The effect of copper toxicity on the growth and morphology of Rhodes grass (*Chloris gayana*) in solution culture

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Abstract
A solution culture experiment was conducted to examine the effect of Cu toxicity on Rhodes grass (*Chloris gayana*), a pasture species used in mine site rehabilitation. The experiment used dilute, solution culture to achieve external nutrient concentrations which were representative of the soil solution, and ion exchange resins to maintain stable concentrations of Cu in solution.

Copper toxicity was damaged plant roots, with symptoms ranging from disruption of the root cuticle and reduced root hair proliferation, to severe deformation of root structure. A reduction in root growth was observed at an external Cu concentration of <1µM, with damage evident from an external concentration of 0.2µM.

Critical to the success of this experiment, in quantitatively examining the relationship between external Cu concentration and plant response, was the use of ion exchange resin to buffer the concentration of Cu in solution. After some initial difficulty with pH control, stable concentrations of Cu in solution were maintained for the major period of plant growth. The development of this technique will facilitate future investigations on the effect of heavy metals on plants.

Introduction
Copper toxicity is a problem of both agricultural and environmental significance. Sources of Cu contamination include mining and smelting, urban, industrial and agricultural wastes, and the use of agrochemicals. Copper is present in many forms in soils, with free Cu^{2+} activity considered to be the best indicator of bioavailability (Sauve *et al.* 1996). Soil solution Cu concentrations are generally extremely low, with more than 98% Cu in solution bound to soluble organic matter, irrespective of pH (Sauve *et al.* 1997). Adsorption of Cu is highly pH dependent and bioavailability of Cu increases with decreasing pH. Due to its high affinity for organic matter, Cu is not readily leached from the soil profile and tends to accumulate in the surface soil (McBride *et al.* 1997).

While Cu is an essential micronutrient, exposure to excess Cu has a detrimental effect on plant growth. The affect of Cu toxicity is largely on root growth and morphology. Copper tends to accumulate in the root tissue with little translocated to the shoots (Marschner 1995). Because the principle effect of Cu toxicity is on root growth, the study of Cu toxicity in a soil environment is difficult. Solution culture provides a model of the interaction between the plant and the soil solution and allows examination of root growth and morphology. A number of difficulties have been observed with experiments conducted in solution culture. Comparison between experiments using nutrient solutions of different ionic strengths is difficult, due to the effect of high ionic strength on ameliorating metal toxicity (Reichman 2002). Most work conducted using solution culture also uses a system of simply replacing the solution at regular intervals. This results in significant variation in the concentration of the nutrient solution (Reichman 2001).

In this study we examined the effect of Cu toxicity on the growth and morphology of Rhodes grass (*Chloris gayana*), using a low ionic strength solution culture, to represent soil solution conditions, with the Cu concentration buffered using ion exchange resin. By using a method which maintains stable concentrations of Cu in solution, this project aims to examine the relationship between the external Cu concentration and the effect on plant growth.

Materials and Methods
Treatments
A dilute renewed solution culture experiment was conducted to determine the response of Rhodes grass to Cu toxicity. Ten Cu rates were used with 4 replications. The Cu concentration in solution was buffered by Amberlite IRC748 Cu specific ion exchange resin. A range of Cu concentrations was achieved by

mixing Cu saturated resin with Ca saturated resin at different ratios (g Cu + g Ca; 0.5+9.5, 1+9, 2+8, 3+7, 4+6, 5+5, 6+4, 7+3, 8+2, 9+1). Zinc saturated resin (0.1 g) was added to ensure that Zn concentrations in solution were maintained at adequate levels for plant growth. Prior to addition to the nutrient solution, the resin for each pot was mixed in a small container with triple deionised water and allowed to equilibrate overnight. After transfer to the nutrient solution, the resin was allowed to equilibrate for 5 days. The solution Cu concentrations were monitored during this time, and the solution pH was adjusted. The nutrient solution was constantly mixed using an air driven pump system which ensured that the water was continually passing over the resin. The entire volume of the pot passed over the resin every 13 minutes.

Solution samples were taken 2-3 times per week for the duration of the experiment to monitor Cu concentration. Once a week solution samples were taken for multi-element analysis by inductively coupled plasma atomic emission spectroscopy (ICPAES). Solution samples were taken approximately 12 hours after pH adjustment and before nutrient addition. Due to the difficulty measuring low Cu concentrations, samples were concentrated prior to analysis using Amberlite IRC748 resin beds. A 40 times concentration was achieved by running 400 mL of solution through a resin bed and eluting with 10 mL 1M HNO_3. Samples were analysed by atomic absorption spectroscopy (AAS). Comparison with standards showed 98% recovery using this method.

Rhodes grass seeds were germinated in sand. After two weeks, seedlings were transplanted into the nutrient solution culture system. Healthy plants were selected for uniformity and allocated randomly to pots. Plants were supported in 20 L pots of nutrient solution by 4 cups with mesh bases held in the lid of each pot. Three seedlings were transplanted into each cup and one week after transplant each cup was thinned to 2 plants leaving 8 plants per pot. The cups were lined with black plastic beads to support the plants and prevent light from entering the pot.

Plants were grown in dilute renewed solution culture. The composition of the basal nutrient solution was (in µM) 1900 N (1700 NO_3, 200 NH_4), 2 P, 502 K, 750 Ca, 200 Mg, 202 S, 500 Cl, 10 Fe, 1 Mn, 1 Zn, 3 B, 0.02 Mo. Iron was added as FeCDTA to minimize the interaction between Cu and chelates in solution. Nutrients were added each week using Programmed Nutrient Addition (Asher and Blamey 1987). A preliminary experiment was conducted to determine the growth rate and nutrient requirement of Rhodes grass. Nutradd was then used to calculate nutrient requirement over the duration of the experiment.

Plants were harvested at four weeks, and plant height and root length recorded. Two plants were randomly selected from each pot for analysis of root morphology. These roots were rinsed in deionised water, stored in 10% ethanol and refrigerated. The remaining plant parts were rinsed in deionised water, then dried at 70°C and weighed. Plant tops and roots were then ground and digested in nitric-perchloric acid and analysed by ICPAES for Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn. Plant roots stored in ethanol were dried and weighed but not included for analysis.

Results

Foliar symptoms

No foliar symptoms were observed as a result of Cu toxicity. Treatments 1-8 (≤0.66 µM Cu) showed little difference in growth although there appeared to be a range of plant sizes within treatments and individual pots. Treatment 9 (1.17 µM Cu) showed reduced growth, while growth in treatment 10 (2.06 µM Cu) was severely reduced.

Root growth

Root growth in treatment 10 plants was severely inhibited. Roots were extremely stunted and the root cuticle was thickened, cracked and brown. No lateral roots or root hairs were present. Treatment 9 plants again showed stunted, deformed root structure. The root cuticle was damaged, with severe damage at the root meristems. No root hairs were present. Large numbers of deformed lateral roots were present. Increased lateral root production was also observed in treatment 8 (0.66 µM). While plants in all other treatments appeared to have well-developed root systems, as the Cu concentration increased there was a decrease in the number of root hairs.

A change in the overall morphology of the root system was also observed. Plants in the lowest Cu treatments had root systems with fewer, longer roots. As the Cu concentration increased, the plants tended to have more, shorter roots.

Plant analysis

There was no significant reduction in dry weight of shoots or roots for the first 8 treatments (Figure 1). Treatment 9 showed a significant reduction in shoot dry weight of more than 60% while treatment 10 had a growth reduction of 98%. Root dry weight was reduced by 60% in treatment 9 and 99% in treatment 10. Similarly, there was no significant reduction in plant height for treatments 1-8 (Figure 2). Treatment 9 had a 23% reduction in height, while treatment 10 had an 81% reduction in height. There was no significant difference in the root lengths of plants in treatments 1-7 (≤0.51 µM Cu). There was a 30% reduction in root length in treatment 8, 50% in Treatment 9, and 99% in treatment 10.

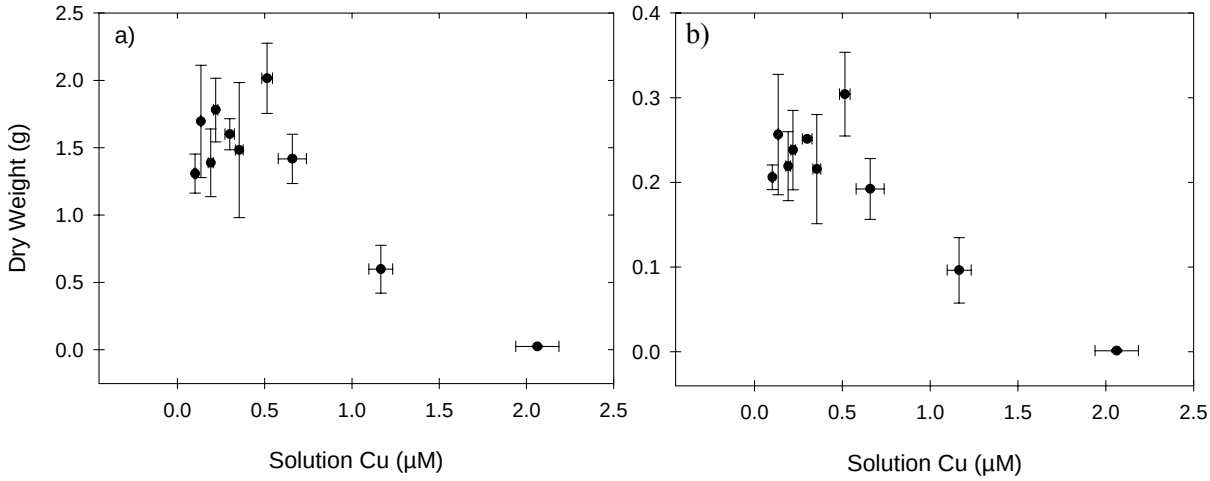

Figure 1. Relationship between solution Cu concentration and mean plant dry weights for a) shoots, and b) roots.

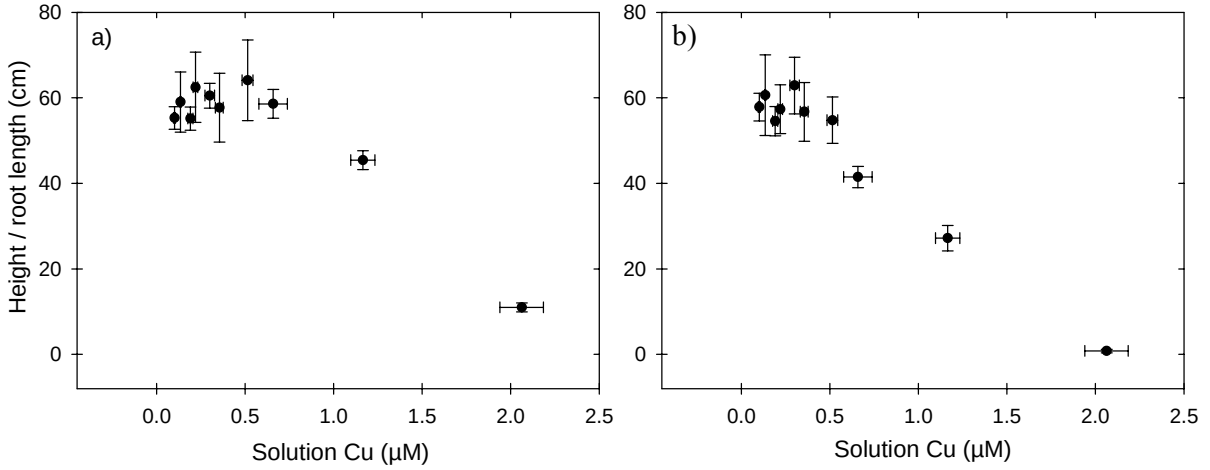

Figure 2. Relationship between solution Cu concentration and mean plant a) height, and b) root length.

Plant nutrient concentration

There was little increase in the Cu concentration of shoot tissue, with concentrations ranging from 13-20 µg/g in the treatment 1-8 (Figure 3). Treatment 9 contained 26 µg/g Cu, while treatment 10 contained 80 µg/g. The Cu concentration in the root tissue increased linearly with solution Cu. Concentrations ranged from 17 µg/g in treatment 1 to 287 µg/g in treatment 9. There was insufficient root tissue in treatment 10 to perform nutrient analysis.

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Plate 1. The effect of Cu toxicity on A) root cuticle, and B) root morphology.

Solution nutrient concentration

The stability of the Cu concentration in solution increased over time, with little variation occurring during the second half of the experiment (Figure 4). Mean Cu concentrations in solution ranged from 0.1-2µM from day 13 on. The concentration of Cu in solution peaked at day 6 with concentrations ranging from 0.2µM for treatment 1 to 7µM for treatment 10. The Cu^{2+} activity calculated using PHREEQC (Parkhurst 1995) was 75±2% of the total Cu concentration in solution as the pH and ionic strength of the solution was held relatively constant throughout the experiment.

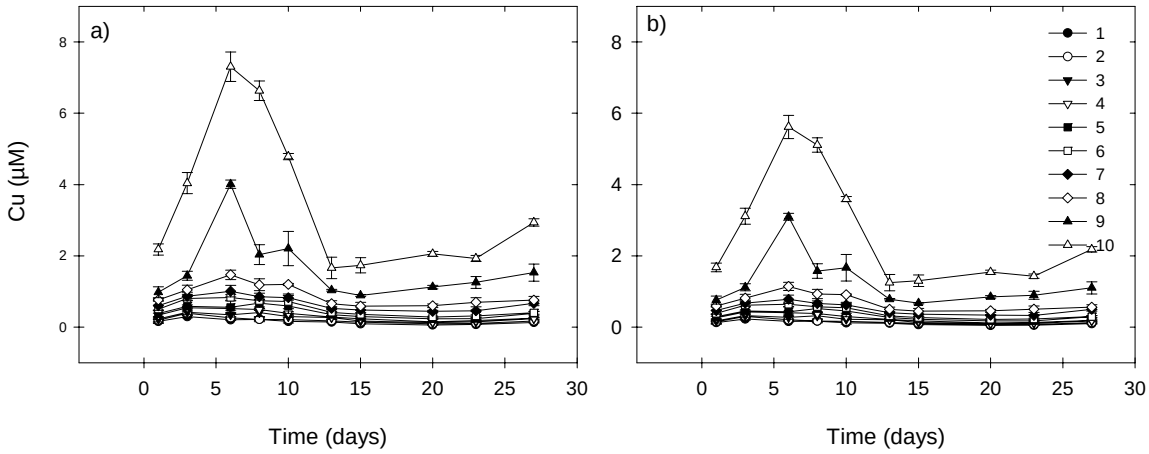

Figure 4. Copper concentration in solution. Figure a) shows Cu concentration in solution; b) shows Cu^{2+} activity.

Discussion

Due to the control over the Cu concentration in solution, this experiment gives an opportunity to examine the incremental effects of Cu toxicity that has not been achieved by previous research. Renewed or non-renewed solution culture methods have been the dominant growth medium used to date. Solution renewal rates range from several days (Lexmond and van der Vorm 1981; Wong and Bradshaw 1982; Lidon and Henriques 1992; 1993; 1994; Mocquot *et al.* 1996; Liao *et al.* 2000), to weekly (Zhu and Alva 1993; Wheeler and Power 1995; Reichman 2001), or not at all. These experiments using renewed solution culture would have produced a saw-toothed pattern of nutrient status, with high values following renewal, gradually falling to low values over time.

Little work has been conducted using resin buffered nutrient solutions. Checkai *et al.* (1992) achieved reasonable control over the concentrations of macronutrients in solution, but the concentrations of micronutrient metals varied over the duration of the experiment and reached up to 30 times initial concentrations. The work of Smolders and McLaughlin (1996) with Cd achieved much better control over solution concentrations, although the basal nutrient solution was of high ionic strength relative to that of the soil solution in non-saline soils.

The concentration of Cu in solution varied considerably over the initial stages of this experiment due to a lack of appreciation of the effect of pH on the ion exchange resin (Figure 3). A change in pH of 0.1 units caused a 3 fold change in solution Cu and daily pH adjustments were not sufficient to account for this. Over the latter part of the experiment, during which most plant growth occurred, better control over solution pH, and hence Cu concentration was achieved. During the second half of the experiment little variation in Cu concentration occurred over time or between treatments. As 90% of plant growth occurred during this time, this experiment allows the effect of external Cu concentration on plant growth to be quantified in a situation which mirrors the nutrient concentration of the soil solution and the pH at which Cu toxicity would occur. As the solution Cu concentrations were stable for the period in which most plant growth occurred, the solution Cu concentrations used for comparison with plant growth are calculated as mean concentration from day 13 on.

Effect of Cu toxicity on plant growth

The effects of Cu toxicity on the growth and morphology of Rhodes grass are generally consistent with the reported symptoms of Cu toxicity in other plants. The plants in treatment 9 showed a reduction in growth of greater than 10%. The concentration of Cu in the shoot tissue of these plants was 26 µg/g

which falls within the range of 20-30 µg/g suggested by Marschner (1995) as a general critical concentration for Cu toxicity. The concentration of Cu in the root tissue of treatment 9 plants was 390 µg/g. No reduction in dry weight was observed for treatment 8 and the concentration of Cu in the shoot tissue was 20 µg/g.

While significant reductions in plant dry weight occurred from treatment 9 on, it was observed that a range of morphological changes occurred in response to increasing levels of Cu toxicity. Thickening and cracking of the root cuticle, damage to the meristem, and a reduction in root length was observed from treatment 7, which corresponds to an external Cu concentration of 0.51µM. Damage to the root cuticle and a reduction in the number and length of root hairs was observed from treatment 4, which had an external Cu concentration of 0.22µM. This shows that while a growth reduction occurred in response to an external Cu concentration of 1.1µM, plants are under stress from elevated Cu concentrations as low as 0.22 µM.

Because this experiment was conducted using solution culture as the growth medium, nutrient availability was high and a reduction in root hair proliferation would not have impacted on the plant's ability to access nutrients. In the soil environment however, where root surface area is critical to a plant's ability to take up nutrients, a reduction in root hair proliferation may have a much greater effect on plant growth. As well as a decrease in root area, a reduction in the number of root hairs may also inhibit nodulation of legumes. Phosphorus nutrition may be particularly affected as root proliferation and mycorrhizal associations are critical to the ability of plants to access P (Marschner 1995). The affect of Cu toxicity on root morphology is similar to that of Al toxicity (Hecht-Buchholz *et al.* 1990). Both affect root proliferation and reduce root hair formation and hence, affect nodulation.

Tissue nutrient concentration

The increase in the concentration of Cu in the shoot tissue from treatment 1-9 was quite small (Figure 3). This is consistent with published observations that Cu does not tend to accumulate in the shoots (Loneragan 1981; Marschner 1995). The concentration of Cu in the shoot tissue of treatment 10 plants was substantially higher than the other treatments. This may be due to a breakdown in the Cu tolerance mechanisms in the roots. The concentration of Cu in the root tissue increased linearly as the Cu concentration in the nutrient solution increased, which is also consistent with published observations (Loneragan 1981; Robson and Reuter 1981; Marschner 1995).

The shoot concentration of nutrient cations Ca, K, Mg and Mn decreased as the concentration of Cu in the nutrient solution increased. In the case of Mn, this occurred despite the solution cultures which contained the highest concentration of Cu also containing the highest concentration of Mn. This inhibition of uptake may be due to increased competition. At high Cu concentrations, where severe root damage was observed, reduced uptake of these elements may be due to breakdown of membrane function. The concentrations of K, P, and Mn in treatment 10 were below the concentrations considered adequate for plant growth (Reuter and Robinson 1997). This was despite the concentration of these elements in solution being within the range considered sufficient for plant growth in solution culture. The effect of Cu toxicity again resembles Al toxicity, in that Al is a strong inhibitor of Ca and Mg uptake (Marschner 1995). A slight reduction in K concentration in the roots was observed. This may be due to K efflux as part of a mechanism of Cu tolerance (Murphy *et al.* 1999).

Critical solution Cu concentration

The critical concentration of Cu in the nutrient solution associated with a 10% reduction in growth is between 0.6 and 1.1µM. This is comparable to the values of 0.5 µM determined by Zhu and Alva (1993) for swingle citrumelo seedlings, 0.7 µM by Wong and Bradshaw (1982) for rye grass, and 1.5 µM by Hill et al. (2000) for taro. Zhu and Alva (1993), used a dilute nutrient solution similar to the one used for this experiment. Wong and Bradshaw (1982), used a solution of 3050 µM $Ca(NO_3)_2$ which would have a similar ionic strength to dilute nutrient solution. The solution used by Hill et al. (2000), was approximately twice the concentration of that used in this experiment.

Conclusion

This experiment has shown that the effect of Cu toxicity on root morphology and plant nutrient composition is, in many ways, similar to the effect of Al toxicity. The effect of Cu on root hair

proliferation suggests that reductions in growth due to nutrient deficiency or inhibition of nodulation may occur at lower Cu concentrations than observed here.

The use of ion exchange resin in maintaining stable concentrations of Cu in solution was critical to the success of this project. Future use of these resins however, would require a better method of pH control, such as automated pH titration, rather than the daily adjustment used here.

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