

RESEARCH ARTICLE

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GROWTH AND HEAVY METAL CONTENT IN TWO ROSELLE VARIETIES CULTIVATED UNDER DIFFERENT TYPES OF SOIL MEDIA

Siti Aishah Mohd Ali, Sahibin Abd. Rahim, Rohana Tair, and Tan Wei Hsiang*

Faculty of Science and Technology, Universiti Malaysia Sabah, Jln UMS, 88400 Kota Kinabalu, Sabah, Malaysia.

***Correspondence:**
tan@ums.edu.my

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ABSTRACT. Recent increases in anthropogenic activities have contributed to elevated levels of heavy metals in the environment. These metals can bioaccumulate within ecosystems and be absorbed by plants. Therefore, this study was carried out to investigate the influences of different soil type treatments (ultrabasic, clay, and organic soil) on plant growth, phenolic constituents' production, and selected heavy metal content (Zn, Cd, Co, Cu, Ni, Cr, Pb, and Mn) in two Roselle varieties (*H. sabdariffa* L. and *H. sabdariffa* var. *UMKL-1*). The growth parameters, including plant height, stem diameter, number of branches, and leaf count, were recorded. Roselle calyx extract was analyzed for heavy metal concentrations using Inductively Coupled Plasma - Optical Emission Spectrometry. The results showed no statistically significant effects of soil type ($p > 0.05$) or variety on growth parameters. Overall, growth parameters increased steadily over the 84 days after transplanting in both varieties. In addition, heavy metal results indicated that Mn and Zn were the predominant heavy metals accumulated in both varieties. Roselle grown in ultrabasic soil showed higher levels of Mn, Co, Ni, and Pb, while clay soil resulted in higher concentrations of Zn, Cu, Cr, and Cd. In contrast, organic soil had the lowest heavy metal content. In conclusion, both roselle varieties grown in organic soil demonstrated better growth and lower heavy metal accumulation in the calyces.

INTRODUCTION

In recent years, the issue of environmental contamination by heavy metals has gained significant attention due to human activities. There is growing concern about the bioaccumulation of these metals in the environment, particularly as they are absorbed by plants. Heavy metals present in water and soil can be taken up by plants, eventually accumulating in their tissues (Nnaji *et al.*, 2023). This accumulation can negatively impact the nutritional value of plants, leading to deficiencies in essential nutrients (Khan *et al.*, 2015). While some heavy metals are required by plants for growth, excessive levels can become highly toxic (Chibuike & Obiora, 2014). Therefore, it is crucial to monitor the heavy metal concentrations in plants to assess potential risks related to their consumption.

Roselle, scientifically known as *Hibiscus sabdariffa* L., is a subtropical plant belonging to the Malvaceae family. In Malaysia, it is commonly referred to as "asam paya," "asam kumbang," or "asam susur" and is widely recognized for its versatile uses. The edible calyces of Roselle are harvested for a variety of food and traditional medicinal applications, including herbal teas, ice creams, jams, and jellies (Avela *et al.*, 2021). Due to its numerous health benefits, Roselle is extensively used for its medicinal

properties. This herbaceous shrub is known for its antioxidant, antibiotic, antihypertensive, antidiabetic, cardioprotective, and anticancer effects (Abou-Sreea *et al.*, 2022). The calyx extract contains a range of beneficial compounds, including phenolic acids, anthocyanins, flavonoids, alkaloids, terpenes, phenols, cardiac glycosides, steroids, saponins, tannins, and anthraquinone (Nerdy *et al.*, 2022).

This study involved two varieties of Roselle: *Hibiscus sabdariffa* L. and *H. sabdariffa* var. UMKL-1. The former, commonly known as the Arab variety, is widely grown in Malaysia and is distinguished by its deep red calyces. It was introduced to the country in the early 1990s by the Department of Agriculture in Terengganu (Mohamad *et al.*, 2011). The UMKL-1 variety, often referred to as the Terengganu variety, was developed by Universiti Malaya (UM) for commercial cultivation. This variety is noted for its dark green leaves and red calyces. Compared to UMKL-1, the Arab variety generally produces a higher yield of calyces per plant and exhibits more vigorous growth (Mohamad *et al.*, 2011).

Currently, there aren't many studies on how Roselle accumulates heavy metals when cultivated using different soil media. It is essential to understand how different types of soil media may affect Roselle's reactions to metal uptake. Therefore, this study aims to investigate the influences of different soil media treatments (ultrabasic, clay, and organic soil) on the growth of two Roselle varieties, calyces (*H. sabdariffa* L. and *H. sabdariffa* var. UMKL-1), and evaluating the selected heavy metals concentrations (Zn, Cd, Co, Cu, Ni, Cr, Pb, Mn) in soil and Roselle calyces using Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES).

MATERIALS AND METHODS

Plant Samples and Treatments

Two roselle varieties were evaluated in three types of soil media (ultrabasic, clay, and organic soil) at Universiti Malaysia Sabah from December 2021 to April 2022. Healthy roselle seeds were first sown in seed trays filled with organic soil. After two weeks, seedlings measuring 6–8 cm in height were selected and transplanted into 20 × 20 cm nursery polyethylene bags containing 15 kg of the respective soil media. Irrigation was provided via a drip system at a rate of 1.72 L per plant per day, applied twice daily, following the optimal recommendation for roselle growth reported by Nur Amirah *et al.* (2015). All plants received the same commercial fertilizer regime (NPK Green 15:15:15 and NPK + Mg Blue 12:12:17:2) along with organic matter/compost. Fertilizers were applied every two weeks after transplanting. Manual weeding was conducted regularly, and pesticides were used when needed. Calyces were harvested 35–40 days after flowering from four individual plants per treatment, serving as biological replicates. Figure 1 illustrates the roselle cultivation process.

Sample Collection

The collection of calyces started at the end of February until April 2022. Fresh calyces were placed in zip-lock bags and kept in a cooler for transport to the laboratory. Each calyx was washed with water, drained, the seeds were removed, and then the calyces were air-dried at room temperature for three days. The dried calyces were sealed in zip-lock bags and stored in a refrigerator at -20 °C until extraction. Roselle calyx extract was prepared following the method of Chumsri *et al.* (2008). Briefly, dried calyces were extracted with water at a 1:10 ratio in a water bath at a constant temperature of 50 °C for 30 minutes. The extracts were then filtered using Whatman No. 1 filter paper, and the filtrates were dried with a freeze dryer.

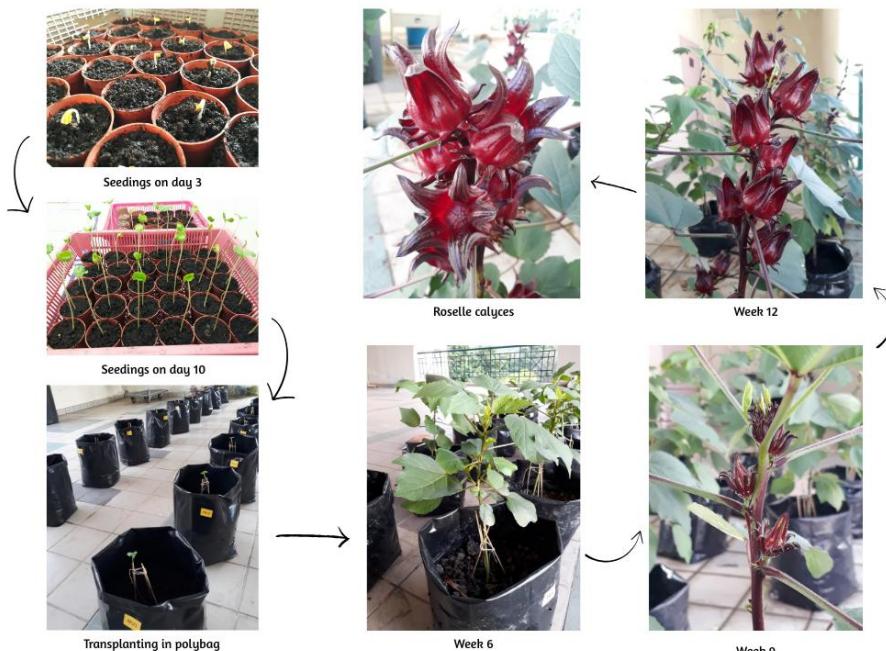


Figure 1. Cultivation process of *H. sabdariffa*.

Determination of Growth Traits

Growth parameters, including stem diameter (mm), plant height (cm), number of branches, and number of leaves (an indicator of physiological age), were recorded at seven-day intervals after transplanting. The number of branches was determined by counting the primary reproductive branches. Plant height was measured from ground level to the shoot tip along the main stem using a measuring tape. The number of leaves was counted and recorded for every visible leaf on the plant, including newly emerging leaf tips.

Heavy Metal Analysis

The soil and dry calyces' samples underwent heavy metals analysis (Zn, Cd, Co, Cu, Ni, Cr, Pb, and Mn). Using the digestion method, the heavy metals were extracted from the soil (Werdegebril *et al.*, 2012). Briefly, 1 g of each soil sample was digested with a mixture of 5 mL HNO₃ and 10 mL HCl for at least 45 minutes at 160 °C. It was removed from the heated plate before it dried, chilled, diluted in a 200 mL volumetric flask with distilled water, agitated, and poured back into the beaker after 30 minutes of settling. The heavy metal concentrations in the samples were determined using ICP-OES, and the results were expressed in milligrams per liter (mg/L). Calibration was performed to ensure accurate and reliable measurements.

The dry calyces samples underwent heavy metal analysis using the wet digestion technique according to US EPA method 3050 B. Briefly, about 1 g of finely powdered dried calyx was combined with 10 mL of 65% nitric acid (HNO₃), then heated to a temperature of 95 °C ± 5 °C and subjected to reflux for a duration of 10 to 15 minutes, ensuring that boiling did not occur. After cooling, a volume of 5 mL of concentrated HNO₃ was introduced and refluxed for 30 minutes. The presence of brown fumes showed that oxidation had taken place due to the addition of HNO₃. Subsequently, 5 mL concentrated nitric acid was added dropwise until no more brown fumes were produced by the sample. After the mixture had cooled down, about 2 mL of water and 3 mL of 30% hydrogen peroxide (H₂O₂) were added to the sample. Subsequently, the flask was sealed with a watch glass and subjected to further heating to trigger the peroxide reaction. The sample combination was heated until the bubbling ceased and then chilled. A 1 mL portion of 30% hydrogen peroxide (H₂O₂) was introduced into the flask. The sample was subjected to heat until the effervescence reached a minimum level. The maximum volume of 30%

H_2O_2 that may be added is 10 mL. The acid-peroxide sample was thereafter placed under a watch glass and subjected to heating at a temperature of $95^\circ\text{C} \pm 5^\circ\text{C}$, avoiding boiling, until the volume was decreased to roughly 5 mL. Subsequently, the sample was allowed to undergo a cooling process. The cooled sample was treated with 10 mL of 37% hydrochloric acid (HCl) solution. The mixture was then heated for 15 minutes. Subsequently, the initial sample combination was diluted by adding 100 mL of water. The particulates in the sample were separated by passing them through a membrane filter paper with a pore size of 0.45 μm . Subsequently, the material that had undergone filtration was subjected to analysis using ICP-OES.

Statistical Analysis

The mean value \pm standard deviation collected data were subjected to analysis of variance using the Statistical Package for Social Science for Windows version 29.0 software (IBM SPSS Statistics). To find significant variations between treatments, a one-way analysis of variance (ANOVA) was used. Significantly different values are defined at the 5% level ($p < 0.05$).

RESULTS AND DISCUSSIONS

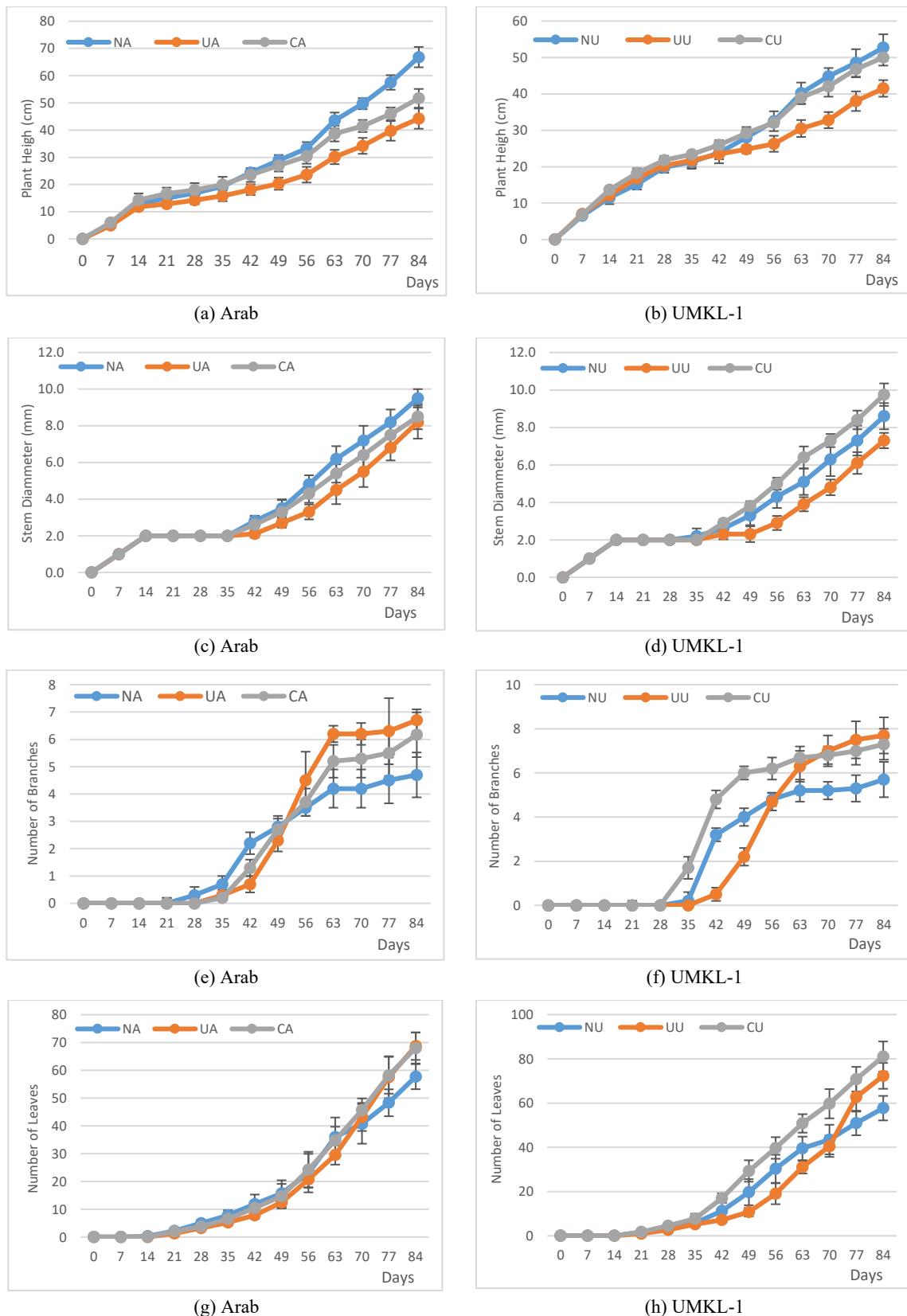
Growth Characteristics

Figure 2 presents the data for each growth parameter measured at seven-day intervals in roselle plants under different irrigation treatments. No statistically significant differences were observed among the irrigation treatments ($p > 0.05$). Overall, all growth parameters showed an increasing trend over the 84 days after transplanting across all treatments. Although the differences were not significant, both Roselle varieties grown in ultrabasic soil exhibited the lowest growth rates for all parameters except the number of branches, when compared to clay and organic soil. This may be attributed to the high mineral content of ultrabasic soil, which often results in increased acidity. Excessively acidic soils can negatively impact soil physical properties and reduce nutrient availability for plant growth (Xia *et al.*, 2024).

These results are consistent with Shuhaimi *et al.* (2019) on *H. sabdariffa* L., Nur Amirah *et al.* (2015) on *H. sabdariffa* var. UMKL-1 and Azza *et al.* (2010) on *Jatropha curcas*. Nur Amirah *et al.* (2015) reported no statistically significant differences in UMKL-1 growth cultivated in Beach Ridges Interspersed with Swales (BRIS) soil with respect to leaf area index, plant height, stem diameter, or post-harvest quality. In contrast, Khalil and Abdel-Kader (2011) found that sandy soil significantly enhanced all growth characteristics in *H. sabdariffa* L. from Egypt compared to clay soil. Azza *et al.* (2010) suggested that sandy soil allows the root system to penetrate deeper and spread more widely than clay soil, promoting better root establishment. Metwally *et al.* (1972) highlighted that soil aeration, nutrient availability, water movement, and microbiological activity are highly dependent on soil pore structure. However, sandy soils may experience rapid leaching of nutrients and chemicals as they move downward with water (Roslan *et al.*, 2010).

Heavy Metal Concentration in Soil

The concentration of heavy metals in the soil followed the order: ultrabasic soil > clay soil > organic soil (Table 1). The highest concentration of heavy metals in ultrabasic soil is in descending order: Cr > Mn > Ni > Co > Cu > Zn > Pb > Cd. The high levels of Cr, Ni, and Mn in ultrabasic soil are likely due to the geological nature of ultrabasic formations, which are known to contain elevated concentrations of these metals (Siebecker, 2010). In contrast, Pb, Cu, and Zn were found at comparatively lower levels, while Cd was below the detection limit.



Arab: NA = Organic soil; UA = Ultrabasic soil; CA = Clay soil

UMKL-1: NU = Organic soil; UU = Ultrabasic soil; CU = Clay soil

Figure 2. The effect of soil media's treatment on Arab and UMKL-1 growth with different parameters: a-b) Plant height; c-d) Stem diameter; e-f) Number of branches, and g-h) Number of leaves. Data points are means of biological replicates ($n = 4$). The vertical bar represents the standard deviation.

Table 1. Heavy metal concentration in soils.

Heavy metal	Soil Sample (mg/kg)		
	Ultrabasic Soil	Clay Soil	Organic Soil
Zn	49.42 ± 4.58	29.23 ± 12.17	7.20 ± 1.73
Cd	BDL	BDL	BDL
Co	306.23±4.50	1.02±0.34	0.14 ± 0.12
Cu	80.57 ± 14.19	4.28 ± 2.41	1.07 ± 0.38
Ni	2023.60 ± 415.50	3.99 ± 1.95	1.00 ± 0.17
Cr	4402.80 ± 462.10	13.24 ± 5.09	7.33 ± 0.79
Pb	0.07 ± 0.12	2.86 ± 1.83	0.10 ± 0.12
Mn	2257±402	23.07±5.67	14.75±2.11

BDL = Below Detection Limit; Values represent the mean of four replicates ± Standard deviation

Ultrabasic soil has a rich mineral composition and generally exhibits a high pH. The findings are consistent with the known influence of soil minerals on metal retention, as the mineral content in ultrabasic soil can affect the availability and mobility of heavy metals in the environment (Wuana & Mbasugh, 2013). In clay soil, heavy metals were present in the descending order: Zn > Mn > Cr > Cu > Ni > Pb > Co > Cd. Clay is characterized by fine particles and high water-holding capacity. Its mineral structure may restrict the accumulation of heavy metals, suggesting that factors beyond mineral composition also influence metal retention (Tiwari & Lata, 2018).

In addition, organic soil contains heavy metals in descending order: Mn > Cr > Zn > Cu > Ni > Co > Pb > Cd. The high organic matter content in this soil type can affect metal availability and binding, contributing to the relatively lower uptake of heavy metals observed (Abubakari *et al.*, 2017). All measured heavy metal concentrations were well below WHO limits, indicating that all soil types fall within acceptable ranges for agricultural use and pose minimal risk related to excessive heavy metal content (U.S. Environmental Protection Agency, 1996; 2002).

Accumulation of Heavy Metals in Roselle Calyces

Tables 2 and 3 show the heavy metal concentrations in the Roselle Arab and UMKL-1 varieties. Both Roselle calyces show similar heavy metal content, in the descending order: ultrabasic soil > clay soil > organic soil. Mn and Zn are the highest in concentration, followed by Cu, Cr, and Ni, while Co, Cd, and Pb are almost below the detection limit. The heavy metal concentrations in Roselle calyces for all elements did not violate the permissible limits of World Health Organization (WHO) guidelines for vegetable crops (Bigdeli & Seilsepour, 2008). Thus, the low amounts of heavy metals in roselle plant tissues show that the fruits are safe to be consumed.

Table 2. Heavy metal concentration in the Roselle Arab variety.

Heavy metal	Soil Sample (mg/kg)		
	Ultrabasic Soil	Clay Soil	Organic Soil
Zn	36.42 ± 3.32	46.01 ± 2.34	39.66 ± 1.03
Cd	BDL	BDL	BDL
Co	1.34±0.01	BDL	BDL
Cu	9.52 ± 0.01	9.31 ± 0.11	7.44 ± 0.32
Ni	5.36 ± 0.01	1.45± 0.01	1.03 ± 0.12
Cr	9.67 ± 0.22	9.47 ± 0.12	4.43 ± 0.32
Pb	BDL	BDL	BDL
Mn	150.45±6.32	97.33±3.67	68.73±2.11

BDL = Below Detection Limit; Values represent the mean of four replicates ± Standard deviation.

Table 3. Heavy metal concentration in the Roselle UMKL-1 variety.

Heavy metal	Soil Sample (mg/kg)		
	Ultrabasic Soil	Clay Soil	Organic Soil
Zn	36.77 ± 3.58	56.11 ± 8.17	50.22 ± 5.73
Cd	BDL	BDL	BDL
Co	1.21±0.01	BDL	BDL
Cu	8.43 ± 0.01	9.39 ± 0.17	8.49 ± 0.52
Ni	5.89 ± 0.01	2.45± 0.01	1.73 ± 0.11
Cr	6.91 ± 0.12	7.23 ± 2.12	3.73 ± 0.12
Pb	BDL	BDL	BDL
Mn	134.45±6.32	102.53±4.69	89.73±1.34

BDL = Below Detection Limit; Values represent the mean of four replicates ± Standard deviation.

Roselle, like many leafy vegetables, is classified as a hyperaccumulator plant. Hyperaccumulators are capable of storing higher concentrations of heavy metal ions in their shoots than in their roots and possess greater tolerance to heavy metals compared to other plant species (Saleem *et al.*, 2020). Roselle is also an important source of minerals, including K, Ca, and Mg, as well as trace elements such as Fe, Mn, Zn, and Cu. These minerals play a crucial role in promoting health by acting as antioxidants or being involved in the functioning of antioxidant enzymes (Mitra *et al.*, 2022). According to Getso *et al.* (2018), the Roselle plant can accumulate a high concentration of Mn from mineral fertilizers, exceeding the tolerable daily intake but within the permissible limit. Similarly, Younes *et al.* (2016) found that roselle can remove up to 8 µM Mn from contaminated soil. Abou El-Seoud *et al.* (1997) observed that certain heavy metals (Fe, Mn, Zn, and Co) accumulated in roselle calyces at varying concentrations when plants were grown in soil amended with organic waste compost. Consistent with these findings, Shuhaimi *et al.* (2019) reported that Pb, Cd, and Cu levels in roselle crops remained very low.

Herrera-Estrella *et al.* (1999), in their study on heavy metal adaptation, explained that genotypic evolution and natural selection enabled accumulator species to adapt over time to habitats with high endogenous metal concentrations. The key mechanisms involved include metabolic adaptability, complexation, and compartmentalization. As a result, metal tolerance in plants may arise through fundamental strategies such as metal exclusion or metal accumulation, depending on the species. Therefore, metal toxicity in plants is often a complex and subtle phenomenon rather than a clearly visible, isolated issue. It may result from intricate interactions among major toxic ions, other essential or non-essential ions, and various environmental factors.

CONCLUSION

Cultivation of both roselle varieties in different soil media showed an overall increasing growth trend over the 84 days after transplanting. However, no statistically significant differences ($P > 0.05$) were observed in most growth parameters. For heavy metal accumulation, both varieties and soil types followed the descending order: ultrabasic soil > clay soil > organic soil. Therefore, it can be concluded that both varieties grown in organic soil may achieve better growth, particularly in height and stem diameter, while also exhibiting lower heavy metal content in the calyces. Future research could explore the influence of environmental variables beyond soil conditions to provide a more comprehensive understanding of the factors affecting plant growth and bioactive compound production. Such an approach could enhance knowledge of Roselle cultivation in Malaysia and help optimize conditions to maximize its health-promoting properties.

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