Office Use Only

Project Code	
Project Type	

FINAL REPORT
2023

PROJECT CODE	S-UA 821
PROJECT TITLE	
Investigating aluminium speciation in wheat roots in alkaline soil	

PROJECT DURATION						
Project start date	1/04/2021					
Project end date	31/03/2023					
SAGIT Funding	2021/22	$35,350				

PRINCIPAL INVESTIGATOR			
Title: Dr	**First Name:** Rhiannon		**Surname:** Schilling
Organisation:	The University of Adelaide		
Mailing address:	Level 3, Rundle Mall Plaza, 50 Rundle Mall, Adelaide SA 5000		
Telephone:		**Email:**	
Mobile:			

ADMINISTRATION CONTACT DETAILS			
Title: Ms	**First Name:** Michelle		**Surname:** Whittle
Organisation:	The University of Adelaide		
Mailing address:	Level 3, Rundle Mall Plaza, 50 Rundle Mall, Adelaide SA 5000		
Telephone:		**Email:**	
Mobile:			

PROJECT REPORT:

Executive Summary

Alkaline soils (pH > 8) are widespread in SA and are known to reduce grain yields. These soils impact on crops by reducing root growth due to the high pH per se (OH-) and indirectly by nutrient deficiencies and toxicities. Increasing evidence suggested aluminium (Al) toxicity in alkaline soils is a major factor contributing to the reduction of root growth. However, unlike acid soils (pH < 5.5), the role of Al in reducing root growth in alkaline soils has been less clear. This was mainly due to a lack of measurements demonstrating the speciation of Al in the alkaline soils surrounding the roots and within the roots of plants grown in alkaline soils. This bottleneck has been restricting progress towards developing crops with tolerance to alkaline soils, as the role of Al in contributing to the reduction in root growth in these soil types has been overlooked.

This SAGIT funded project utilised the Canadian Light Source (CLS) synchrotron facilities to, for the first time, demonstrate the forms of Al in an alkaline, sodic soil from Roseworthy, SA and the form of Al that occurs in roots of wheat plants grown under high pH and high pH + Al.

The findings discovered:

- The presence of spectra with a clear double peak indicating close alignment to the boehmite Al form in the alkaline, sodic soil from Roseworthy.
- The presence of Al in spectra aligned with the boehmite form in wheat root tips tested in the Al K-Edge indicating this form occurred within the wheat plant roots under high pH + Al conditions. Both Janz and Mace under the high pH + Al treatment showed these spectra indicating alignment with the presence of boehmite, which was not present in the root tips of either variety under the control or high pH only treatments.

Project objectives

Recent advances in synchrotron-based x-ray technology provided a new opportunity to investigate the form of aluminium in alkaline soils and the form of aluminium within wheat roots of plants grown in alkaline soils. This technology was used to determine if a toxic form of aluminium influences wheat root growth in alkaline soils.

The aims of this project were:

1. To determine the form of aluminium in an alkaline SA soil profile (to 1 m deep).
2. To measure the form of aluminium and spatial distribution of this aluminium in wheat roots grown under control, high pH and high pH with aluminium.

Overall Performance

The objectives of this project were successfully achieved despite long delays due to COVID shutdowns. Both the soil from Roseworthy SA and the root tips of two wheat varieties grown under control, high pH and high pH + Al conditions were tested on Spherical Grating Monochromator (SGM) and the VLS-PGM beamline (Variable Line Spacing Plane Grating Monochromator) = Al L-edge and Al K-Edge (XANES respectively.

List of personnel
Dr Rhiannon Schilling (lead CI, SARDI)
Dr Cassandra Schefe, AgriSci Pty Ltd
Dr Daniel Menadue (SARDI, assisted with growth chamber experiment)
Dr Jay Dynes, Canadian Light Source
Dr Lucia Zuin, Canadian Light Source

Difficulties encountered
This project was delayed by COVID as the Canadian Light Source (CLS) was forced into a non-scheduled shutdown and use of the synchrotron was central to the project. The CLS allocated beamtime to COVID vaccine development as a priority. R Schilling and C Schefe were able to secure

beamtime to complete the soil testing in Aim 1 in 2021 and the root tissue testing for Aim 2 in 2022, this occurred due to established collaborations with colleagues at the facility and following a successful competitive grant re-application for beamtime following the re-opening of the CLS facility. The CLS facility managed the difficult time of COVID shutdowns very professionally and we are grateful for the support of Dr Lucia Zuin, Dr Jay Dynes and CLS colleagues for their assistance with this project. The shipment of both soil and plant tissue to Canada was also another difficulty encountered due quarantine export requirements. The CLS helped to ensure this exporting of samples went smoothly and the root tissue was freeze-dried to ensure it was suitable for scientific testing following transport.

KEY PERFORMANCE INDICATORS (KPI)

KPI	Achieved	If not achieved, please state reason.
1. Preliminary testing of soil samples to 1 m for Al speciation using synchrotron.	Yes ☒ No ☐	N/A
2. Complete controlled growth chamber experiment and prepare root samples for synchrotron.	Yes ☒ No ☐	N/A
3. Complete synchrotron testing of root samples and finalise data analysis.	Yes ☒ No ☐	N/A

TECHNICAL INFORMATION

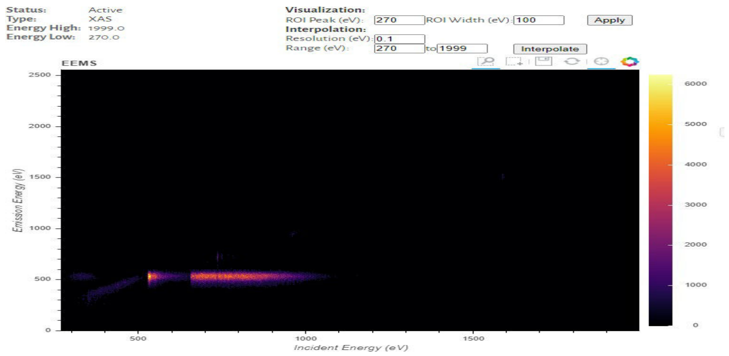
Aim 1 - To determine the form of aluminium in an alkaline SA soil profile (to 1 m deep).

Soil samples from Roseworthy SA collected at depths 0-20, 20-40, 40-60, 60-80 and 80-100 cm (Table 1) were prepared using an agate mortar and pestle and 5 g of prepared soil per depth was sent following quarantine and export requirements to the CLS.

Table 1: The salinity (EC) and pH and the range of depths of the soil samples collected at Roseworthy, SA used for analysing at the CLS. This profile represents a classic sodic subsoil profile and is from a sodic trial site that has been extensively characterised for wheat variety root growth. A full ICP analysis of the soil profile is available.

Site	Depth (cm)	$EC_{1:5}$ (µS/cm)	pH (water)
Roseworthy	0-20	101	7.42
Roseworthy	20-40	303	9.39
Roseworthy	40-60	359	10.00
Roseworthy	60-80	934	10.07
Roseworthy	80-100	1443	9.81

The 5× soil samples, $AlPO_4$, Al Oxalate and Cu Tape (control samples) were tested on the Spherical Grating Monochromator (SGM) beamline by the CLS team (Jay Dynes) (Figure 1, 2 and 3). The XAS spectra showed shifts in the aluminium peaks (slopes) of the samples with a clearly distinguishable double peak in each sample (Figure 2) and that these peaks did not correspond to aluminium oxalate or aluminium phosphate (Figure 3). This data suggests that the spectra is aligned to the boehmite aluminium form (Figure 4) due to the unique double peaks of boehmite.



Status: Active
Type: XAS
Energy High: 1999.0
Energy Low: 270.0

Visualization:
ROI Peak (eV): 270 ROI Width (eV): 100
Interpolation:
Resolution (eV): 0.1
Range (eV): 270 to 1999

Figure 1: Raw data for the Roseworthy sample 1 at 0-20 cm. This is available for all the soil samples and is here to show the actual sample result from the beamline as an example.

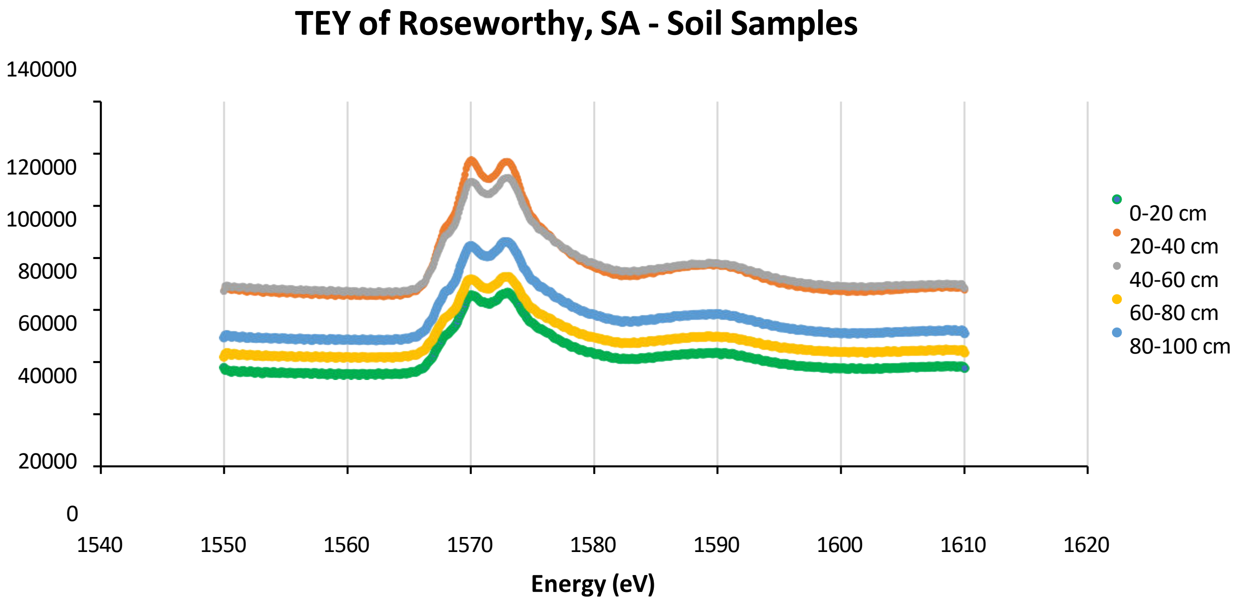


Figure 2: The spectra total energy yield (TEY) for Roseworthy soil samples collected at 0-20, 20-40, 40-60, 60-80 and 80-100 cm depth.

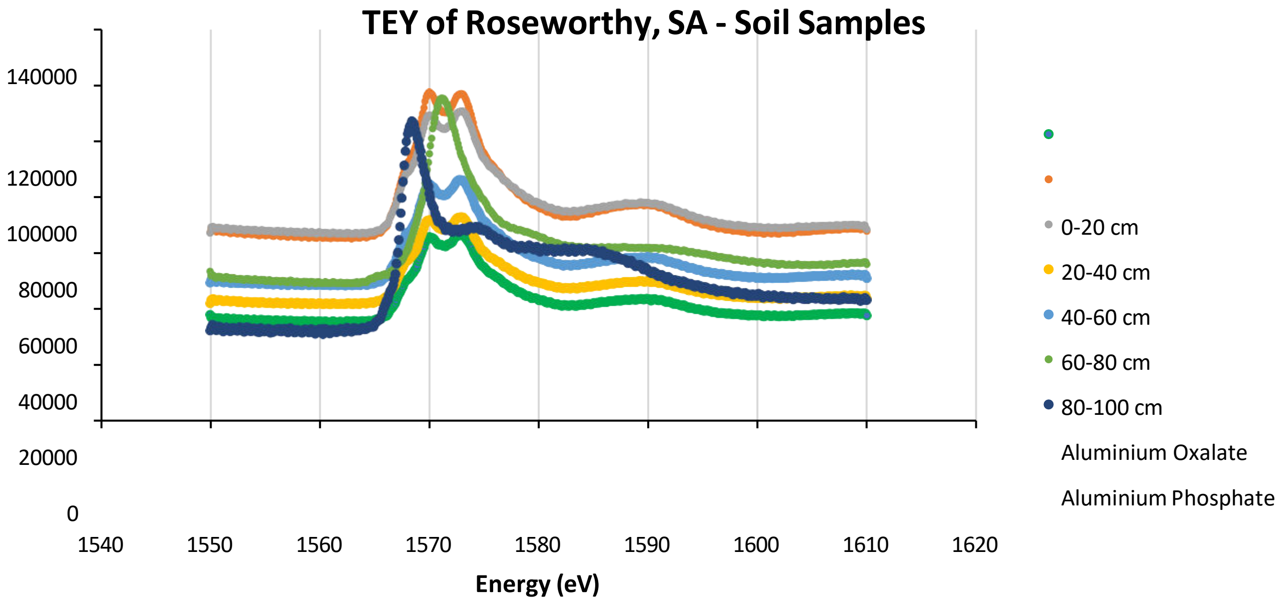


Figure 3: The spectra total energy yield (TEY) for Roseworthy soil samples collected at 0-20, 20-40, 40-60, 60-80 and 80-100 cm depth with aluminium oxalate and aluminium phosphate standard check indicating these forms were not aligned with the spectra in the soil samples.

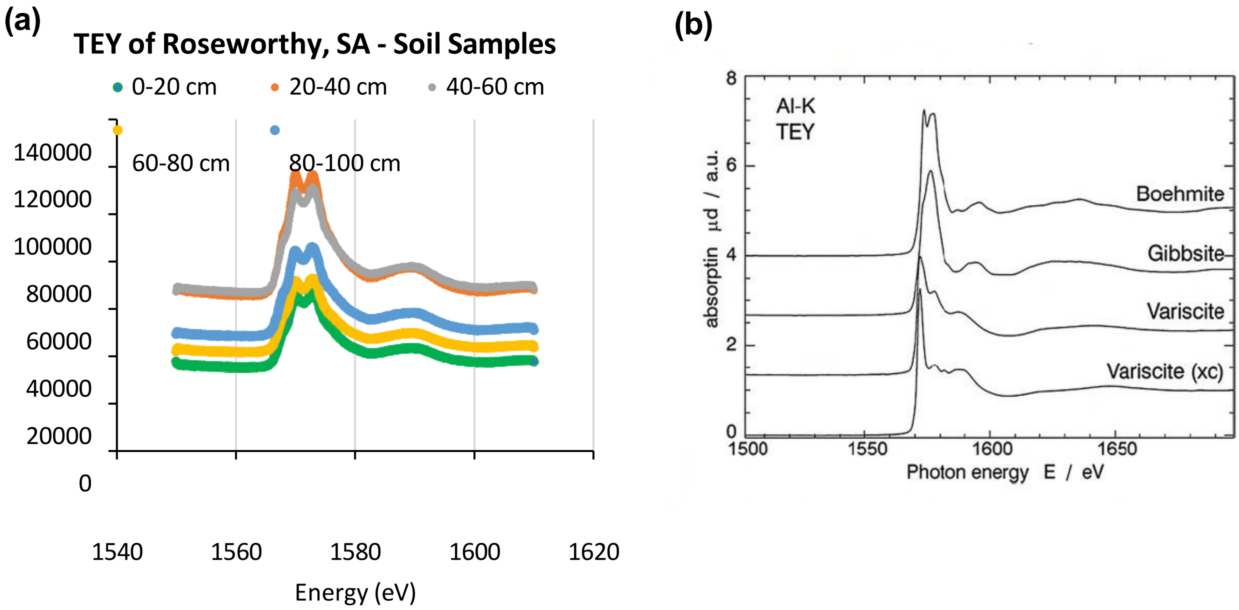


Figure 4: The (a) spectra total energy yield (TEY) for Roseworthy soil samples collected at 0-20, 20-40, 40-60, 60-80 and 80-100 cm depth compared to (b) previously published XANES spectra of aluminium K-edge reference compounds boehmite, gibbsite, variscite and variscite (xc) aluminium forms (Schefe, Kappen, Zuin, Pigram, Christensen, Journal of Colloid and Interface Science 330 (2009), 51-59) showing the similarities in double peak between the soil samples and the boehmite form of aluminium.

Aim 2 - To measure the form of aluminium and spatial distribution of this aluminium in wheat roots grown under control, high pH and high pH with aluminium.

Two wheat varieties differing in their tolerance to high pH with high aluminium (Janz - sensitive and Mace - tolerant) were selected based on previous root screenings of 52 bread wheat cultivars (Schilling, unpublished). A hydroponics experiment following the established protocols by Ma and Rathjen with nutrient solution containing sufficient calcium, zinc and boron were used including 3 treatments (control, high pH, high pH + Al) with control = pH 7.5 and high pH = 9.2 and aluminium treatment established at 1 mg/L of Al. The pH of the solution was adjusted morning and night using Na_2CO_3 or HCl and values recorded. Plants were grown for 5 days of treatment (to match previous screening methods). Ten replicates for each treatment and cultivar were used in a completely randomised block design. Following this, the ten plants were weighed for shoot and root fresh weight and then 5 plants were rinsed in 1mM $CaCl_2$ and 1 cm tips of 4 plants roots from 5 of the replicates were frozen in liquid nitrogen before storage in a -80C freezer. Samples were then freeze dried overnight and transported to CLS where they were tested on both the VLS-PGM beamline (Variable Line Spacing Plane Grating Monochromator) = Al L-edge and Al K-Edge (XANES).

Results of the plant experiment showed uniform growth amongst the replicates within each cultivar and treatment (Figure 5, 6a,b). The shoot biomass of Mace was higher than Janz in the control and high pH treatments, but the shoot biomass was similar in the high pH + Al treatment (Figure 6a). The shoot biomass tolerance (relative to control) was around 0.8 of the control for both treatments (Figure 6 c). As expected, the root biomass was most affected by the treatments with a significant reduction in root growth under both the high pH and high pH + Al treatment compared to the control for both Janz and Mace with Mace having larger roots under both high pH and high pH + Al compared to Janz. (Figure 6b). This was also observed in the root biomass tolerance (relative to control) with relative growth for Janz at 0.296 and 0.25 for high pH and high pH + Al respectively and the relative growth for Maze at 0.5 and 0.359 for high pH and high pH + Al respectively (Figure 6d).

Janz – Control ***Mace – Control***

Janz – High pH ***Mace – High pH***

Janz – High pH + High Al ***Mace – High pH + High Al***

Figure 5: The ten replicates shown for both Janz and Mace under control, high pH and high pH + high aluminium (Al). Plant growth was very uniform between replicates and the experimental growth conditions of the plants were ideal for this synchrotron experiment.

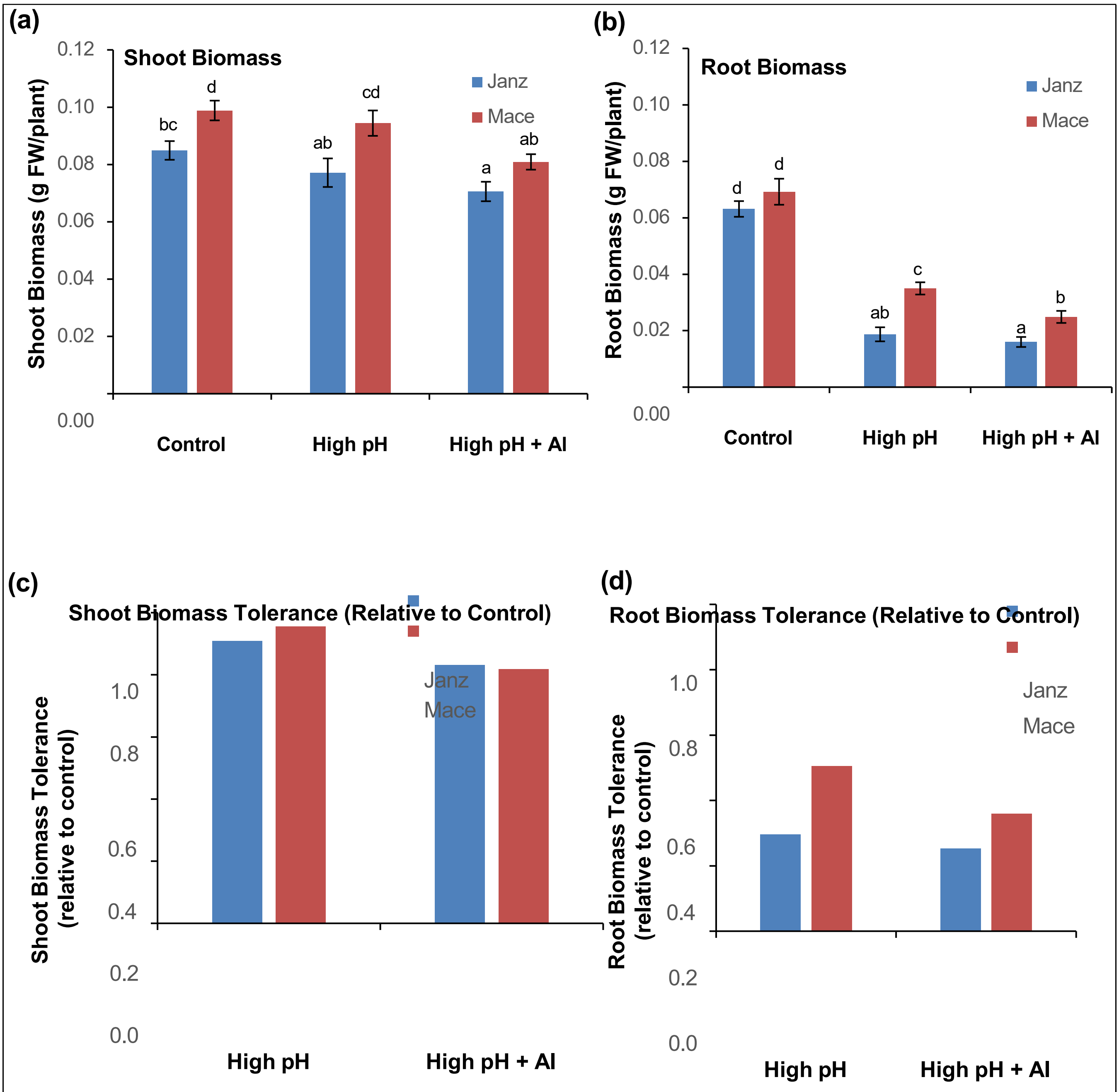
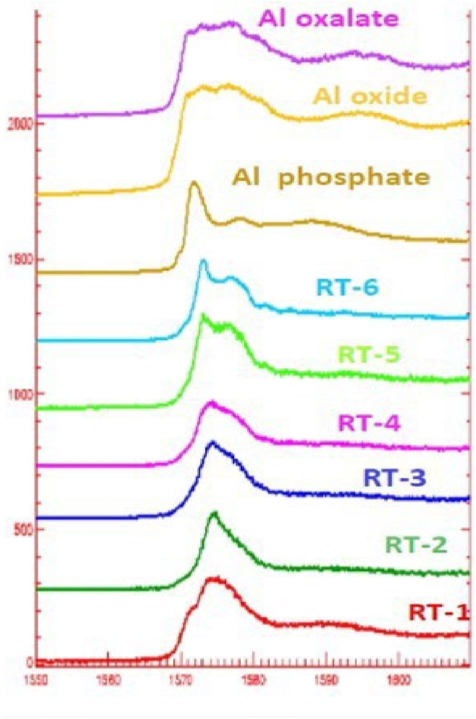


Figure 6: (a) Shoot and (b) root biomass of Janz (blue) and Mace (red) after 5 days of treatment in control (pH 7.5), high pH (pH 9.2) and high pH (9.2) + 1mg/kg Al. Biomass values are presented as mean ± SEM (*n* = 10) with a significant difference *p* ≤ 0.001 shown with a different letter. The relative (c) shoot and (d) root biomass tolerance is shown compared to the control for each cultivar and treatment.

Results of the Al L-edge data for the root tips completed by Dr Lucia Zuin was found to be below the detector limit at 16 sec dwell time and was not able to distinguish between the different treatments (data not shown). Results of the Al K-Edge (XANES) was completed by Dr Jay Dynes and are promising. We detected differences between established treatments in the roots with very clear spectra for the control, high pH and high pH + Al treatments (Figure 7). The spectra showed two distinct peaks in the rt-5 and rt-6 sample (root tissue of Mace and Janx from the high pH + high Al treatments) that does not align with Al oxalate, Al oxide or Al phosphate but shows alignment to the distinguishable peaks of boehmite (Figure 4).



Root sample #	Sample name
rt-1	Janz Control
rt-2	Mace Control
rt-3	Janz High pH
rt-4	Mace High pH
rt-5	Janz High pH + Aluminium
rt-6	Mace High pH + Aluminium

Figure 7: The results of the Al K-Edge testing of the six root samples (rt-1, rt-2, rt-3, rt-4, rt-5 and rt-6) from the hydroponic experiment. These results show the speciation of aluminium within the root tissue samples for each treatment compared to some of the known aluminium forms.

In summary, the spectra generated for both the soil and root tissue samples (rt-5 and rt-6) indicate the presence of two dominant peaks. In this case, both the soil and root tissue spectra results indicate a close alignment with boehmite. The presence of the double peak is a distinguishable feature of boehmite and is present in both the soil and root tissue samples. The slight post edge shoulder presence in both sample types also suggests that other forms or combinations of aluminium along with boehmite in the samples may be likely.

CONCLUSIONS REACHED &/OR DISCOVERIES MADE

The key findings of this research were:

Aim 1 – form of Al in alkaline soil

- The presence of spectra with a clear double peak indicating close alignment to the boehmite Al form in the alkaline, sodic soil from Roseworthy.

Aim 2 – form of Al in wheat roots grown under high pH with Al

- A hydroponics experiment was conducted to obtain root tissue samples of two wheat cultivars under control, high pH and high pH + Al conditions and demonstrated a reduction in root biomass of both varieties under the high pH and high pH + Al treatments compared to the control.
- Both Al L-edge and Al K-Edge (XANES) were completed on the root tissue samples. The Al L-edge was below detection limits and no results were obtained. However, the Al K-Edge (XANES) has generated some promising results. The spectra obtained indicates the presence of double peaks that has close alignment with boehmite present in the root tissue samples of both Janz and Mace under the high pH + Al treatment that is not present under the other treatments (control, high pH only).

The growth chamber experiment was successful and replicated a large volume of data demonstrating the toxic effects of both high pH and high pH + Al on reducing wheat root growth. Having these exact root tips for use in the synchrotron experiment was highly valuable and makes the direct link between the treatments, root growth and Al speciation form detected. Up to now, there had been a suggestion that the toxic form of Al may be present in the soil at high pH but that this form would change once it enters the plant root tips and it would no longer be toxic to plant roots due to the change in pH within the plant cells. This was before technology like the synchrotron was available for use and now, we have the first evidence to suggest that boehmite may be present within these wheat roots under the high pH + Al treatment. This project determined that the use of Al L-edge for the root tips was below the detector limit at 16 sec dwell time and was not able to distinguish between the different control, high pH and high pH + Al treatments. This is important for future work as it suggests that the low concentrations make Al K-Edge (XANES) more suitable for the aluminum studies.

INTELLECTUAL PROPERTY

The intellectual property generated includes the soil and root tissue synchrotron datasets and growth chamber experiment datasets. There are no outputs suitable for commercialisation.

APPLICATION / COMMUNICATION OF RESULTS

Potential industry impact

This project involved blue-sky research with applied outcomes for the management of crops in alkaline soils of South Australia. Together the findings of both Aim 1 and 2 have demonstrated the alignment of spectra to the boehmite form of Al in soil with high pH (Aim 1) and within the plant roots when grown in a high pH + Al treatment (Aim 2). This initial evidence establishes the need to focus on improving the tolerance of cereal crops to aluminium at high pH – this has the potential to have a significant impact on industry in a similar way as to the improvements in yield following the development of acid tolerant crops that tolerant the aluminium in acid soils.

Publications and extension activities

This research was blue-sky and findings are novel with the synchrotron results detecting, for the first time, a form of Al in the root tissue samples as well as the soil samples with a direct link to the roots from wheat plants with reduced root growth under the high pH + Al conditions. The findings will be combined with R Schilling unpublished data from a GRDC-funded project focused on sodic soils and are intended to be published in a high impact peer-reviewed scientific journal before distribution. Extension of findings will occur following this publication and R Schilling plans to present this work at the next Australian Agronomy Conference.

Suggested path to market

By combining this SAGIT project results with unpublished work from a past GRDC project and Yitpi Foundation project (with past findings by Prof Tony Rathjen who first considered aluminum may be toxic to crop roots at high pH), there is now a growing body of evidence to demonstrate the need to focus on improving tolerance to aluminium in high pH soils. There is a significant opportunity to improve the growth of our cereal crops through better management of this aluminum within the calcareous soils and sodic soil types. There is also a clear path to market through plant breeding companies with this evidence from within the soil and plant root tips combined with the previous findings providing a business case for focusing on this trait for selections.

POSSIBLE FUTURE WORK

Future work should focus on ways to reduce the toxicity effects of the Al at high pH on the root growth of cereal and pulse crops. R Schilling has developed wheat germplasm with enhanced tolerance to multiple subsoil constraints (high pH, Al, sodium, root growth through compacted soil) through introduction of tolerance alleles from Focused Identification Germplasm Set (FIGS) in a previous GRDC-funded project (including new wheat pre-breeding lines that continue to grow long roots without any effects of the high pH or high pH + Al) and R Schilling and M Pallotta have developed genetic markers for Al tolerance at high pH in wheat identified through a recent Yitpi Foundation project.

Based on the findings in this SAGIT project, the presence of the toxic Al in the high pH soils should be focused on in future work. Testing the recently developed germplasm across different environments of calcareous soil or sodic sites to evaluate the ability of new lines to better tolerate the soil constraints is needed and combining these lines with subsoil amelioration of constraints has the potential to improve crop growth in these soils types.