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Response of wheat (*Triticum aestivum* L.) and cowpea (*Vigna unguiculata* L.) to foliar wetting with low pH mine waters containing acid-generating metal cations

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ABSTRACT

Acid mine drainage (AMD) contains metals that have detrimental effects on crop growth if present in excess in plant-available form. The use of untreated AMD from coal mines for crop irrigation on strategically limed soils is considered a potential option for mine water management, especially in remote areas without access to water treatment infrastructure. However, leaf scorching and trace element enrichment of plant tissue are of potential concern, as these waters often contain high concentrations of potentially toxic, acid-generating metals (Al^{3+}, Fe^{2+} and Mn^{2+}) which may cause foliar damage and hyper-accumulate in plant tissue. The objectives of this trial were to quantify any foliar injury and metal accumulation in wheat (*Triticum aestivum* L.) and cowpea (*Vigna unguiculata* L.) vegetative material after foliar wetting with simulated AMD enriched with metal cations, and to ascertain if biomass production will be affected. In this trial, crop water requirements were not met through mine water irrigation, as this was only a foliar wetting investigation. Two pot experiments were set up, with wheat grown in the winter and cowpea in the summer season of 2021. Sulphuric acid was used to lower pH to 2, after the addition of low, intermediate and high concentrations of individual acid-generating metal cations, as well as a combination of all three cations. Areal foliar injury was greatest for cowpea (18.7%) with a combination of Al, Fe and Mn at the highest concentration. The crop was not able to recover at this injury level. No injury was recorded in wheat. Both crops accumulated only limited quantities of the metal cations. Calculated hazard quotient for cattle ranged from 0.022 to 0.53, indicating that such fodder would be safe to consume. It is concluded that there are large differences in crop specie susceptibility to leaf scorching after foliar wetting with acidic metal cation-rich mine waters. It is recommended, therefore, that because large volumes of mine water need to be managed, and centre pivot overhead irrigation is likely to be utilized to this end, that studies to screen more species to select crops tolerant to scorching through foliage wetting with mine water irrigation be conducted. Thereafter, studies to better understand root zone effects of irrigation with such waters need to be conducted on species that can tolerate foliar wetting with these waters.

1. Introduction

Upon exposure to water and oxygen, pyrite, which is present in many mining deposits, reacts to form sulphuric acid (H_2SO_4) and ferric hydroxide [$Fe(OH)_3$] (Sparks, 2003). This is generally known as acid mine drainage (AMD). It is common for AMD to also be enriched with Fe, Al, and Mn. Because of its acidity and high metal content, AMD is usually neutralized in an expensive High Density Sludge (HDS) plant, before being released to the environment or used for irrigation (Jovanovic et al., 2004). Given the perceived resistance by consumers to use untreated mine waters for domestic purposes, using them for crop irrigation seems to be only viable solution. Using AMD as a source of water for the irrigation of crops on strategically limed soil profiles has been suggested as a potentially beneficial use of these problematic waters that would negate the need for expensive HDS treatment, and is therefore worth investigating (Annandale et al., 2023).

Metal cations (Fe, Al and Mn) commonly found in AMD, contribute indirectly to water acidity through hydrolysis under low pH conditions (Brown and Ekberg, 2016). Acidity increases with the increasing oxidation state of these elements. Al^{3+} applied through water would

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undergo hydrolysis and precipitation, thereby generating H^+ (Eq. 1). Mn would predominantly be present as Mn^{2+} in acidic mine water. However, when the water is applied to an aerated leaf surface, it would first oxidize to its trivalent or tetravalent forms, which then precipitate as MnOOH (Eq. 2). MnOOH, over time will oxidize to the more stable MnO_2 resulting in net acid generation. In acidic mine water, the dominant Fe species will be Fe^{2+}. When iron rich water is applied to aerated leaf surfaces, it will oxidize to Fe^{3+} which in turn will undergo hydrolysis and precipitate as α-FeOOH, a thermodynamically stable metal. The oxidation of Fe^{2+} and subsequent hydrolysis of Fe^{3+} also generates protons (Eq. 3) (Gazea et al., 1996).

$$Al^{3+} + 3H_2O = Al(OH)_3 + 3H^+ \tag{1}$$

$$4Mn^{2+} + O_2 + 6H_2O = 4MnOOH + 8H^+ \tag{2}$$

$$2Fe^{2+} + O_2 + 2H_2O = \alpha{-}2FeOOH + 2H^+ \tag{3}$$

Preliminary crop growth studies performed in a glasshouse, where crops were grown in limed soils that were surface irrigated with AMD, indicated that strategic liming could overcome, at least in the short-term, any effects of acidic mine waters in the rooting environment. However, considering the fact that there are very large volumes of AMD available, and such mine waters are inherently unsuitable for micro-irrigation due to their propensity to precipitate metal oxides that would cause system clogging, it would be necessary to make use of overhead irrigation to efficiently utilise these waters. A logical question that then arises, is whether or not crop foliage will be scorched by overhead irrigation, thereby reducing photosynthetic leaf area, and reducing production to below economic levels, or whether foliar absorption of metals could result in phytotoxicity. In this study, sulphuric acid together with acid generating cations (Fe, Al and Mn), was used to simulate the effect AMD may have when wetting the foliage of cowpea and wheat. Leaf scorching due to acidity has been reported by some researchers (Keever and Jacobson, 1983, Lal, 2016)In a previous study by Mabuza et al. (2023) it was reported that there was limited leaf scorching due to AMD simulated with sulphuric acid only. It emerged from the same study, that more conclusive results should be achieved if acid generating metal cations were included in such synthetic acid mine waters. While no studies have been found considering foliar wetting with metal rich waters, some authors have reported a linear relationship between foliar Mn concentration and soil solution Mn levels ($R^2 = 0.89$) (Reichman, 2002).

The trial reported on here, had the objective of examining the effects of simulated AMD containing acid generating metal cations, on leaf scorching. To achieve this, two consecutive pot trials were carried out in the winter and summer seasons of 2021. The specific objectives were 1) to determine if AMD containing metal cations will cause leaf scorching in two selected crops (wheat and cowpea), and 2) to determine whether metal cations commonly found in AMD (Al, Fe or Mn) would accumulate to toxic levels in plant leaves of these crops due to foliar absorption.

2. Materials and methods

2.1. Location of the study

The study was carried out during 2021 at the University of Pretoria, Innovation Africa Farm, South Africa. It involved two glasshouse pot trials. Acid mine waters were synthesized in the Soil Science Laboratory of the University of Pretoria.

2.2. Experimental design

The trials were laid out in a split-plot design. Main plot consisted of four metal cations (Al, Fe, Mn and a cocktail of these metals. Subplots consisted of four levels of each metal (control, low, medium and high). Each treatment was represented by one flowerpot. The experiment was replicated four times (Fig. 1). Wheat was planted for the winter trial with 13 plants per pot while cowpeas were planted in summer with three plants per pot.

2.3. Methodology

Flowerpots (6 L by volume) with a top diameter and height, both of 22 cm, were filled with soil and coir in the ratio of 5:1. Coir assisted with free drainage. The soil was a sandy loam, classified as a deep Hutton loamy, kaolinitic, mesic, typic, highly weathered soil (Soil Classification Working Group, 1991). The soil pH ($CaCl_2$) was 5.49, and no limestone was added. The wheat planted soil received the recommended amounts of nitrogen (100 kg/ha), phosphorus (40 kg/ha) and potassium (40 kg/ha), assuming an effective soil depth of 150 mm. Cowpea planted soil received similar amounts, but the nitrogen application was halved, as this is a leguminous crop. Cowpea seeds were not inoculated.

Domestic tap water was used for irrigation for the duration of the trial. Crops were irrigated to field capacity once a week after emergence. Irrigation frequency was gradually increased to three times a week, as crop water demand increased. Before each irrigation, pots were weighed to determine the amount of water depleted. Irrigation water was applied to the soil surface using a beaker to avoid wetting the foliage. All treatments were allowed to drain freely.

Daily wetting of crop foliage with simulated AMD was started a week after crop emergence. Leaves were wetted with a spray bottle to just below the point of runoff. To prevent acidic water from dripping onto the growth media, the soil was covered with plastic discs. These discs were strategically sliced to allow plants to grow through them and were not removed during irrigation as water was able to seep past the sides and through the slits. Leaf wetting with simulated acid waters continued until the trial was terminated five weeks after crop emergence.

2.4. Synthesis of mine waters

The following salts were used to synthesize the metalliferous “mine waters”; $FeSO_4 0.7\ H_2O$, $MnSO_4 0.7\ H_2O$, and $Al_2(SO_4)_3 0.18\ H_2O$. The ion concentrations used were based on analysis of AMD from a colliery in the Mpumalanga Coalfields of South Africa. This water was reported to contain on average 154 mg/L Al, 57 mg/L Mn, and 266 mg/L Fe, with an electrical conductivity of 377 mS/m and a pH of 2.6 (Maree et al., 2013, Van Der Laan et al., 2014). Based on these average values, and considering the inherently variable nature of constituent concentrations, three concentration levels spanning these average values were used for each metal (Al; 100, 150 and 200 mg/L: Mn; 40, 60 and 80 mg/L and Fe; 150, 250 and 300 mg/L). Firstly, the amount of salt required to obtain the desired concentrations of specific elements was calculated. The hydrochemistry software program Aqion, version 7.3.5 (www.aqion.de), was then used to assess the pH after the salts were dissolved in water and to determine how much H_2SO_4 was needed to get the pH down to 2. Aqion makes use of the well-known United States Geological Survey (U.S.G.S.) program PhreeqC (Ball and Nordstrom, 1991, Parkhurst and Appelo, 1999). The Aqion calculated pH was confirmed with a pH meter. A target pH of 2 was selected because a pH of 2 was found in a previous pot trial (Mabuza et al., 2023), to cause maximum scorching (but not death),.

In addition to the single metal “mine water” solutions, “mine water” cocktails consisting of a mixture of all three metals, at their low, medium and high concentrations were also synthesized. Due to restricted laboratory access during the Covid19 lockdown, waters were synthesized once and used for the entire duration of the trial (32 days). These were stirred each time before use. A summary of the characteristics of the synthetic mine waters are presented in Table 1.

2.5. Data collection and analysis

Crops were visually inspected daily after treatment commenced for

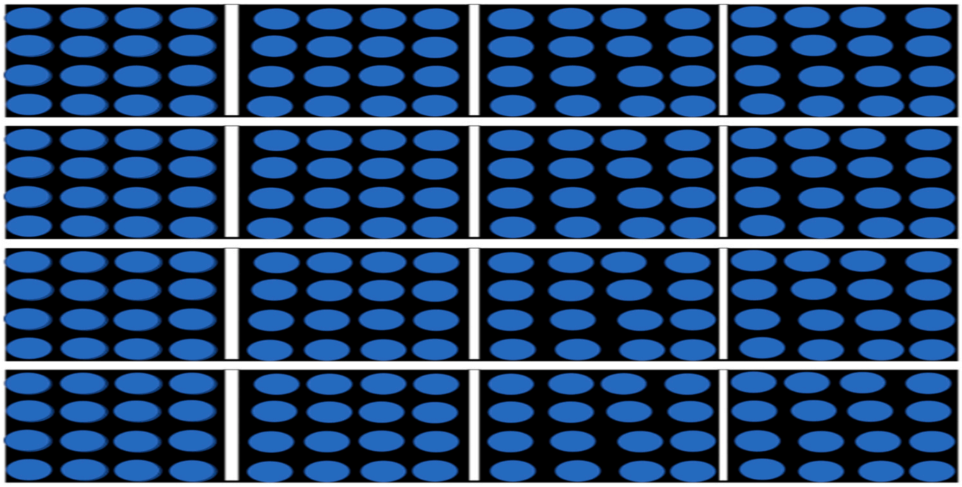

Fig. 1. Plot layout of the experiment.

Table 1
Characteristics of synthetic metalliferous mine waters used in the foliage scorching trial.

Synthetic mine water	Metal concentration (mg/L)	Molar mass of element (g/mol)	H_2SO_4 for lowering pH (ml/L)	Final calc. pH	Final calc. EC (mS/m)
Control	0	0	0.38	2.12	434.0
Al Low	100	26.98	0.33	2.19	381.5
Al Medium	150	26.98	0.33	2.14	382.2
Al High	200	26.98	0.33	2.16	382.9
Fe Low	150	54.94	0.38	2.02	433.4
Fe Medium	250	54.94	0.38	2.07	433.0
Fe High	300	54.94	0.38	2.04	432.9
Mn Low	40	55.845	0.38	2.00	435.3
Mn Medium	60	55.845	0.38	2.20	436.0
Mn High	80	55.845	0.38	2.03	436.7
Cocktail Low	100, 150,40	26.98, 54.94,55.845	0.27	2.08	435.8
Cocktail Medium	150,250,60	26.98, 54.94,55.845	0.27	2.13	436.1
Cocktail High	200,300,80	26.98, 54.94,55.845	0.27	2.13	438.1

the inception of foliar damage. If symptoms were observed, control crops were checked for similar symptoms. If the control crops did not display similar foliar damage, the damage was attributed to the water quality sprayed on foliage. Damaged or scorched leaves were photographed, and images were imported into ImageJ to determine fractional leaf area damage (Alheeti et al., 2021).

Destructive sampling was done at the end of the experiment for dry mass determination. Ten plants for wheat and all three cowpea plants were cut off at ground level. The plants were washed with tap water before oven-drying at 65 °C until a constant mass was achieved.

2.6. Determination of metal concentrations in plant tissue

Collected plant samples were washed while still fresh. Tap water was used to wash leaves, rubbing them gently by hand. A mild liquid detergent was used for this exercise. This was done to make sure that the metal concentrations measured in plant tissue represented absorbed metals, and not just those deposited onto the leaf surface. Samples were then rinsed three times with deionized water. Excess water was removed by shaking the leaves. Samples were then transferred into paper bags which were left open at the top and placed in a drying room before oven drying at 65 °C for 72 hours. Samples were ground with a coffee grinder and analysed for metal content using the procedure of Ondo et al. (2013).

Acid Digestion was used to determine the elemental composition of plant tissues. The analysis was done for leaves and stems at the end of the trial using the EPA (US Environmental Protection Agency) method (Usepa, 1996). Among the various EPA procedures, the microwave assisted acid digestion of siliceous and organically based matrices procedure was used. This procedure allows total sample decomposition for general use with judicious choice of acid combinations and is recommended for plant tissue analysis.

2.7. Assessing zoo-toxicity of metal elements in crop leaves

Non-cancer adverse health effects for animals are routinely assessed using Hazard Quotients (HQs) (Fowles et al., 2020). For each exposure scenario, an exposure dosage (mg per kg body mass per day) was computed, and this was divided by the acute, intermediate or chronic MRL (maximum residue limits) to yield the HQ for the particular exposure. HQs of less than 1.0 generally signal that adverse health consequences are unlikely to occur as a result of exposure, even for sensitive animal species, depending on the magnitude of the HQ and the uncertainty factors employed in determining the MRL. HQs greater than 1.0 may indicate a higher risk of negative health impacts in those who are exposed. Eq. 4 by Chary et al. (2008) was used to calculate HQs.

$$HQ = \frac{\mathbf{Wplant}\left(\frac{\mathbf{kg}}{\mathbf{day}}\right)X \quad Mplant\left(\frac{mg}{kg}\right)}{RfD \quad (mg/kg \quad body \quad mass/day)X \quad B(kg \quad body \quad mass)} \tag{4}$$

In equation 5, W_{plant} is the dry mass of plant material consumed per day (kg/day), the assumption was 2% of body mass. M_{plant} is the measured concentration of the potentially toxic element in plant material (mg/kg), RfD is the oral reference dosage for the potentially toxic element (mg per kg body mass per day), and B is the consumer's body mass. Calculations were made for an average cow with a body mass of 410 kg. Reference dosages for Al, Fe and Mn are 0.0007, 0.007 and 0.14 mg per kg body mass per day respectively (WHO. 1974). Food safety was not evaluated because the trial did not run until grain formation and the foliage of these crops is not consumed by people.

2.8. Statistical analysis

Data collected were subjected to statistical analysis using Microsoft Excel and R Statistical software, version 2.15.3 (Team, 2013). The experimental data were tested for the null hypothesis that foliar applied, acid generating metalliferous mine waters will not cause foliar injury as compared to the sulphuric acid only control. Where permissible, the effects of the treatments were tested against the interaction of crop x treatments. Each sample analysis was replicated four times. Separation of means of significant AMD treatments was assessed with the DNMRT Least Significant Difference (LSD) test at the 0.05 P level.

3. Results

3.1. Dry biomass

The total biomass produced per pot was measured on a dry mass basis. The variation in average dry biomass of the leaves and shoots for both wheat and cowpea can be seen in Fig. 2. Biomass for wheat was not significantly affected by the foliar wetting with different synthetic acid mine waters. However, it was noted that the medium Mn level mine water produced the marginally highest biomass for wheat. For cowpeas, the control treatment produced a marginally higher biomass than any of the metalliferous mine waters. Among the metalliferous mine waters, Mn enriched waters produced the highest biomass. The highest concentration of the Al, Fe & Mn mine water cocktail, produced the lowest cowpea biomass.

3.2. Leaf scorching

There was no scorching recorded when metalliferous mine waters were sprayed onto wheat. This was not the case for cowpea. Fig. 3 shows the degree of leaf scorching experienced by cowpea throughout the experiment. At 16 days after planting (i.e. two days after the first wetting of foliage with mine water), the high level of the Al, Mn and Fe mine water cocktail already produced significantly (LSD test, $P<0.05$) greater areal leaf damage (6.3%) than any other treatment while the Mn enriched water exhibited the least damage at only 0.9% (Fig. 4). It should be noted that as time progressed and leaf wetting continued, the magnitude of foliar injury increased until it reached a peak 24 days after planting. At this peak, the high-level mine water cocktail continued to exhibit significantly greater injury (15.4%) than the other treatments, whilst the medium level of Mn continued to be the lowest at 3.0%. It was also noted that the control treatment without metal cations (sulphuric acid alone) recorded an injury of 5.0% during the peak period. After the peak, there was a steady decline in leaf scorching across all treatments. This is interpreted to indicate that more mature foliage is relatively resistant to foliar damage by acid mine waters. It was observed that injury started with chlorosis which then progressed to rusty lesions across all treatments.

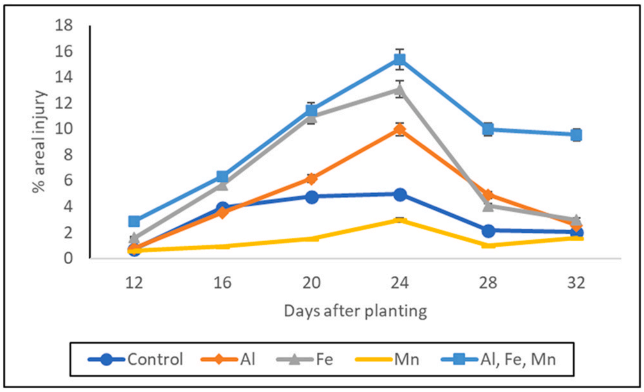

Fig. 3. Fractional leaf area damage (%) experienced by cowpea after foliar spraying with acid mine waters containing metal cations showing highest levels of all treatments.

3.3. AMD salinity response of cowpeas

Salinity of the mine waters (EC_w) measured during the course of the experiment shows that it increased over time. This increase may be attributed to slow kinetics of the metal cations when dissolved in water. The cocktail treatment produced the highest final salinity of 398 mS/m. It is unclear what effect such a high salinity level may have had on the observed leaf scorching. No reference could be found about cowpea's susceptibility to foliar scorching. Published criteria linking leaf scorching to foliar damage seems to identify chloride and sodium ions as the active agents, whereas sulphate ions were used in this study.

3.4. Influence of wetting foliage with metalliferous mine waters on plant uptake of Al, Fe and Mn

3.4.1. Aluminium

As expected, Al content was significantly (LSD test, $P<0.05$) higher in treatments that received Al, than those that did not. The amount of Al

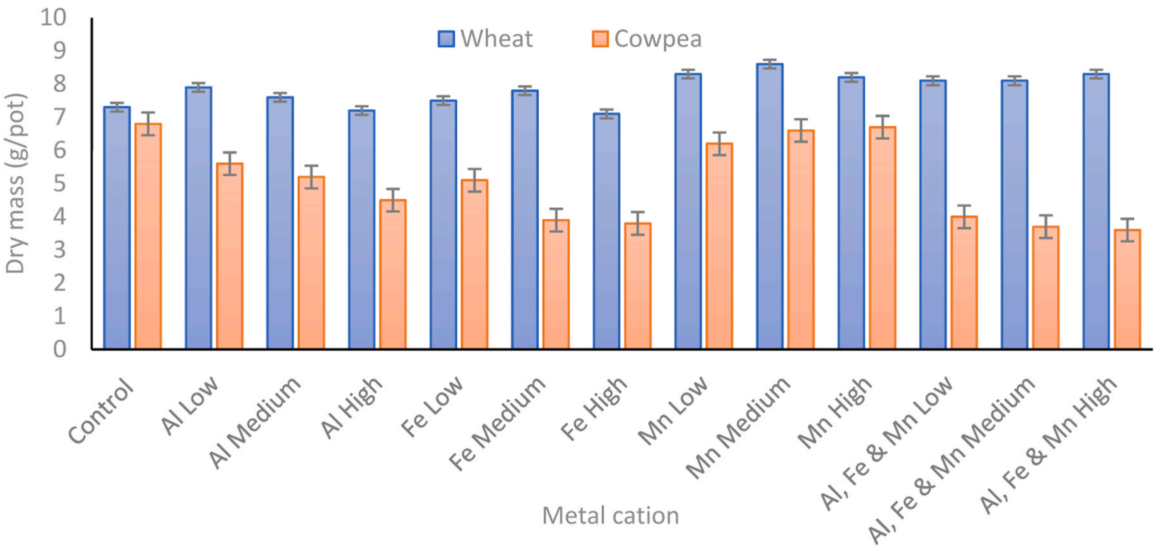

Fig. 2. Dry mass (g/pot) for wheat and cowpeas when sprayed with synthetic metalliferous mine water at a pH of 2. Columns indicate mean ± SE based on four replicates.

Fig. 4. Scorched cowpea leaves at 24 days after planting showing; **A** scorching from highest concentration cocktail treatment, **B** highest level of Mn, and **C** sulphuric acid control.

in plant tissue increased with increasing application level while the lower Al concentrations in plant tissue were, in turn, associated with higher dry mass (Fig. 5A). Al content was lower in wheat than cowpeas. This may be due to lack of pooling of waters on wheat leaves and thus less opportunity for absorption, than was the case with cowpeas.

3.4.2. Iron

As expected, Fe content was significantly (LSD test, $P<0.05$) higher in treatments that received Fe, than those that did not. Lower quantities of Fe for untreated plants would have come from soil uptake. As was the case with Al, Fe content was higher in cowpeas than in wheat (Fig. 5B). This suggests that cowpea leaves were able to absorb more metals than wheat because of the pooling of waters on the cowpea leaves.

3.4.3. Manganese

Generally, foliar application of manganese on both wheat and cowpeas significantly (LSD test, $P<0.05$) increased plant tissue contents. It can further be seen from Fig. 5C that even treatments that were not exposed to Mn enriched waters, had substantial amounts of Mn in their tissues. The high Mn content may be due to Mn being an essential element, and Mn uptake from the soil.

3.5. Assessing zoo-toxicity of Al, Fe and Mn concentrations in crop leaves

Neither wheat nor cowpeas showed any signs of zoo-toxicity to cattle due to the toxicity of Fe, Mn and Al, when wetted with metalliferous mine waters. The highest HQ was 0.53 for cowpeas, while it was only 0.10 for wheat, both observed for the cocktail mine water. The lowest HQ was recorded with wheat when tested for Al (Table 2). HQs greater than 1.0 suggest an increased hazard of adverse health effects for animals fed with crop residues.

4. Discussion

4.1. Dry biomass

Our study demonstrated that biomass accumulation in wheat was not affected by foliar wetting with acid waters. However, it was observed that there was a linear reduction in dry mass for cowpeas with increase in Al concentration. This was due to leaf desiccation and partial leaf chlorosis at the leaf edges observed at the early stages of crop growth.

Mn treatments yielded higher biomass because Mn is an essential micro-element, a precursor for enzymatic structures that are responsible for the synthesis of proteins, carbohydrates, lipids and nucleic acids (Shahid et al., 2015). It is also responsible for the optimal utilization of major plant nutrients. Mousavi et al. (2011) stated that increase in plant biomass after foliar-applied Mn can increase the efficiency of photosynthesis and the synthesis of carbohydrates like starch. A deficiency of Mn, being an essential element, will lead to a reduced photosynthetic activity which will consequently lead to reduced plant growth. However, in this trial, it is envisaged that plants absorbed sufficient Mn from the soil. Consistent with the findings of this study, some authors have reported that foliar application of a 5% Mn micro nutrient solution to wheat increased biomass yield (Pahlavan-Rad and Pessarakli, 2009). The same authors also reported increased Fe concentration in wheat grains after foliar application of Fe. This study reported lower concentration s of Fe in wheat than cowpeas due to differences in leaf orientation of the two crops.

4.2. Leaf scorching

No foliar injury was observed in wheat throughout the study period. Cowpeas on the other hand were severely injured, especially with the cocktail solution of metal cations. Pooling of acid waters on the surface of cowpea leaves was observed, while this was not the case for wheat. This may be partly attributed to the differences in leaf morphology and angle orientation of these two crops. Cowpea leaves are almost horizontally oriented, thereby holding more water while also encouraging its pooling on the leaf surface. The more acute orientation of wheat leaves meant that water just ran off upon foliar spraying. Since there was no foliar injury in wheat, all reference to leaf scorching henceforth refers to cowpeas.

4.2.1. Mn injury

The least injury was recorded with Mn treatments. This is attributed to Mn being an essential element, hence it was likely used in physiological processes in the plant, which appears to have led to the significantly ($P<0.05$) low injury recorded in this trial. Consistent with the observations made in this trial, Millaleo et al. (2010) stated that common symptoms of Mn injury include necrotic spots in leaves as well as chlorosis. According to El-Jaoual and Cox (1998), symptoms of Mn toxicity are very diverse between plant species. They include yellowing of leaf margins, followed by leaf necrosis in some crops like alfalfa *(Medicago sativa* L.) Interveinal and marginal chlorosis, together with brown necrotic spotting has been reported in sweet potato *(Ipomoea batatas* L.) and snap bean *(Phaseolus vulgaris* L.). Soybean (*Glycine max* L.) has exhibited crumpled, distorted small leaves with irregular interveinal chlorosis (Wu, 1994). Small reddish-purple spots on the underside of leaves in cowpea *(Vigna unguiculata* L.) have been reported (Clark and Baligar, 2000). While expression of Mn toxicity varies appreciably within plant species, brown spots on older leaves surrounded by chlorotic zones are typical symptoms. Such necrotic brown spots are reported to be localized accumulations of oxidized Mn. In severe cases of Mn toxicity, plant roots turn brown, usually after the shoots have been severely injured. The discolouration of leaves is caused by the plant creating enzymes to control free radicals that are present with high iron and manganese levels (Kumar et al., 2022).

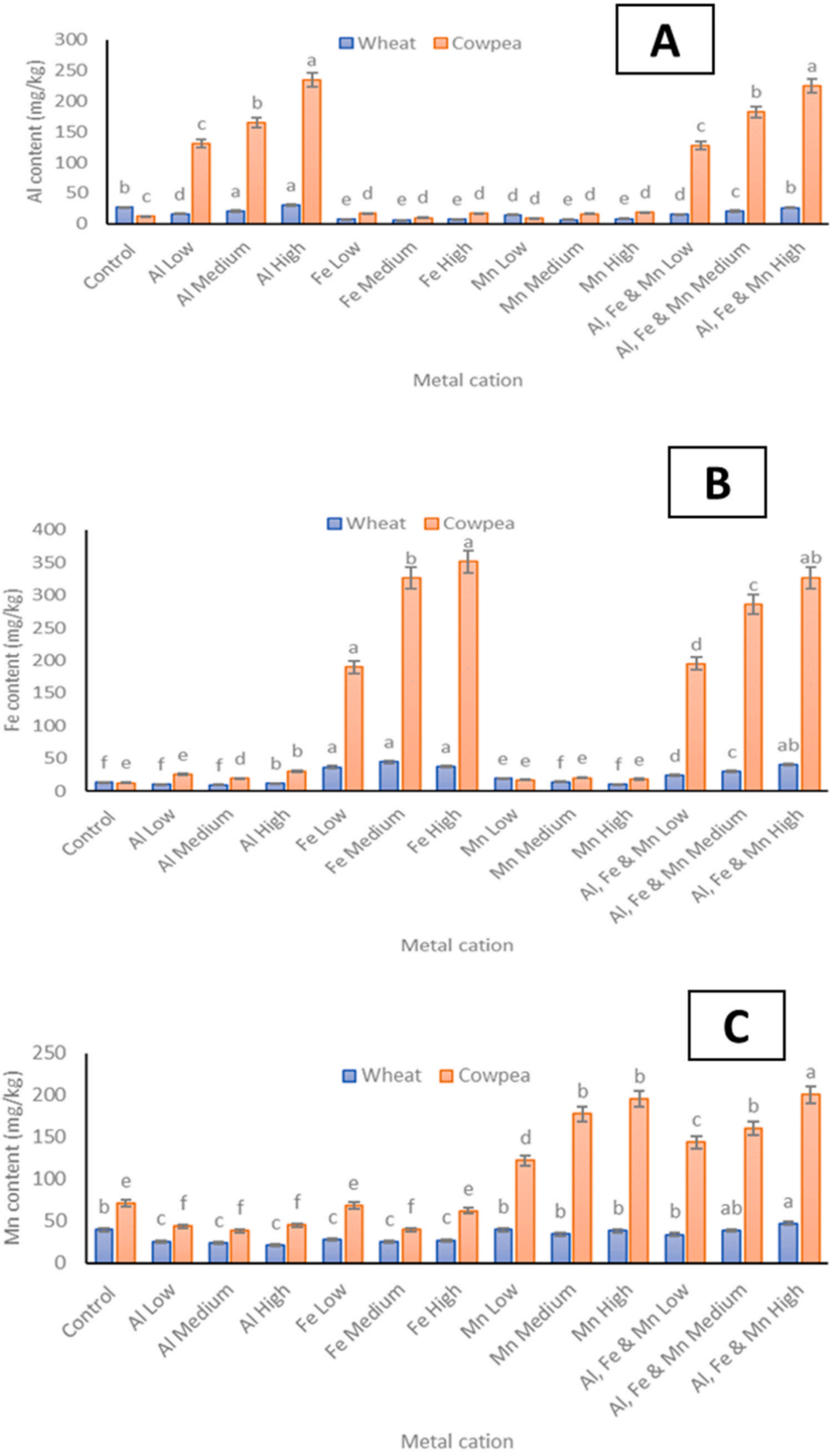

Fig. 5. Metal cation concentration (mg/kg) in wheat and cowpea for Al (**A**), Fe (**B**) and Mn (**C**) after foliar wetting with acid mine waters containing different levels of Al, Fe and Mn. Columns indicate mean ± SE based on four replicates. Means with different letters indicate a significant difference at $P<0.05$ using the LSD test.

Table 2
Hazard quotients of Al, Fe and Mn in wheat and cowpeas after foliar wetting with acid mine waters containing different levels of Al, Fe and Mn.

Element	Metal cation hazard quotient			
	Wheat			
	Al	Fe	Mn	Al, Fe, Mn
Al	0.022	0.0888	0.079	0.046b
Fe	0.058	0.0584	0.096	0.039b
Mn	0.046	0.0423	0.096	0.100a
Significance	ns	ns	ns	*
	Cowpeas			
	Al	Fe	Mn	Al, Fe, Mn
Al	0.25b	0.038c	0.09	0.37
Fe	0.027c	0.42b	0.11	0.53
Mn	0.030c	0.038c	0.31	0.37
Significance	*	*	ns	ns

Columns indicate mean ± SE based on four replicates. Means in columns followed by the same letters are not significantly different at 5% level of significance according to LSD test; * = Significant at 5%; and ns-not significant.

4.2.2. *Fe injury*

In this trial, leaf scorching due to Fe was seen by browning, bronzing, discoloration of foliage, and leaf curling. These observations are consistent with those of Yamauchi and Peng (1995) who reported similar symptoms after over-head irrigating crops with water containing Fe. The mild scorching from Fe compared to Al may be attributed to Fe being an essential micro element. The symptoms of iron toxicity include bronzing and stippling or mottling of leaves (Krstic et al., 2012). Pahlavan-Rad and Pessarakli (2009) report increased Fe concentration in wheat grains after foliar iron application. Affected plants create enzymes that will neutralize free radicals associated with elevated iron levels (Shahid et al., 2015).

4.2.3. *Al injury*

It was observed in this trial that Al treatments showed the highest leaf scorching. This was apparent by leaf chlorosis starting from leaf tips. At the highest level, it led to collapse of growth tips and leaf petioles of older leaves. However, Rout et al. (2001) stated that Al toxicity symptoms are difficult to identify on shoots because they often resemble those of phosphorus deficiency (Shahnawaz et al., 2016). Sometimes Al toxicity will mimic calcium deficiency that is evident with the curling up of young leaves. However, for this trial, these are dismissed because no P deficiencies were noted in control treatments. When evident, aluminium toxicity typically shows overall stunting, small dark green leaves, and with late maturity, purpling of stems, leaves and leaf veins, as well as yellowing and death of leaf tips (Shahnawaz et al., 2016).

4.3. *AMD salinity*

The source of metal cations used in this trial were sulphate-based, hence there were concerns about salinity also contributing to leaf injury. However, foliar injury was not attributed to salinity injury whose symptoms are often confused with those of acid scorching (Maas and Grattan, 1999). According to Maas and Grattan (1999), symptoms of excess salt include wilting of plants together with leaf burn leading to leaf drying. The appearance of toxicity symptoms implies that the crop has already passed the tolerance threshold and will potentially suffer yield losses. Losses due to salt injury are higher in crops irrigated by overhead irrigation which causes chlorosis and then necrosis in leaves (Paraskevopoulou et al., 2020). Crops accumulate salts in their leaves and become injured in the process. Crops irrigated through overhead irrigation are injured by both salt spray and salinity from the soil.

4.4. *Uptake of metal cations by crops*

The concentrations of Al, Fe and Mn in both cowpea and wheat leaves was found to be below toxic levels when assessed against zootoxicity threshold values. The published maximum tolerable levels (MTL) in plant dry biomass for beef cattle are 1000 mg Al/day/kg consumed, 750 mg Fe/day/kg consumed and 2000 mg Mn/day/kg consumed (National Research Council 2005). These findings show that these crops are not likely to pose toxic hazards related to Al, Fe and Mn in cattle upon ingestion of their vegetative material. The nonhazardous quantities of metal cations recorded may be due to plants having evolved a detoxification process based on chelation and subcellular compartmentalization to reduce the negative effects of heavy metal exposure and accumulation (Singh et al., 2011).

5. Conclusion

It can be concluded from this study that there are large specie variations in susceptibility to leaf scorching upon foliar wetting with acid mine waters containing acid generating metal cations. Wheat is resistant to foliar wetting with such waters. The highest foliar injury, together with death of older leaves and leaf curling were noted for cowpea with the metal cocktail treatment. However, plants recovered over time although growth had already been significantly compromised. Crops were safe for livestock feeding as metal cation levels were within acceptable levels. A screening trial is recommended to screen a wide range of crops for foliar wetting with AMD containing metal cations. Root zone effect studies of irrigation with such waters is then recommended on species that can tolerate foliar wetting with these waters.

CRediT authorship contribution statement

Mzwandile Petros Mabuza: Investigation, Writing – original draft. **Phil Tanner:** Methodology, Supervision, Writing – review & editing. **Meiring Du Plessis:** Conceptualization, Investigation, Writing – review & editing. **John George Annandale:** Conceptualization, Funding acquisition, Writing – review & editing. **Joachim Martin Steyn:** Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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