has an abundance of succulents (Meadows, 1985). Steinkopf lies in the northern half of Namaqualand, north of O'Kiep. It is transversed from north to south by the Ikosis mountains. Steinkopf is divided into a winter-rainfall area in the west and a summer rainfall area in the east. The area is also characterised by a poor rainfall, generally poor soils and frequent droughts. Mining has been a lucrative industry in the Steinkopf area for many years. The copper mining industry was established in the mid-nineteenth century and has been a major source of employment (Emmet, 1987).

The devastation created by these mining activities is visually overwhelming since the mine dump at Koiingnaas is visible from the Kamiesberg, 80 kilometres away. In the gale-lashed area north of Port Nolloth, sand whipped from the mine dumps has killed all vegetation in places and the combination of salt and clay water-repellent complexes means that little moisture penetrates the surface, resulting in a sterile, arid soil (Cowling and Pierce, 1992). It has been recognised that mine tailings are one of the important pollutant sources; they are characterised by a high content of metals, few nutrients and weak water holding capacity (Hao *et al*, 2003). Agricultural soils may accumulate As, Cd, Cu and Zn originating from industrial areas, animal manures, pesticides and fertilizers (Chen *et al*, 1997). The disturbance to the biological

equilibrium due to excess heavy metals mayhave an unfavourable influence on soil fertility, plant development and yield (Maliszewska et al, 1985). The mining industry accounts for more than half of the region's economic capacity and is its largest employer, but the fact that it operates in an area of spectacular biodiversity means its impact carries a huge ecological cost (Cowling and Pierce, 1992). Heavy metal contamination of arable soil is an international issue for resource sustainability and food quality assurance (Rayment et al, 2002). Heavy metals can persist in soils for long periods of time, while some are mobilised and relocated by harvesting and transport, thereby posing a threat to long-term resource sustainability and nutrient supply (Rayment et al, 2002). According to Hopkins and Hüner (2004) the required copper concentration considered adequate for normal growth of higher plants is 10 mg.kg<sup>-1</sup> plant dry matter. However the average copper concentration found in the soil is 30 mg.kg<sup>-1</sup> (Larcher, 2003). The Council of Geoscience compiled geochemical maps in 1981 in the Springbok and Steinkopf regions of the Northern Cape using an X-ray Fluorescence Spectrophotometer to analyse the concentrations of several elements including copper. Five kg samples were taken from the top 20 cm of the sampling medium with a sampling density of 1.km<sup>-2</sup>. In Table 2.7 astonishing average soil copper readings of 312.3 mg.kg<sup>-1</sup> and 29.6 mg.kg<sup>-1</sup> were obtained for Springbok and Steinkopf respectively, which certainly suggest that the soil copper concentration of the Springbok region is above the 30 mg.kg<sup>-1</sup>Cu average as a point of reference (Elsenbroek, 1995). The complete geochemical data obtained from this survey is included as an appendix.

Agriculture, or more specifically stock farming, originally formed the economic basis of these communities, but has steadily declined with the increase of population and the deterioration of the farming resources. Farming provides a very small part of the total income of the contemporary community of Steinkopf, thus the majority of the farmers are classified as conservative farmers. Conservative farmers mainly farm for their own use (Emmet, 1987). The central aim of this study was to assess the copper tolerance of the local dryland wheat cultivars in the Steinkopf area, since the same cultivars have been used for many years in a copper contaminated district (Emmet, 1987). According to Muftah Adam (Personal Communication) SST 29, SST 33 and SST 34 were the least salt tolerant, while SST 17, SST 35 and SST 36 were the most salt tole rant. Therefore, in addition to investigating the toxic effects of copper, another objective was to establish whether there is a parallel between copper and salt tolerance. Some selection for copper tolerance may have occurred.

#### **<u>2.2 Materials and Methods:</u>**

Two screenings were conducted to select the most suitable dryland wheat cultivars to be used in the subsequent two experiments (see Chapters 3 and 4). The experimental design of the first screening included six cultivars, five treatments and two replicates. The Paper Roll method of Hampton and TeKrony (1995) was used to determine at which copper concentration germination was inhibited and at which concentration it was optimal. The dampened sheets were pre-treated with distilled water as control and solutions containing 4, 8, 16 and 32 mg Cu.L<sup>-1</sup>, prepared from analytically pure CuSO<sub>4</sub>.7H<sub>2</sub>O. The six wheat cultivars used were Flameks (SST 17), Knoppies (SST 29), Rooiwol (SST 33), Rooigys (SST 34), Yecoro Royo (SST 35) and Charchia (SST

36). The healthiest seeds (caryopses) of more or less the same size were carefully selected. The seeds were first surface sterilized with 3.5% sodium hypochlorite for three minutes to prevent fungal growth and afterwards they were rinsed off six-eight times with distilled water.

The Paper Roll Method consists of dampened sheets of special germination paper on which five seeds were placed in a straight line two cm apart with their orientation such that the shoots would emerge at the 'top' and the roots at the 'bottom' of each seed. This was covered with another sheet of dampened paper and then rolled. Two replicates were placed in a plastic zip lock bag to keep the moisture inside. The towel rolls were prepared under sterile conditions by working in a laminar flow cabinet. The plastic bags were then randomised and placed in an upright position inside a growth chamber at 25 °C during the day for 12 hours and 10 °C at night for 12 hours (Hampton and TeKrony, 1995). After nine days in the growth chamber, the roots and shoots of the seedlings were dissected just below the remainder of the caryopsis so that the additional weight would not be included. All the seedlings could not be dissected and measured on the same day, therefore the rest were placed in a freezer at -10 °C to prevent any further growth.

The experimental design for the second screening was almost the same but with changes to the copper concentrations and a new set of six wheat cultivars. The experimental design of the second screening again consisted of six cultivars, five treatments and two replicates. The Paper Roll Method was again employed to assure the most effective germination in nine days. The two cultivars that were the most and

least copper tolerant in the previous screening, Rooiwol and Knoppies respectively, were used again, while Kariega and Witwol were also screened because they come from the same study area and are also used by subsistence farmers. Thus the six wheat cultivars used were Kariega (SST 13), Rooiwol (SST 33), Rooigys (SST 34), Charchia (SST 36), Losper (SST 37), and Witwol. Two paper rolls of different copper concentrations and of a different cultivar were placed in one plastic bag to ensure that the rolls were exposed to the same ambient conditions in the growth chamber. The copper concentrations were also changed to a logarithmic scale and the new copper concentrations were 4, 12.5, 40 and 125 mg Cu.L<sup>-1</sup>. Critical values coinciding with the onset of phytotoxicity range from 60 to 125 mg Cu.L<sup>-1</sup> for copper (Mantovi *et al*, 2003) were thus included.

#### 2.3 <u>Results :</u>

The results showing the growth of the different cultivars are given in Tables 2.1 (lower copper ranges) and 2.3 (higher copper ranges). Tables 2.2 (lower copper range) and 2.4 (higher copper range) show how the different copper concentrations affected growth. Tables 2.5 and 2.6 show how the cultivars ranked with respect to copper tolerance.

## 2.4 <u>Discussion :</u>

The first six Dryland wheat cultivars assessed were Flameks, Knoppies, Rooiwol, Rooigys, Yecoro Royo and Charchia. These wheat cultivars exhibited clear differences in terms of the following growth responses: root length, root mass, shoot mass, shoot length, percentage germination and shoot-to-root ratio. The ranking of the various cultivars was determined by employing a point-system ranging from one to six, whereby six was assigned to the cultivar with the highest tolerance, while one was assigned the cultivar bearing the least tolerance to copper toxicity. It was established that Rooigys was the most tolerant to the elevated copper levels, while Charchia, Flameks and Knoppies were the most susceptible (Table 2.1 and Table 2.5). Rooigys is a winter-rainfall wheat cultivar originally from the Northern Cape and is thus better adapted to these adverse conditions, while Charchia, Flameks and Knoppies are not. It is thus also evident that there does not exist a parallel between copper and salt tolerance since the results obtained in this screening is contradictory to those of Muftah Adam as mentioned in Section 2.2. If there were a parallel Rooigys would have been expected to be one of the three least tolerant cultivars and Charchia one of the three most tolerant cultivars.

The second screening again produced significant differences between Rooiwol, Witwol, Kariega, Rooigys, Charchia and Losper in terms of all the growth parameters investigated. The same trend was obtained since Rooigys and Witwol did the best of the six cultivars, while Kariega and Rooiwol did the worst (Table 2.3 and Table 2.6). Witwol's excellent performance can again be attributed to the fact that Witwol and Rooigys originated in the Northern Cape and are still used by subsistence farmers in this region. Rooiwol has been used for the past 50 years in the Northern Cape and is

currently still used by subsistence farmers only because of its excellent baking properties (Personal Communication).

Table 2.2 shows significant differences for root length, root mass, shoot mass and shoot-to-root ratio of the first six cultivars tested as the copper concentrations were raised from 0 to 32 mg Cu.L<sup>-1</sup>. Percentage germination and shoot length were unaffected by increasing copper concentrations. At 32 mg Cu.L<sup>-1</sup> the mass and length of the roots declined more than that of the shoots. Root length or mass is commonly used to indicate heavy metal inhibition since roots are the preferential accumulation sites when the external copper supply is high (Marschner, 1995).

The second screening again showed marked effects with increasing copper levels. Root lengths, root mass, shoot length and shoot-to-root ratio differed significantly as the copper concentrations were raised from 0 to 125 mg Cu.L<sup>-1</sup> (Table 2.4). Percentage germination and shoot mass were unaffected by these copper levels. This time, however, clear visual symptoms of the copper toxicity were observed. The roots were severely reduced in length and mass, while the leaves were partially discoloured and were desiccated. It can thus be concluded that for optimum growth, 125 mg Cu.L<sup>-1</sup> should not be exceeded. The critical level where toxicity sets in was most probably reached long before 125 mg Cu.L<sup>-1</sup>.

The common denominator for both screenings, unaffected by the detrimental effects of the excess copper, was the percentage germination. The treatment affects germination rate more than it does germination percentage (Ashraf and Iram, 2002).

This does not come as a surprise as the caryopsis has nutrients in store and only requires moisture from the ambient environment for germination to take place.

**Table 2.1:** Root length, root mass, shoots length, shoot mass, percentage germination and shoot/root ratio of the seedlings of six dryland wheat cultivars treated with different copper concentrations (low arithmetic copper range).

Cultivar	Root length (1	Shoot nm) mass (g)	Root mass (g)	Germination (%) <sup>b</sup>	Shoot SR Ratio <sup>c</sup> length (mm)
Flameks	15.2243	bс 1.3272 с	0.66670	c 99.000 a	14.1136 bc 2.1860 b
Knoppies	16.0073	bc 1.3464 c	0.53260	d 95.250 bc	15.3889 a 2.5962 a
Rooiwol	19.2645	a 1.2970 c	0.73740	c 96.500 ab	13.0305 d 1.7884 d
Rooigys	19.2069	a 1.6402 a	0.88690	b 98.500 ab	14.8164 ab 1.6550 d
Yecoro Royo	16.6248	b 1.3701 bc	1.19290	a 90.000 d	9.6839 e 1.2464 e
Charchia	14.6242	c 1.6259 ab	0.86170	b 92.500 cd	13.8609 cd 2.0298 c

<sup>a</sup> Means followed by the same letter are not significantly different (P=0.05)

Germination (%)<sup>b</sup> = Percentage germination

<sup>c</sup> S/R Ratio = Shoot/root ratio (mass basis)

Table 2.2:The effect of five different copper concentrations on the growth<br/>of the seedlings of six dryland wheat cultivars (low arithmetic<br/>copper range).

Concentration	Root	Root	Shoot
	Shoot	Germination	SR Ratio <sup>c</sup>
$(mg Cu.L^{-1})$	length (mm) <sup>a</sup>	mass (g)	length (mm)
	mass (g)	(%) <sup>b</sup>	
	21.0859.2	1 20117 a	13 7805 a
0	1.8128 a	94.167 a	1.62742 d
4	20.4839 a	1.15183 a	13.2928 a
	1.8144 a	96.250 a	1.70658 d
8	10.1150 c	0.65133 b	13.1938 a
	1.3813 b	94.792 a	2.29842 a
16	16.6283 b	0.61133 b	13.6517 a
	1.1183 bc	94.167 a	1.88992 c
32	15.8105 b	0.44950 c	13.4932 a
	1.0457 c	97.083 a	2.06250 b

<sup>a</sup> Means followed by the same letter are not significantly different (P=0.05) Germination (%)<sup>b</sup>= Percentage germination <sup>c</sup> S/R Ratio = Shoot/root ratio (mass basis)

**Table 2.3:** Root length, root mass, shoot length, shoot mass, percentage germination and shoot/root ratio of the seedlings of 6 dryland wheat cultivars treated with different copper concentrations (high logarithmic copper range).

Cultivar	Root Shoot length (mm) <sup>a</sup>	Root Germination mass (g)	Shoot SR Ratio <sup>c</sup> length (mm)
	mass (g)	(%) <sup>b</sup>	
Rooiwol	8.850 cd	0.31300 d 43 750 d	10.531 c 3 3700 bc
	11 221 aba	1 15600 c	16 700 0

Kariega	12.436 ab	0.08200 e	6.310 d
	0.1100 e	9.000 e	1.3960 d
Rooigys	13.512 a	0.91300 b	15.752 ab
	1.9160 b	87.500 b	2.2510 cd
Charchia	10.120 bc	0.60200 c	17.566 a
	1.4590 c	49.500 d	3.7120 b
Losper	7.029 d	0.44800 cd	13.170 bc
	1.7310 b	65.000 c	6.1400 a

<sup>a</sup> Means followed by the same letter are not significantly different (P=0.05) Germination (%)<sup>b</sup> = Percentage germination

<sup>c</sup> S/R Ratio = Shoot/root ratio (mass basis)

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Table 2.4: The effect of five different copper concentrations on the growth of the seedlings of six dryland wheat cultivars (high logarithmic copper range).

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Concentration (mg Cu.L <sup>-1</sup> )	Root Shoot length (mm) <sup>a</sup> mass (g)	Root Germination mass (g) (%) <sup>b</sup>	Shoot SR Ratio <sup>c</sup> length (mm)
0	17.8364 a	1.0209 a	13.5991 b
	1.5336 a	67.273 a	1.5209 b
4	14.9773 b	0.8182 ab	14.4445 ab
	1.4518 a	59.545 a	1.8909 b
12.5	9.3564 c	0.6255 bc	15.1500 a
	1.6073 a	63.864 a	3.0900 b
40	4.9964 d	0.3855 cd	13.6218 b
	1.4027 a	60.227 a	5.1145 a
125	4.6155 d	0.3073 d	13.1518 b
	1.4327 a	65.227 a	5.5382 a

<sup>a</sup> Means followed by the same letter are not significantly different (P=0.05) Germination (%)<sup>b</sup> = Percentage germination <sup>c</sup> S/R Ratio = Shoot/root ratio (mass basis)

<u> Table 2.5:</u>	A comparison of the growth and germination of the first set of
	six dryland wheat cultivar seedlings in order of their
	performance where $1 = \text{best}$ and $6 = \text{worst}$ ).

Cultivar	Root length	Germin (mm) (%)	Root nation mass (g)	SR Rati	Shoot o <sup>a</sup> length (	Overall mm) Ranking	ţ	Shoot mass (g)
Flameks	5	1	5	5	3	4.0		5
Knoppies	4	4	6	6	1	4.2	(worst)	4
Rooiwol	1	3	4	3	5	3.7		6
Rooigys	2	2	2	2	2	1.8	(best)	1
Yecoro Royo	3	6	1	1	6	3.3		3
Charchia	6	5	3	4	4	4.0		2

<sup>a</sup> S/R Ratio = Shoot/root ratio (mass basis); low ratio considered best

Cultivar	Root Germi	Root nation SR R	Shoot atio <sup>a</sup> Rank	Shoot ng	
	length (mm) <sup>a</sup>	mass (g)	length (mm)	mass (g)	(%)
Rooiwol	5	5 4	5 4.8	5 (worst)	5
Witwol	3	1 3	2 1.8	1 (best)	1
Kariega	2	6 1	6 4.5	6	6
Rooigys	1	2 2	3 2.0	2	2
Charchia	4	3 5	1 3.5	4	4
Losper	6	4 6	4 4.3	3	3

**Table 2.6:**A comparison of the growth and germination of the second set<br/>of six dryland wheat cultivar seedlings in order of their<br/>performance where 1 = best and 6 = worst).

<sup>a</sup> S/R Ratio = Shoot/root ratio (mass basis); low ratio considered best

**<u>Table 2.7:</u>** Geo-chemical copper data from the Namaqualand region.

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READINGS

## REGIONS

$(mg.kg^{-1})$	<u>SPRINGBOK</u> <u>STEINKOPF</u>	
Minimum	10 6	
Maximum	7 120 137	
Average	312.3 29.6	
Standard Deviation	183.5 3.8	

## 2.5 <u>References :</u>

Ashraf M, Iram A (2002) Optimisation and influence of seed priming with salts of potassium or calcium in two spring wheat cultivars differing in salt tolerance, at the initial growth stages. Agrochimica **10**: 47-55

Chen TB, Wong JWC, Zhou HY, Wong MH (1997) Assessment of trace element distribution and contamination in surface soils of Hong Kong. Environmental Pollution **96**: 61-68

Cowling R, Pierce S (1992) Namaqualand: a succulent desert. Fernwood Press, South Africa, pp 138-142

Elsenbroek, JH (1995) Instrumentation and Analytical technique for the analysis of regional geochemical samples used at the South African Council for Geoscience. Analyst **120**: 1535-1541

Emmet AB (1987) Steinkopf: a study of a community in decline. Human Science Research Council, Pretoria, South Africa, pp 114-115

Hampton JG, TeKrony DM (1995) Handbook of vigour test methods. (3<sup>rd</sup> edition) The International Seed Testing Association, Zurich, Switzerland

Hao X, Wang Y, Cang L, Chen H (2003) Effect of different amendments on ryegrass growth in copper mine tailings. Pedosphere **13**: 299-308

Hopkins, WG, Hüner, NPA (2004) Introduction to Plant Physiology (3<sup>rd</sup> Edition) John Wiley and Sons Inc., United States of America

Larcher, W (2003) Physiological Plant Ecology (4<sup>th</sup> Edition) Springer Publishers, Germany

Maliszewska W, Dec S, Wierzbicka H, Wozniakowska A (1985) The influence of various heavy metal compounds on the development and activity of soil microorganisms. Environmental Pollution **37**: 195-215

Mantovi P, Bonazzi G, Maestri E, Marmiroli N (2003) Accumulation of copper and zinc from liquid manure in agricultural soils and crop plants. Plant and Soil **250**: 249-257

Marschner H (1995) Mineral Nutrition of higher plants. ( $2^{nd}$  edition) Academic Press, London

Meadows ME (1985) Biogeography and ecosystems of South Africa. Juta, Cape Town

Rayment GE, Jeffrey AJ, Barry GA (2002) Heavy metals in Australian sugarcane. Communications in Soil Science and Plant Analysis **33**: 3203-3212

#### ABSTRACT

#### DOES CALCIUM ENHANCE COPPER UPTAKE?

#### **J.GORDON**

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Calcium (Ca) is essential to membrane properties and the maintenance of its structure. Previous studies found that a high calcium level abolishes the toxic effects of metals such as zinc. The 'Viets Effect' occurs when the presence of one ion accelerates the absorption of another ion. In contrast to antagonism, the 'Viets Effect' is an example of synergism. The four wheat cultivars (*Triticum aestivum*) assessed were Witwol, Rooiwol, Rooigys and Losper, employing the Paper Doll Method. The wheat seeds were germinated at copper concentrations ranging from 0 to 125 mg Cu.L<sup>-1</sup> with and without 0.5mM Ca. The outcome contradicted all expectations since the copper accumulation increased significantly in the presence of calcium.

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#### **Chapter 3:** Does calcium enhance copper tolerance?

## **3.1 Introduction:**

Most soils contain enough calcium for adequate plant growth and it is absorbed as divalent Ca<sup>2+</sup> (Salisbury and Ross, 1992). Calcium, in the form of calcium pectate, is an important component of plant cell walls. Calcium salts of phosphatidic acids occur in membranes and are essential to the maintenance of their structure and properties. Much calcium within the cytosol becomes reversibly bound to a small protein called calmodulin. This binding changes the structure of calmodulin in such a way that it then activates several enzymes (Salisbury and Ross, 1992). The enzyme, amylase, is specifically activated by calcium, while other enzymes such as ATP -ases require magnesium and to a lesser extent calcium to be activated. The presence of large amounts of insoluble calcium salts of organic acids (e.g. oxalic acid) in many plants suggests that it may have a role in regulating the acidity of the cell sap (Sutcliffe and Baker, 1974). Crystals of calcium oxalate are found in many cells to neutralise organic acids which might otherwise be toxic. Some plants, calcifuge species, are confined to acid soils where the amount of calcium is low, while calcicole species occur where calcium abounds (Sutcliffe, 1962).

The interactions between trace elements and the widespread calcium carbonate minerals are of geochemical interest since they influence the distribution of the ions in the aquatic environment as well as the reactivity of the minerals. The association with carbonate minerals can provide important pathways for scavenging potentially toxic metals like  $Cu^{2+}$ ,  $Cd^{2+}$  and  $Pb^{2+}$  (Schosseler *et al*, 1999). Numerous studies report on the ameliorating effect of calcium on heavy metal toxicity. One of these studies

investigated the involvement of calcium in zinc uptake and detoxification in  $Zn^{2+}$ tolerant and non-tolerant populations of *Silene maritima*. It was established that increasing calcium concentrations reduced Zn-toxicity and led to a higher level of zinc accumulation by the roots of the tolerant plants, but decreased transport to the shoots of both types. Thus it was concluded that a higher calcium concentration abolishes the toxic effects of zinc (Antosiewicz and Hennig, 2004).

When the presence of one ion accelerates the absorption of another ion, it is called the "Viets Effect". According to Epstein (1972). Viets immersed excised barley roots in aerated solutions of 5mM KBr and found that progressively more K and Br ions were absorbed at increasingly higher levels of calcium salt. The absence of calcium cause rapid disruption of membrane structure and function, thus the "plus calcium" treatment is considered the control. Only when a high calcium concentration accelerates the absorption of an ion beyond the rate observed at low and moderate calcium concentrations, might a true "Viets Effect" be inferred (Epstein, 1972).

The "Viets Effect" is an example of synergism. Calcium ions stimulate the net uptake of K<sup>+</sup> at low pH, by counteracting the negative effects of high H<sup>+</sup> concentrations on plasma membrane integrity and functioning of the proton efflux pump. At low external pH, Ca<sup>2+</sup> not only enhances the net influx of K<sup>+</sup>, but also of anions such as Cl<sup>-</sup> (proton-anion co-transport). Due to its stabilising effect on the plasma membrane Ca<sup>2+</sup> also plays an important role in the selection of ion uptake and K<sup>+</sup>/Na<sup>+</sup> selectivity of roots in particular (Marschner, 1995).
The objective of this study was to determine whether calcium sulphate promoted or inhibited copper uptake in four Dryland wheat cultivars since Perfus -Barbeoch *et al* (2002) obtained results which suggested that  $Cd^{2+}$  might permeate the plasma membrane of the guard cells through calcium channels. The same study also confirmed that  $Ca^{2+}$  channels from wheat roots were  $Cd^{2+}$  permeable.

## **<u>3.2 Materials and Methods:</u>**

The paper doll method of Hampton and TeKrony (1995) was used to assure the most effective germination in a short period (in this case nine days). The experimental design for this test consisted of five copper concentrations, four Dryland wheat cultivars and three replicates. Each paper doll contained five seeds of each cultivar. The four wheat cultivars used were Witwol, Rooiwol, Rooigys and Losper. Half of the 30 paper dolls were treated with 0.5 mM calcium (prepared from  $CaSO_4.2H_2O$ ) and the rest were left without the calcium treatment. All the paper towels were treated with different copper solutions prepared from analytically pure CuSO<sub>4</sub>.7H<sub>2</sub>O. The copper concentrations were 4, 12.5, 40 and 125 mg Cu.L<sup>-1</sup> with distilled water as control. The seeds were first surface sterilized with 3.5% sodium hypochlorite for three minutes to prevent fungal growth and afterwards they were washed off six-eight times with distilled water. The paper dolls were prepared under sterile conditions in a laminar flow cabinet. Three replicates of each treatment were labelled and placed in separate zip lock plastic bags to minimise evaporation and thus the copper concentration remained more or less stable. The plastic bags were then randomised by drawing a number out of a hat. In total there were 30 plastic bags in the growth cabinet with temperatures of 25 °C during the day for 12 hours and 10 °C at night for

12 hours. It was necessary to dissect the seedling just below the remaining caryopsis on harvesting to avoid including the additional weight of the caryopsis. All the seedlings could not be dissected and measured at once, thus the rest were placed in the freezer (at -10  $^{\circ}$ C) to prevent any further growth.

# 3.3 Results :

**Table 3.3.1:** A comparison of the tolerance of four dryland wheat cultivar seedlings to copper during the germination and early growth stages. (Include 'plus' and minus calcium treatments).

Cultivar	Root Shoot length (mm) <sup>a</sup> mass (g	Root Germination mass (g) ) (%) <sup>b</sup>	Shoot S/R Ratio <sup>c</sup> length (mm) <sup>a</sup>
Witwol	7.21a	0.102 a	17.41 a
	0.355 a	88.0 a	4.61 a
Rooiwol	7.71a	0.035 c	9.73 c
	0.089 d	35.3 c	3.22 b
Rooigy s	6.44 ab	0.067 b	14.12 b
	0.278 b	70.0 b	5.39 a
Losper	5.75 b	0.038 c	12.93 b

<sup>a</sup> Means followed by the same letter are not significantly different (P=0.05)

Germination  $(\%)^{b}$  = Percentage germination

<sup>c</sup> S/R Ratio = Shoot/root ratio (mass basis)

**<u>Table 3.3.2</u>**: The effect of copper on the germination and early growth stages of four dryland wheat cultivar seedlings (Including 'plus' and minus calcium treatments).

Concentration		Deet	Chart
Concentration	KOOL	Germi	nation S/R Ratio <sup>c</sup>
	Bhoot	Gerini	ination b/ it itatio

(mg Cu.L <sup>-1</sup> )	length (mm) <sup>a</sup> mass (g)	mass (g) (%) <sup>b</sup>	length (mm)
0	18.31 a	0.093 a	15.29 a
	0.229 a	69.5 a	2.44 c
4	9.57 b	0.077 ab	15.75 a
	0.240 a	59.6 a	3.15 b
12.5	2.99 c	0.064 b	14.75 a
	0.256 a	70.0 a	4.52 b
40	1.30 d	0.041 c	14.01 a
	0.252 a	71.4 a	8.30 a
125	0.74 d	0.031 c	8.96 b
	0.164 b	61.1 a	7.14 a

<sup>a</sup> Means followed by the same letter are not significantly different (P=0.05) Germination (%)<sup>b</sup> = Percentage germination <sup>c</sup> S/R Ratio = Shoot/root ratio (mass basis)

#### Calcium amelioration of the effects of copper on the <u>Table 3.3.3:</u> germination and early growth stages of four dryland wheat cultivar seedlings.

Concentration	Root	Ro	pot	Shoot
(mg Cu.L <sup>-1</sup> )	She length (mm) ma	oot <sup>a</sup> ma ss (g)	Germination ass (g) (%) <sup>b</sup>	SR Ratio <sup>c</sup> length (mm)
+ C a	6.8946 a	0.0	060784 a	14.3464 a
- Ca	0.2 6 4902 a	3250 a	71.250 a	4.9925 a
Cu	0.23100 a	61.176 b	4.5828 a	13.5157 d

 $^{a}$  Means followed by the same letter are not significantly different (P=0.05)

Germination (%)<sup>b</sup> = Percentage germination <sup>c</sup> S/R Ratio = Shoot/root ratio (mass bas is)

## 3.4 Discussion:

According to Table 3.3.1 there were significant differences for all six growth parameters (root length, root mass etc.) in this comparative study of four dryland wheat (*Triticum aestivum*) cultivars. Witwol germinated and developed the best as expected, since this was also the case with the two previous screenings (Section 2.3). In second place was SST 34 also called Rooigys and then SST 37 (Losper). Rooiwol did worst by far of all four dryland wheat cultivars (Table 3.3.1). These particular Rooiwol seeds were collected from the farm Lang Bank (District Steinkopf). Most experimental studies of heavy metal tolerance in dryland plants confirm the fundamental tenet that populations growing in metal-contaminated habitats are differentiated from populations of the same species growing in clean sites by possessing genetically based tolerance (Ye *et al*, 2003). Cultivars differ from each other genetically and even regionally within the cultivars, which suggests that the germination of Rooiwol from this region may be worse or better than Rooiwol from another region.

At low copper concentrations all the recorded parameters were more or less maximal and minimal at high copper concentrations (Table 3.3.2). Anatomical changes accompanied this decline at high copper concentrations. Long, healthy and less branched roots developed at low copper concentrations and short, branched roots developed at high copper concentrations for both "plus " and "minus" calcium treatments. Another observation at extreme copper concentrations was seeds developing a hypocotyl with no roots whatsoever. According to Marschner (1995) a large copper supply usually inhibits root growth before shoot growth. This does not imply that roots are more sensitive than shoots, but roots are the preferential copper

accumulation sites when the external copper supply is high. Only at 125 mg Cu.L<sup>-1</sup> was a reduction in chlorophyll content observed, causing evenly distributed yellowing over the surface of the coleoptiles, which is a characteristic symptom of copper toxicity (Table 3.3.2). Nine days were sufficient for chlorosis to set in. At 40 mg Cu.L<sup>-1</sup> Rooiwol and Witwol caryopses showed advanced *Penicillium* and *Aspergillus* sp. growth on the paper dolls without calcium: no fungi occurred on any of the paper dolls with the 0.5mM calcium treatment. Fungi could be living on sugars leached from seeds where calcium is absent and membrane integrity not as well maintained. This does not necessarily mean that the seeds were contaminated since they were thoroughly sterilised as discussed in the "Materials and Methods". Their spores are airborne and can even occur within the seeds and are generally all over (Personal communication: Jeremy Klaasen, ARC). At 125 mg Cu.L<sup>-1</sup> Penicillium again occurred on the Rooiwol seeds which is strange since high copper concentrations would be expected to inhibit fungal infestation. In 1878 vine downy mildew, which is caused by the plant pathogenic fungus Plasmopara viticola, was introduced to the southwest of France. The vine downy mildew spread like wildfire across all European vineyards. In order to control the fungus a mixture of Ca (OH)<sub>2</sub> and CuSO4 was systematically applied to successfully combat the infestation by the fungus (Brun et al, 2000).

In most studies on contaminated soils the determination of plant copper uptake has been restricted to measurements of the copper content in the aerial parts of the plants. However, in contaminated soils copper can substantially accumulate in the roots of a plant without any significant increase in the copper content of the aerial parts, since divalent copper atoms have a high affinity for the negatively charged components of

the root cell walls. Therefore, estimating copper availability on the basis of the copper content in the above ground portion of the plants can lead to largely underestimated values (Brun *et al*, 2000). Root elongation is 'almost' the universal method for assessing degrees of tolerance to toxic metals (Marschner, 1995). It is evident from Table 3.3.2 that root de velopment was severely inhibited since there are significant differences at each of the four copper concentration increments, while the shoots only showed toxic affects at the highest copper concentration (125 mg Cu.L<sup>-1</sup>).

Statistically Table 3.3.3 and Figure 3.3.1 do not indicate significant differences for any of the growth parameters except for percentage germination. Percentage germination may exhibit a significant difference since calcium is actively involved in cell division and the absence of exogenous calcium could result in the cessation of root growth (Marschner, 1995). The tentative deduction that can be made, is that calcium does not enhance nor does it inhibit copper uptake. A possible reason could be that much of the calcium precipitated out as  $CaSO_4$ ; perhaps  $CaC_b$  and  $CuCl_2$ should have been used instead of  $CaSO_4$  and  $CuSO_4$ . The outcome of this study is contradictory to a similar study done by Bharti *et al* (1996) on *Sesamum indicum*, where the seedlings were treated with 10 mM calcium chloride. The  $Cu^{2+}$ accumulation was significantly enhanced and again it was observed that the roots accumulated more  $Cu^{2+}$  than the leaves in the presence of a high calcium concentration.



**Figure 3.1**: The effect of the plus and minus calcium treatments on germination.

# **3.5 References:**

Antosiewicz DM, Hennig J (2004) Overexpression of *LCT*1 in tobacco enhances the protective action of calcium against cadmium toxicity. Environmental Pollution **129**: 237-245

Bharti N, Singh RP, Sinha SK (1996) Effect of calcium chloride on heavy metal induced alteration in growth and nitrate assimilation of *Sesamum indicum* seedlings. Phytochemistry **41**: 105-109

Brun LA, Maillet J, Hinsinger P, Pépin M (2000) Evaluation of copper availability to plants in copper-contaminated vineyard soils. Environmental Pollution **111**: 293-302

Epstein E (1972) Mineral nutrition of plants: principles and perspectives. John Wiley and Sons, Toronto

Hampton JG, TeKrony DM (1995) Handbook of vigour test methods. (3<sup>rd</sup> edition) The International Seed Testing Association, Zurich, Switzerland

Marschner H (1995) Mineral nutrition of higher plants. (2nd edition) Academic Press, London

Perfus-Barbeoch L, Leonhardt N, Vavasseur A, Forestier C (2002) Heavy metal toxicity: cadm ium permeates through calcium channels and disturbs the plant water status. The Plant Journal **32**: 539-548

Salisbury FB, Ross CW (1992) Plant Physiology. (4<sup>h</sup> Edition) Wadsworth Publishing Company, Belmont, California

Schosseler PM, Wehrli B, Schweiger A (1999) Uptake of Cu<sup>2+</sup> by the calcium carbonates vaterite and calcite as studied by continuous wave (cw) and pulse electron paramagnetic resonance. Geochimica et Cosmochimica Acta **63**: 1955-1967

Sutcliffe JF (1962) Mineral salts absorption in plants. Pergamon Press, London

Sutcliffe JF, Baker DA (1974) Plants and mineral salts. Edward Arnold, London

Ye ZH, Baker AJM, Wong MH, Willis AJ (2003) Copper tolerance, uptake and accumulation by *Phragmites australis*. Chemosphere **50**: 795-800

### ABSTRACT

# WHAT DO PLANTS DO WITH COPPER?

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Rooiwol, Rooigys, Losper and Witwol were cultivated in a greenhouse at copper concentrations varying from 0 to 125 mg Cu.kg<sup>-1</sup>. Four cultivars, three replicates and five copper concentrations were used. The concentration of the following elements were determined in the shoots and roots : Cu, Fe, Zn, Mn, K, Mg, Ca, Na, N and P. Witwol performed the best. Witwol achieved this tolerance by excluding copper uptake at the rhizosphere and further inhibiting translocation to the shoots. Shoot copper levels in the copper tolerant Witwol reached 0.144 mg Cu.kg<sup>-1</sup> whereas in the copper sensitive Rooiwol they reached 0.175 mg Cu.kg<sup>-1</sup>.

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# **<u>Chapter 4:</u>** What do wheat plants do with copper?

#### **4.1. Introduction:**

Pollution sources can have a natural or a human origin and can be site-specific or diffuse (Lehoczky and Kiss, 2002). Natural sources of copper contamination include wind-blown dust, decaying vegetation, forest fires and sea spray, while human activities contributing to copper release include mining and metal production (Anonymous a, 2002). Heavy metals are also deposited in soils by atmospheric input, the use of mine ral fertilizers or compost and sewage sludge disposal. Agricultural chemicals used decades ago resulted in zinc, copper, lead and silver accumulation (Lehoczky and Kiss, 2002). It has long been established that Co, Cr and Cu, like other pollutant elements, are relatively toxic to plants when given in supranormal doses. Copper uptake is a metabolically mediated process and the element is taken up either as a divalent cation  $(Cu^{2+})$  or as Cu chelate (Chatteriee and Chatteriee, 2000). The ready availability of copper can adversely influence plant diversity (Anonymous a, 2002). Sewage sludge compost adds organic matter and improves soil properties such as bulk density, porosity, water holding capacity and it can also increase aggregate stability. However, sewage sludge can introduce potentially toxic trace elements, including heavy metals (Korboulewsky et al, 2002). The disposal of copper containing waste water results in sewage sludge contaminated with copper (Anonymous a, 2002).

The combustion of fossil fuels releases copper into the air, which remains in the air for a period of time. When it starts to rain, large quantities may end up in the soil. As a result soil may contain high levels of copper after copper from the air has settled. (Anonymous a, 2002).

Contamination of the soil-water-air-plant-animal-human system with toxic heavy metals is a form of the chemical environmental load which has health, economic and ecologic importance (Lehoczky and Kiss, 2002). The heavy metals pose a health hazard to humans as well as plants and animals, often requiring soil remediation practices. Conventional remediation methods usually involve excavation, removal of contaminated soil layers or the washing of contaminated soils with strong acids or heavy metal chelators. However, conventional technologies used for small areas of heavily contaminated sites are not economically feasible for larger areas, resulting in less arable land for cultivation. Copper contaminated soils are not limited to the copper mining areas (Greman *et al*, 2001). Meerkotter (2003) and Meerkotter *et al* (2003) found the inner city farming area of Philippi near Cape Town to have soil copper levels that exceeded the guidelines (WRC, 1997). Data on the soil copper status of two regions in the Northern Cape is included as an appendix as mentioned in Section 2.1.

## **4.2 Materials and Methods:**

### **4.2.1** Cultivation and treatment of the wheat plants:

Two weeks before cultivation started, the greenhouse was fumigated with 3.5 % sodium hypochlorite and Terminex, a fungicide used by the Agricultural Research

Council at The Nietvoorbij Research Station, Stellenbosch (Personal Communication – J.Claasen). Four winter rainfall wheat cultivars (Rooiwol, Witwol, Rooigys and Losper) were previously selected using the Paper Doll Method as described in Chapter 2. Sixty pots with a diameter of seven cm and a total volume of 250 ml were appropriately labelled according to the cultivars, copper treatment and replicate number using a permanent marker. The pots were filled with clean quartz sand. Free copper ions were removed from the sand with deionised water to ensure that the soil was as free as possible from unknown ions, which could affect growth. The 60 pots were randomly placed into three blocks of 20 pots each, to allow each treatment to enjoy an equal chance of experiencing favourable as well as unfavourable conditions, on an asbestos surface in the experimental greenhouse. In each pot 15 seeds of a specific wheat cultivar were planted at a depth of three cm and watered daily with 100 ml deionised water for the first week. The following week each pot daily received 100 ml half-strength Hoagland solution as described in Epstein (1972). During week three the majority of the plants reached the two-leaf stage and each pot received 100 ml full-strength Hoagland solution. The first of the five copper treatments was started in week four and was applied as CuSO<sub>4</sub>.5H<sub>2</sub>O. The twelve control pots were given 100 ml of Hoagland solution only, while the rest (48 pots) were given a 100 ml solution containing Hoagland solution as well as 4 mg  $Cu.L^{-1}$ . The last two days of week 4, 12 pots remained as the control, 12 pots remained as 4 mg Cu.L<sup>-1</sup>, while the rest (36 pots) received a 100 ml solution containing Hoagland solution and 12.5 mg Cu.L<sup>-1</sup>. The first three days of week five the soil copper concentration was raised to 40 mg Cu.L<sup>-1</sup> for 24 of the 60 pots. Eventually the sand copper concentration was raised to the highest copper treatment, 125 mg Cu.L<sup>-1</sup>, for the last 12 pots at the end of week five. Thus the copper treatments were increased incrementally until every concentration

had been reached. The full treatments were continually applied on a daily basis for an additional two weeks before the plants were harvested. The waterholding capacity of each sand-filled pot was 100 ml and therefore each application was also 100 ml in order to replace the solution in the sand-filled pot as completely as possible

# **4.2.2 Harvesting and preparation of Material:**

The plants were harvested seven weeks after the seeds were planted and two weeks after the highest Cu-concentration was applied. At harvesting, the plants were separated into shoots and roots by dissecting the plants just below the caryopsis. In total there were now 120 samples and the caryopses were removed to avoid the addition of pre-treatment plant material. The roots were carefully but vigorously washed with deionised water to remove sand particles. The fresh mass of the shoots of the wheat plants were recorded and the material was placed in labelled brown paper bags. Dry Mass of the roots and shoots were also recorded after drying the material for 72 hours at 70 °C to a constant weight in an oven. The material was then ground using a Wiley Intermediate Mill.

# 4.2.3 Acid Digestion:

The samples were digested using the sulphuric-acid/hydrogen peroxide procedure of Allen (1989). Preferably 0.4g of each sample was weighed out exactly and neatly folded inside two Rizzla cigarette papers before being placed in a digestion tube. Samples of less than 0.4g were recorded accurately for consideration in future calculations. A 4.4 ml aliquot of the digestion mixture was dispensed into each glass

digestion tube before it was placed in the Buchi aluminium digestion block and heated to a temperature of 200 °C. The temperature was raised hourly by 50 °C up to 350 °C. After another hour the temperature was raised to 380 °C at which digestion was continued until a clear and colourless solution was obtained. The glass tubes were removed from the digestion block before it was turned off. The samples were cooled, filtered and diluted with deionised water into a 100 ml volumetric flask and made up to volume with deionised water. Blank solutions were prepared using the same method.

# **4.2.4 Nitrogen Determination:**

Standard microkjeldahl distillations and titrations were carried out as described by Allen (1989). A Buchi 320 Nitrogen distillation apparatus was used.

# **4.2.5 Phosphorus Determination:**

The Murphy and Riley (1962) Method was used to determine the phosphorus content of the plant samples. A Shimadzu UV-160A UV-visible recording spectrophotometer was used.

### **4.2.6 Atomic Absorption Spectrophotometry:**

A Unicam Solaar M Series Atomic Absorption Spectrophotometer was used to analyse the extracts for cations. An air-acetylene flame was used for all the analyses. Standards were produced using stock solutions of 1000 mg.L<sup>-1</sup> of Saarchem (Ca), Riedel-de Haën (K) and Spectrosol (Mg, Cu, Zn and Na). Dilutions were made with a 1% H<sub>2</sub>SO<sub>4</sub> solution to get the required concentrations.

#### 4.3. Results

Table 4.1 shows the effect of copper on germination and early growth of four Dryland wheat cultivars. Witwol performed better than Losper in all respects but in the shoot-to-root ratio no differences were found. Rooigys also had the highest fresh and dry shoot mass as well as high percentage germination.

Table 4.2 shows the effects of copper treatments on growth. There were fewer significant differences with the copper concentration series than between the cultivars. The dry and the fresh shoot masses were lower at the highest copper concentrations. Thus clearly shoot growth was affected more than root growth

Table 4.3 shows the overall chemical composition of cultivars under copper treatment. Losper plants contained high amounts of heavy metals such as Cu (0.595 mg.kg<sup>-1</sup>DW) and Fe (0.481 mg.kg<sup>-1</sup>DW). According to Larcher (2003) the average content of mineral nutrients in the phytomass of wheat plants should have been between 0.004 to 0.02 mg.kg<sup>-1</sup>DW for copper and between 0.002 to 0.7 mg.kg<sup>-1</sup>DW for iron. The mineral nutrient concentrations of the rest of the cultivars were evaluated

on the same criteria. Losper plants also contained high levels of cations like Ca, Mg, Na and in P, but low levels of N and K. Excess copper caused an increase in the phosphorus concentration, which is in agreement with the findings for other plants (Chatterjee and Chatterjee, 2000). Witwol was fairly low in Fe, Ca and Na.

The chemical composition of the roots of cultivars under copper treatment is given in Table 4.4. Cu, Fe, Mg, Ca and P levels were high in the roots of Losper, but they were low in K and N. Witwol was fairly low in Fe, while Rooigys and Rooiwol were often intermediate.

Table 4.5 shows the chemical composition of shoots of different cultivars. The heavy metals (Cu, Fe, Zn) in the shoots of Losper were high as well as the cations (Mg, Ca, Na). The nitrogen levels were also high in the shoots of Losper. The shoot copper concentrations were again low in Witwol, but concentrations of Mn and K were high in the shoots. Rooiwol and Rooigys were again intermediate.

The overall effect of copper treatments on the chemical composition is shown in Table 4.6. There was a significant increase in copper at the two highest copper levels. The heavy metals, cations and N were not affected by the copper increments. Phosphorus was at its highest at low copper levels.

Table 4.7 represents the effects of copper treatments on the chemical composition of roots. The root copper concentrations were higher at the two highest copper treatments. Potassium and Mn decreased with increased copper concentrations. The other elements studied were not affected.

Table 4.8 shows the effect of copper treatment on the chemical composition of shoots. The shoot copper levels were higher at the two highest Cu treatments. The Zn, Ca and Na levels increased with the copper increments. The P levels were high at low copper treatments. The other elements studied were not affected.

A comparison of the copper content of roots and shoots is given in Table 4.9. The roots had higher levels of heavy metals like Cu, Fe and Zn, but not Mn. The roots had higher levels of Ca and Na, but lower levels of K. There were no significant differences for Mn, Mg, N and P.

Cultivar	Shoot mass (g) (%) <sup>b</sup>	Shoot Germina length (mm)	Shoot ation SR Rat freshmass (g)	Root tio <sup>c</sup> dry mass (g)	dry
Witwol	3.824 a	0.541 a 89.67 a	6.154 a 11.918	0.541 a a	
Rooiwol	1.273 b	0.377 ab 37.33 c	2.617 bc 12.618	0.377 ab a	
Rooigys	1.916 b	0.358 ab 77.33 b	3.629 b 27.552	0.358 ab a	
Losper	0.713 c	0.105 b 44.33 c	1.084 с 7.744 г	0.105 b a	

# **Table 4.1:**The effect of copper on germination and growth of four wheat<br/>cultivars over a seven week period

<sup>a</sup> Means followed by the same letter are not significantly different (P=0.05) Germination (%)<sup>b</sup> = Percentage germination <sup>c</sup> S/R Ratio = Shoot/root ratio (mass basis)

**Table 4.2:** The effect of different copper concentrations on the growth and germination of wheat cultivars.

Concentration	Shoot	Shoot	Germina	Shoot tion	SR Ratio	Root	
(mg Cu.kg <sup>-1</sup> )	mass (g) (%) <sup>b</sup>	length (1	mm)	fresh ma	ass (g)	dry mass (g)	dry 
0	2.872 a	0.880 a	63.33 a	4.782 a	32.030 a	0.491 a a	
4	1.908 a	0.560 a bc	62.50 a	3.467 a	b 9.600 a	0.385 a	
12.5	2.207 a	0.627 a b	63.75 a	3.650 a	b 17.430 a	0.339 a a	
40	1.578 t	0.495 a	62.92 a	2.847 a	b 6.670 a	0.277 a	
125	1.093 c	0.333 a	58.33 a	2.109 b	9.050 a	0.233 a	

<sup>a</sup> Means followed by the same letter are not significantly different (P=0.05) Germination (%)<sup>b</sup> = Percentage germination <sup>c</sup> S/R Ratio = Shoot/root ratio (mass basis)

#### Comparative concentration levels (mg.kg<sup>-1</sup>DM) of 10 **Table 4.3:** elements in four dryland wheat cultivars under copper treatment.

# **Dryland Wheat Cultivars**

	Witwol Rooigys	Rooiwol Losper	
Copper	0.267 b 0.385 b	0.298 b 0.595 a	
Iron	0.100 c 0.230 b	0.152 bc 0.481 a	

Zinc	0.1 0.0	.02 a 093 a	0	).108 a ).114 a
Manganese 0.054 a	0.056 a	0.045 a	0.055 a	
Potassium	42.078 a 39.040 a		28.565 I	36.820 a b
Magnesium	2.174 b 2.568 b		2 4.168 a	2.531 b
Calcium	22.240 c 40.500 bc	78.690	a	49.150 b
Sodium	3.5 4.2	i47 c 289 bc	5	5.607 ab 5.785 a
Nitroger	n 32. 37	.702 b .802 b	6 4	50.004 a 15.509 b
Phospho	<b>Drus</b> 9.0 10	034 b .402 b	1 1	0.964 ab 4.230 a

# Table 4.4:The effect of copper treatments on the root concentration of<br/>ten elements in four wheat cultivars.

_		Duvland Wheat
(	Cultivars	Di yiand wheat
	Witwol Rooigys	Rooiwol Losper
-		
Copper	0.389 b 0.499 ab	0.421 ab 0.586 a
Iron	0.173 c	0.267 bc
	0.417 b	0.843 a
Zinc	0.177 a	0.182 a
	0.152 a	0.178 a
Manganese	0.041 a	0.055 a
-	0.054 a	0.060 a
Potassium	17.487 a	17.205 a
	16.743 a	11.678 b
Magnesium	1.517 b	2.701 b
-	2.523 b	4.836 a

Calcium	34.790 b 68.440 ab	84.120 a 80.190 a
Sodium	6.033 b 7.387 b	9.761 ab 11.257 a
Nitrogen	29.930 b 35.570 b	81.070 a 45.130 b
Phosphorus	8.371 b 8.321 b	9.338 b 19.512 a

# Table 4.5:The effect of copper treatments on the shoot concentration<br/>of ten elements in four wheat cultivars.

# **Dryland Wheat Cultivars**

	Witwol Rooigys	Rooiwol Losper	
<b>Copper</b> 0.271 b	0.144 c	0.175 bc 0.604 a	
Iron	0.027 b 0.044 b	0.037 b 0.119 a	
Zinc	0.028 b 0.035 b	0.034 b 0.051 a	
Manganese 0.054 b	0.070 a	0.055 b 0.031 c	
<b>Potassium</b> 61.336 a	66.669 a	56.435 ab 45.451 b	
Magnesium 2.6	2.832 b 113 b	2.360 b 3.499 a	
<b>Calcium</b> 12.	9.684 b 559 b	14.166 b 77.187 a	

Sodium	1.061 b 1.191 b	1.453 b 2.312 a
Nitrogen	35.475 b 40.034 ab	38.941 ab 45.888 a
Phosphorus	9.697 ab 12.484 a	12.591 a 8.948 b

# Table 4.6:The effect of copper treatments on element levels (mg.kg <sup>-1</sup>DM) of four dryland whe at cultivars.

		Сорр			
		0	40	4 125	12.5
Copper		0.018 c	0.08	38 c	0.163 c
	0.504 b		1.158 a		
[ron	0.206 0.208 a	a 0.339	0.229 a a	0.22	3 a
Zinc	0.091 0.098 a	a 0.138	0.080 a a	0.11	5 a
	Manganese	0.059 a	0.047 a	0.056 a 0.048	0.053 a
Potass	<b>sium</b> 38.952 a	40.552 a	30.880 a	38.194 a	34.551 a
Magn	<b>esium</b> 2.791 a	2.487 a 3.100	a.19	99 a	2.724 a
Calciu	<b>im</b> 46.919 a	35.783 a	50.426 a	51.791 a	53.296 a
Sodiu	<b>m</b> 5.351 a	4.214 a 5.447	5.64 a	47 a	4.624 a
Nitro	<b>gen</b> 45.216 a	38.890 a	45.230 a	44.366 a	46.320 a

Phosphorus	10.761 b		14.212 a	
10.492 b		9.964 b		10.360 b

# Table 4.7:The effect of copper treatments on element levels (mg.kg<sup>1</sup>DM) in the roots of wheat cultivars.

	C 0		Coppe 0	er Treatm		12.5			
				40			125		
Сорре	<b>r</b> 0.668 b	0.023 c		0. 1.273 a	153 c			0.253 c	;
Iron	0.370	b 0.365 t	)	0.4	409 a	b 0.595 a			0.386 ab
Zinc	0.152 a 0.161 a	1	0.225 a	0.128 a 1			0.195 a	l	
	Manganese		0.066 a	0.045 bc		0.059 a	0.039 c		0.054 ab
Potassi	<b>ium</b> 17.674 a	20.778	a	11.702 b		18.899	a	9.840	b
Magne	e <b>sium</b> 2.942 a	2.335 a	2.951 a	3.: 1	578 a			2.665 a	l
Calciu	<b>m</b> 64.210 a	58.410	a	63.260 a		88.910	a	59.650	a
Sodiur	<b>n</b> 9.269 a	7.210 a	8.780 a	9.5 1	862 a			7.926 a	l
Nitrog	<b>en</b> 49. 950 a	38.250	a	54.470 a		47.680	a	49.280	a
Phosph	<b>10rus</b> 10.339 a	10.564	a	9.666 a		13.992	a 12.367	a	

<sup>a</sup> Means followed by the same letter are not significantly different (P=0.05)

<u>Table 4.8:</u>	The effect of copper treatments on element levels (mg.kg
	<sup>1</sup> DM) in the shoots of wheat cultivars.

		Co 0	opp	er Treatment		12.5			
Coppe	e <b>r</b> 0.340 b	0.013 c		0.023 1.044 a	c		0.073 c	2	
Iron	0.042 4			0.048 2		0.050			
II UII	0.042 a	0.0	84 :	0.048 a a		0.0397	ı		
Zinc	0.030	b		0.033			0.035 ab		
		0.034 ab			0.051 a	a			
	Manganese	0.05	53 a	0.050 a	0.054 a	0.056.a		0.051 a	
				0.020 u		0.050 u			
Potass	ium	60.326 a		50.059 -	57.489	a	50.261	_	
	60.230 a			50.058 a			59.261	а	
Magne	esium	2.639 a		2.820	a		2.783 a	ι	
	2.639 a	3.2	49 :	a					
Calciu	m	13.159 b			14.668	b			
	29.633 ab	37.	596	a		46.940	a		
Sodim	m	1 218 h			1 /32	h			
Soura	1.323 b	1.210 0		1.434 b	1.452	0	2.113 a	l	
<b>.</b>		20 521			44.055				
Nitrog	g <b>en</b> 40.484 a	39.531 a		35 988 a	41.055	a	43 365	я	
	-010- a			55.700 d			+5.505	a	
Phospl	horus	10.958 b		10.060.1	14.432	a	0 252 1		
	10.645 b			10.262 b			8.353 1	D	

	Org	gans		
	Roots	Shoots		
 Copper	0.474 a	0.299 b		
Iron	0.425 a	0.057 b		
Zinc	0.172 a	0.037 b		
Manganese	0.053 a	0.053 a		
Potassium	15.778 b	57.473 a		
Magnesium	2.894 a	2.826 a		
Calcium	66.887 a	28.399 b		
Sodium	8.609 a	1.504 b		
Nitrogen	47.924 a	40.084 a		
Phosphorus	11.386 a	10.930 a		

# Table 4.9:The effect of copper treatments on element levels (mg.kg <sup>-1</sup>DM) in the organs of four dryland wheat cultivars.

<sup>a</sup> Means followed by the same letter are not significantly different (P=0.05)

#### 4.3.2 Discussion:

### <u>Cultivars –</u>

Witwol, Rooiwol, Rooigys and Losper exhibited clear differences in percentage germination and yield productivity as expected (Table 4.1). Losper differed significantly from the rest of the cultivars and appeared to be the least resistant to the stress effects of excess copper. Witwol was the most copper tolerant followed by Rooigys and then Rooiwol. This order can be attributed to the fact that Witwol, Rooigys and Rooiwol were historically used by subsistence farmers in the Northern Cape (Steinkopf and Concordia) as mentioned in chapter two. The Northern Cape is well-known for its copper mines and thus its soil content has high copper levels. This is sufficient evidence to conclude that Witwol and Rooigys have adapted defence mechanisms over the years to cope more efficiently with copper toxicity. Rooigys grew well under these extreme environmental conditions since it is also from the Northern Cape Region, unlike Losper.

It's evident from Table 4.3 that Losper acquired more heavy metals such as copper and iron from the soil than the rest of the cultivars. Witwol acquired the least amounts of copper from the soil (0.267 mg Cu.kg<sup>-1</sup>) and had the highest percentage germination and yield productivity. In contrast, Losper, acquired 0.595 mg Cu.kg<sup>-1</sup> from the soil and yielded the least plant material. Except for Losper all the cultivars had higher copper concentrations in the roots than the shoots, which in turn may explain Losper's poor growth performances (Table 4.4 and 4.5). Losper displayed smaller, more chlorotic leaves and was thus shown to be more sensitive to copper toxicity. According to Marschner (1995) a large supply of copper usually inhibits root growth before shoot growth since roots are the sites for preferential copper accumulation when the external copper supply is high. As in the preceding experiments (Chapter 2 and 3) it was evident that the roots were smaller and less developed than the shoots. Heavy metals become concentrated near the soil surface without soil mixing therefore the roots of plants growing in non-tilled soils are mostly concentrated at shallower depths (Düring *et al*, 2003). It is clear that Losper is less able to cope with copper stress since it did not restrict or exclude copper uptake by the roots and even allowed substantial amounts of copper (0.604 mg Cu.kg<sup>-1</sup>) to be transported to the shoots.

## <u>Copper Treatments-</u>

The amount of copper taken up by the plant is directly proportional to the amount of copper available to the plant (Marschner, 1995). This statement is substantiated in Tables 4.6, 4.7 and 4.8 since more copper was acquired with increasing copper levels. Table 41 shows a significant decrease in fresh and dry shoot mass as the copper concentration increased. After six weeks at 125 mg Cu.L<sup>-1</sup> the shoot growth of all the cultivars was strongly inhibited, showing toxic symptoms such as partial discolouration of the kaves, desiccated appearance and an early harvest was necessary to ensure that there would be sufficient fresh plant material for analysis. Optimal fresh and dry mass were obtained below 12.5 mg Cu.L<sup>-1</sup> (Table 4.2). The copper treatments in general had a significant influence on the growth, development and chemical composition of *Triticum aestivum*.

The copper treatments caused no significant decreases in the nitrogen concentration of roots and shoots. It was anticipated that the elevated copper levels would decrease the nitrogen levels since copper toxicity interferes with nitrogen metabolism in

cauliflower (Chatterjee and Chatterjee, 2000, Table 4.7 and 4.8). The phosphorus concentration in roots and shoots showed no significant differences with increase in copper concentration, while the phosphorus concentration in Losper was significantly higher than for the rest.

In conclusion, there is ample evidence confirming that copper tolerance in Witwol and Rooigys were achieved through the exclusion of copper at the rhizosphere and the inhibition of its translocation from roots to shoots. The latter is evident from Table 4.9 where the copper content in the roots and shoots is given as 0.474 mg Cu.kg<sup>-1</sup> and 0.299 mg Cu.kg<sup>-1</sup> respectively. These large differences indicate an important restriction of the internal transport of copper from the roots towards shoots. Chatterjee and Chatterjee (2000) stated that the displacement of several ions from root exchange sites by copper has been reported because copper is very strongly bound in the rootfree spaces. Roots are frequently higher in copper content than other plant tissues. According to Table 4.3 the copper content in Witwol (0.267 mg Cu.kg<sup>-1</sup>) is less than half that of Losper (0.595 mg Cu.kg<sup>-1</sup>). This suggests that Witwol employed the exclusion mechanism to minimise its root copper concentration, while Losper did not. Witwol can be classified as a pseudometallophyte since it avoided metal uptake and restricted metal transport to the shoots, unlike hyperaccumulators that absorb metal contaminants from the soil and transport them to the aerial parts (Dahmani-Muller et al, 2000). The next step in understanding this evolutionary adaptation to these adverse conditions would be to investigate the assessed cultivars at the mole cular level. This will paint the bigger physiological picture as to why some cultivars are better equipped than others to survive metal toxicity.

# **4.4 References:**

Allen SE (1989) Chemical Analysis of Ecological Material (2<sup>nd</sup> edition). Blackwell Scientific Publications, Oxford

Anonymous a (2002): http://www.eco-use.net/toxics/copper.shtml

Chatterjee J, Chatterjee C (2000) Phytotoxicity of cobalt, chromium and copper in cauliflower. Environmental Pollution **109**: 69-74

Dahmani-Muller H, van Oort F, Gélie, Balabane M (2000) Strategies of heavy metal uptake by three plant species growing near a metal smelter. Environmental Pollution **109**: 231-238

Düring R, Hoß T, Gäth S (2003) Sorption and bioavailibity of heavy metals in longterm differently tilled soils amended with organic wastes. The science of the total environment **313**: 227-234

Epstein E (1972) Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, Toronto, Canada

Grcman H, Velikonja-Bolta Š, Vodnik D, Kos B, Leštan D (2001) EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. Plant and Soil **235**: 105-114 Korboulewsky N, Dupouyet S, Bonin G (2002) Environmental risks of applying sewage sludge compost to vineyards: carbon, heavy metals, nitrogen, and phosphorus accumulation. J. Environ. Qual **31**: 1522-1527

Larcher W (2003) Physiological plant ecology (4<sup>th</sup> edition). Springer-Verlag Publishers, Berlin, Germany

Lehoczky E, Kiss Z (2002) Cadmium and Zinc uptake by ryegrass (Lolium *perenne L.*) in relation to soil metals. Communication in Soil Science and Plant Analysis **33**: 3177-3187

Marschner H (1995) Mineral Nutrition of higher plants (2<sup>nd</sup> edition). Academic Press, London

Meer kotter M (2003) Heavy Metals, and vegetable farming in Cape Town. Unpublished M.Sc.Thesis, University of the Western Cape, Bellville

Meerkotter M, Aalbers J, Raitt LM, Cook B (2003) A survey of heavy metal accumulation in irrigation water, soil and vegetables of the Philippi area of the Cape Flats. South African Journal of Botany **69**: 242

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Analytical Chemistry Acta **27**: 31-36

WRC (Water Research Commission) (1997) Permissible utilization and disposal of sewage sludge. WRC, Pretoria

## APPENDIX:

GEOCHEMICAL DATA: SPRINGBOK

Мар	No.	Loy	Lox	Cu	Мар	No	Loy	Lox	Cu	Мар	No	Loy	Lox	Cu
4TL	2B	73377.21	-3265851.06	87	4TL	27I	80284.48	-3290954.46	92	4TL	23Q	88662.87	-3286736.08	215
4TL	3B	73416.68	-3266098.77	97	4TL	281	80159.21	-3291137.83	36	4TL	24Q	88686.57	-3287436.68	232
4TL	4B	73207.97	-3267463.7	49	4TL	2J	81134.4	-3265944.15	271	4TL	25Q	88021.35	-3288292.21	150
4TL	5B	73866.84	-3268788.75	421	4TL	ЗJ	81453.8	-3266923.72	375	4TL	26Q	88887.5	-3289375.38	221
4TL	6B	73838.42	-3269775.19	1222	4TL	4J	81108.22	-3267686.09	110	4TL	28Q	88372.97	-3291100.72	186
4TL	7B	72956.78	-3270620.55	363	4TL	5J	81428.34	-3268635.46	155	4TL	2R	89521.57	-3265180.88	48
4TL	8B	73754.65	-3271183.37	655	4TL	6J	81682.25	-3269401.96	337	4TL	3R	89682.62	-3266463.92	1559
4TL	10B	72983.6	-3273527.14	174	4TL	7J	81006.91	-3270896.85	298	4TL	4R	89750.68	-3267205.87	361
4TL	11B	73574.31	-3274326.79	76	4TL	8J	81172.06	-3271369.14	99	4TL	5R	89751.64	-3268228.26	143
4TL	12B	73569.37	-3275172.78	1539	4TL	9J	81480.81	-3272585.17	542	4TL	6R	89429.73	-3269479.71	162
4TL	13B	73193.05	-3276594.17	872	4TL	10J	81324.61	-3273860.69	335	4TL	7R	89105.05	-3270635.41	121
4TL	14B	73538.41	-3277116.06	348	4TL	11J	81288.45	-3274323.18	543	4TL	8R	89117.25	-3271396.18	254
4TL	15B	73020.73	-3278549.21	349	4TL	12J	81623.15	-3275932.65	227	4TL	9R	89183.95	-3272833.11	100
4TL	16B	73663.37	-3279496.15	357	4TL	13J	81676.68	-3276437.55	180	4TL	10R	89387.84	-3273371.78	79
4TL	17B	73634.45	-3280291.2	152	4TL	14J	81870.76	-3277177.44	183	4TL	11R	89249.8	-3274093.74	182
4TL	18B	73140.82	-3281347.21	94	4TL	15J	81589.76	-3278616.21	230	4TL	12R	89668.36	-3275141.14	95
4TL	19B	73099.03	-3282897.4	187	4TL	16J	81770.95	-3279899.72	182	4TL	13R	88978.58	-3276182.42	88
4TL	20B	73425.23	-3283590.06	89	4TL	17J	81125.27	-3280780.89	273	4TL	14R	89725.18	-3277207.37	72
4TL	21B	73460.84	-3284638.46	98	4TL	18J	81420.93	-3281699.46	89	4TL	15R	89736.33	-3278224.96	112
4TL	22B	73613.89	-3285196.08	52	4TL	19J	81105.34	-3282684.14	108	4TL	16R	89231.54	-3279114.51	113
4TL	23B	73254.95	-3286522.2	81	4TL	20J	81371.69	-3283138.68	60	4TL	17R	89068.24	-3280263.95	89
4TL	24B	73073.92	-3287782.02	44	4TL	21J	81040.47	-3284782.74	76	4TL	18R	89668.12	-3281527.17	100
4TL	25B	73194.87	-3288630.99	49	4TL	22J	81439.47	-3285592.97	51	4TL	19R	89853.98	-3282825.9	85
4TL	26B	73617.18	-3289945.39	21	4TL	23J	81088.47	-3286158.79	50	4TL	20R	89214.54	-3283656.85	99
4TL	27B	73344.99	-3290800.15	40	4TL	24J	81595.52	-3287298.94	89	4TL	21R	89289.72	-3284736.4	84
4TL	28B	73141.79	-3291082.41	46	4TL	25J	81702.16	-3288751.92	86	4TL	22R	89891.98	-3285898.94	150
4TL	3C	74464.69	-3266949.55	122	4TL	26J	81281.52	-3289492.36	82	4TL	23R	89451.67	-3286618.78	210
4TL	4C	74250.9	-3267891.31	122	4TL	27J	81176.17	-3290960.47	57	4TL	24R	89274.92	-3287485.87	235
4TL	5C	74507.46	-3268758.6	851	4TL	28J	81103.97	-3291880.4	10	4TL	25R	89071.43	-3288417.81	205
4TL	6C	74697.74	-3269871.09	551	4TL	2K	82439.31	-3265310.27	324	4TL	26R	89249.97	-3289600.53	136
4TL	7C	74130.73	-3270834.71	962	4TL	ЗK	82694.43	-3266237.95	303	4TL	27R	89442	-3290214.47	140
4TL	8C	74737.81	-3271156.3	1490	4TL	4K	82480.52	-3267184.75	270	4TL	28R	89346.75	-3291894.34	159
4TL	9C	74222.25	-3272287.33	741	4TL	5K	82852.57	-3268281.4	482	4TL	2S	90084.02	-3265259.68	45
4TL	10C	74680.81	-3273985.35	1185	4TL	6K	82884.76	-3269898.82	208	4TL	3S	90638.98	-3266929.77	178

4TL	11C	74613.15 -	3274714.01	1261	4TL	7K	82319.88	-3270560.3	65	4TL	4S	90255	-3267399.12	212
4TL	12C	74577.47 -	3275156.36	2221	4TL	8K	82164.56	-3271160.98	356	4TL	5S	90563.01	-3268433.82	125
4TL	13C	74125.23 -	3276379.55	1603	4TL	9K	82691.52	-3272311.67	131	4TL	6S	90449.98	-3269801.02	204
4TL	14C	74100.87 -	3277194.85	1011	4TL	10K	82778.15	-3273970.67	441	4TL	7S	90696.11	-3270683.17	474
4TL	15C	74427.09 -	3278099.04	205	4TL	11K	82723.64	-3274357.17	276	4TL	8S	90284.13	-3271695.78	98
4TL	16C	74338.69 -	3279915.05	467	4TL	12K	82793.16	-3275250.24	516	4TL	9S	90588.57	-3272881.49	127
4TL	17C	74628.42 -	3280445.69	97	4TL	13K	82764.26	-3276256.82	321	4TL	10S	90080.15	-3273498.98	123
4TL	18C	74606.61 -	3281578.34	213	4TL	14K	82676.5	-3277408.06	879	4TL	11S	90157.62	-3274694.42	63
4TL	19C	74477.49 -	3282773.93	132	4TL	15K	82142.26	-3278689.73	159	4TL	12S	90218.81	-3275088.7	178
4TL	20C	74826.15 -	3283794.48	154	4TL	16K	82873.37	-3279517.89	115	4TL	13S	89996.59	-3276810.89	76
4TL	21C	74467.73 -	3284460.86	115	4TL	17K	82350.51	-3280744.44	162	4TL	14S	90147.99	-3277650.5	180
4TL	22C	74266.68 -	3285715.17	59	4TL	18K	82715.64	-3281070.38	122	4TL	15S	90735.55	-3278157.98	727
4TL	23C	74464.62 -	3286717.05	72	4TL	19K	82407.64	-3282372.52	150	4TL	16S	90378.42	-3279620.12	54
4TL	24C	74331.71 -	3287222.57	66	4TL	20K	82108.05	-3283745.37	97	4TL	17S	90649.08	-3280104.98	102
4TL	25C	74643.78 -	3288086.13	99	4TL	21K	82392.99	-3284265.82	78	4TL	18S	90678.06	-3281858.3	163
4TL	26C	74041.86 -	3289884.94	49	4TL	22K	82671.15	-3285284.71	79	4TL	19S	90589.85	-3282178.54	120
4TL	27C	74081.74 -	3290540.61	51	4TL	23K	82094.49	-3286867.56	56	4TL	20S	90584.6	-3283462.67	103
4TL	28C	74157.96 -	3291363.33	39	4TL	24K	82063.51	-3287536.65	39	4TL	21S	90077.03	-3284256.46	100
4TL	2D	75577.38 -	3265072.18	241	4TL	25K	82746.97	-3288887.46	77	4TL	22S	90686.02	-3285560.18	244
4TL	3D	75488.93 -	3266465.15	266	4TL	26K	82894.96	-3289233.44	45	4TL	23S	90113.88	-3286740.23	210
4TL	4D	75221.87 -	3267103.47	151	4TL	27K	82120.19	-3290459.05	58	4TL	24S	90048.58	-3287156.69	388
4TL	5D	75062.06 -	3268318.47	454	4TL	28K	82730.35	-3291500.91	38	4TL	25S	90277.15	-3288567.24	243
4TL	6D	75055.97 -	3269637.84	532	4TL	2L	83681.63	-3265828.22	138	4TL	26S	90117.91	-3289545.55	313
4TL	7D	75155.47 -	3270541.69	1208	4TL	3L	83408.2	-3266310.26	113	4TL	27S	90519.72	-3290874.58	90
4TL	8D	75665.22 -	3271143.01	2407	4TL	4L	83798.46	-3267276.4	330	4TL	28S	90446.38	-3291205.23	79
4TL	9D	75064.58 -	3272674.93	690	4TL	5L	83735.14	-3268246.91	279	4TL	2T	91524.44	-3265923.33	82
4TL	10D	75175.34 -	3273528.69	897	4TL	6L	83611.39	-3269215.99	485	4TL	3T	91273.32	-3266526.78	202
4TL	11D	75070.69 -	3274755.07	5131	4TL	7L	83413.83	-3270535.85	320	4TL	4T	91663.55	-3267281.4	38
4TL	12D	75040.4 -	3275182.44	1520	4TL	8L	83294.76	-3271520.15	59	4TL	5T	91660.3	-3268268.44	132
4TL	13D	75550.73 -	3276609.73	417	4TL	9L	83803.13	-3272604.92	305	4TL	6T	91354.24	-3269701.57	175
4TL	14D	75785.69 -	3277325.41	1525	4TL	10L	83488.69	-3273116.22	355	4TL	7T	91463.83	-3270817.19	79
4TL	15D	75013.17 -	3278243.87	657	4TL	11L	83765.3	-3274200.54	448	4TL	8T	91362.64	-3271897.6	108
4TL	16D	75006.82 -	3279361.78	455	4TL	12L	83767.73	-3275585.58	874	4TL	9T	91478.77	-3272524.85	524
4TL	17D	75100.8 -	3280285.65	88	4TL	13L	83231.48	-3276101.69	275	4TL	10T	91080.33	-3273391.73	79
4TL	18D	75304.57 -	3281892.02	86	4TL	14L	83850.23	-3277843.8	300	4TL	11T	91483.19	-3274463.93	64
4TL	19D	75420.39 -	3282957.42	57	4TL	15L	83841.83	-3278835.75	202	4TL	12T	91719.46	-3275124.25	247
4TL	20D	75354.66 -	3283817.07	135	4TL	16L	83856.39	-3279284.33	235	4TL	13T	91199.42	-3276869.6	111
4TL	21D	75298.12	-3284289.15	191	4TL	17L	83610.78	-3280930.42	148	4TL	14T	91412.32	-3277665.34	71
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4TL	22D	75214.78	-3285254.14	74	4TL	18L	83833.62	-3281519.91	73	4TL	15T	91092.3	-3278624.73	872
4TL	23D	75071.06	-3286640.76	63	4TL	19L	83664.98	-3282044.73	52	4TL	16T	91708.45	-3279626.44	239
4TL	24D	75717.81	-3287839.61	63	4TL	20L	83419.33	-3283267.77	85	4TL	17T	91634.21	-3280420.42	693
4TL	25D	75550.93	-3288077.4	42	4TL	21L	83530.69	-3284096.36	62	4TL	18T	91554.35	-3281450.97	70
4TL	26D	75471.12	-3289531	48	4TL	22L	83115.37	-3285249.91	60	4TL	19T	91550.55	-3282886.23	632
4TL	27D	75255.85	-3290110.12	52	4TL	23L	83161.35	-3286923.05	66	4TL	20T	91112.47	-3283087.38	161
4TL	28D	75273.85	-3291263.86	144	4TL	24L	83779.49	-3287416.14	53	4TL	22T	91862.31	-3285250.61	250
4TL	2	76478.92	-3265300.03	237	4TL	25L	83737.19	-3288774.94	35	4TL	23T	91020.03	-3286348.71	74
4TL	4	76027.51	-3267550.64	280	4TL	26L	83234.91	-3289558.78	45	4TL	24T	91475.61	-3287109.92	142
4TL	5	76120.52	-3268303.25	538	4TL	27L	83902.61	-3290511.35	38	4TL	25T	91378.4	-3288447.27	157
4TL	6	76006.6	-3269282.63	651	4TL	28L	83833.37	-3291093.92	93	4TL	26T	91078.57	-3289830.19	109
4TL	7	76083.07	-3270095.29	52	4TL	2M	84227.98	-3265523.87	113	4TL	27T	91158.88	-3290693.3	87
4TL	8	76290.16	-3271349.2	3411	4TL	ЗM	84130.82	-3266221.61	150	4TL	28T	91518.09	-3291693.96	50
4TL	9	76168.56	-3272227.67	647	4TL	4M	84596.27	-3267416.17	111	4TL	2U	92354.52	-3265127.12	268
4TL	10	76329.94	-3273284.08	2025	4TL	5M	84303.39	-3268718.67	64	4TL	3U	92757.86	-3266179.18	53
4TL	11	76773.7	-3274543.59	2877	4TL	6M	84428.3	-3269613.06	310	4TL	4U	92805.02	-3267590.47	244
4TL	12	76748.31	-3275827.25	2253	4TL	7M	84175.08	-3270730.16	257	4TL	5U	92598.86	-3268210.09	186
4TL	13	76209.94	-3276645.49	953	4TL	8M	84637.82	-3271189.35	340	4TL	6U	92269.87	-3269335.46	636
4TL	14	76289.72	-3277105.68	1207	4TL	9M	84767.51	-3272945.06	302	4TL	8U	92679.64	-3271178.38	94
4TL	15	76250.39	-3278127.12	964	4TL	10M	84412.79	-3273455.39	1416	4TL	9U	92463.63	-3272638.83	49
4TL	16	76358.14	-3279957.85	58	4TL	12M	84298.63	-3274444.84	853	4TL	10U	92468.19	-3273721.75	72
4TL	17	76769.78	-3280234.53	202	4TL	13M	84205.58	-3275394.5	332	4TL	11U	92140.98	-3274771.62	222
4TL	18	76405.82	-3281772.05	199	4TL	14M	84459.08	-3276815.7	324	4TL	12U	92208.76	-3275100.58	100
4TL	19	76555.34	-3282903.73	176	4TL	15M	84482.9	-3277511.28	61	4TL	13U	92215.52	-3276515.95	44
4TL	20	76345.23	-3283477.92	144	4TL	15M	84518.56	-3278982.72	63	4TL	14U	92904.17	-3277222.23	55
4TL	21	76089.98	-3284680.59	70	4TL	16M	84797.38	-3279548.36	171	4TL	15U	92829.47	-3278247.87	91
4TL	22	76535.29	-3285874.67	156	4TL	17M	84412.16	-3280707.66	168	4TL	16U	92187.06	-3279416.18	161
4TL	23	76513.69	-3286785.73	0	4TL	18M	83996.6	-3281871.27	60	4TL	17U	92124.58	-3280351.46	188
4TL	24	76625.6	-3287166.1	47	4TL	19M	84043.45	-3282658.05	53	4TL	18U	92348.9	-3281303.6	115
4TL	25	76046.49	-3288215.05	81	4TL	20M	84436.48	-3283719.94	88	4TL	19U	92735.24	-3282647.37	1000
4TL	26	76152.29	-3289703.26	39	4TL	21M	84130.01	-3284745.13	108	4TL	20U	92824.67	-3283550.98	474
4TL	27	76621.59	-3290948.27	55	4TL	22M	84339.84	-3285883.27	96	4TL	21U	92586.7	-3284874.93	500
4TL	28	76770.2	-3291480.61	41	4TL	23M	84128.6	-3286079.72	59	4TL	22U	92420.71	-3285500.54	283
4TL	2F	77075.71	-3265842.98	495	4TL	24M	84342.58	-3287892.82	53	4TL	23U	92129.42	-3286098	456
4TL	3F	77779.07	-3266141.67	515	4TL	25M	84622.1	-3288216.73	69	4TL	24U	92168.89	-3287408.37	44
4TL	4F	77336.64	-3267163.63	705	4TL	26M	84204.99	-3289445.78	100	4TL	25U	92626.79	-3288496.99	32

4TL	5F	77580.07	-3268584.6	440	4TL	27M	84348.88	-3290602.5	56	4TL	. 26U	92235.57	-3289696.43	40
4TL	6F	77421.27	-3269119.72	521	4TL	28M	84476.94	-3291789.06	57	4TL	. 27U	92183.19	-3290843.47	53
4TL	7F	77010.86	-3270278.42	1931	4TL	2N	85440.28	-3265607.98	69	4TL	. 28U	92804.92	-3291397.08	58
4TL	8F	77460.76	-3271704.28	1678	4TL	ЗN	85298.08	-3266717.64	33	4TL	2V	93059.31	-3265365.41	130
4TL	9F	77243.46	-3272368.96	1094	4TL	4N	85313.16	-3267569.13	70	4TL	. 3V	93348.64	-3266550.76	92
4TL	10F	77225.39	-3273768.63	2756	4TL	5N	85355.79	-3268109.03	32	4TL	4V	93290.24	-3267526.42	220
4TL	11F	77186.04	-3274578.54	1826	4TL	6N	85688.27	-3269174.53	61	4TL	. 5V	93747.01	-3268237.28	7120
4TL	12F	77676.79	-3275768.66	1372	4TL	7N	85575.14	-3270758.28	243	4TL	. 6V	93611.86	-3269049.96	1527
4TL	13F	77603.36	-3276315.88	1266	4TL	8N	85275.74	-3271698.02	146	4TL	. 7V	93298.33	-3270160.6	71
4TL	14F	77752.14	-3277266.23	930	4TL	9N	85714.92	-3272725.75	114	4TL	. 8V	93077.61	-3271394.3	187
4TL	15F	77832.4	-3278768.95	748	4TL	10N	85610.94	-3273498.88	730	4TL	. 9V	93073.51	-3272205.05	126
4TL	16F	77391.71	-3279292.36	254	4TL	11N	85367.31	-3274636.35	1776	4TL	. 10V	93083.74	-3273685.96	49
4TL	17F	77551.45	-3280630.76	221	4TL	13N	85850.76	-3276984.65	98	4TL	. 11V	93161.76	-3274433.18	85
4TL	18F	77358.3	-3281764.39	199	4TL	14N	85100.53	-3277812.98	392	4TL	. 12V	93484.64	-3275478.31	67
4TL	19F	77894.95	-3282718.9	46	4TL	15N	85679.57	-3278254.78	26	4TL	. 13V	93427.05	-3276207.21	37
4TL	20F	77047.49	-3283610.39	169	4TL	16N	85161.6	-3279274.95	137	4TL	. 14V	93340.94	-3277076.45	79
4TL	21F	77678.65	-3284829.02	83	4TL	17N	85101.55	-3280532.61	197	4TL	. 15V	93159.66	-3278134.82	42
4TL	22F	77571.25	-3285108.51	112	4TL	18N	85662.88	-3281296.31	156	4TL	. 16V	93062.07	-3279275.75	178
4TL	23F	77059.11	-3286733.18	58	4TL	19N	85152.82	-3282195.8	167	4TL	. 17V	93465.74	-3280101.19	124
4TL	24F	77262.21	-3287730.14	78	4TL	20N	85165.94	-3283767.44	83	4TL	. 19V	93770.86	-3282107.83	386
4TL	25F	77731.56	-3288335.54	108	4TL	21N	85369.93	-3284089.56	117	4TL	20V	93647.78	-3283686.32	619
4TL	26F	77540.32	-3289388.64	59	4TL	22N	85805.72	-3285897.84	45	4TL	. 21V	93638.83	-3284063.83	1415
4TL	27F	77652.32	-3290615.11	57	4TL	23N	85440.49	-3286851.13	75	4TL	22V	93085.93	-3285707.68	621
4TL	28F	77435.04	-3291491.32	52	4TL	24N	85945.1	-3287457.36	95	4TL	. 23V	93385.83	-3286021.99	366
4TL	2G	78090.28	-3265766.28	323	4TL	25N	85681.68	-3288579.26	65	4TL	. 24V	93471.84	-3287494.63	63
4TL	3G	78120.79	-3266179.98	452	4TL	26N	85537.94	-3289754.35	118	4TL	. 25V	93895.87	-3288098.96	40
4TL	4G	78444.5	-3267189.87	475	4TL	27N	85513.57	-3290569.65	129	4TL	. 26V	93643.55	-3289815.4	55
4TL	5G	78442.31	-3268982.75	1020	4TL	28N	85517.6	-3291249.65	158	4TL	. 27V	93073.08	-3290924.98	30
4TL	6G	78299.06	-3269286.57	589	4TL	20	86692.03	-3265728.29	65	4TL	28V	93545.15	-3291203.09	32
4TL	7G	78314.65	-3270329.45	473	4TL	30	86741.24	-3266202.87	95	4TL	2W	94703.09	-3265102.17	104
4TL	8G	78237.1	-3271687.42	2415	4TL	40	86519.26	-3267914.99	85	4TL	. 3W	94834.26	-3266157.86	289
4TL	9G	78245.91	-3272166.08	2045	4TL	50	86247.4	-3268543.12	286	4TL	- 4W	94750.56	-3267137.96	511
4TL	10G	78765.31	-3273210.82	674	4TL	60	86449.52	-3269368.83	168	4TL	. 5W	94283.21	-3268360.78	2611
4TL	11G	78302.9	-3274650.32	484	4TL	70	86354.08	-3270419.16	234	4TL	- 6W	94265.93	-3269302.16	505
4TL	12G	78112.02	-3275688.32	1759	4TL	8O	86047.52	-3271660.9	195	4TL	. 7W	94077.43	-3270239.49	153
4TL	13G	78162.52	-3276958.66	1596	4TL	90	86107.35	-3272750.16	403	4TL	- 8W	94338.79	-3271116.96	75
4TL	14G	78328.39	-3277400.75	1555	4TL	100	86348.28	-3273214.17	331	4TL	. 9W	94039.34	-3272696.3	77

4TL	15G	78456.76	-3278149.17	131	4TL	110	86100.26	-3274749.4	1104	4TL	10W	94332.42	-3273086	42
4TL	16G	78582.29	-3279229.91	252	4TL	120	86270.85	-3275629.76	1530	4TL	11W	94111.23	-3274339.84	78
4TL	17G	78239.43	-3280727.64	189	4TL	130	86135.2	-3276251.05	98	4TL	12W	94314.06	-3275135.34	80
4TL	18G	78890.4	-3281110.71	51	4TL	140	86090.02	-3277519.13	94	4TL	13W	94033.04	-3276362.58	81
4TL	19G	78624.8	-3282962.82	92	4TL	150	86204.67	-3278846.39	230	4TL	14W	94413.49	-3277529.94	49
4TL	20G	78870.07	-3283668.67	127	4TL	160	86274.55	-3279724.37	127	4TL	15W	94445.92	-3278074.63	66
4TL	21G	78179.86	-3284090.48	73	4TL	170	86700.51	-3280459.68	101	4TL	16W	94530.48	-3279396.15	89
4TL	22G	78933.67	-3285236.47	99	4TL	180	86374.09	-3281051.27	865	4TL	17W	94129.76	-3280358.66	186
4TL	23G	78930.04	-3286027.09	86	4TL	190	86683.33	-3282247.16	360	4TL	18W	94613.72	-3281835.69	631
4TL	24G	78060.69	-3287416.66	65	4TL	200	86124.88	-3283699.5	102	4TL	19W	94362.5	-3282655.7	505
4TL	25G	78125.39	-3288088.02	53	4TL	210	86825.61	-3284108.93	91	4TL	20W	94668.18	-3283151.46	468
4TL	26G	78908.82	-3289259.89	51	4TL	220	86296.26	-3285184.24	101	4TL	21W	94250.83	-3284390.58	525
4TL	27G	78865.07	-3290467.57	50	4TL	230	86446.83	-3286059.08	79	4TL	22W	94109.07	-3285057.05	113
4TL	28G	78156.92	-3291221.35	41	4TL	240	86240.56	-3287746.4	161	4TL	23W	94464.34	-3286223.81	81
4TL	2H	79912.22	-3265426.68	279	4TL	250	86589.67	-3288535.29	133	4TL	24W	94756.15	-3287091.93	91
4TL	ЗH	79830.07	-3266341.34	257	4TL	260	86741.81	-3289556.23	163	4TL	25W	94955.86	-3288869.44	40
4TL	4H	79909.36	-3267672.8	184	4TL	270	86688.78	-3290305.38	310	4TL	26W	94950.91	-3289715.42	49
4TL	5H	79839.81	-3268693.53	250	4TL	280	86630.76	-3291477.47	103	4TL	27W	94158.03	-3290854.99	45
4TL	6H	79851.93	-3269882.38	919	4TL	2P	87648.68	-3265544.46	135	4TL	28W	94725.83	-3291558.42	122
4TL	7H	79864.34	-3270421.56	457	4TL	3P	87132.98	-3266469	141	4TL	2X	95399.5	-3265481.28	180
4TL	8H	79886.04	-3271419.26	83	4TL	4P	86995.65	-3267160.76	95	4TL	3X	95690.95	-3266364.5	138
4TL	9H	79220.03	-3272733.07	1128	4TL	5P	87161.76	-3268655.44	150	4TL	4X	95245.13	-3267104.35	218
4TL	10H	79815.8	-3273744.37	314	4TL	6P	87282.5	-3269725.99	99	4TL	5X	95079.98	-3268757.38	153
4TL	11H	79105.59	-3274372.2	442	4TL	7P	87045.98	-3270138.4	224	4TL	6X	95366.28	-3269645.51	362
4TL	12H	79505.82	-3275555.14	12	4TL	8P	87853.82	-3271980.68	91	4TL	7X	95258.78	-3270141.56	258
4TL	13H	79073.82	-3276562.24	1320	4TL	9P	87017.8	-3272177.44	229	4TL	8X	95650.4	-3271475.39	216
4TL	14H	79521.76	-3277645.58	357	4TL	10P	87669.24	-3273603.04	113	4TL	9X	95072.39	-3272690.55	96
4TL	15H	79589.33	-3278196.14	385	4TL	11P	87256.62	-3274217.76	458	4TL	10X	95657.18	-3273102.27	40
4TL	16H	79005.13	-3279884.57	241	4TL	12P	87500.39	-3275412.11	451	4TL	11X	95561.88	-3274359.09	36
4TL	17H	79082.38	-3280238.94	123	4TL	13P	87126.95	-3276924.23	83	4TL	12X	95058.16	-3275203.33	102
4TL	18H	79032.43	-3281708.36	203	4TL	14P	87333.66	-3277981.71	130	4TL	13X	95317.61	-3276161.34	86
4TL	19H	79177.75	-3282804.68	110	4TL	15P	87019.58	-3278477.9	255	4TL	17X	95080.85	-3280834.46	67
4TL	20H	79469.7	-3283879.29	99	4TL	16P	87416.23	-3279811.84	109	4TL	18X	95884.45	-3281155.67	84
4TL	21H	79283.76	-3284071.3	129	4TL	17P	87268.36	-3280523.5	125	4TL	19X	95582.59	-3282624.16	232
4TL	22H	79396.15	-3285494.2	146	4TL	18P	87814.82	-3281276.78	161	4TL	20X	95110.11	-3283000.76	443
4TL	23H	79651.39	-3286416.86	54	4TL	19P	87037.38	-3282190.08	183	4TL	21X	95756.14	-3284229.82	112
4TL	24H	79224.88	-3287192.42	63	4TL	20P	87282.03	-3283772.24	219	4TL	22X	95912.94	-3285054.45	104

4TL	25H	79647.86	-3288053.57	87	4TL	21P	87425.06	-3284752.67	96	4TL	23X	95866.3	-3286171.4	88
4TL	26H	79096.69	-3289198.87	65	4TL	22P	87618.56	-3285729.26	152	4TL	24X	95170.53	-3287252.84	55
4TL	27H	79295.14	-3290392.14	72	4TL	23P	87764.55	-3286372.33	102	4TL	25X	95402.7	-3288723.9	60
4TL	28H	79170.68	-3291391.41	96	4TL	24P	87908.89	-3287297.39	81	4TL	26X	95156.52	-3289544.02	44
4TL	21	80695.89	-3265525.82	51	4TL	25P	87111.81	-3288401.61	250	4TL	27X	95811.02	-3290415.7	47
4TL	31	80813.4	-3266732.28	606	4TL	26P	87802.71	-3289863.39	200	4TL	28X	95894.94	-3291339.33	36
4TL	41	80692	-3267177.64	268	4TL	27P	87062.55	-3290691.96	251	4TL	3Y	96055.44	-3266292.55	134
4TL	51	80237.01	-3268516.59	437	4TL	28P	87093.36	-3291730.17	151	4TL	4Y	96164.69	-3267634.79	81
4TL	61	80543.36	-3269621.76	333	4TL	2Q	88270.92	-3265226.8	152	4TL	5Y	96746.88	-3268368.78	236
4TL	71	80815.74	-3270459.2	306	4TL	3Q	88710.72	-3266440.9	241	4TL	6Y	96034.88	-3269072.1	135
4TL	81	80843.78	-3271401.66	518	4TL	4Q	88268.81	-3267865.78	355	4TL	7Y	96458.27	-3270341.21	231
4TL	91	80657.7	-3272449.84	119	4TL	5Q	88218.79	-3268700.62	167	4TL	8Y	96713.68	-3271681.88	116
4TL	101	80828.34	-3273753.25	639	4TL	6Q	88553.01	-3269267.57	214	4TL	9Y	96343.9	-3272614.91	75
4TL	111	80108.89	-3274345.6	475	4TL	7Q	88031.32	-3270232.25	183	4TL	10Y	96580.89	-3273245.02	73
4TL	121	80475.36	-3275890.35	382	4TL	8Q	88513.53	-3271145.17	112	4TL	11Y	96036.6	-3274737.99	66
4TL	131	80148.44	-3276290.55	398	4TL	9Q	88737.92	-3272731.89	103	4TL	12Y	96193.49	-3275346.06	81
4TL	141	80103.33	-3277130.54	754	4TL	10Q	88309.26	-3273598.05	190	4TL	13Y	96054.5	-3276108.29	88
4TL	15I	80083.95	-3278585.58	339	4TL	11Q	88336.8	-3274349.11	126	4TL	14Y	96677.73	-3277236.07	24
4TL	161	80334.14	-3279296.59	276	4TL	12Q	88511.5	-3275481.38	804	4TL	15Y	96335.16	-3278084.13	44
4TL	171	80782.36	-3280792.91	1124	4TL	13Q	88771.11	-3276857.41	93	4TL	16Y	96305.83	-3279533.89	52
4TL	181	80406.47	-3281771.13	104	4TL	14Q	88579.35	-3277507.59	353	4TL	17Y	96768.76	-3280622.63	67
4TL	191	80159.2	-3282425.02	116	4TL	15Q	88088.21	-3278246.36	105	4TL	18Y	96423.91	-3281566.32	70
4TL	201	80067.14	-3283757.46	103	4TL	16Q	88696.15	-3279806.91	116	4TL	20Y	96441.98	-3283354.64	45
4TL	211	80073.66	-3284120.23	121	4TL	17Q	88798.58	-3280161.88	138	4TL	21Y	96269.33	-3284473.64	53
4TL	221	80906.01	-3285353.7	74	4TL	18Q	88733.88	-3281615.83	188	4TL	22Y	96811.16	-3285634.75	82
4TL	231	80609.74	-3286374.09	73	4TL	19Q	88770.27	-3282205.94	90	4TL	23Y	96477.94	-3286513.25	53
4TL	241	80713.65	-3287091.7	40	4TL	20Q	88389.15	-3283617.16	77	4TL	24Y	96360.81	-3287628.54	53
4TL	251	80906.8	-3288083.39	41	4TL	21Q	88785.35	-3284120.1	134	4TL	25Y	96490.92	-3288941.06	31
4TL	261	80464.44	-3289739.93	25	4TL	22Q	88887.05	-3285356.4	131	4TL	26Y	96682.66	-3289142.01	23
4TL	271	80284.48	-3290954.46	92	4TL	23Q	88662.87	-3286736.08	215	4TL	27Y	96278.42	-3290678.59	23
4TL	281	80159.21	-3291137.83	36	4TL	24Q	88686.57	-3287436.68	232	4TL	28Y	96655.6	-3291558.8	14

			APPENDIX											
			GEOCHEMI	CAL DATA: ST	EINKOPF									
Мар	No.	Loy	Lox	Cu (ppm)	Мар	No.	Loy	Lox	Cu (ppm)	Мар	No.	Loy	Lox	Cu (ppm)
4TG	1A	49495.55	-3237078.02	2 34	4TG	11	57091.68	-3237260.84	24	4TG	25P	64164.72	-3261667.89	33
4TG	2A	49621.1	-3238153.26	6 17	4TG	21	57881.79	-3238108.03	25	4TG	26P	64492.15	-3262353.78	43
4TG	ЗA	49074.04	-3239425.82	2 28	4TG	31	57806.66	-3239401.58	21	4TG	27P	64477.76	-3263948.27	41
4TG	4A	49246.09	-3240559.48	3 30	4TG	41	57074.29	-3240888.42	15	4TG	28P	64087.89	-3264133.99	45
4TG	5A	49089.59	-3241253.79	9 17	4TG	51	57161.48	-3241246.47	23	4TG	1Q	65679.37	-3237373.01	19
4TG	6A	49069.4	-3242706.98	3 35	4TG	61	57857.29	-3242837.36	24	4TG	2Q	65171.32	-3238129.75	22
4TG	7A	49243.04	-3243740.09	9 12	4TG	71	57264	-3243970.99	26	4TG	3Q	65800.49	-3239967.9	22
4TG	8A	49060.88	-3244135.77	7 23	4TG	81	57631.94	-3244347.51	34	4TG	4Q	65702.35	-3240922.73	26
4TG	9A	49788.22	-3245859.59	9 27	4TG	91	57537.5	-3245322.71	38	4TG	5Q	65470.58	-3241536.59	27
4TG	10A	49513.76	-3246586.4	4 34	4TG	10I	57846.77	-3246284.22	25	4TG	6Q	65749.49	-3242959.13	37
4TG	11A	49415.89	-3247460.72	2 26	4TG	111	57640.97	-3247191.7	26	4TG	7Q	65579.8	-3243471.39	26
4TG	12A	49720.95	-3248180.37	7 28	4TG	12I	57247.08	-3248585.06	23	4TG	9Q	65811.79	-3245147.52	25
4TG	13A	49398.88	-3249935.8	3 18	4TG	13I	57661.29	-3249941.01	18	4TG	10Q	65192.54	-3246828.02	15
4TG	14A	49143.22	-3250759.47	7 15	4TG	14I	57289.62	-3250309.12	20	4TG	11Q	65147.78	-3247660.54	31
4TG	15A	49611.68	-3251444.58	3 24	4TG	15I	57197.32	-3251863.25	24	4TG	12Q	65087.64	-3248497.08	28
4TG	16A	49806.45	-3252156.97	7 18	4TG	16I	57053.21	-3252598.64	17	4TG	13Q	65802.94	-3249409.82	25
4TG	17A	49801.71	-3253299.13	3 21	4TG	17I	57745.16	-3253560.16	21	4TG	14Q	65103.25	-3250400.55	22
4TG	18A	49196.47	-3254537.66	6 27	4TG	18I	57225.64	-3254185.29	26	4TG	15Q	65039.94	-3251438.2	23
4TG	19A	49331.91	-3255845.07	7 35	4TG	19I	57495.46	-3255134.13	29	4TG	16Q	65068.2	-3252079.24	35
4TG	20A	49474.23	-3256589.24	4 21	4TG	201	57741.98	-3256589.77	29	4TG	17Q	65093.78	-3253907.87	15
4TG	21A	49663.09	-3257849.83	3 15	4TG	211	57267.11	-3257454.38	26	4TG	18Q	65403.71	-3254859.36	22
4TG	22A	49499.08	-3258352.4	4 29	4TG	221	57530.87	-3258342.43	28	4TG	19Q	65909.25	-3255516.71	31
4TG	23A	49248.39	-3259941.4	4 17	4TG	231	57435.49	-3259408.17	26	4TG	20Q	65313.05	-3256770.93	30
4TG	24A	49670.37	-3260185.58	3 26	4TG	241	57595.27	-3260651.74	18	4TG	21Q	65767.85	-3257510.5	34
4TG	25A	49265.48	-3261210.82	2 29	4TG	251	57423.19	-3261506.08	17	4TG	22Q	65544.05	-3258462.1	25
4TG	27A	49054.48	-3263496.98	3 27	4TG	261	57187.04	-3262492.09	28	4TG	23Q	65389.15	-3259896.35	30
4TG	28A	49844.38	-3264500.18	3 23	4TG	271	57093.65	-3263527.76	31	4TG	24Q	65100.77	-3260758.13	33
4TG	1B	50351.8	-3237073.98	3 27	4TG	281	57042.55	-3264379.99	34	4TG	25Q	65558.98	-3261981.09	19
4TG	2B	50802.01	-3238724.2	2 26	4TG	1J	58194.96	-3237710.91	42	4TG	26Q	65075.47	-3262900.49	31
4TG	3B	50239.8	-3239462.27	7 19	4TG	2J	58550.09	-3238358.36	49	4TG	27Q	65357.73	-3263125.41	37
4TG	4B	50443.65	-3240648.36	6 12	4TG	3J	58306.95	-3239374.11	23	4TG	28Q	65163	-3264476.52	32
4TG	5B	50443.39	-3241951.87	7 22	4TG	4J	58079.71	-3240607.32	34	4TG	1R	66123.38	-3237588.45	36
4TG	6B	50122.95	-3242841.74	4 6	4TG	5J	58178.74	-3241549.96	32	4TG	2R	66878.83	-3238961.82	15
4TG	7B	50162.11	-3243317.41	1 35	4TG	6J	58765.72	-3242882.04	11	4TG	3R	66444.94	-3239663.04	18

4TG	8B	50151.74 -	-3244927.26	34	4TG	7J	58804.14	-3243674.74	16	4TG	4R	66161.94 -3240978.	14 41
4TG	9B	50560.23	-3245834.9	25	4TG	8J	58802.64	-3244691.29	9	4TG	5R	66833.91 -3241631.3	34 30
4TG	10B	50188.8	-3246505	35	4TG	9J	58583.93	-3245336.21	32	4TG	6R	66645.45 -3242887.2	24 61
4TG	11B	50338.24 -	-3247370.43	31	4TG	10J	58727.34	-3246598.85	31	4TG	7R	66832.23 -3243644	.4 19
4TG	12B	50105.71 -	-3248683.82	27	4TG	11J	58071	-3247466.61	12	4TG	8R	66643.44 -3244905	.3 39
4TG	13B	50505.47 -	-3249953.26	35	4TG	12J	58858.2	-3248434.41	26	4TG	9R	66325 -3245382.0	61 47
4TG	14B	50257.67 -	-3250581.16	28	4TG	13J	58663.73	-3249705	29	4TG	10R	66085.95 -3246489.2	22 23
4TG	15B	50525.87 -	-3251172.57	26	4TG	14J	58328.21	-3250135.88	17	4TG	11R	66873.35 -32473	01 34
4TG	16B	50227.19	-3252114.2	18	4TG	15J	58211.23	-3251758.85	24	4TG	12R	66916.64 -3248325.	54 17
4TG	17B	50208.73 -	-3253235.32	23	4TG	16J	58794.06	-3252924.58	22	4TG	13R	66275.59 -3249496.2	28 16
4TG	18B	50849.92 -	-3254661.57	54	4TG	17J	58566.25	-3253478.31	25	4TG	14R	66372.63 -3250851.4	49 20
4TG	19B	50102.94 -	-3255452.91	33	4TG	18J	58144.03	-3254155.13	26	4TG	15R	66348.06 -3251453.8	32 28
4TG	20B	50672.34	-3256134.6	20	4TG	19J	58729.93	-3255809.25	29	4TG	16R	66586.23 -3252118.	74 24
4TG	21B	50432.86 -	-3257935.72	27	4TG	20J	58270.91	-3256433.33	28	4TG	17R	66801.41 -3253667.9	94 18
4TG	22B	50326.75 -	-3258552.82	17	4TG	21J	58338.73	-3257620.52	23	4TG	18R	66849.98 -3254612	
4TG	23B	50163.36 -	-3259810.36	35	4TG	22J	58169.27	-3258817.27	33	4TG	19R	66753.14 -3255929.	58 41
4TG	24B	50392.58 -	-3260152.58	71	4TG	23J	58207.15	-3259770.99	29	4TG	20R	66914.48 -3256232.	11 50
4TG	25B	50230.77 -	-3261309.57	12	4TG	24J	58743.02	-3260732.31	37	4TG	21R	66280.07 -3257760.0	63 17
4TG	26B	50375.86 -	-3262164.65	22	4TG	25J	58722.85	-3261802.99	77	4TG	22R	66808.02 -3258842.2	22 35
4TG	27B	50677.31 -	-3263779.92	13	4TG	26J	58315.22	-3262334.82	36	4TG	23R	66760.24 -3259261.8	84 27
4TG	28B	50532.23 -	-3264147.85	18	4TG	27J	58602.99	-3263164.06	28	4TG	24R	66095.75 -3260788.3	38 30
4TG	1C	51785.55 -	-3237857.84	17	4TG	28J	58715.27	-3264364.25	43	4TG	25R	66658.06 -3261806	i.8 39
4TG	2C	51890.73 -	-3238554.27	22	4TG	1K	59580.59	-3237696.4	35	4TG	26R	66156.99 -3262916	5.3 54
4TG	3C	51853.48 -	-3239578.53	19	4TG	2K	59635.37	-3238470.04	20	4TG	27R	66634.08 -3263929.	12 34
4TG	4C	51777.45 -	-3240197.61	17	4TG	ЗK	59538.55	-3239404.83	22	4TG	28R	66326.4 -3264090.0	23 23
4TG	5C	51602.78 -	-3241550.04	17	4TG	4K	59872.56	-3240373	29	4TG	1S	67434.87 -3237629.4	46 18
4TG	6C	51603.4 -	-3242305.02	30	4TG	5K	59833.31	-3241809.83	25	4TG	2S	67771.17 -3238945.0	26
4TG	8C	51907.08 -	-3244192.21	104	4TG	6K	59475.07	-3242661.98	19	4TG	3S	67328.83 -3239545.	06 33
4TG	9C	51356.58 -	-3245746.39	37	4TG	7K	59887.19	-3243514.5	25	4TG	4S	67915.82 -3240494.0	64 32
4TG	10C	51642.99 -	-3246137.67	34	4TG	8K	59243.68	-3244951.83	24	4TG	5S	67502.87 -3241871.0	65 51
4TG	11C	51163.09 -	-3247384.45	24	4TG	9K	59091.38	-3245888	31	4TG	6S	67738.07 -3242581.0	67 24
4TG	12C	51637.64 -	-3248970.85	36	4TG	10K	59354.37	-3246252.57	28	4TG	7S	67767.98 -3243580.	16 18
4TG	13C	51356.34 -	-3249495.89	24	4TG	11K	59628.68	-3247362.77	37	4TG	8S	67134.37 -3244867.	15 15
4TG	14C	51911.7 -	-3251002.05	34	4TG	12K	59184.53	-3248601.84	9	4TG	9S	67083.35 -3245412.3	38 13
4TG	15C	51551.23 -	-3251582.28	12	4TG	13K	59153.88	-3249525.87	24	4TG	10S	67456.65 -3247007.2	22 16
4TG	16C	51873.9 -	-3252569.84	41	4TG	14K	59366.22	-3250888.67	13	4TG	11S	67656.22 -3247875.9	95 31
4TG	17C	51381.09 -	-3253171.55	28	4TG	15K	59111.74	-3251923.8	29	4TG	12S	67613.97 -3248517.3	38 20

4TG	18C	51509.28 -3254206.7	′ 13	4TG	16K	59047.04	-3252906	11	4TG	13S	67651.32 -3249632.11	15
4TG	19C	51388.51 -3255275.8	22	4TG	17K	59414.52	-3253519.03	25	4TG	14S	67241.62 -3250501.01	25
4TG	20C	51136.53 -3256502.35	22	4TG	18K	59204.23	-3254647.67	29	4TG	15S	67169.15 -3251371.97	19
4TG	21C	51533.59 -3257736.38	23	4TG	19K	59224.49	-3255715.98	25	4TG	16S	67137.24 -3252391.55	14
4TG	22C	51521.62 -3258223.78	19	4TG	20K	59161.26	-3256446.62	43	4TG	17S	67374.8 -3253524.49	41
4TG	24C	51883.8 -3260522.5	28	4TG	21K	59524.8	-3257960.29	39	4TG	18S	67515.12 -3254681.23	31
4TG	25C	51025.21 -3261326.62	18	4TG	22K	59385.91	-3258540	27	4TG	19S	67488.47 -3256003.13	34
4TG	26C	51651.26 -3262218.39	27	4TG	23K	59129.51	-3259680.7	35	4TG	20S	67480.39 -3256737.41	34
4TG	27C	51157.88 -3263746.12	32	4TG	24K	59115.46	-3260887.67	38	4TG	21S	67877.47 -3257588.94	20
4TG	28C	51377.26 -3264314.18	34	4TG	25K	59223.76	-3261307.5	39	4TG	22S	67114.89 -3258922.81	15
4TG	1D	52393.23 -3237958.22	40	4TG	26K	59846.24	-3262329.88	137	4TG	23S	67503.12 -3259985.14	32
4TG	2D	52414.03 -3238865.52	22	4TG	27K	59872.37	-3263615	10	4TG	24S	67578.14 -3260986.59	30
4TG	3D	52371 -3239365.98	18	4TG	28K	59119.45	-3264496.54	18	4TG	25S	67178.37 -3261322.65	50
4TG	4D	52214.29 -3240216.29	28	4TG	1L	60429.97	-3237873.09	20	4TG	26S	67050.07 -3262582.5	42
4TG	5D	52512.13 -3241504.19	24	4TG	2L	60487.54	-3238375.14	39	4TG	27S	67906.2 -3263650.46	30
4TG	6D	52819.61 -3242722.26	14	4TG	3L	60215.89	-3239670.85	35	4TG	28S	67424.62 -3264081.8	110
4TG	7D	52678.2 -3243493.05	23	4TG	4L	60560.25	-3240634.67	25	4TG	1T	68750.77 -3237756.32	34
4TG	8D	52166.51 -3244687.41	90	4TG	5L	60100.8	-3241953.25	30	4TG	2T	68837.07 -3238662.89	24
4TG	9D	52232.16 -3245678.18	46	4TG	6L	60662.27	-3242755.21	14	4TG	3T	68499.81 -3239425.83	30
4TG	10D	52168.02 -3246116.86	33	4TG	7L	60213.47	-3243223.93	41	4TG	4T	68927.62 -3240651.82	26
4TG	11D	52645.16 -3247587.67	<sup>′</sup> 19	4TG	8L	60059.98	-3244331.14	22	4TG	5T	68678.06 -3241994.28	56
4TG	12D	52438.51 -3248278.68	31	4TG	9L	60424.07	-3245301.29	17	4TG	6T	68754.18 -3242291.2	23
4TG	13D	52496.09 -3250003.73	56	4TG	10L	60409.7	-3246513.27	25	4TG	7T	68169.18 -3243757.55	18
4TG	14D	52480.49 -3250546.26	14	4TG	11L	60482.93	-3247771.29	19	4TG	8T	68440.94 -3244218.33	13
4TG	15D	52431.29 -3251675.43	25	4TG	12L	60874.19	-3248864.02	17	4TG	9T	68810.54 -3245792.79	32
4TG	16D	52303.34 -3252547.77	32	4TG	13L	60854.1	-3249627.7	21	4TG	10T	68422.24 -3246260.46	25
4TG	17D	52569.78 -3253853.73	23	4TG	14L	60616.23	-3250945.77	42	4TG	11T	68283.95 -3247977.65	16
4TG	18D	52611.52 -3254213.81	13	4TG	15L	60297.25	-3251584.09	30	4TG	12T	68941.14 -3248931.86	24
4TG	19D	52712.13 -3255438.4	19	4TG	16L	60710.75	-3252492.06	23	4TG	13T	68531 -3249272.27	27
4TG	20D	52218.3 -3256820.15	6	4TG	17L	60032.08	-3253851.58	23	4TG	14T	68893.9 -3250413.46	16
4TG	21D	52397.7 -3257536.56	26	4TG	18L	60691.97	-3254841.2	19	4TG	15T	68594.54 -3251747.61	22
4TG	22D	52707.38 -3258186.06	17	4TG	19L	60562.49	-3255889.59	34	4TG	16T	68192.97 -3252340.24	32
4TG	23D	52166.04 -3259906.92	27	4TG	20L	60398.53	-3256467.65	40	4TG	17T	68826.06 -3253660.26	36
4TG	24D	52347.23 -3260366.78	43	4TG	21L	60094.33	-3257187.47	44	4TG	18T	68055.6 -3254349.4	15
4TG	25D	52405.02 -3261553.32	21	4TG	22L	60246.51	-3258546.31	40	4TG	19T	68166.42 -3255801.14	25
4TG	27D	52737.93 -3263608.52	39	4TG	23L	60179.38	-3259412.58	33	4TG	20T	68411.71 -3256587.32	26
4TG	28D	52053.95 -3264207.72	28	4TG	24L	60377.51	-3260150.36	38	4TG	21T	68470.58 -3257451.82	28

4TG		1	53652.16 -3237497.52	35	4TG	25L	60307.5	-3261977.74	39	4TG	22T	68549.05 -3258	171.66	30
4TG		2	53455.14 -3238501.2	19	4TG	26L	60275.54	-3262539.32	11	4TG	23T	68678.87 -3259	564.25	27
4TG		3	53410.58 -3239177.71	24	4TG	27L	60267.4	-3263198.1	21	4TG	24T	68076.27 -3260	762.69	32
4TG		4	53628.08 -3240309.33	28	4TG	28L	60436.27	-3264532.87	36	4TG	25T	68102 -3261	977.32	36
4TG		5	53366.07 -3241535.22	29	4TG	1M	61382.48	-3237477.78	34	4TG	26T	68185.67 -3262	923.98	35
4TG		6	53131.83 -3242798.17	21	4TG	2M	61558.56	-3238626.8	30	4TG	27T	68418.62 -3263	744.58	45
4TG		7	53340.37 -3243607.09	24	4TG	ЗM	61390.5	-3239496.5	12	4TG	28T	68786.24 -3264	126.11	51
4TG		8	53923.16 -3244314.82	23	4TG	4M	61547.66	-3240397.67	25	4TG	1U	69865.55 -3	237573	21
4TG		9	53381.5 -3245658.19	97	4TG	5M	61063.46	-3241709.6	24	4TG	2U	69630.05 -3238	931.49	39
4TG		10	53580.56 -3246305.44	54	4TG	6M	61094.75	-3242763.54	19	4TG	3U	69641.48 -323	9828.1	36
4TG		11	53056.14 -3247463.73	38	4TG	7M	61040.2	-3243897.39	30	4TG	4U	69391.35 -3240	873.59	34
4TG		12	53291.13 -3248329.76	27	4TG	8M	61604.71	-3244729.74	29	4TG	5U	69753.81 -3241	486.29	28
4TG		13	53088.54 -3249876.63	49	4TG	9M	61541.89	-3245148.37	23	4TG	6U	69934.77 -3242	484.65	25
4TG		14	53722.37 -3250879.63	27	4TG	10M	61075.81	-3246949.68	35	4TG	7U	69132.15 -3243	891.38	15
4TG		15	53485.13 -3251347.18	17	4TG	11M	61181.4	-3247334.09	34	4TG	8U	69317.49 -3244	517.59	24
4TG		16	53308 -3252966.19	19	4TG	12M	61373.57	-3248544.58	10	4TG	9U	69227.54 -3245	271.64	27
4TG		17	53434.29 -3253724.4	7	4TG	13M	61154.72	-3249421	30	4TG	10U	69360.73 -3246	689.62	34
4TG		18	53341.38 -3254141.05	30	4TG	14M	61649.3	-3250168.22	16	4TG	11U	69877.99 -3247	780.58	19
4TG		19	53879.86 -3255827.29	23	4TG	15M	61916.86	-3251610.14	31	4TG	12U	69790.72 -3248	952.54	18
4TG		20	53449.26 -3256478.39	26	4TG	16M	61063.59	-3252792.09	12	4TG	13U	69819.96 -3249	961.04	28
4TG		21	53281.06 -3257579.6	24	4TG	17M	61479.4	-3253664.98	26	4TG	14U	69103.8 -3250	437.34	26
4TG		22	53353.79 -3258233.63	20	4TG	18M	61911.89	-3254896.31	24	4TG	15U	69361.4 -325	1954.1	31
4TG		23	53139.39 -3259653.9	30	4TG	19M	61880.65	-3255523.37	21	4TG	16U	69102.06 -325	2374.9	33
4TG		24	53631.4 -3260516.71	18	4TG	20M	61893.86	-3256545.92	29	4TG	17U	69476.03 -3253	577.21	56
4TG		25	53239.77 -3261417	21	4TG	21M	61256.46	-3257279.05	27	4TG	18U	69613.28 -3254	245.55	42
4TG		26	53821.22 -3262527.27	23	4TG	22M	61122.9	-3258236.58	45	4TG	19U	69099.98 -3255	922.97	39
4TG		27	53663.56 -3263850.62	18	4TG	23M	61144.01	-3259521.36	20	4TG	20U	69596.34 -3256	796.13	25
4TG		28	53064.9 -3264148.43	35	4TG	24M	61598.72	-3260950.43	46	4TG	21U	69268.75 -3257	947.24	41
4TG	1F		54488.36 -3237492.15	20	4TG	25M	61814.79	-3261568.6	74	4TG	22U	69422.22 -3258	828.03	36
4TG	2F		54733.2 -3238132.34	23	4TG	26M	61236.33	-3262859.23	40	4TG	23U	69893.71 -3259	543.54	48
4TG	3F		54883.13 -3239601.76	22	4TG	27M	61639.76	-3263308.54	58	4TG	24U	69773.52 -3260	909.62	57
4TG	4F		54838.1 -3240514.78	24	4TG	28M	61191.47	-3264381.25	37	4TG	25U	69314.58 -3261	226.69	29
4TG	5F		54070.47 -3241390.32	25	4TG	1N	62216.31	-3237431.99	30	4TG	26U	69313.9 -3262	:842.21	57
4TG	6F		54113.55 -3242570.86	55	4TG	2N	62640.5	-3238330.6	15	4TG	27U	69486.97 -3263	578.34	54
4TG	7F		54152.22 -3243665.55	27	4TG	ЗN	62103.86	-3239674.3	9	4TG	28U	69568.94 -3264	092.06	46
4TG	8F		54797.02 -3244196.18	51	4TG	4N	62909.32	-3240899.98	12	4TG	1V	70429.62 -3237	876.87	26
4TG	9F		54110.58 -3245444.46	42	4TG	5N	62567.11	-3241738.09	21	4TG	2V	70197.7 -3238	722.23	37

4TG	10F	54699.82 -3246283.47	39	4TG	6N	62669.4	-3242172.62	54	4TG	3V	70113.51 -3239159.59	41
4TG	11F	54297.08 -3247887.63	13	4TG	7N	62146.22	-3243617.87	19	4TG	4V	70358.29 -3240947.29	36
4TG	12F	54195.47 -3248283.58	32	4TG	8N	62210.49	-3244553.19	24	4TG	5V	70400.53 -3241911.35	30
4TG	13F	54868.19 -3249842.76	37	4TG	9N	62204.9	-3245478.87	16	4TG	6V	70301.81 -3242569.2	32
4TG	14F	54197.8 -3250694.5	27	4TG	10N	62449.46	-3246582.08	24	4TG	7V	70234.13 -3243979	54
4TG	15F	54670.08 -3251550.98	24	4TG	11N	62065.86	-3247895.59	18	4TG	8V	70368.54 -3244460.94	14
4TG	16F	54531.19 -3252130.69	29	4TG	12N	62576.93	-3248774.75	20	4TG	9V	70095.64 -3245852.19	26
4TG	17F	54136.62 -3253916.58	21	4TG	13N	62834.93	-3249979.5	31	4TG	10V	70575.84 -3246588.4	31
4TG	18F	54371.19 -3254254.12	12	4TG	14N	62897.76	-3250783.87	26	4TG	11V	70353.15 -3247675.96	40
4TG	19F	54442.17 -3255622.71	23	4TG	15N	62463.33	-3251646.11	32	4TG	12V	70094.21 -3248167.24	48
4TG	20F	54666.66 -3256724.59	18	4TG	16N	62230.94	-3252727.99	31	4TG	13V	70783.97 -3249314.84	40
4TG	21F	54774.68 -3257607.43	30	4TG	17N	62813.83	-3253969.22	25	4TG	14V	70348.46 -3250499.12	27
4TG	22F	54663.54 -3258606.7	23	4TG	18N	62497.14	-3254954.96	25	4TG	15V	70894.44 -3251154.09	26
4TG	23F	54311.36 -3259519.65	23	4TG	19N	62208.24	-3255595.26	21	4TG	16V	70061.39 -3252946.35	29
4TG	24F	54242.73 -3260179.47	27	4TG	20N	62391.19	-3256563.55	28	4TG	17V	70218.48 -3253772.02	34
4TG	25F	54469.8 -3261165.77	8	4TG	21N	62596.37	-3257347.09	22	4TG	18V	70112.5 -3254540.12	28
4TG	26F	54815.27 -3262648.05	24	4TG	22N	62628.63	-3258768.5	28	4TG	19V	70113.95 -3255894.07	80
4TG	27F	54591.79 -3263594.63	28	4TG	23N	62691.54	-3259265.86	25	4TG	20V	70344.08 -3256528.26	19
4TG	28F	54514.98 -3264072.74	19	4TG	24N	62834.93	-3260911	34	4TG	21V	70556.7 -3257810.55	26
4TG	1G	55639.35 -3237447.1	22	4TG	25N	62789.92	-3261441.52	39	4TG	22V	70125.48 -3258929.68	23
4TG	2G	55495.88 -3238554.97	21	4TG	26N	62403.25	-3262266.64	36	4TG	23V	70708.8 -3259476.39	34
4TG	3G	55494.78 -3239642.01	20	4TG	27N	62200.47	-3263587.02	49	4TG	24V	70511.9 -3260631.07	57
4TG	4G	55101.65 -3240335.84	28	4TG	28N	62126.69	-3264095.52	42	4TG	25V	70892.54 -3261808.66	52
4TG	5G	55440.88 -3241148.34	29	4TG	10	63279.62	-3237647.92	16	4TG	26V	70399.18 -3262953.89	69
4TG	6G	55384.79 -3242840.74	29	4TG	20	63083.46	-3238485.57	23	4TG	27V	70279.75 -3263620.44	33
4TG	7G	55713.58 -3243964.58	38	4TG	30	63332.24	-3239448.13	27	4TG	28V	70630.86 -3264252.53	25
4TG	8G	55034.25 -3244111.13	48	4TG	40	63499.34	-3240656.96	27	4TG	1W	71910.07 -3237340.14	45
4TG	9G	55645.23 -3245384.4	36	4TG	50	63589.42	-3241276.91	16	4TG	2W	71366.11 -3238718.6	22
4TG	10G	55109.54 -3246255.07	45	4TG	6O	63779.88	-3242436.95	14	4TG	3W	71580.06 -3239598.34	54
4TG	11G	55654.49 -3247690.09	16	4TG	70	63800.08	-3243429.76	18	4TG	4W	71740.78 -3240368.89	27
4TG	12G	55828.81 -3248330.67	26	4TG	8O	63178.29	-3244460.85	28	4TG	5W	71631.09 -3241499.11	43
4TG	13G	55491.99 -3249622.1	25	4TG	90	63117.37	-3245156.42	20	4TG	6W	71219.68 -3242317.56	26
4TG	14G	55358.57 -3250348.12	25	4TG	10O	63106.41	-3246851.79	14	4TG	7W	71112.63 -3243407.69	21
4TG	15G	55136.52 -3251808.16	30	4TG	110	63328.22	-3247535.77	30	4TG	8W	71684.73 -3244124.79	29
4TG	16G	55404.52 -3252173.06	23	4TG	120	63406.21	-3248492.12	31	4TG	9W	71955.84 -3245818.59	25
4TG	17G	55838.77 -3253912.83	26	4TG	13O	63509.48	-3249294.12	14	4TG	10W	71078.92 -3246289.34	36
4TG	18G	55790.45 -3254493.47	22	4TG	140	63248.7	-3250806.96	23	4TG	11W	71144.82 -3247582.1	44

130	55112.9 -325596	88.96 25	41G	150	63214.48	-3251861.62	20	4TG	1200	71137.17 -3248844.86	30
20G	55583.42 -32563	36.99 13	4TG	16O	63241.43	-3252522.71	24	4TG	13W	71201.94 -3249161.16	52
21G	55302.22 -32573	95.53 19	4TG	170	63527.02	-3253538.02	24	4TG	14W	71459.45 -3250984.93	25
22G	55796.87 -32582	18.25 28	4TG	18O	63290.4	-3254760.55	28	4TG	15W	71562.67 -3251328.93	20
23G	55659.53 -32594	62.41 10	4TG	19O	63194.49	-3255987.3	29	4TG	16W	71921.54 -3252454.75	29
24G	55514.23 -32603	68.84 13	4TG	200	63547.85	-3256508.81	39	4TG	17W	71110.71 -3253221.76	56
25G	55734.58 -32613	04.37 24	4TG	210	63222.87	-3257161.84	24	4TG	18W	71091.12 -3254589.42	54
26G	55640.59 -326242	25.55 36	4TG	220	63572.64	-3258196.47	26	4TG	19W	71763.03 -3255167.13	33
27G	55839.22 -32637	67.31 72	4TG	230	63682.86	-3259733.73	30	4TG	20W	71590.41 -3256182.48	32
28G	55134.28 -32640	73.23 17	4TG	240	63427.06	-3260788.91	33	4TG	21W	71275.5 -3257294.17	31
1H	56135.41 -323750	60.28 20	4TG	250	63401.71	-3261250.27	34	4TG	22W	71558.67 -3259034.06	50
2H	56056.28 -32384	55.96 24	4TG	260	63512.75	-3262163.5	39	4TG	23W	71105.11 -3259804.44	32
3H	56389.15 -32396	70.67 33	4TG	270	63198.5	-3263265.16	32	4TG	24W	71395.15 -3260140.61	73
4H	56808.58 -32408	70.94 18	4TG	28O	63535.4	-3264495.23	68	4TG	25W	71916.01 -3261941.44	65
5H	56214.99 -324124	44.57 29	4TG	1P	64665.11	-3237864.92	71	4TG	26W	71350.77 -3262649.11	94
6H	56185.58 -32424	55.57 31	4TG	2P	64126.05	-3238710.21	16	4TG	27W	71300.79 -3263637.31	35
7H	56062.42 -32438	66.75 36	4TG	3P	64732.91	-3239434.62	19	4TG	28W	71169.22 -3264182.27	64
8H	56347.03 -32445	14.59 29	4TG	4P	64084.33	-3240796.12	22	4TG	1X	72826.15 -3237345.07	23
9H	56827.3 -3245	326.3 55	4TG	5P	64724.22	-3241859.91	33	4TG	2X	72456.46 -3238906.11	14
10H	56068.23 -32464	54.05 40	4TG	6P	64540.46	-3242738.65	13	4TG	3X	72423.06 -3239336.74	22
11H	56526.96 -32478	98.49 32	4TG	7P	64809.85	-3243159.01	9	4TG	4X	72458.02 -3240793.56	63
12H	56307.26 -32485	58.44 42	4TG	8P	64734.26	-3244306.57	21	4TG	5X	72167.86 -3241529.4	44
13H	56118.56 -324912	29.84 23	4TG	9P	64657.07	-3245937.19	51	4TG	6X	72532.36 -3242187.53	32
14H	56368.09 -32509	98.38 24	4TG	10P	64660.52	-3246878.57	22	4TG	7X	72298.37 -3243752.47	19
15H	56265.68 -32516	35.85 19	4TG	11P	64642.82	-3247300.17	42	4TG	8X	72470.48 -3244961.63	36
16H	56514.92 -3252	744.4 19	4TG	12P	64191.59	-3248875.98	26	4TG	9X	72204.28 -3245563.16	36
17H	56832.32 -325342	29.63 19	4TG	13P	64909.72	-3249975.12	30	4TG	10X	72417.62 -3246910.92	32
18H	56755.86 -325474	43.22 25	4TG	14P	64602.73	-3250966.53	34	4TG	11X	72666.54 -3247641.98	26
19H	56624.2 -32559	77.69 23	4TG	15P	64238.88	-3251903.87	22	4TG	12X	72635.15 -3248883.04	25
20H	56798 -32563	96.79 29	4TG	16P	64373.87	-3252682.79	32	4TG	13X	72227.33 -3249188.38	18
21H	56299.38 -32572	39.71 23	4TG	17P	64094.88	-3253172.76	25	4TG	14X	72096.04 -3250875.83	36
22H	56704.43 -325842	28.97 19	4TG	18P	64867.87	-3254738.54	29	4TG	15X	72185.58 -3251656.79	14
23H	56113.59 -32592	96.01 15	4TG	19P	64262.24	-3255906.58	39	4TG	16X	72406.94 -3252194.78	41
24H	56727.57 -326014	41.68 36	4TG	20P	64554.96	-3256660.65	36	4TG	17X	72130.15 -3253339.17	22
25H	56130.04 -32614	15.95 32	4TG	21P	64161.09	-3257671.51	38	4TG	18X	72527.2 -3254955.7	22
26H	56771.98 -326252	25.17 30	4TG	22P	64456.99	-3258224.47	29	4TG	19X	72655.55 -3255376.85	47
	7H 8H 9H 10H 11H 12H 13H 14H 15H 16H 17H 18H 19H 20H 21H 22H 23H 24H 25H 26H	7H 56062.42 -324380   8H 56347.03 -32445   9H 56827.3 -32445   10H 56068.23 -324464   11H 56526.96 -324789   12H 56307.26 -324859   13H 56118.56 -324912   14H 56368.09 -325099   15H 56265.68 -325163   16H 56514.92 -32522   17H 56832.32 -325342   18H 56755.86 -325474   19H 56624.2 -325592   20H 56798 -325639   21H 56299.38 -325639   22H 56704.43 -325842   23H 56113.59 -32592   24H 56727.57 -326014   25H 56130.04 -32614   26H 56771.98 -326525	7H   56062.42   -3243866.75   36     8H   56347.03   -3244514.59   29     9H   56827.3   -3245326.3   55     10H   56068.23   -3246454.05   40     11H   56526.96   -3247898.49   32     12H   56307.26   -3248558.44   42     13H   56118.56   -3249129.84   23     14H   56368.09   -3250998.38   24     15H   56265.68   -3251635.85   19     16H   56514.92   -3252744.4   19     17H   56832.32   -3254743.22   25     19H   56624.2   -3255977.69   23     20H   56798   -3256396.79   29     21H   566299.38   -3257239.71   23     22H   56704.43   -3258428.97   19     23H   56113.59   -3259296.01   15     24H   56727.57   -3260141.68   36     25H   56130.04   -3261415.95   3	7H 56062.42 -3243866.75 36 4TG   8H 56347.03 -3244514.59 29 4TG   9H 56827.3 -3245326.3 55 4TG   10H 56068.23 -3246454.05 40 4TG   11H 56526.96 -3247898.49 32 4TG   12H 56307.26 -3248558.44 42 4TG   13H 56118.56 -3249129.84 23 4TG   14H 56368.09 -3250998.38 24 4TG   15H 56265.68 -3251635.85 19 4TG   16H 56514.92 -3252744.4 19 4TG   17H 56832.32 -3253429.63 19 4TG   18H 56755.86 -3254743.22 25 4TG   19H 56624.2 -3255977.69 23 4TG   20H 56798 -3256396.79 29 4TG   21H 56299.38 -325729.71 23 4TG   22H 56704.43 -3258428.97 19 4TG   23H	7H 56062.42 -3243866.75 36 4TG 3P   8H 56347.03 -3244514.59 29 4TG 4P   9H 56827.3 -3245326.3 55 4TG 5P   10H 56068.23 -3245326.3 55 4TG 6P   11H 56526.96 -3247898.49 32 4TG 7P   12H 56307.26 -3248558.44 42 4TG 8P   13H 56118.56 -3249129.84 23 4TG 10P   14H 56368.09 -3250998.38 24 4TG 10P   15H 56265.68 -3251635.85 19 4TG 11P   16H 56514.92 -3252744.4 19 4TG 13P   17H 56832.32 -3253429.63 19 4TG 13P   18H 56755.86 -3254743.22 25 4TG 14P   19H 56624.2 -3255977.69 23 4TG 15P   20H 56798 -3256396.79 29 4TG 16P   21	7H 56062.42 -3243866.75 36 4TG 3P 64732.91   8H 56347.03 -3244514.59 29 4TG 4P 64084.33   9H 56827.3 -3245326.3 55 4TG 5P 64724.22   10H 56068.23 -3246454.05 40 4TG 6P 64540.46   11H 56526.96 -3247898.49 32 4TG 7P 64809.85   12H 56307.26 -3248558.44 42 4TG 8P 64734.26   13H 56118.56 -3249129.84 23 4TG 10P 64660.52   15H 56265.68 -3251635.85 19 4TG 11P 6442.82   16H 56514.92 -3252744.4 19 4TG 12P 64191.59   17H 56832.32 -3253429.63 19 4TG 13P 64909.72   18H 56755.86 -3254743.22 25 4TG 14P 64602.73   19H 56624.2 -3255977.69 23 4TG 15P 64238.88   2	7H 56062.42 -3243866.75 36 4TG 3P 64732.91 -3239434.62   8H 56347.03 -3244514.59 29 4TG 4P 64084.33 -3240796.12   9H 56827.3 -3245326.3 55 4TG 5P 64724.22 -3241859.91   10H 56068.23 -3246454.05 40 4TG 6P 64540.46 -3242738.65   11H 56526.96 -3247898.49 32 4TG 7P 64809.85 -3243159.01   12H 56307.26 -3248558.44 42 4TG 8P 64734.26 -3244306.57   13H 56118.56 -3249129.84 23 4TG 9P 64657.07 -3245937.19   14H 56368.09 -3250998.38 24 4TG 10P 64660.52 -3246878.57   15H 56265.68 -3251735.85 19 4TG 11P 6442.82 -3247300.17   16H 56514.92 -3252744.4 19 4TG 13P 64909.72 -3249975.12   18H 56755.86 -3254743.22 <td< td=""><td>7H 56062.42 -3243866.75 36 4TG 3P 64732.91 -3239434.62 19   8H 56347.03 -3244514.59 29 4TG 4P 64084.33 -3240796.12 22   9H 56827.3 -3245326.3 55 4TG 5P 64724.22 -3241859.91 33   10H 56068.23 -3246454.05 40 4TG 6P 64540.46 -3242738.65 13   11H 56526.96 -3247898.49 32 4TG 7P 64809.85 -3243159.01 9   12H 56307.26 -3248558.44 42 4TG 8P 64734.26 -3244306.57 21   13H 56118.56 -3249129.84 23 4TG 10P 64660.52 -3245878.75 22   15H 56265.68 -3251635.85 19 4TG 11P 6460.52 -3248875.98 26   17H 56832.32 -3252744.4 19 4TG 12P 64191.59 -3248875.98 26   17H 56832.32 -32554743.22 25 4TG</td><td>7H 56062.42 -3243866.75 36 4TG 3P 64732.91 -3239434.62 19 4TG   8H 56347.03 -3244514.59 29 4TG 4P 64084.33 -3240796.12 22 4TG   9H 56827.3 -3246526.3 55 4TG 6P 64742.22 -3241859.91 33 4TG   10H 56068.23 -3247888.49 32 4TG 6P 64540.46 -3242738.65 13 4TG   11H 56526.66 -3247898.49 32 4TG 7P 64809.85 -3243159.01 9 4TG   12H 56307.26 -3248558.44 42 4TG 8P 64734.26 -3244306.57 21 4TG   13H 56118.56 -3249129.84 23 4TG 10P 64665.27 -3248075.98 22 4TG   14H 56368.09 -325098.38 24 4TG 10P 64660.52 -324875.98 26 4TG   15H 56265.68 -3251635.85 19 4TG 11P 6462.82 -3247300.17</td><td>TH 561062.0 3243866.75 36 4TG 3P 64732.91 -3226474.62 19 4TG 28W   8H 56347.03 -3244514.59 29 4TG 4P 64084.33 -3240796.12 22 4TG 1X   9H 56827.3 -3245326.3 55 4TG 5P 64724.22 -3241859.91 33 4TG 3X   10H 56068.23 -3246454.05 40 4TG 6P 64540.46 -3242738.65 13 4TG 3X   11H 56526.96 -3247898.49 32 4TG 7P 64809.85 -3243159.01 9 4TG 4X   12H 56307.26 -3248558.44 42 4TG 8P 64734.26 -324306.57 21 4TG 5X   13H 56118.56 -3249129.84 23 4TG 10P 64660.52 -324878.57 22 4TG 7X   15H 56265.68 -3251635.85 19 4TG 11P 64642.82 -324730.17 42 4TG 8X   16H 565</td><td>617H 56062.42 3243866.75 36 4TG 3P 64732.91 3239434.62 19 4TG 1X 72826.15 -3264182.27   8H 56347.03 -3244514.59 29 4TG 5P 64724.22 -3241859.91 33 4TG 2X 72456.46 -3239336.74   10H 56082.42 -3246454.05 40 4TG 6P 64450.46 -3242738.65 13 4TG 3X 72428.06 -3239336.74   11H 5656.69 -3247898.49 32 4TG 6P 64460.46 -3242738.65 13 4TG 3X 72428.06 -3239336.74   12H 56307.26 -3248558.44 42 4TG 8P 64734.26 -324396.57 21 4TG 5X 72458.06 -3241729.48 -3244172.93 -3244172.93 -3244175.33 -3244175.33 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.33 -3244175.33 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34</td></td<>	7H 56062.42 -3243866.75 36 4TG 3P 64732.91 -3239434.62 19   8H 56347.03 -3244514.59 29 4TG 4P 64084.33 -3240796.12 22   9H 56827.3 -3245326.3 55 4TG 5P 64724.22 -3241859.91 33   10H 56068.23 -3246454.05 40 4TG 6P 64540.46 -3242738.65 13   11H 56526.96 -3247898.49 32 4TG 7P 64809.85 -3243159.01 9   12H 56307.26 -3248558.44 42 4TG 8P 64734.26 -3244306.57 21   13H 56118.56 -3249129.84 23 4TG 10P 64660.52 -3245878.75 22   15H 56265.68 -3251635.85 19 4TG 11P 6460.52 -3248875.98 26   17H 56832.32 -3252744.4 19 4TG 12P 64191.59 -3248875.98 26   17H 56832.32 -32554743.22 25 4TG	7H 56062.42 -3243866.75 36 4TG 3P 64732.91 -3239434.62 19 4TG   8H 56347.03 -3244514.59 29 4TG 4P 64084.33 -3240796.12 22 4TG   9H 56827.3 -3246526.3 55 4TG 6P 64742.22 -3241859.91 33 4TG   10H 56068.23 -3247888.49 32 4TG 6P 64540.46 -3242738.65 13 4TG   11H 56526.66 -3247898.49 32 4TG 7P 64809.85 -3243159.01 9 4TG   12H 56307.26 -3248558.44 42 4TG 8P 64734.26 -3244306.57 21 4TG   13H 56118.56 -3249129.84 23 4TG 10P 64665.27 -3248075.98 22 4TG   14H 56368.09 -325098.38 24 4TG 10P 64660.52 -324875.98 26 4TG   15H 56265.68 -3251635.85 19 4TG 11P 6462.82 -3247300.17	TH 561062.0 3243866.75 36 4TG 3P 64732.91 -3226474.62 19 4TG 28W   8H 56347.03 -3244514.59 29 4TG 4P 64084.33 -3240796.12 22 4TG 1X   9H 56827.3 -3245326.3 55 4TG 5P 64724.22 -3241859.91 33 4TG 3X   10H 56068.23 -3246454.05 40 4TG 6P 64540.46 -3242738.65 13 4TG 3X   11H 56526.96 -3247898.49 32 4TG 7P 64809.85 -3243159.01 9 4TG 4X   12H 56307.26 -3248558.44 42 4TG 8P 64734.26 -324306.57 21 4TG 5X   13H 56118.56 -3249129.84 23 4TG 10P 64660.52 -324878.57 22 4TG 7X   15H 56265.68 -3251635.85 19 4TG 11P 64642.82 -324730.17 42 4TG 8X   16H 565	617H 56062.42 3243866.75 36 4TG 3P 64732.91 3239434.62 19 4TG 1X 72826.15 -3264182.27   8H 56347.03 -3244514.59 29 4TG 5P 64724.22 -3241859.91 33 4TG 2X 72456.46 -3239336.74   10H 56082.42 -3246454.05 40 4TG 6P 64450.46 -3242738.65 13 4TG 3X 72428.06 -3239336.74   11H 5656.69 -3247898.49 32 4TG 6P 64460.46 -3242738.65 13 4TG 3X 72428.06 -3239336.74   12H 56307.26 -3248558.44 42 4TG 8P 64734.26 -324396.57 21 4TG 5X 72458.06 -3241729.48 -3244172.93 -3244172.93 -3244175.33 -3244175.33 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.33 -3244175.33 -3244175.34 -3244175.34 -3244175.34 -3244175.34 -3244175.34

4TG	28H	56630.6	-3264136.47	42	4T	G 2	24P	64078.21	-3260917.32	133
4TG	11	57091.68	-3237260.84	24	4T	G 2	25P	64164.72	-3261667.89	33
4TG	21	57881.79	-3238108.03	25	4T	G 2	26P	64492.15	-3262353.78	43
4TG	31	57806.66	-3239401.58	21	4T	G 2	27P	64477.76	-3263948.27	41
4TG	41	57074.29	-3240888.42	15	4T	G 2	28P	64087.89	-3264133.99	45
4TG	51	57161.48	-3241246.47	23	4T	G 1	Q	65679.37	-3237373.01	19
4TG	61	57857.29	-3242837.36	24	4T	G 2	Q	65171.32	-3238129.75	22
4TG	71	57264	-3243970.99	26	4T	G 3	Q	65800.49	-3239967.9	22
4TG	81	57631.94	-3244348	34	47	G 4	1Q	65702.35	-3240923	26
4TG	91	57537.5	-3245323	38	47	G 5	5Q	65470.58	-3241537	27
4TG	10I	57846.77	-3246284	25	47	G 6	SQ SQ	65749.49	-3242959	37
4TG	111	57640.97	-3247192	26	41	G 7	7Q	65579.8	-3243471	26
4TG	12I	57247.08	-3248585	23	47	G 9	Q	65811.79	-3245148	25
4TG	13I	57661.29	-3249941	18	47	G 1	10Q	65192.54	-3246828	15
4TG	14I	57289.62	-3250309	20	47	G 1	1Q	65147.78	-3247661	31
4TG	15I	57197.32	-3251863	24	47	G 1	l2Q	65087.64	-3248497	28
4TG	16I	57053.21	-3252599	17	41	G 1	13Q	65802.94	-3249410	25
4TG	17I	57745.16	-3253560	21	41	G 1	14Q	65103.25	-3250401	22
4TG	18I	57225.64	-3254185	26	47	G 1	15Q	65039.94	-3251438	23
4TG	19I	57495.46	-3255134	29	47	G 1	16Q	65068.2	-3252079	35
4TG	201	57741.98	-3256590	29	47	G 1	17Q	65093.78	-3253908	15
4TG	211	57267.11	-3257454	26	47	G 1	8Q	65403.71	-3254859	22
4TG	221	57530.87	-3258342	28	47	G 1	9Q	65909.25	-3255517	31
4TG	231	57435.49	-3259408	26	47	G 2	20Q	65313.05	-3256771	30
4TG	241	57595.27	-3260652	18	47	G 2	21Q	65767.85	-3257511	34
4TG	251	57423.19	-3261506	17	41	G 2	22Q	65544.05	-3258462	25
4TG	261	57187.04	-3262492	28	47	G 2	23Q	65389.15	-3259896	30
4TG	271	57093.65	-3263528	31	47	G 2	24Q	65100.77	-3260758	33
4TG	281	57042.55	-3264380	34	47	G 2	25Q	65558.98	-3261981	19
4TG	1J	58194.96	-3237711	42	47	G 2	26Q	65075.47	-3262900	31
4TG	2J	58550.09	-3238358	49	47	G 2	27Q	65357.73	-3263125	37
4TG	3J	58306.95	-3239374	23	47	G 2	28Q	65163	-3264477	32
4TG	4J	58079.71	-3240607	34	47	G 1	IR	66123.38	-3237588	36
4TG	5J	58178.74	-3241550	32	41	G 2	2R	66878.83	-3238962	15
4TG	6J	58765.72	-3242882	11	41	G 3	BR	66444.94	-3239663	18
4TG	7J	58804.14	-3243675	16	41	G 4	1R	66161.94	-3240978	41
4TG	8J	58802.64	-3244691	9	41	G 5	5R	66833.91	-3241631	30

21X	72124.22	-3257480.87	30
22X	72673.85	-3258921.23	59
23X	72142.73	-3259263.75	23
24X	72592.9	-3260455.97	25
25X	72399.34	-3262018.54	24
26X	72287.5	-3262569.83	18
27X	72444.04	-3263939.02	22
28X	72391.06	-3264131.82	24
	21X 22X 23X 24X 25X 26X 27X 28X	21X   72124.22     22X   72673.85     23X   72142.73     24X   72592.9     25X   72399.34     26X   72287.5     27X   72444.04     28X   72391.06	21X72124.22-3257480.8722X72673.85-3258921.2323X72142.73-3259263.7524X72592.9-3260455.9725X72399.34-3262018.5426X72287.5-3262569.8327X72444.04-3263939.0228X72391.06-3264131.82

4TG	9J	58583.93	-3245336	32	4TG	6R	66645.45	-3242887	61
4TG	10J	58727.34	-3246599	31	4TG	7R	66832.23	-3243644	19
4TG	11J	58071	-3247467	12	4TG	8R	66643.44	-3244905	39
4TG	12J	58858.2	-3248434	26	4TG	9R	66325	-3245383	47
4TG	13J	58663.73	-3249705	29	4TG	10R	66085.95	-3246489	23
4TG	14J	58328.21	-3250136	17	4TG	11R	66873.35	-3247301	34
4TG	15J	58211.23	-3251759	24	4TG	12R	66916.64	-3248326	17
4TG	16J	58794.06	-3252925	22	4TG	13R	66275.59	-3249496	16
4TG	17J	58566.25	-3253478	25	4TG	14R	66372.63	-3250851	20
4TG	18J	58144.03	-3254155	26	4TG	15R	66348.06	-3251454	28
4TG	19J	58729.93	-3255809	29	4TG	16R	66586.23	-3252119	24
4TG	20J	58270.91	-3256433	28	4TG	17R	66801.41	-3253668	18
4TG	21J	58338.73	-3257621	23	4TG	18R	66849.98	-3254612	19
4TG	22J	58169.27	-3258817	33	4TG	19R	66753.14	-3255930	41
4TG	23J	58207.15	-3259771	29	4TG	20R	66914.48	-3256232	50
4TG	24J	58743.02	-3260732	37	4TG	21R	66280.07	-3257761	17
4TG	25J	58722.85	-3261803	77	4TG	22R	66808.02	-3258842	35
4TG	26J	58315.22	-3262335	36	4TG	23R	66760.24	-3259262	27
4TG	27J	58602.99	-3263164	28	4TG	24R	66095.75	-3260788	30
4TG	28J	58715.27	-3264364	43	4TG	25R	66658.06	-3261807	39
4TG	1K	59580.59	-3237696	35	4TG	26R	66156.99	-3262916	54
4TG	2K	59635.37	-3238470	20	4TG	27R	66634.08	-3263929	34
4TG	3K	59538.55	-3239405	22	4TG	28R	66326.4	-3264090	23
4TG	4K	59872.56	-3240373	29	4TG	1S	67434.87	-3237629	18
4TG	5K	59833.31	-3241810	25	4TG	2S	67771.17	-3238945	26
4TG	6K	59475.07	-3242662	19	4TG	3S	67328.83	-3239545	33
4TG	7K	59887.19	-3243515	25	4TG	4S	67915.82	-3240495	32
4TG	8K	59243.68	-3244952	24	4TG	5S	67502.87	-3241872	51
4TG	9K	59091.38	-3245888	31	4TG	6S	67738.07	-3242582	24
4TG	10K	59354.37	-3246253	28	4TG	7S	67767.98	-3243580	18
4TG	11K	59628.68	-3247363	37	4TG	8S	67134.37	-3244867	15
4TG	12K	59184.53	-3248602	9	4TG	9S	67083.35	-3245412	13
4TG	13K	59153.88	-3249526	24	4TG	10S	67456.65	-3247007	16
4TG	14K	59366.22	-3250889	13	4TG	11S	67656.22	-3247876	31
4TG	15K	59111.74	-3251924	29	4TG	12S	67613.97	-3248517	20
4TG	16K	59047.04	-3252906	11	4TG	13S	67651.32	-3249632	15
4TG	17K	59414.52	-3253519	25	4TG	14S	67241.62	-3250501	25

4TG	18K	59204.23	-3254648	29	4T	G 15S	67169.15	-3251372	19
4TG	19K	59224.49	-3255716	25	4T	G 16S	67137.24	-3252392	14
4TG	20K	59161.26	-3256447	43	4T	G 17S	67374.8	-3253524	41
4TG	21K	59524.8	-3257960	39	4T	G 18S	67515.12	-3254681	31
4TG	22K	59385.91	-3258540	27	4T	G 19S	67488.47	-3256003	34
4TG	23K	59129.51	-3259681	35	4T	G 20S	67480.39	-3256737	34
4TG	24K	59115.46	-3260888	38	4T	G 21S	67877.47	-3257589	20
4TG	25K	59223.76	-3261308	39	4T	G 22S	67114.89	-3258923	15
4TG	26K	59846.24	-3262330	137	4T	G 23S	67503.12	-3259985	32
4TG	27K	59872.37	-3263615	10	4T	G 24S	67578.14	-3260987	30
4TG	28K	59119.45	-3264497	18	4T	G 25S	67178.37	-3261323	50
4TG	1L	60429.97	-3237873	20	4T	G 26S	67050.07	-3262583	42
4TG	2L	60487.54	-3238375	39	4T	G 27S	67906.2	-3263650	30
4TG	3L	60215.89	-3239671	35	4T	G 28S	67424.62	-3264082	110
4TG	4L	60560.25	-3240635	25	4T	G 1T	68750.77	-3237756	34
4TG	5L	60100.8	-3241953	30	4T	G 2T	68837.07	-3238663	24
4TG	6L	60662.27	-3242755	14	4T	G 3T	68499.81	-3239426	30
4TG	7L	60213.47	-3243224	41	4T	G 4T	68927.62	-3240652	26
4TG	8L	60059.98	-3244331	22	4T	G 5T	68678.06	-3241994	56
4TG	9L	60424.07	-3245301	17	4T	G 6T	68754.18	-3242291	23
4TG	10L	60409.7	-3246513	25	4T	G 7T	68169.18	-3243758	18
4TG	11L	60482.93	-3247771	19	4T	G 8T	68440.94	-3244218	13
4TG	12L	60874.19	-3248864	17	4T	G 9T	68810.54	-3245793	32
4TG	13L	60854.1	-3249628	21	4T	G 10T	68422.24	-3246260	25
4TG	14L	60616.23	-3250946	42	4T	G 11T	68283.95	-3247978	16
4TG	15L	60297.25	-3251584	30	4T	G 12T	68941.14	-3248932	24
4TG	16L	60710.75	-3252492	23	4T	G 13T	68531	-3249272	27
4TG	17L	60032.08	-3253852	23	4T	G 14T	68893.9	-3250413	16
4TG	18L	60691.97	-3254841	19	4T	G 15T	68594.54	-3251748	22
4TG	19L	60562.49	-3255890	34	4T	G 16T	68192.97	-3252340	32
4TG	20L	60398.53	-3256468	40	4T	G 17T	68826.06	-3253660	36
4TG	21L	60094.33	-3257187	44	4T	G 18T	68055.6	-3254349	15
4TG	22L	60246.51	-3258546	40	4T	G 19T	68166.42	-3255801	25
4TG	23L	60179.38	-3259413	33	4T	G 20T	68411.71	-3256587	26
4TG	24L	60377.51	-3260150	38	4T	G 21T	68470.58	-3257452	28
4TG	25L	60307.5	-3261978	39	4T	G 22T	68549.05	-3258172	30
4TG	26L	60275.54	-3262539	11	4T	G 23T	68678.87	-3259564	27

4TG	27L	60267.4	-3263198	21	4	4TG	24T	68076.27	-3260763	32
4TG	28L	60436.27	-3264533	36	4	4TG	25T	68102	-3261977	36
4TG	1M	61382.48	-3237478	34	4	4TG	26T	68185.67	-3262924	35
4TG	2M	61558.56	-3238627	30	4	4TG	27T	68418.62	-3263745	45
4TG	ЗM	61390.5	-3239497	12	4	4TG	28T	68786.24	-3264126	51
4TG	4M	61547.66	-3240398	25	4	4TG	1U	69865.55	-3237573	21
4TG	5M	61063.46	-3241710	24	4	4TG	2U	69630.05	-3238931	39
4TG	6M	61094.75	-3242764	19	4	4TG	3U	69641.48	-3239828	36
4TG	7M	61040.2	-3243897	30	4	4TG	4U	69391.35	-3240874	34
4TG	8M	61604.71	-3244730	29	4	4TG	5U	69753.81	-3241486	28
4TG	9M	61541.89	-3245148	23	4	4TG	6U	69934.77	-3242485	25
4TG	10M	61075.81	-3246950	35	4	4TG	7U	69132.15	-3243891	15
4TG	11M	61181.4	-3247334	34	4	4TG	8U	69317.49	-3244518	24
4TG	12M	61373.57	-3248545	10	4	4TG	9U	69227.54	-3245272	27
4TG	13M	61154.72	-3249421	30	4	4TG	10U	69360.73	-3246690	34
4TG	14M	61649.3	-3250168	16	4	4TG	11U	69877.99	-3247781	19
4TG	15M	61916.86	-3251610	31	4	4TG	12U	69790.72	-3248953	18
4TG	16M	61063.59	-3252792	12	4	4TG	13U	69819.96	-3249961	28
4TG	17M	61479.4	-3253665	26	4	4TG	14U	69103.8	-3250437	26
4TG	18M	61911.89	-3254896	24	4	4TG	15U	69361.4	-3251954	31
4TG	19M	61880.65	-3255523	21	4	4TG	16U	69102.06	-3252375	33
4TG	20M	61893.86	-3256546	29	4	4TG	17U	69476.03	-3253577	56
4TG	21M	61256.46	-3257279	27	4	4TG	18U	69613.28	-3254246	42
4TG	22M	61122.9	-3258237	45	4	4TG	19U	69099.98	-3255923	39
4TG	23M	61144.01	-3259521	20	4	4TG	20U	69596.34	-3256796	25
4TG	24M	61598.72	-3260950	46	4	4TG	21U	69268.75	-3257947	41
4TG	25M	61814.79	-3261569	74	4	4TG	22U	69422.22	-3258828	36
4TG	26M	61236.33	-3262859	40	4	4TG	23U	69893.71	-3259544	48
4TG	27M	61639.76	-3263309	58	4	4TG	24U	69773.52	-3260910	57
4TG	28M	61191.47	-3264381	37	4	4TG	25U	69314.58	-3261227	29
4TG	1N	62216.31	-3237432	30	4	4TG	26U	69313.9	-3262842	57
4TG	2N	62640.5	-3238331	15	4	4TG	27U	69486.97	-3263578	54
4TG	3N	62103.86	-3239674	9	4	4TG	28U	69568.94	-3264092	46
4TG	4N	62909.32	-3240900	12	4	4TG	1V	70429.62	-3237877	26
4TG	5N	62567.11	-3241738	21		4TG	2V	70197.7	-3238722	37
4TG	6N	62669.4	-3242173	54		4TG	3V	70113.51	-3239160	41
4TG	7N	62146.22	-3243618	19	4	4TG	4V	70358.29	-3240947	36

4TG	8N	62210.49	-3244553	24	4T	G 5V	70400.53	-3241911	30
4TG	9N	62204.9	-3245479	16	4T	G 6V	70301.81	-3242569	32
4TG	10N	62449.46	-3246582	24	4T	G 7V	70234.13	-3243979	54
4TG	11N	62065.86	-3247896	18	4T	G 8V	70368.54	-3244461	14
4TG	12N	62576.93	-3248775	20	4T	G 9V	70095.64	-3245852	26
4TG	13N	62834.93	-3249980	31	4T	G 10V	70575.84	-3246588	31
4TG	14N	62897.76	-3250784	26	4T	G 11V	70353.15	-3247676	40
4TG	15N	62463.33	-3251646	32	4T	G 12V	70094.21	-3248167	48
4TG	16N	62230.94	-3252728	31	4T	G 13V	70783.97	-3249315	40
4TG	17N	62813.83	-3253969	25	4T	G 14V	70348.46	-3250499	27
4TG	18N	62497.14	-3254955	25	4T	G 15V	70894.44	-3251154	26
4TG	19N	62208.24	-3255595	21	4T	G 16V	70061.39	-3252946	29
4TG	20N	62391.19	-3256564	28	4T	G 17V	70218.48	-3253772	34
4TG	21N	62596.37	-3257347	22	4T	G 18V	70112.5	-3254540	28
4TG	22N	62628.63	-3258769	28	4T	G 19V	70113.95	-3255894	80
4TG	23N	62691.54	-3259266	25	4T	G 20V	70344.08	-3256528	19
4TG	24N	62834.93	-3260911	34	4T	G 21V	70556.7	-3257811	26
4TG	25N	62789.92	-3261442	39	4T	G 22V	70125.48	-3258930	23
4TG	26N	62403.25	-3262267	36	4T	G 23V	70708.8	-3259476	34
4TG	27N	62200.47	-3263587	49	4T	G 24V	70511.9	-3260631	57
4TG	28N	62126.69	-3264096	42	4T	G 25V	70892.54	-3261809	52
4TG	10	63279.62	-3237648	16	4T	G 26V	70399.18	-3262954	69
4TG	20	63083.46	-3238486	23	4T	G 27V	70279.75	-3263620	33
4TG	30	63332.24	-3239448	27	4T	G 28V	70630.86	-3264253	25
4TG	40	63499.34	-3240657	27	4T	G 1W	71910.07	-3237340	45
4TG	50	63589.42	-3241277	16	4T	G 2W	71366.11	-3238719	22
4TG	60	63779.88	-3242437	14	4T	G 3W	71580.06	-3239598	54
4TG	70	63800.08	-3243430	18	4T	G 4W	71740.78	-3240369	27
4TG	80	63178.29	-3244461	28	4T	G 5W	71631.09	-3241499	43
4TG	90	63117.37	-3245156	20	4T	G 6W	71219.68	-3242318	26
4TG	10O	63106.41	-3246852	14	4T	G 7W	71112.63	-3243408	21
4TG	110	63328.22	-3247536	30	4T	G 8W	71684.73	-3244125	29
4TG	120	63406.21	-3248492	31	4T	G 9W	71955.84	-3245819	25
4TG	130	63509.48	-3249294	14	4T	G 10W	/ 71078.92	-3246289	36
4TG	140	63248.7	-3250807	23	4T	G 11W	/ 71144.82	-3247582	44
4TG	150	63214.48	-3251862	20	4T	G 12W	/ 71137.17	-3248845	30
4TG	160	63241.43	-3252523	24	4T	G 13W	/ 71201.94	-3249161	52

4TG	170	63527.02	-3253538	24	4	4TG	14W	71459.45	-3250985	25
4TG	18O	63290.4	-3254761	28	4	4TG	15W	71562.67	-3251329	20
4TG	19O	63194.49	-3255987	29	4	4TG	16W	71921.54	-3252455	29
4TG	200	63547.85	-3256509	39	2	4TG	17W	71110.71	-3253222	56
4TG	210	63222.87	-3257162	24	4	4TG	18W	71091.12	-3254589	54
4TG	220	63572.64	-3258196	26	4	4TG	19W	71763.03	-3255167	33
4TG	230	63682.86	-3259734	30	4	4TG	20W	71590.41	-3256182	32
4TG	240	63427.06	-3260789	33	4	4TG	21W	71275.5	-3257294	31
4TG	25O	63401.71	-3261250	34	4	4TG	22W	71558.67	-3259034	50
4TG	26O	63512.75	-3262164	39	2	4TG	23W	71105.11	-3259804	32
4TG	270	63198.5	-3263265	32	2	4TG	24W	71395.15	-3260141	73
4TG	28O	63535.4	-3264495	68	2	4TG	25W	71916.01	-3261941	65
4TG	1P	64665.11	-3237865	71	2	4TG	26W	71350.77	-3262649	94
4TG	2P	64126.05	-3238710	16	2	4TG	27W	71300.79	-3263637	35
4TG	3P	64732.91	-3239435	19	4	4TG	28W	71169.22	-3264182	64
4TG	4P	64084.33	-3240796	22	4	4TG	1X	72826.15	-3237345	23
4TG	5P	64724.22	-3241860	33	4	4TG	2X	72456.46	-3238906	14
4TG	6P	64540.46	-3242739	13	4	4TG	3X	72423.06	-3239337	22
4TG	7P	64809.85	-3243159	9	4	4TG	4X	72458.02	-3240794	63
4TG	8P	64734.26	-3244307	21	4	4TG	5X	72167.86	-3241529	44
4TG	9P	64657.07	-3245937	51	4	4TG	6X	72532.36	-3242188	32
4TG	10P	64660.52	-3246879	22	4	4TG	7X	72298.37	-3243752	19
4TG	11P	64642.82	-3247300	42	4	4TG	8X	72470.48	-3244962	36
4TG	12P	64191.59	-3248876	26	4	4TG	9X	72204.28	-3245563	36
4TG	13P	64909.72	-3249975	30	4	4TG	10X	72417.62	-3246911	32
4TG	14P	64602.73	-3250967	34	4	4TG	11X	72666.54	-3247642	26
4TG	15P	64238.88	-3251904	22	2	4TG	12X	72635.15	-3248883	25
4TG	16P	64373.87	-3252683	32	2	4TG	13X	72227.33	-3249188	18
4TG	17P	64094.88	-3253173	25	2	4TG	14X	72096.04	-3250876	36
4TG	18P	64867.87	-3254739	29	2	4TG	15X	72185.58	-3251657	14
4TG	19P	64262.24	-3255907	39	2	4TG	16X	72406.94	-3252195	41
4TG	20P	64554.96	-3256661	36	4	4TG	17X	72130.15	-3253339	22
4TG	21P	64161.09	-3257672	38	2	4TG	18X	72527.2	-3254956	22
4TG	22P	64456.99	-3258224	29	2	4TG	19X	72655.55	-3255377	47
4TG	23P	64243.01	-3259333	33	2	4TG	20X	72170.51	-3256472	47
4TG	24P	64078.21	-3260917	133	2	4TG	21X	72124.22	-3257481	30
4TG	25P	64164.72	-3261668	33	2	4TG	22X	72673.85	-3258921	59

4TG	26P	64492.15	-3262354	43
4TG	27P	64477.76	-3263948	41
4TG	28P	64087.89	-3264134	45
4TG	1Q	65679.37	-3237373	19
4TG	2Q	65171.32	-3238130	22
4TG	3Q	65800.49	-3239968	22
4TG	4Q	65702.35	-3240923	26
4TG	5Q	65470.58	-3241537	27
4TG	6Q	65749.49	-3242959	37
4TG	7Q	65579.8	-3243471	26
4TG	9Q	65811.79	-3245148	25
4TG	10Q	65192.54	-3246828	15
4TG	11Q	65147.78	-3247661	31
4TG	12Q	65087.64	-3248497	28
4TG	13Q	65802.94	-3249410	25
4TG	14Q	65103.25	-3250401	22
4TG	15Q	65039.94	-3251438	23
4TG	16Q	65068.2	-3252079	35
4TG	17Q	65093.78	-3253908	15
4TG	18Q	65403.71	-3254859	22
4TG	19Q	65909.25	-3255517	31
4TG	20Q	65313.05	-3256771	30
4TG	21Q	65767.85	-3257511	34
4TG	22Q	65544.05	-3258462	25
4TG	23Q	65389.15	-3259896	30
4TG	24Q	65100.77	-3260758	33
4TG	25Q	65558.98	-3261981	19
4TG	26Q	65075.47	-3262900	31
4TG	27Q	65357.73	-3263125	37
4TG	28Q	65163	-3264477	32
4TG	1R	66123.38	-3237588	36
4TG	2R	66878.83	-3238962	15
4TG	3R	66444.94	-3239663	18
4TG	4R	66161.94	-3240978	41
4TG	5R	66833.91	-3241631	30
4TG	6R	66645.45	-3242887	61
4TG	7R	66832.23	-3243644	19

4TG	23X	72142.73	-3259264	23
4TG	24X	72592.9	-3260456	25
4TG	25X	72399.34	-3262019	24
4TG	26X	72287.5	-3262570	18
4TG	27X	72444.04	-3263939	22
4TG	28X	72391.06	-3264132	24

4TG	8R	66643.44	-3244905	39
4TG	9R	66325	-3245383	47
4TG	10R	66085.95	-3246489	23
4TG	11R	66873.35	-3247301	34
4TG	12R	66916.64	-3248326	17
4TG	13R	66275.59	-3249496	16
4TG	14R	66372.63	-3250851	20
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4TG	16R	66586.23	-3252119	24
4TG	17R	66801.41	-3253668	18
4TG	18R	66849.98	-3254612	19
4TG	19R	66753.14	-3255930	41
4TG	20R	66914.48	-3256232	50
4TG	21R	66280.07	-3257761	17
4TG	22R	66808.02	-3258842	35
4TG	23R	66760.24	-3259262	27
4TG	24R	66095.75	-3260788	30
4TG	25R	66658.06	-3261807	39
4TG	26R	66156.99	-3262916	54
4TG	27R	66634.08	-3263929	34
4TG	28R	66326.4	-3264090	23
4TG	1S	67434.87	-3237629	18
4TG	2S	67771.17	-3238945	26
4TG	3S	67328.83	-3239545	33
4TG	4S	67915.82	-3240495	32
4TG	5S	67502.87	-3241872	51
4TG	6S	67738.07	-3242582	24
4TG	7S	67767.98	-3243580	18
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4TG	9S	67083.35	-3245412	13
4TG	10S	67456.65	-3247007	16
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4TG	13S	67651.32	-3249632	15
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4TG	15S	67169.15	-3251372	19
4TG	16S	67137.24	-3252392	14

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4TG	19S	67488.47	-3256003	34
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4TG	21S	67877.47	-3257589	20
4TG	22S	67114.89	-3258923	15
4TG	23S	67503.12	-3259985	32
4TG	24S	67578.14	-3260987	30
4TG	25S	67178.37	-3261323	50
4TG	26S	67050.07	-3262583	42
4TG	27S	67906.2	-3263650	30
4TG	28S	67424.62	-3264082	110
4TG	1T	68750.77	-3237756	34
4TG	2T	68837.07	-3238663	24
4TG	3T	68499.81	-3239426	30
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4TG	5T	68678.06	-3241994	56
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4TG	8T	68440.94	-3244218	13
4TG	9T	68810.54	-3245793	32
4TG	10T	68422.24	-3246260	25
4TG	11T	68283.95	-3247978	16
4TG	12T	68941.14	-3248932	24
4TG	13T	68531	-3249272	27
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4TG	16T	68192.97	-3252340	32
4TG	17T	68826.06	-3253660	36
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4TG	21T	68470.58	-3257452	28
4TG	22T	68549.05	-3258172	30
4TG	23T	68678.87	-3259564	27
4TG	24T	68076.27	-3260763	32
4TG	25T	68102	-3261977	36

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4TG	27T	68418.62	-3263745	45
4TG	28T	68786.24	-3264126	51
4TG	1U	69865.55	-3237573	21
4TG	2U	69630.05	-3238931	39
4TG	3U	69641.48	-3239828	36
4TG	4U	69391.35	-3240874	34
4TG	5U	69753.81	-3241486	28
4TG	6U	69934.77	-3242485	25
4TG	7U	69132.15	-3243891	15
4TG	8U	69317.49	-3244518	24
4TG	9U	69227.54	-3245272	27
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4TG	11U	69877.99	-3247781	19
4TG	12U	69790.72	-3248953	18
4TG	13U	69819.96	-3249961	28
4TG	14U	69103.8	-3250437	26
4TG	15U	69361.4	-3251954	31
4TG	16U	69102.06	-3252375	33
4TG	17U	69476.03	-3253577	56
4TG	18U	69613.28	-3254246	42
4TG	19U	69099.98	-3255923	39
4TG	20U	69596.34	-3256796	25
4TG	21U	69268.75	-3257947	41
4TG	22U	69422.22	-3258828	36
4TG	23U	69893.71	-3259544	48
4TG	24U	69773.52	-3260910	57
4TG	25U	69314.58	-3261227	29
4TG	26U	69313.9	-3262842	57
4TG	27U	69486.97	-3263578	54
4TG	28U	69568.94	-3264092	46
4TG	1V	70429.62	-3237877	26
4TG	2V	70197.7	-3238722	37
4TG	3V	70113.51	-3239160	41
4TG	4V	70358.29	-3240947	36
4TG	5V	70400.53	-3241911	30
4TG	6V	70301.81	-3242569	32

4TG	7V	70234.13	-3243979	54
4TG	8V	70368.54	-3244461	14
4TG	9V	70095.64	-3245852	26
4TG	10V	70575.84	-3246588	31
4TG	11V	70353.15	-3247676	40
4TG	12V	70094.21	-3248167	48
4TG	13V	70783.97	-3249315	40
4TG	14V	70348.46	-3250499	27
4TG	15V	70894.44	-3251154	26
4TG	16V	70061.39	-3252946	29
4TG	17V	70218.48	-3253772	34
4TG	18V	70112.5	-3254540	28
4TG	19V	70113.95	-3255894	80
4TG	20V	70344.08	-3256528	19
4TG	21V	70556.7	-3257811	26
4TG	22V	70125.48	-3258930	23
4TG	23V	70708.8	-3259476	34
4TG	24V	70511.9	-3260631	57
4TG	25V	70892.54	-3261809	52
4TG	26V	70399.18	-3262954	69
4TG	27V	70279.75	-3263620	33
4TG	28V	70630.86	-3264253	25
4TG	1W	71910.07	-3237340	45
4TG	2W	71366.11	-3238719	22
4TG	3W	71580.06	-3239598	54
4TG	4W	71740.78	-3240369	27
4TG	5W	71631.09	-3241499	43
4TG	6W	71219.68	-3242318	26
4TG	7W	71112.63	-3243408	21
4TG	8W	71684.73	-3244125	29
4TG	9W	71955.84	-3245819	25
4TG	10W	71078.92	-3246289	36
4TG	11W	71144.82	-3247582	44
4TG	12W	71137.17	-3248845	30
4TG	13W	71201.94	-3249161	52
4TG	14W	71459.45	-3250985	25
4TG	15W	71562.67	-3251329	20

4TG	16W	71921.54	-3252455	29
4TG	17W	71110.71	-3253222	56
4TG	18W	71091.12	-3254589	54
4TG	19W	71763.03	-3255167	33
4TG	20W	71590.41	-3256182	32
4TG	21W	71275.5	-3257294	31
4TG	22W	71558.67	-3259034	50
4TG	23W	71105.11	-3259804	32
4TG	24W	71395.15	-3260141	73
4TG	25W	71916.01	-3261941	65
4TG	26W	71350.77	-3262649	94
4TG	27W	71300.79	-3263637	35
4TG	28W	71169.22	-3264182	64
4TG	1X	72826.15	-3237345	23
4TG	2X	72456.46	-3238906	14
4TG	3X	72423.06	-3239337	22
4TG	4X	72458.02	-3240794	63
4TG	5X	72167.86	-3241529	44
4TG	6X	72532.36	-3242188	32
4TG	7X	72298.37	-3243752	19
4TG	8X	72470.48	-3244962	36
4TG	9X	72204.28	-3245563	36
4TG	10X	72417.62	-3246911	32
4TG	11X	72666.54	-3247642	26
4TG	12X	72635.15	-3248883	25
4TG	13X	72227.33	-3249188	18
4TG	14X	72096.04	-3250876	36
4TG	15X	72185.58	-3251657	14
4TG	16X	72406.94	-3252195	41
4TG	17X	72130.15	-3253339	22
4TG	18X	72527.2	-3254956	22
4TG	19X	72655.55	-3255377	47
4TG	20X	72170.51	-3256472	47
4TG	21X	72124.22	-3257481	30
4TG	22X	72673.85	-3258921	59
4TG	23X	72142.73	-3259264	23
4TG	24X	72592.9	-3260456	25

4TG	25X	72399.34	-3262019	24
4TG	26X	72287.5	-3262570	18
4TG	27X	72444.04	-3263939	22
4TG	28X	72391.06	-3264132	24