CORRELATION ANALYSIS OF GROWTH PARAMETERS AND HEAVY METALS IN POTATO (ROYAL POTATO CULTIVAR) IRRIGATED WITH TREATED ACID MINE DRAINAGE WATER

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Abstract. Wastewater can be used for the irrigation of field crops if international water quality guidelines are followed, and it has been suggested as an alternative solution in water-scarce areas, such as South Africa. The aim of the study was to assess the correlation between crop physiology, and heavy metal accumulation in *Solanum tuberosum* L. (Royal cultivar) irrigated with treated acid mine drainage (AMD) water. Through a randomized complete block design (RCBD), two treatments were applied, namely raw AMD (control), AMD + quicklime (1 g). After 90 days of planting (DAP), the growth indices (plant height, number of tubers, fresh weight, and dry weight) and the compositions of selected heavy metals (Al, As, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, and Zn) were examined. The paper also identified the synergistic and antagonistic effects between the metals, with relevance to the mobility of metals and their uptake by the plants. The analysis showed a strong positive link between plant height and fresh tuber weight (+0.97, $p < 0.01$), suggesting that taller plants tend to produce heavier tubers under good growing conditions. The heavy metals Al, As, and Cr were, however, found to be negatively correlated with plant height and tuber yield. However, there is a positive correlation between Cd and fresh tuber weight. These results highlight the complex interactions between water quality, treated AMD irrigation, and crop health, and emphasize the need for careful management of water sources, soil conditions, and cultivar selection when using treated AMD for agricultural irrigation. Although treated AMD can be an alternative irrigation resource, it should be utilized under strict segmentation models to ensure environmental sustainability and food safety.
Keywords: *heavy metal uptake, wastewater reuse, potato physiology, AMD treatment, irrigation water quality*

Introduction

Acid mine drainage (AMD) is high in heavy metals, sulfates, and has low pH that contributes to considerable environmental problems in the mining areas all over the world (An et al., 2023). On how the AMD can be treated to reduce its ecological impacts, there has been a development towards its potential use as an alternative source of water to be used in irrigating agricultural land in areas that are water deficient (Sheridan et al., 2017). Nonetheless, utilization of treated AMD water in crop farming elicits fears concerning its impacts on plant growth, and possibility of development of contaminants such as heavy metals in edible plant products, which present threats to food security and human health (Oberholster et al., 2014). Using acid mine drainage (AMD) as irrigation water has been an issue of interest particularly, in water-deficient areas such as in South Africa. AMD is a widespread environmental pollutant produced by active and abandoned mine sites, with low pH (typically < 4) and heavy metal concentration (generally cadmium (Cd), lead (Pb) arsenic (As) and iron (Fe)) (Johnson and Hallberg, 2005; Simate and Ndlovu, 2014;

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Ojonimi et al., 2019). Uncontrolled AMD release into the water supply systems is of catastrophic ecological and agricultural danger, especially when related to sources of freshwater shortage regions, where farmers are compelled to utilize polluted waters in agriculture (Naidoo, 2022; Ingrao, 2023; Mapukata et al., 2024). As a result of using untreated AMD in agriculture, soil acidification, aggravated nutrient availability, and the accretion of heavy metals to crops are identified, eventually posing a threat to food security and human health (Hou et al., 2020; Alengebawy et al., 2021; Angon et al., 2024). As an example, potatoes are especially sensitive to heavy metal stress because of its shallow root system and high-water requirements (Stasinos et al., 2014; Bedoya-Perales et al., 2023). Munyai and Modise (2024) conducted a greenhouse experimental trial in South Africa, which showed that, when potatoes were watered using AMD contaminated water, there was a 30-70 percent decrease in the tuber yield. Plant physiology is also impacted because AMD heavy metals slow the growth and lead to chlorosis and result in a low amount of biomass (Nagajyoti et al., 2010; Ghori et al., 2019; Speduolli, 2022; Hafeez et al., 2023). Studies indicate that the Cd, Pb and As in AMD are readily absorbed by the roots of potato and edible tuber as well as posing a higher risk to health (Chen et al., 2014). Moreover, it is also noted that exposure to untreated AMD severely reduces shoot length, root development, and tuber yield related to metal-mediated oxidative stress and the inhibition of nutrient uptake (Nemutanzhela et al., 2017). Antoniadis et al. (2017) in different research, described a case where lead (Pb) is accumulated in potato skins more than WHO/FAO safety levels (0.1 mg/kg), caused by an irrigation water contaminated by potato crop. According to Huang et al. (2022), tuberization and starch content were inhibited and even low quantities of cadmium (Cd) at 0.1-0.5 mg/kg of soil can stop tuber formation. As an example, Nemutanzhela et al. (2017) showed a decrease of 40% in the potato tuber yield in the raw AMD-irrigated system compared to clean water.

Exposure to heavy metals induces physiological stress in plants such as potato, leading to alterations in plant growth and chlorophyll content (Emamverdian et al., 2015). This stress also poses a risk to food safety due to the uptake and translocation of heavy metals into edible plant parts, a process influenced by factors such as soil pH, metal speciation, and plant genotype (McLaughlin et al., 2011). Since potatoes are tuberous crops, they are specifically prone to suffer accumulation of Cd and Pb beyond the acceptable threshold as established by World Health Organization (WHO, 2011). Peralta-Videa et al. (2009) carry out a study which has shown that potato tubers cultivated in soils contaminated with AMD had high levels of Cd and Pb of up to 5 to 10 times more than the safety limits, which could be very hazardous to human health once ingested. Moreover, the study conducted by Gitari et al. (2018) found out that the AMD irrigation in the long term contributes to ongoing metal accumulation in the soils, which decrease the productivity of agriculture in the future and requiring expensive cleaning actions to be implemented. The reuse of treated AMD and agricultural reuse strategies are however being considered as a long-term solution even in water scanty areas. Chemical amendments, such as quicklime (CaO) and fly ash, can be applied to neutralize acid mine drainage (AMD). These materials help mitigate the negative environmental impacts of AMD by increasing the pH, which facilitates the precipitation of dissolved metals (Gitari et al., 2009; Masindi and Gitari, 2016; Raletsena et al., 2023; Munyai et al., 2024). The treatment of quicklime was proven to reduce the concentration of soluble metals via precipitation of hydroxides, whereas fly ash contributed to higher immobilization of metals since it has a high adsorption capacity (Kumar et al., 2020). Ensuring that the bioavailability of Cd and Pb in irrigation water was decreased by more than 70% using three combinations of

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quicklime and fly ash, namely, 2 g quicklime plus 75% fly ash, could significantly contribute to crop growth as proved by experimental study conducted by Munyai and Modise (2021). Nevertheless, the relationship between crop physiology and accumulation of heavy metal is poorly explored. The Correlation analysis offer a sound statistical method to explain interactions between such variables and furnished the ideas of how the treated AMD water affects the physiology and metabolism of plants. The proposed study will compare growth parameters (e.g., plant height, tuber yield, and biomass) and the concentration of heavy metals (e.g., Fe, Cu, Zn, Pb) in Royal potato cultivar to find patterns and dependencies that might be used to reduce the aggravation of irrigation. This knowledge would play a pivotal role in striking a balance on agricultural productivity and the environment and food safety in areas subjected to mining. This research assesses the relationship between growth parameters and heavy metals of potato cultivars (Royal) irrigated using treats AMD. This knowledge of correlations is significant to determine the possibility of the utilization of treated AMD in agriculture.

Materials and methods

Experimental area and design

The study was conducted at the UNISA Science campus, University of South Africa (UNISA), Johannesburg, Gauteng Province. Acid mine drainage water samples were collected from the Gold Mine in Gauteng (S -26.1480995, E 27.9267078), using sterile 50 L plastic containers (that had been cleaned with 20% sodium hypochlorite and UV-sterilized for 1 h). A randomized complete block design (RCBD) was employed to evaluate the effects of acid mine drainage (AMD) treatments on potato cultivation, using three distinct treatments. The 3 irrigation treatments applied were: TW1 (tap water), TW2 (100% raw mine water), and TW3 (100% raw mine water amended with 2 g/L of quicklime). The quicklime solution was prepared at a ratio of 1 gram per liter of acid mine drainage (AMD), based on its documented effectiveness in AMD treatment as reported by Othman et al. (2017). For the experiment, 25 Royal potato cultivar plants were grown in 25 cm pots, spaced 30 cm apart within rows and 60 cm between rows. The experimental setup consisted of five pots per treatment, totaling 15 pots for TW1, TW2, and TW3, with an additional 10 pots serving as guard rows. Each treatment was replicated five times to ensure statistical reliability. Irrigation was conducted twice weekly, depending on weather conditions, with 500 ml of water applied per plant per session.

Growth parameters measurements

The experiment was conducted for a period of 90 days after planting (DAP). At harvest, three pots were sampled, key growth parameters such as the plant height, number of tubers per plant, and fresh and dry tuber yield were assessed to measure the impact of each treatment on plant development and productivity. Fresh and dry tuber weights were measured using high-capacity laboratory balances with a readability range of 100 mg to 1000 mg. Dry weights were recorded after freeze-drying the samples for five days using the Free Zone Plus 2.5 L Cascade Benchtop Freeze Dry System (Vacutec, USA).

Heavy metals concentration analysis

The effect of heavy metals on potato tubers was evaluated using a hydrochloric-nitric acid (HNO_3-HCl) digestion method, chosen for its effectiveness in liberating metal ions

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from the complex tuber matrix and reducing detection noise. Concentrations of micronutrients (Cu, Fe, Mn, Zn) and heavy metals (As, Cd, Co, Cr, Ni, Mg, Mn, Mo) were then measured using an inductively coupled plasma optical emission spectrometer (ICP-OES), as per USEPA Method 6020.

Correlation between growth parameters and selected heavy metals

The relationship between growth parameters and selected heavy metal content was identified to determine whether treated AMD affects the reduction of heavy metals during the composting process. The relationships between growth parameters and selected heavy metal concentrations were assessed using a correlation matrix, with significance levels of the correlation coefficients determined through STATISTICA version 12 (StatSoft Inc., Tulsa, OK, USA). All pairwise correlations are presented in *Table 1*. Positive correlation coefficients (+) indicate that as one variable increases, the other tends to increase as well, while negative coefficients (–) suggest that an increase in one variable corresponds to a decrease in the other. Strong positive and negative correlations were graphically visualized, and a correlation diagram was created using the EzCorrGraph application (https://ezcorrgraph.firebaseapp.com) (de Campos and Licht, 2021). Because this application was created with HTML5 and JavaScript, it can easily and simply create a correlation diagram from a list of pairs or a correlation matrix (Ogugua et al., 2023).

Results

The result of this correlation analysis highlighted a strong positive or negative connection on the potato plant growth parameters (Plant height, Number of tubers, Fresh tuber weight and Dry tuber weight) and selected heavy metals (Al, As, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb and Zn) (*Table 1*). Plant height exhibited a strong positive correlation with fresh tuber weight (r = +0.97, $p < 0.01$), suggesting that optimal vegetative growth supports higher tuber biomass. Cadmium (Cd) also correlated positively with fresh tuber weight (r = +0.99, $p < 0.01$), indicating potential hormetic effects. However, metals such as Al, As, and Cr were strongly negatively correlated with both plant height and yield metrics (r values between -0.91 and -0.99, $p < 0.05$), confirming their phytotoxicity (*Fig. 1*; *Table 1*).

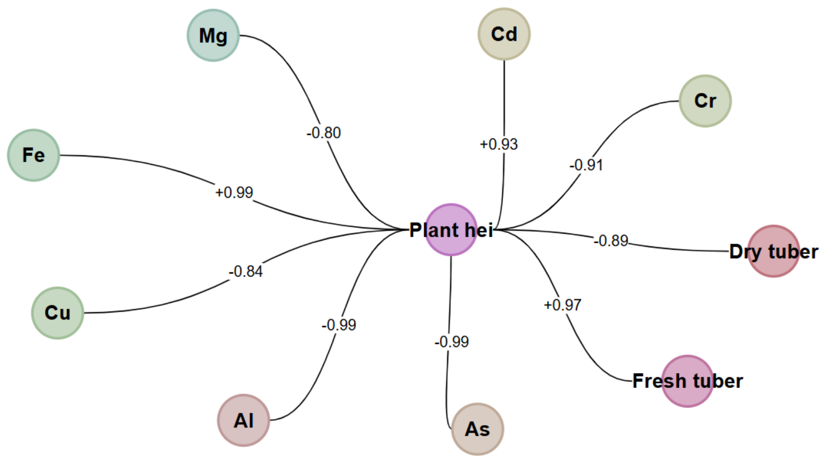

Figure 1. *Plant height vs fresh tuber weight, plant height vs dry tuber weight, plant height vs Al, plant height vs As, plant height vs Cd, plant height vs Cr, plant height vs Cu, plant height vs Fe, plant height vs Mg*

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Also, number of potato tubers showed a strong positive correlation on Mo (+0.99, $p < 0.01$), Ni (+0.90, $p < 0.05$) which suggests the metals might enhance the tuber formation, whereas a strong negative correlation on Mn (-0.97, $p < 0.05$), Pb (-0.79, $p < 0.01$), Zn (-0.99, $p < 0.01$) which indicates a potential inhibitory effect on the potato tuber formation (*Fig. 2*; *Table 1*).

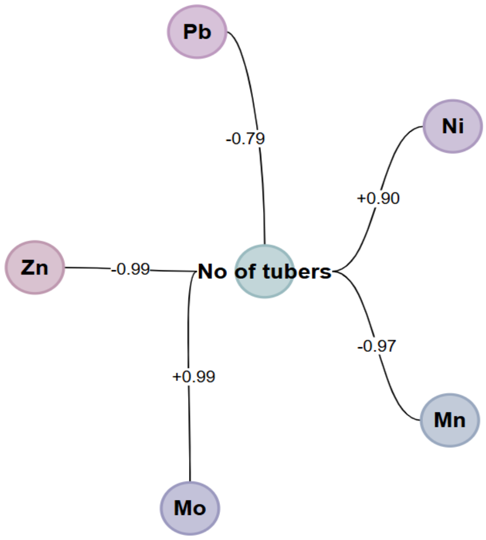

Figure 2. *No. of tubers vs Mn, no. of tubers vs Mo, no. of tubers vs Ni, no. of tubers vs Pb, no. of tubers vs Zn*

Furthermore, Fresh potato tuber weight showed a strong negative correlation on the tuber weight (-0.97, $P < 0.01$), Al (-0.93, $P < 0.05$), As (-0.99, $P < 0.001$), Cr (-0.98, $P < 0.05$), Cu (-0.79, $P < 0.01$) and Mg (-091, $P < 0.05$) whereas Cd (+0.99, $P < 0.05$) and Fe (+0.99, $P < 0.01$) showed a strong positive correlation respectively (*Fig. 3*; *Table 1*) which suggest these metals might contribute to tuber weight.

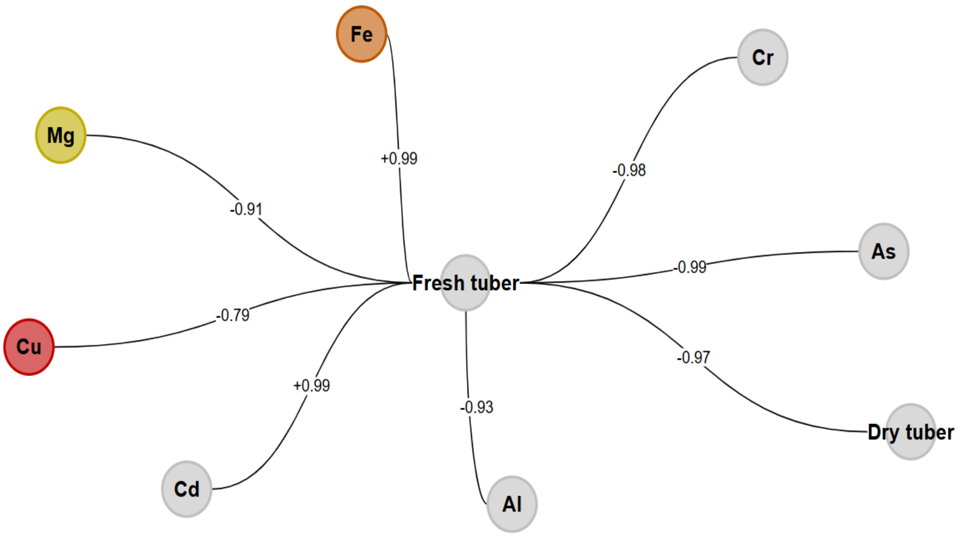

Figure 3. *Fresh tuber weight vs dry tuber weight, fresh tuber weight vs Al, fresh tuber weight vs As, fresh tuber weight vs Cd, fresh tuber weight vs Cr, fresh tuber weight vs Cu, fresh tuber weight vs Fe, fresh tuber weight vs Mg*

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Correlation analysis presented in *Figure 4* and *Table 1* shows a strong positive relationship between the potato dry tuber weight vs Al (+0.82, $P < 0.05$), As (+0.94, $P < 0.05$), Cr (+0.99, $P < 0.01$), and Mg (+0.99, $P < 0.05$), whereas Cd (-0.99 $P < 0.05$) and Fe (-0.93, $P < 0.01$).

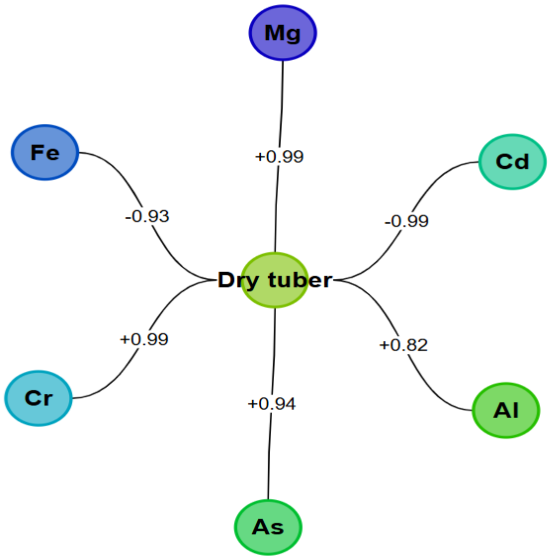

Figure 4. *Dry tuber weight vs Al, dry tuber weight vs As, dry tuber weight vs Cd, dry tuber weight vs Cr, dry tuber weight vs Fe, dry tuber weight vs Mg*

Heavy metal interactions were evident from the correlation analysis in our study. Al demonstrated a strong positive correlation with As (+0.97, $P < 0.05$), Cr (+0.85, $P < 0.05$), Cu (+0.90, $P < 0.05$) and Mg (+0.71, $P < 0.05$) which depicts the metal might co-accumulate while Cd (-0.88, $P < 0.01$), Fe (-0.97, $P < 0.05$) and Pb (-0.71, $P < 0.01$) showed a strong negative relationship which suggests potential antagonistic interactions (*Fig. 5*; *Table 1*).

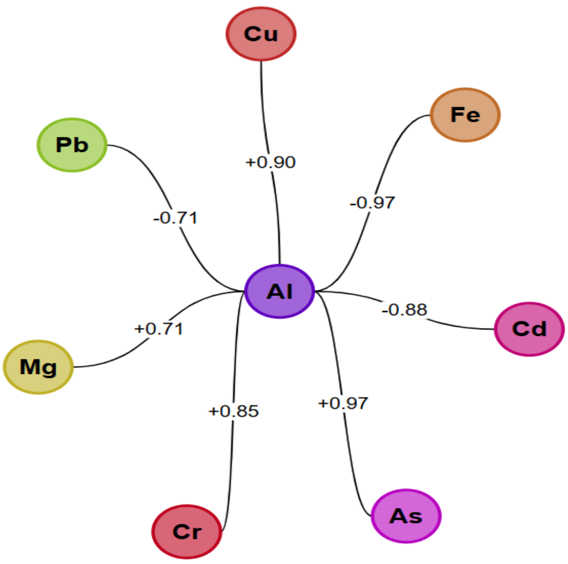

Figure 5. *Al vs As, Al vs Cd, Al vs Cr, Al vs Cu, Al vs Fe, Al vs Mg, Al vs Pb*

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Table 1. *Correlation analysis between potato growth parameters and selected heavy metals in the tubers*

Royal	Plant height	No of tubers	Fresh tuber weight (g)	Dry tuber weight (g)	Al	As	Cd	Cr	Cu	Fe	Mg	Mn	Mo	Ni	Pb	Zn
Plant height	1.00	+0.00*	+0.97**	-0.89**	-0.99*	-0.99*	+0.93**	-0.91*	-0.84**	+0.99**	-0.80**	+0.26*	-0.12*	-0.43**	+0.61**	-0.04*
No of tubers		1.00	+0.23*	-0.46*	+0.13*	-0.13**	+0.35*	-0.41*	+0.54**	+0.11*	-0.60*	-0.97**	+0.99**	+0.90*	-0.79**	-0.99**
Fresh tuber weight (g)			1.00	-0.97**	-0.93*	-0.99***	+0.99*	-0.98*	-0.79**	+0.99**	-0.91*	+0.03**	+0.11*	-0.21*	+0.41*	-0.27*
Dry tuber weight (g)				1.00	+0.82*	+0.94*	-0.99*	+0.99**	+0.50*	-0.93**	+0.99*	+0.22**	-0.35*	-0.03*	-0.17*	+0.49*
Al					1.00	+0.97*	-0.88**	+0.85*	+0.90*	-0.97*	+0.71*	-0.38**	+0.25**	+0.55*	-0.71**	-0.09*
As						1.00	-0.97**	+0.96**	+0.76**	-0.99**	+0.87*	-0.13***	-0.01*	+0.32*	-0.50**	+0.16*
Cd							1.00	-0.99**	-0.59*	+0.97***	-0.96*	-0.10*	0.24*	-0.09*	0.29*	-0.39*
Cr								1.00	+0.54*	-0.95**	+0.97*	+0.16*	-0.30*	+0.03*	-0.23*	+0.44**
Cu									1.00	-0.77*	+0.34*	-0.74**	0.64*	+0.85**	-0.94*	-0.51*
Fe										1.00	-0.86*	+0.15**	-0.01*	-0.33**	+0.51*	-0.15*
Mg											1.00	+0.38*	-0.51*	-0.20*	+0.00*	+0.63*
Mn												1.00	-0.99**	-0.98**	+0.92*	+0.96*
Mo													1.00	+0.95*	-0.86***	-0.99*
Ni														1.00	-0.98**	-0.88*
Pb															1.00	+0.77*
Zn																1.00

Significance is indicated by asterisks. *P < 0.05, **P < 0.01, ***P < 0.001
+0.70 or higher (very strong positive); +0.40 to +0.69 (strong positive); +0.30 to +0.39 (moderate positive) +0.20 to +0.29 (weak positive); +0.01 to +0.19 (no correlation); 0 (zero correlation); − 0.10 to − 0.19 (no relationship); − 0.20 to − 0.29 (weak negative); − 0.30 to − 0.39 (moderate negative); − 0.40 to − 0.69 (strong negative); − 0.70 or higher (very strong negative)

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Heavy metal As showed a strong positive correlation with Cr (+0.96, P < 0.01), Cu (+0.76, P < 0.01) and Mg (+0.87, P < 0.05); a strong negative correlation was recorded on Cd (-0.97, P < 0.01), Fe (-0.99, P < 0.01); Cd vs Cr (+0.97, P < 0.01) and Cr vs Mg (+0.97, P < 0.05); a strong negative correlation was recorded between Cd vs Cr (-0.99, P < 0.01), Mg (-0.96, P < 0.05), Cr vs Fe (-0.95, P < 0.01) as presented (*Fig. 6*; *Table 1*).

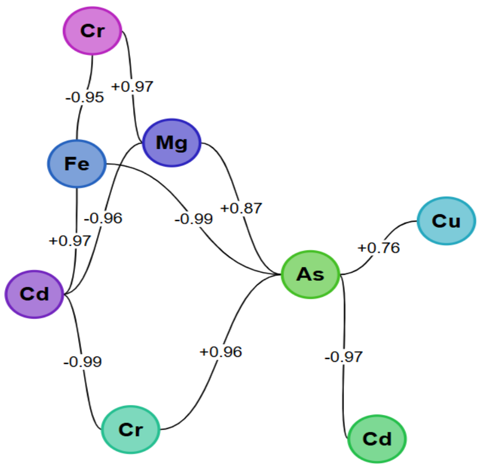

Figure 6. *As vs Cd, As vs Cr, As vs Cu, As vs Fe, As vs Mg; Cd vs Cr, Cd vs Fe, Cd vs Mg; Cr vs Fe, Cr vs Mg*

Conversely, Cu vs Ni (+0.85, P < 0.01); Mn vs Pb (+0.92, P < 0.05); Mo vs Ni (+0.95, P < 0.05) and Pb vs Zn (+0.77, P < 0.05) showed a strong positive relationship. A strong negative correlation was recorded on between Cu vs Fe (-0.77, P < 0.05), Cu vs Mn (-0.74, P < 0.01), Cu vs Pb (-0.94, P < 0.05); Fe vs Mg (-0.86, P < 0.05); Mn vs Mo (-0.99, P < 0.01), Mn vs Ni (-0.98, P < 0.01); Mo vs Pb (-0.86, P < 0.001), Mo vs Zn (-0.99, P < 0.05); Ni vs Pb (-0.98, P < 0.01) and Ni vs Zn (-0.88, P < 0.05) (*Fig. 7*; *Table 1*). Understanding these interactions between metal can help in developing strategies to mitigate heavy metal toxicity in crops.

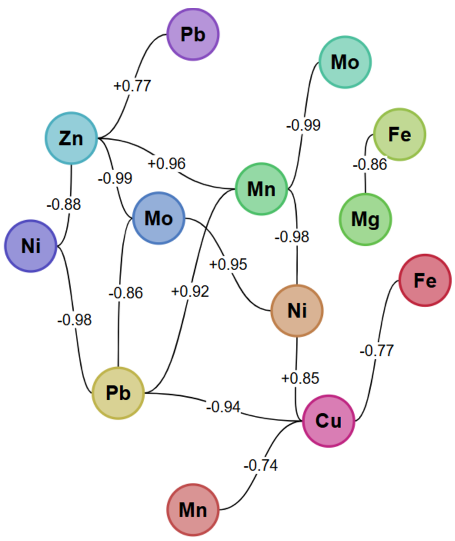

Figure 7. *Cu vs Fe, Cu vs Mn, Cu vs Ni, Cu vs Pb; Fe vs Mg; Mn vs Mo, Mn vs Ni, Mn vs Pb, Mn vs Zn; Mo vs Ni. Mo vs Pb, Mo vs Zn, Mo vs Zn; Ni vs Pb, Ni vs Zn; Pb vs Zn*

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Discussion

The findings of this study confirm established knowledge on the negative effects of Al and As on plant physiology through inhibition of nutrient transport and oxidative stress. The observed positive correlation of Cd with fresh tuber weight aligns with previous reports of low-dose stimulation but must be interpreted with caution due to Cd's known human health risks. Compared with Munyai et al. (2024), this study adds new insights by quantifying metal-metal interactions and highlighting co-accumulation patterns unique to the Royal cultivar under specific AMD treatment regimes. The correlation study of growth parameters and concentrations of heavy metals in the Royal potato cultivar demonstrated complex relationships, identifying the inhibitory and stimulating impact of some metals. Among the results, a positive association between fresh tuber weight and plant height that was considerable (more than 0.97) was one of the greatest. This was a value indicating that higher plant height is correlated with better tuber production. This observation is in like earlier studies by Farrell et al. (2020) that found that vigorous canopy growth in potatoes has significant associations with high translocation of assimilates to the tubers in the case of favorable physiological conditions. Nevertheless, the growth of the plant was significantly reduced by heavy metal stress. As an example, the height of the plants displayed a negative relationship with dry tuber weight (-0.89) and was highly suppressed by both metals (aluminum (Al) and arsenic (As)) in -0.99 each. These findings correspond with established phytotoxic implications of Al and As that have been found to negatively affect root elongation, water imbibition, and nutrient transportation, thus bearing the consequences of poor biomass and yields (Ghori et al., 2019; Alengebawy et al., 2021). The Arsenic (As) intoxication, especially, has been associated with disregulated ATP synthesis and oxidative stress that can be consistent with the stunted growth and the reduced dry matter build-up (Emamverdian et al., 2015). Interestingly, cadmium (Cd) was positively correlated with plant height (+0.93) and fresh tuber weight (+0.99), which within a brief environment seems an antithetical direction. Nevertheless, these trends can be part of a threshold or hormetic relationship in which low levels of some heavy metals initially induce temporary growth, or enzyme-type reactions, until they become dangerous at high doses (Salinitro et al., 2021; Wang et al., 2023; Dhiman et al., 2024). However, this objective advantage should be cautioned because Cd is a well-known carcinogen, and its translocation into edible plants is a significant health hazard to society (WHO, 2011). Previous observations by Chen et al. (2014) and Nemutanzhela et al. (2017) reveal that, even at sub-lethality, Cd results in bioaccumulation in tubers, which is a danger of contamination of the food chain. Molybdenum (Mo) and nickel (Ni) were strongly and positively correlated with the number of tubers per plant (accounting for +0.99 and +0.90, respectively), indicating a possible connection of these microelements in facilitating processes of tuberization. The nitrate reductase and nitrogen assimilation are obligatory for Mo and very important when initiating tubers (Bedoya-Perales et al., 2023). But Mn, Pb, and Zn displayed close negative relations with the number of tubers (-0.97, -0.79, and -0.99, respectively), meaning that they could be too much in the cells, interfering with the cell differentiation or hormonal balance needed to initiate tubers. A comparable result was observed by Antoniadis et al. (2017), with the level of Pb accrued in potato skins moving beyond the safety limits of WHO/FAO and linked to the repressed manufacture of tubers. Regarding metal-metal relationships, it was observed that Al formed strong positive relations with As, Cr, and Cu, indicating a possible co-accumulation in tubers during treated AMD irrigation. The sources of this co-accumulation may be a co-existent uptake pathway or

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synergistic solubility at low pH, which can occur in AMD environments (Hou et al., 2020). Instead, Cd demonstrated antagonistic relationships with Cr, Fe, and Mg (e.g., Cd vs. Fe -0.97), which is in line with the observations by McLaughlin et al. (2011) based on a competitive effect on metal uptake at the root-soil interface of pH and forms of ions. Overall, the interrelation in the correlations indicates the idea that treated AMD water, irrespective of changes in pH as well as metals, nevertheless influences metal accumulation and physiological effects in potatoes. The work notes the urgent necessity of national governments addressing the potentiality behind the reuse of water as a resource in agricultural productions, through the balance of risk of heavy metal build-up in crops with the potentiality of translocation of said metals into edible tissues such as potatoes (Stasinos et al., 2014).

Conclusion

The study results revealed that the treated acid mine drainage (AMD) water has substantial impacts on the potato growth parameters and the pattern of the heavy metal loads, as put in perspective by their substantial correlation. The fact that the plant height is positively correlated with the fresh weight of the tuber, and that some of the trace metals present in AMD, such as Mo and Ni, enhance the effects, suggests that, under the controlled conditions, the treated AMD would assist in crop cultivation. The very high negative correlations given with the metals Al, As, Pb, and Zn, though, also cause some concern about their toxicological effect and bioaccumulation in food crops. These data are comparable to those described in the literature on metal-induced oxidative stress, nutrient imbalances, and lowered crop production in farms using polluted water sources. Since the trend has been toward an expanded scarcity of water in mines that have undergone mining in such areas as South Africa, treated acid mine drainage significantly influences the growth and heavy metal accumulation in the Royal potato cultivar. While quicklime treatment reduced phytotoxicity and enhanced growth in some metrics, persistent accumulation of metals like Cd, Pb, and Zn poses long-term food safety concerns. The results advocate for cautious reuse of treated AMD in agriculture, emphasizing the need for cultivar-specific research, periodic monitoring, and soil amendments.

Author contributions. RM and DMM planned the experiments, UVO interpreted the results, RM, DMM and UVO made the write up and UVO statistically analyzed the data and made illustrations

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Conflict of interests. All authors declare no conflict of interests.

Data availability. Data presented in this study will be available on a fair request to the corresponding author.

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