Bulgarian Journal of Agricultural Science, 32 (No 3) 2026, 546–558

Ultra-trace alkali metals and geographic origin of Pak choi growing in the Northern Vietnam

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Abstract

Mac, Th. V., Truong, N. M. & Nguyen, Th. M. T. (2026). Ultra-trace alkali metals and geographic origin of Pak choi growing in the Northern Vietnam. *Bulg. J. Agric. Sci., 32*(3), 546–558

Metal content in soils of pak choi *(Brassica rapa* L. *ssp. chinensis)* from four different districts in Northern Vietnam (Me Linh, Long Bien, Dan Phuong, and Chuong My district) was investigated for 26 metals (Li, B, Mg, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Rb, Nb, Mo, Ag, Cd, Sb, Cs, Ba, Hg, Tl, Pb, Bi), using inductively coupled plasma mass spectrometry (ICP-MS). Samples were collected during six consecutive planting periods from September 2022 to December 2022, and from January 2023 to April 2023. The obtained data were processed with discriminant analysis, demonstrating the ability to identify the geographic origin of pak choi based on ultra-trace alkali metals (UTAM) (including Lithium (Li), Cesium (Cs) and Rubidium (Rb).

Ultra-trace alkali metals content in pak choi and soils of the three main cultivated areas in two different districts of Northern Vietnam (Me Linh and Dan Phuong District) was investigated using the ICP-MS method during three consecutive planting periods from January 2023 to April 2023. The obtained data were processed with MANOVA (multivariate analysis of variance), and described by graphs. The results showed relationships between UTAM levels in pak choi edible parts (stems and leaves), inedible parts (roots), and soil, with Rb appearing to play a significant role in pak choi growth. Among the three metals of UTAM, Rb has the potential to become a biomarker for tracing geographic origin of pak choi.

Keywords: Pak choi; ultra-trace alkali metals (UTAM); rubidium; ICP-MS; Northern Vietnam

Introduction

Fruits and vegetables play an important role in a healthy and balanced human diet due to their indispensable nutritional components, such as vitamins, minerals, fiber, sugars, as well as essential trace elements. According to recent results from the recent National Institute of Nutrition of Vietnam, the per capita consumption of vegetables and fruits has increased from 190.5g g/person/day to 231 g/person/day for vegetables, and to 140.7 g/person/day for ripe fruits over the past 10 years (2020) (Ministry of Health, 2022). In recent years, consumers have faced numerous food safety issues, such as food fraud, pesticide residues, preservatives, chemical contaminants, wrong geographical origin,... With the development of food tracebility and authentication systems, consumers can now more easily access information and make informed decisions when selecting fruits and vegetables.

In Vietnam, pak choi (*Brassica rapa* L.) is one of the most popular vegetables, alongside cabbage and broccoli, etc. Pak choi is known for its high content of Ca, Mn, K, Zn, Fe, Na, Mg, Se, etc. (Hanson et al., 2009). Therefore, consumers are highly concerned about its cultivation origin.

Metals are present in irrigation water, rain, soil and air. Therefore, this study aims to investigate the relationship between metal content in pak choi and soil in two main vegetable supply regions of Hanoi.

Materials and Methods

Samples

Soil samples: Soil samples were collected in the fifth week of six consecutive harvests from the districts of Me Linh, Long Bien, Dan Phuong, and Chuong My during the periods from September to December 2022 (autumn-winter) and from January to April 2023 (spring-summer).

Pak choi samples: Among the four districts, Dan Phuong and Me Linh are the two major pak choi-producing areas supplying the Northern Vietnam market. Therefore, pak choi samples and their corresponding soils samples were collected from six cultivated areas in the two districts of Me Linh and Dan Phuong (three areas per district). Sampling was conducted continuously from the first week (seeding) to the fifth week (harvest for market sale) (Table 1; Figure 1). Pak choi seeds (*Brassica rapa* L. ssp. *chinensis*) were provided by VECOSEED Co. (Viet Fruit and Vegetable Seeds Company).

The pak choi samples were collected in accordance with TCVN 4046:1985 ("TCVN 4046:1985: Soil-Method of sampling," 1985) and TCVN 8551:2010 ("TCVN 8551:2010: Plants – Method for sampling and preparing sample," 2010), and were subsequently washed several times with distilled water. The soil samples were collected in accordance with TCVN 9016:2011 ("TCVN 9016:2011 Fresh vegetables – Sampling method on the field," 2011) and TCVN 8551:2010.

Chemicals and reagents

Ultrapure deionized water (18.2 MΩ·cm) was obtained from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA). Hydrogen peroxide (30%, H_2O_2) and nitric acid (65%, HNO_3) were purchased from Merck (USA). A multi-element standard solution (TraceCERT) containing Li, B, Mg, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Rb, Nb, Mo, Ag, Cd, Sb, Cs, Ba, Hg, Tl, Pb, and Bi was used in this study.

These elements were classified into five groups: alkali

Table 1. Six different cultivated areas of Dan Phuong and Me Linh District

Dan Phuong District			Me Linh District		
Location 1	Location 2	Location 3	Location 4	Location 5	Location 6
21° 5.068' N 105° 42.098' E	21° 5.166' N 105° 42.440' E	21° 5.211' N 105° 42.453' E	21° 10.470' N 105° 45.406' E	21° 10.388' N 105° 45.529' E	21° 10.360' N 105° 45.652' E

Source: Authors' own elaboration

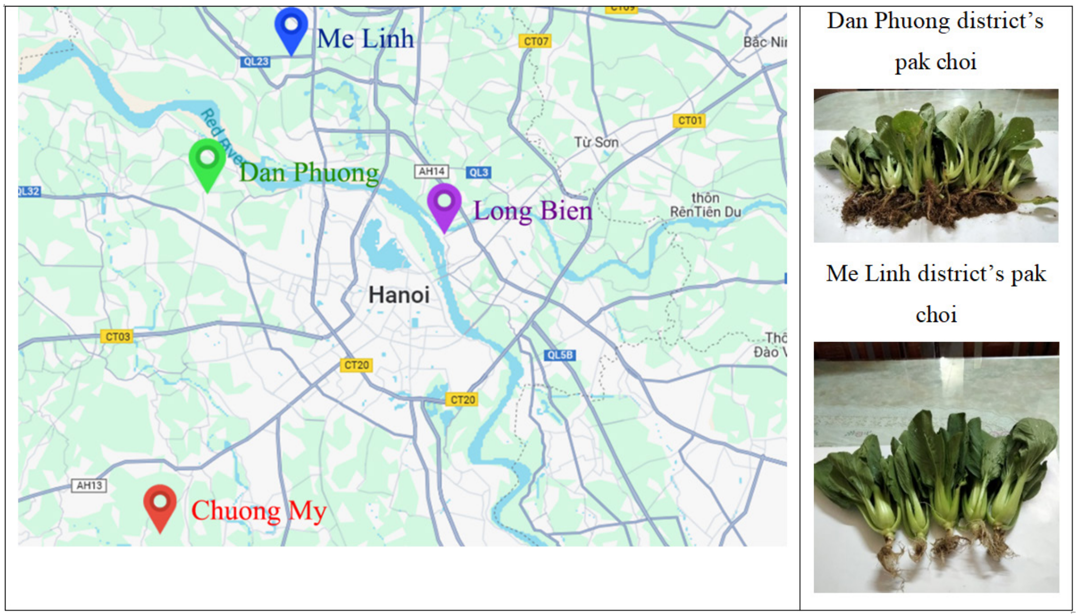


Fig. 1. The geographical locations of the pak choi cultivated areas and samples
Source: Authors' own elaboration

metals (Li, Rb, Cs); alkaline earth metals (Mg, Ba); amphoteric metals (Al, Cr, Zn); heavy metals (As, Cd, Hg, Pb); and other metals (Ti, B, V, Mn, Fe, Co, Ni, Cu, Nb, Mo, Ag, Sb, Tl, Bi).

Sample preparation

Sampling and storing samples

Pak choi samples were separated into edible parts (stems and leaves) and inedible parts (roots), and then dried at 70 °C until the moisture content reached approximately 10-11% (Figure 2a). All samples were subsequently ground and pulverized into powder (<400 µm particle size), and stored in clean plastic bags.

Cultivated soil samples were collected from the top 5 cm layer of soil at the same time as pak choi sampling (Figure 2b). All samples were then dried at 70 °C until reaching approximately 15% moisture content, ground into powder, and stored in clean plastic bags.

Digestion of samples

Pak choi samples: After determining the absolute moisture content, 0.1 g of pak choi samples was pre-digested with 4 mL of HNO_3 (65%) and 2 mL of H_2O_2 (30%) for at least 24 hours in a laboratory hood.

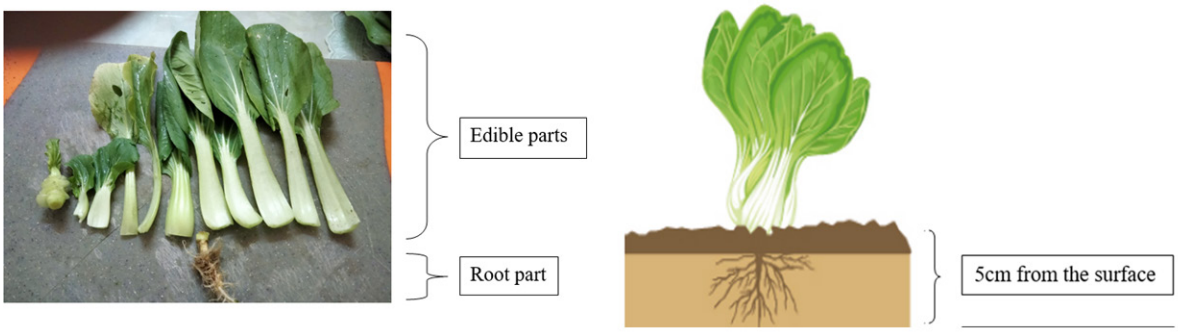
Soil samples: A 0.1 g soil sample was weighed into a polytetrafluoroethylene (PTFE) vessel, then 10 mL of HNO_3 (65%) was added, and allowed to pre-digest for at least 24 hours in a laboratory hood.

After 24 hours, the samples were digested using a Mars X-Press Plus microwave digestion system (Mars 6, CEM, NC, USA).

The digestion programs for pak choi and soil are shown in Figures 3a and 3b, respectively. The digested samples were then analyzed fỏ metal content using ICP-MS.

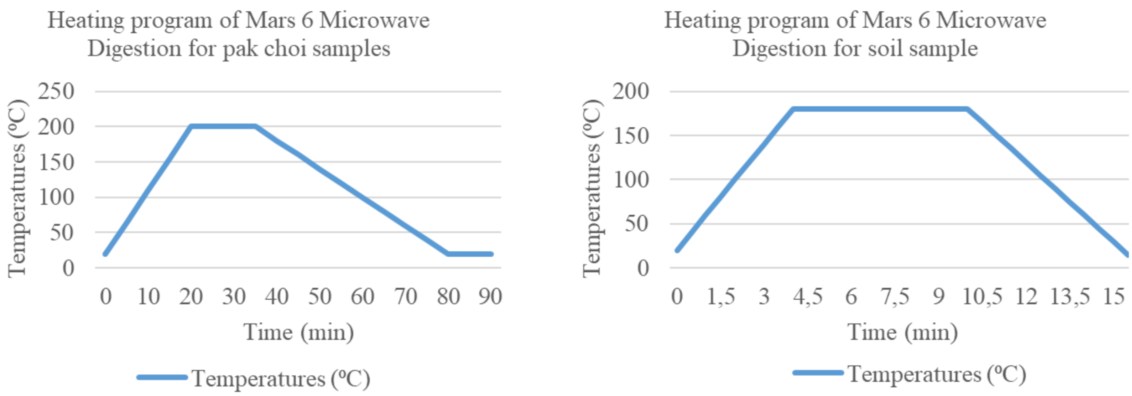
ICP-MS measurements

An Agilent 7900 ICP-MS instrument (Agilent Technolo-



2a 2b

Fig. 2. Preparation of pak choi samples (2a) and soil samples (2b)
Source: Authors' own elaboration



3a 3b

Fig. 3. Heating program of Mars 6 Microwave Digestion for pak choi (3a) and soil samples (3b)
Source: Authors' own elaboration

gies, Tokyo, Japan) was used to measure 26 elements (Li, B, Mg, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Rb, Nb, Mo, Ag, Cd, Sb, Cs, Ba, Hg, Tl, Pb, and Bi). The ICP-MS operating parameters were as follows: RF power, 1550 W; RF matching, 2.0 V; cell entrance, −40 V; cell exit, −60 V; cell energy discrimination, 5.0 V; spray chamber temperature, 2 °C; argon as carrier gas at a flow rate of 1.09 L/min; and helium as auxiliary gas at 4.3 L/min. Data quantitation was performed using matrix-matched multi-element standards prepared in 1% HNO_3.

Instrument detection limits (IDLs) in this study were calculated using the raw intensity data from the standards and blanks (prepared in ultrapure 2% nitric acid), according to the following equation:

$$IDL = \frac{3\ SD_{blank} \times C_x}{S_x - S_{blank}}$$

where: SD_{blank} is the standard deviation of multiple blank measurements, C_x is the mean signal of the standard, S_x is the signal of C_x and S_{blank} is the signal of the blank.

Data analysis

Data obtained from triplicate analyses were expressed as mean metal concentrations of the samples. Discriminant analysis (DA) was performed to evaluate the effects of metal groups on soils from different areas. Multivariate analysis of variance (MANOVA) was used to test the statistical significance of the effect of growth time on metal content in pak choi parts and soil. Data analysis was performed using Microsoft Excel 2023, supplemented with XLSTAT software (version 2023).

Results and Discussion

Survey on soil conditions of some growing areas of the Northern Vietnam

Pak choi is grown in northern regions of Vietnam, including Me Linh, Long Bien, Dan Phuong, and Chuong My districts. Among these, Dan Phuong and Me Linh are the two main districts supplying pak choi to the Northern Vietnam market throughout the year. Long Bien and Chuong My districts were selected for the soil metal survey due to their similar cultivation practices.

In Me Linh and Long Bien districts, pak choi was cultivated over a six-week period, including one week of sowing followed by five weeks of growth until harvest. In these districts, foliar fertilization was applied via spray irrigation during the second week after sowing. In the Dan Phuong and Chuong My districts, pak choi was cultivated in a similar manner; however, fertilization was applied directly to the soil during the second week after sowing (Figure 4).

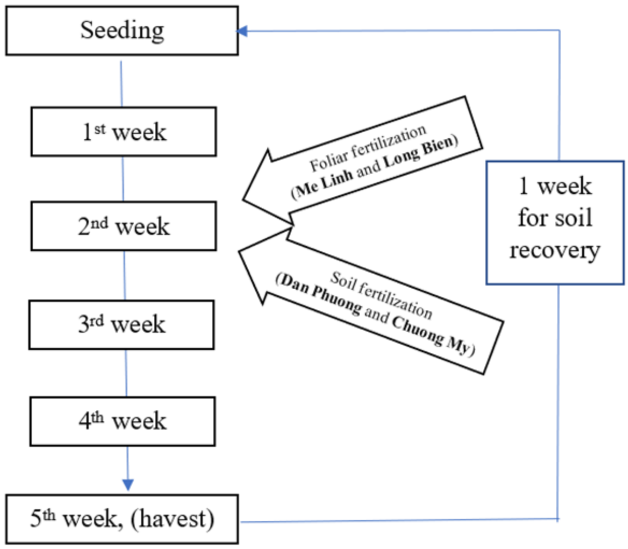


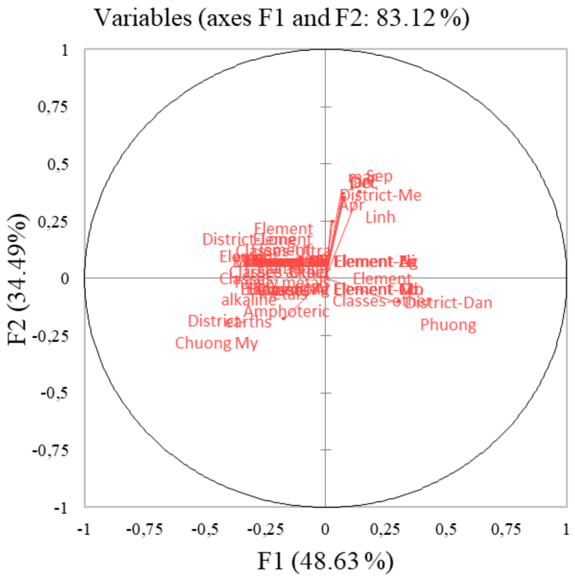
Fig. 4. Pak choi planting in Me linh, Long Bien, Dan Phuong and Chuong My district
Source: Authors' own elaboration

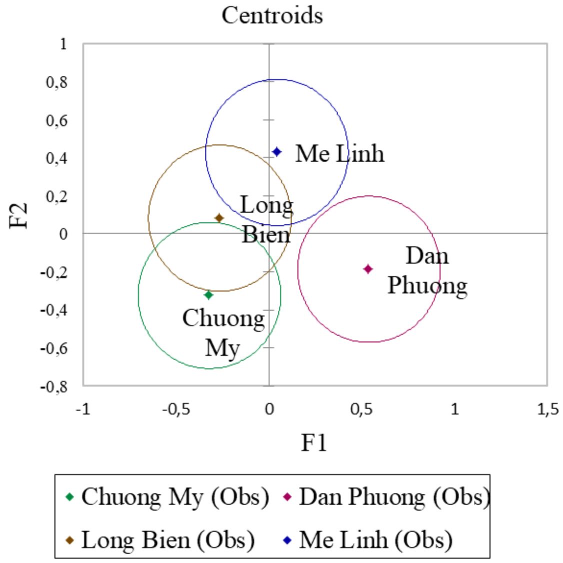
Soil metal composition

The metal composition of soils in the final week of growth, over six consecutive months from September 2022 to April 2023, was determined for four pak choi-growing districts: Me Linh, Long Bien, Dan Phuong, and Chuong My. Discriminant analysis was applied to examine the relationships between metal groups and soils from different geographical areas. The soil metal data from these four districts were further used to assess the association between soil metal groups and the geographical locations where pak choi was cultivated.

The dual-function discriminant plots obtained from discriminant analysis are shown in Figure 5a. This figure illustrates the metal content in soils from Me Linh, Long Bien, Dan Phuong, and Chuong My, with sampling locations treated as "observations", while Figure 5b presents the centroids for the four locations. The results indicate that soil metal content can be used for geographic discrimination, with F1 accounting for 48.63% and F2 for 34.49% of the total variance.

Discriminant plots for both functions were obtained by discriminant analysis, with metal concentrations in soils from Long Bien, Chuong My, Dan Phuong, and Me Linh as variables and "metal groups" as observations. The first and second discriminant functions (F1 and F2) accounted for 43.16% and 22.07% of the total variance, respectively (Figure 6a). Among the metal groups, ultra-trace alkali met-

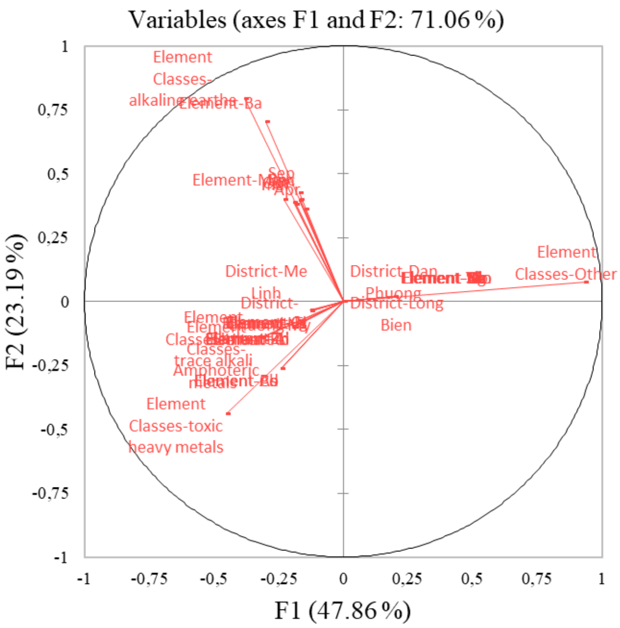


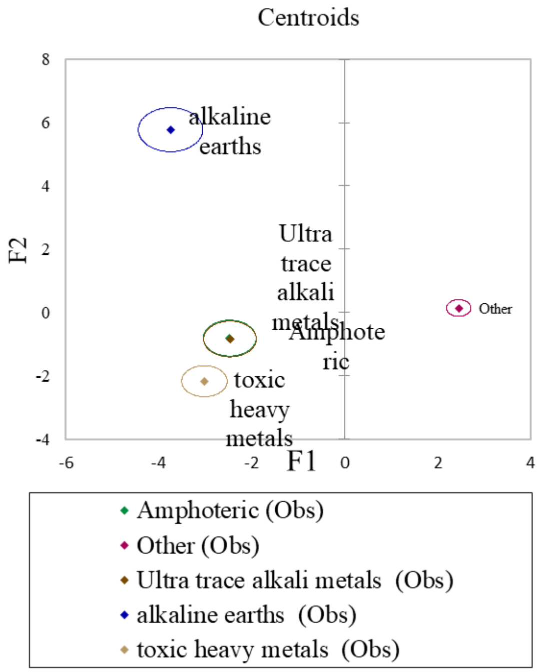


5a 5b

Fig. 5. a) Observation of Discriminant Analysis in Long Bien, Chuong My, Dan Phuong and Me Linh soil using "areas" as observation; b) Centroid of 4 different area

Source: Authors' own elaboration





6a 6b

Fig. 6. a) Observation of Discriminant Analysis in Long Bien, Chuong My, Dan Phuong and Me Linh soil using "metal groups" as observation; b) Centroid of metal groups

Source: Authors' own elaboration

als (UTAM) showed no significant variation in content and were located closest to F1 (Figure 6b). Within the alkali trace metals, Li, Rb, and Cs are classified as UTAM, which have been reported to exhibit regional geographical characteristics (Trace element geochemistry) (Sobolev and Karkach, 2019; Muller et al., 2010; Anke et al., 2005; Prasad, 2008).

Observation of UTAM content by months and weeks during growing time

To understand the dynamic changes of UTAM content during pak choi growth, Dan Phuong and Me Linh districts were selected due to their continuous cultivation throughout the year. Multivariate analysis of variance (MANOVA) was applied, with UTAM content in pak choi and soil over three months as the dependent variables. The independent variables included "month," "location," and UTAM "element" in pak choi edible parts, roots, and soil (Figure 7).

In MANOVA, the null hypothesis (H_0) stated that "the variable or the interaction of the corresponding factor has no significant effect on the dependent variables," whereas the alternative hypothesis (H_a) stated that "the variable or the interaction of the corresponding factor has a significant effect on the dependent variables." The p-values for the variables "location" and "element" in pak choi edible parts, roots, and soil were all less than 0.05, leading to rejection of H_0 (Figure 7b, 7d, and 7f). Thus, at the 95% confidence level, "location" and "element" had significant effect on the average UTAM content in each pak choi part and soil. In Figure 7d, the p-value for the "month" variable was below the significance level ($\alpha = 0.05$), indicating a significant effect of "month" on the average UTAM content of pak choi roots. In contrast, the p-values for "month" in pak choi edible parts and soil were greater than 0.05, indicating no significant effect on average UTAM content in these samples (Figure 7b and 7f).

Figure 8 shows the MANOVA results, with the dependent variable was the average UTAM content in pak choi parts and soil from two districts (Dan Phuong and Me Linh; three locations per district) over five consecutive growth weeks. The independent variables were "week," "location," "part" of pak choi, and UTAM elements in pak choi edible parts, roots, and soil.

MANOVA results showed that the p-values for the variable "week" in pak choi edible parts, roots, and soil were all less than 0.05 (Figure 8b, 8d, 8f). Thus, at the 95% confidence level, "week" had a significant effect on ultra-trace alkali metal (UTAM) content in pak choi edible parts, roots, and soil. Therefore, to observe changes in metal content across different growth stages, the average metal content for each week should be considered.

UTAM content in pak choi parts and soil

UTAM content in pak choi soil

Cesium and lithium contents in pak choi soils per week ranged from approximately 0.1 to 1.6 mg kg^{-1} DW (dry weight), while the average rubidium content per week was much higher, ranging from 20 to 100 mg kg^{-1} DW (Figure 9a and 9b). Differences in UTAM content were observed between the two districts, Dan Phuong and Me Linh, likely due to different farming practices; however, no significant differences were found between locations within the same district (Figure 9a and 9b). Therefore, ultra-trace alkali metals in soil have potential as markers for authenticating pak choi origin at the district level.

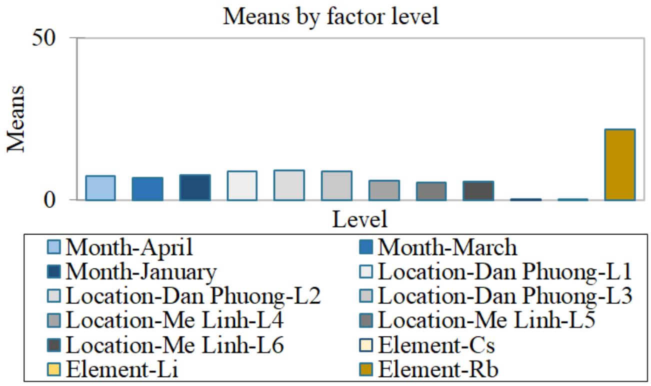
During the five weeks of growth, the average Cs content in soils from locations within the same region (Dan Phuong or Me Linh) varied only slightly, whereas Rb content in soils gradually decreased over time – from approximately 100 mg kg^{-1} DW to 30 mg kg^{-1} DW in Dan Phuong and from 40 mg kg^{-1} DW to 10 mg kg^{-1} DW in Me Linh. This decrease can be explained by the accumulation of Rb in pak choi roots over the growing period. Pak choi, a plant of the genus *Brassica*, has long been recognized for its ability to accumulate metals, making it useful for phytoextraction due to its tolerance of high levels of heavy metals (Baczek-Kwinta et al., 2011; Szczyglowska et al., 2011).

UTAM content in pak choi root

Figure 10a shows a consecutive increase in the average Cs and Li contents per week in Dan Phuong pak choi roots. In contrast, in Me Linh, the average Cs and Li contents in roots reached a maximum in the second week (approximately 0.5 mg kg^{-1} DW for Cs and 2.5 mg kg^{-1} DW for Li) and then slightly decreased until the end of the growing period. Overall, the weekly average Li and Cs contents in roots were low, ranging from 0.05 to 3.2 mg kg^{-1} DW, and did not exhibit consistent trends across growth stages. In the final week, the average Cs and Li contents in pak choi roots differed markedly between the two districts. In Dan Phuong, Cs and Li were approximately 0.5 mg kg^{-1} DW and 2.5 mg kg^{-1} DW, respectively, whereas in Me Linh both values were below 0.5 mg kg^{-1} DW. This difference can be attributed to the differences fertilization methods applied during the second week of growth (see Section 3.1). In Figure 10b, the average Rb content per week in pak choi roots from both districts increased steadily over the five-week growth period, from approximately 5 mg kg^{-1} DW to 20 mg kg^{-1} DW.

UTAM content in pak choi edible part

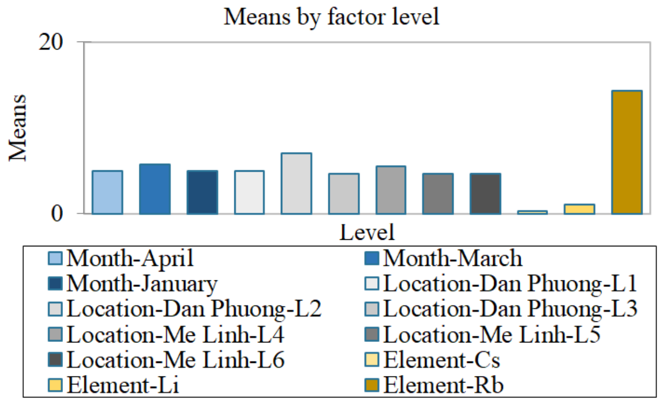
The average Rb content in pak choi edible parts was higher, whereas Li and Cs contents were very low (Figure



a)

	Month	Location	Element
Lambda	0.971	0.692	0.055
F Observed values	2.285	13.514	1308.438
DF1	2	5	2
DF2	152	152	152
F Critical value	3.056	2.274	3.056
p-value	0.105	<0.0001	<0.0001

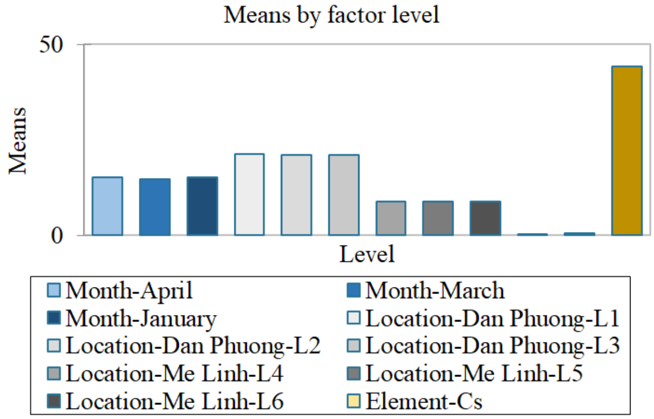
b)



c)

	Month	Location	Element
Lambda	0.954	0.801	0.066
F Observed values	3.643	7.546	1082.092
DF1	2	5	2
DF2	152	152	152
F Critical value	3.056	2.274	3.056
p-value	0.028	<0.0001	<0.0001

d)



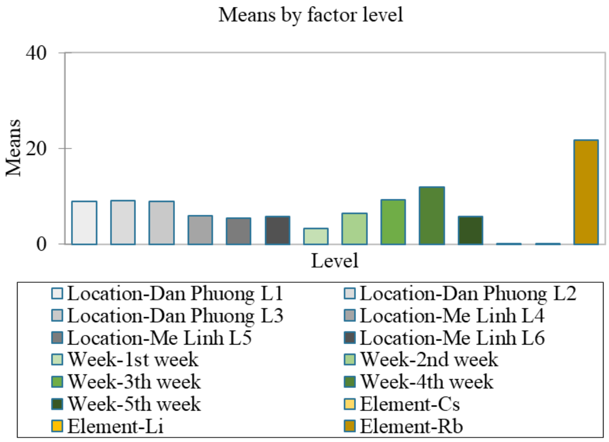
e)

	Month	Location	Element
Lambda	0.999	0.667	0.150
F Observed values	0.060	15.185	429.629
DF1	2	5	2
DF2	152	152	152
F Critical value	3.056	2.274	3.056
p-value	0.942	<0.0001	<0.0001

f)

Fig. 7. a) Mean by factor lever of MANOVA with the average of UTAM content of the month as the dependent variable on pak choi edible part; b) Wilks' test on MANOVA on pak choi edible part; c) Mean by factor lever of MANOVA with the average of UTAM content of the month as the dependent variable on pak choi root; d) Wilks' test on MANOVA on pak choi root; e) Mean by factor lever of MANOVA with the average of UTAM content of the month as the dependent variable on pak choi soil; f) Wilks' test on MANOVA on pak choi soil

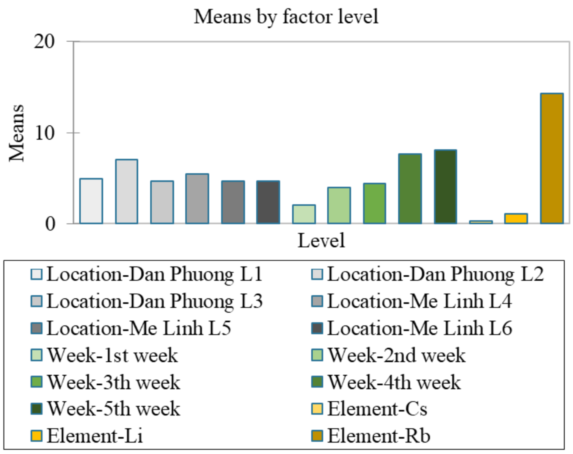
Source: Authors' own elaboration



a)

	Location	Week	Element
Lambda	0.940	0.825	0.288
F Observed values	0.996	4.143	96.408
DF1	5	4	2
DF2	78	78	78
F Critical value	2.332	2.489	3.114
p-value	0.426	0.004	<0.0001

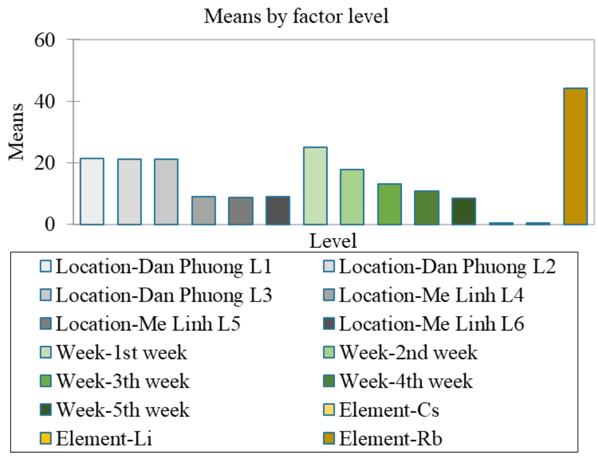
b)



c)

	Location	Week	Element
Lambda	0.961	0.769	0.299
F Observed values	0.639	5.846	91.597
DF1	5	4	2
DF2	78	78	78
F Critical value	2.332	2.489	3.114
p-value	0.671	0.000	<0.0001

d)



e)

	Location	Week	Element
Lambda	0.809	0.820	0.272
F Observed values	3.688	4.283	104.332
DF1	5	4	2
DF2	78	78	78
F Critical value	2.332	2.489	3.114
p-value	0.005	0.003	<0.0001

f)

Fig. 8. a) Mean by factor lever of MANOVA with the average of UTAM content of the growth week as the dependent variable on pak choi edible part; b) Wilks' test on MANOVA on pak choi edible part; c) Mean by factor lever of MANOVA with the average of UTAM content of the growth week as the dependent variable on pak choi root; d) Wilks' test on MANOVA on pak choi root; e) Mean by factor lever of MANOVA analyzing with the average of UTAM content of the growth week as the dependent variable on pak choi soil; f) Wilks' test on MANOVA on pak choi soil

Source: Authors' own elaboration

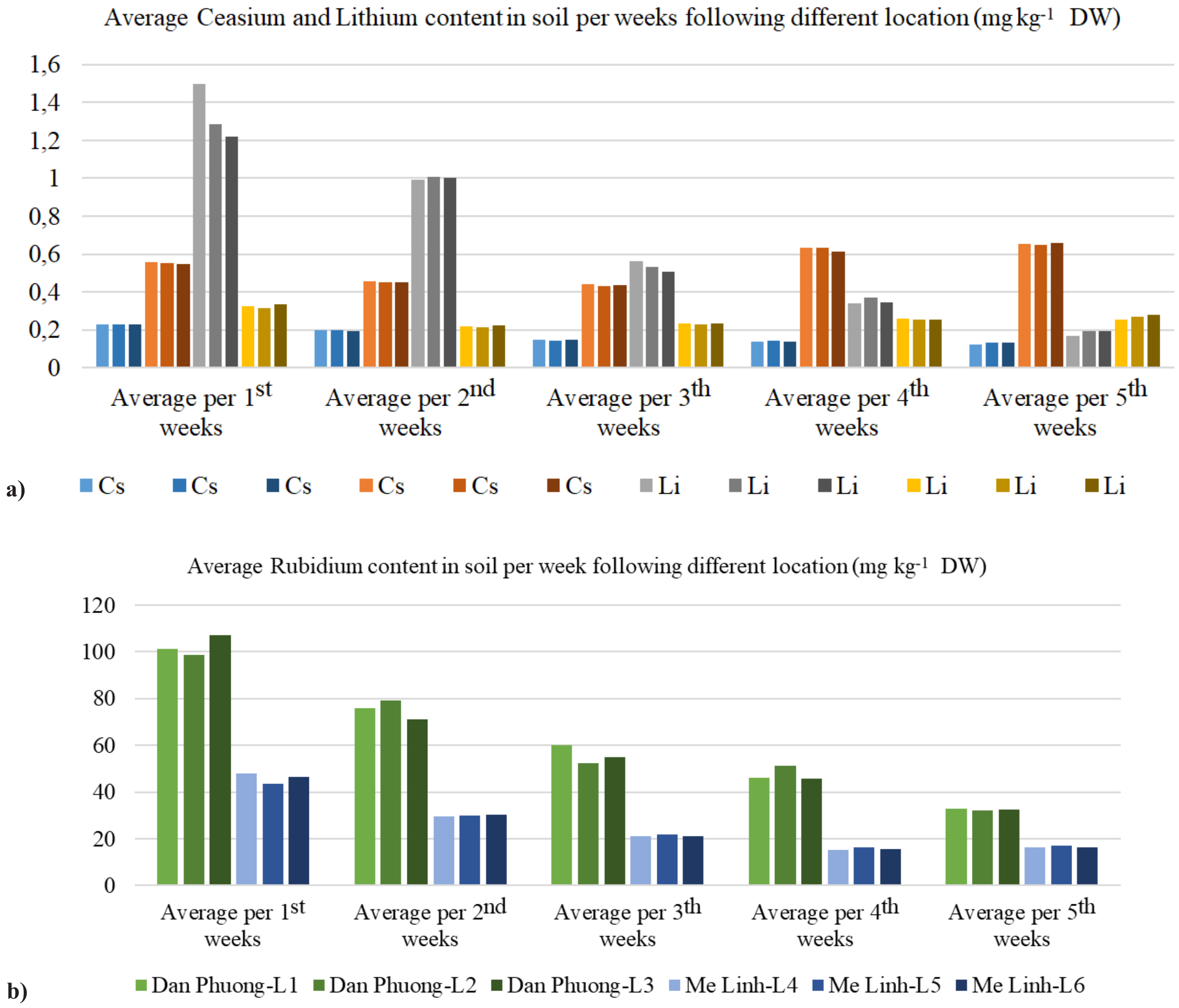


Fig. 9. a) Average Cs and Li content in soil per week following different location (mg kg^{-1} DW); b) Average Rb content in soil per week following different location (mg kg^{-1} DW)
Source: Authors' own elaboration

11), consistent with the trends observed in roots from both Dan Phuong and Me Linh districts. In Dan Phuong, Cs and Li contents in edible parts increased steadily until the final week. In contrast, in Me Linh, average Cs and Li contents in edible parts increased sharply during the second week and then decreased toward the end of the growing period.

The average Rb content in pak choi edible parts from both districts increased steadily and appeared less affected by fertilization method (Figure 11b). Unlike Cs and Li, whose average contents in edible parts were very low from the first growth week (approximately 0.1 mg kg^{-1} DW in both districts), the average Rb content already reached 10 mg kg^{-1} DW in the first week and remained relatively stable in both edible parts and roots throughout the growing period in both districts.

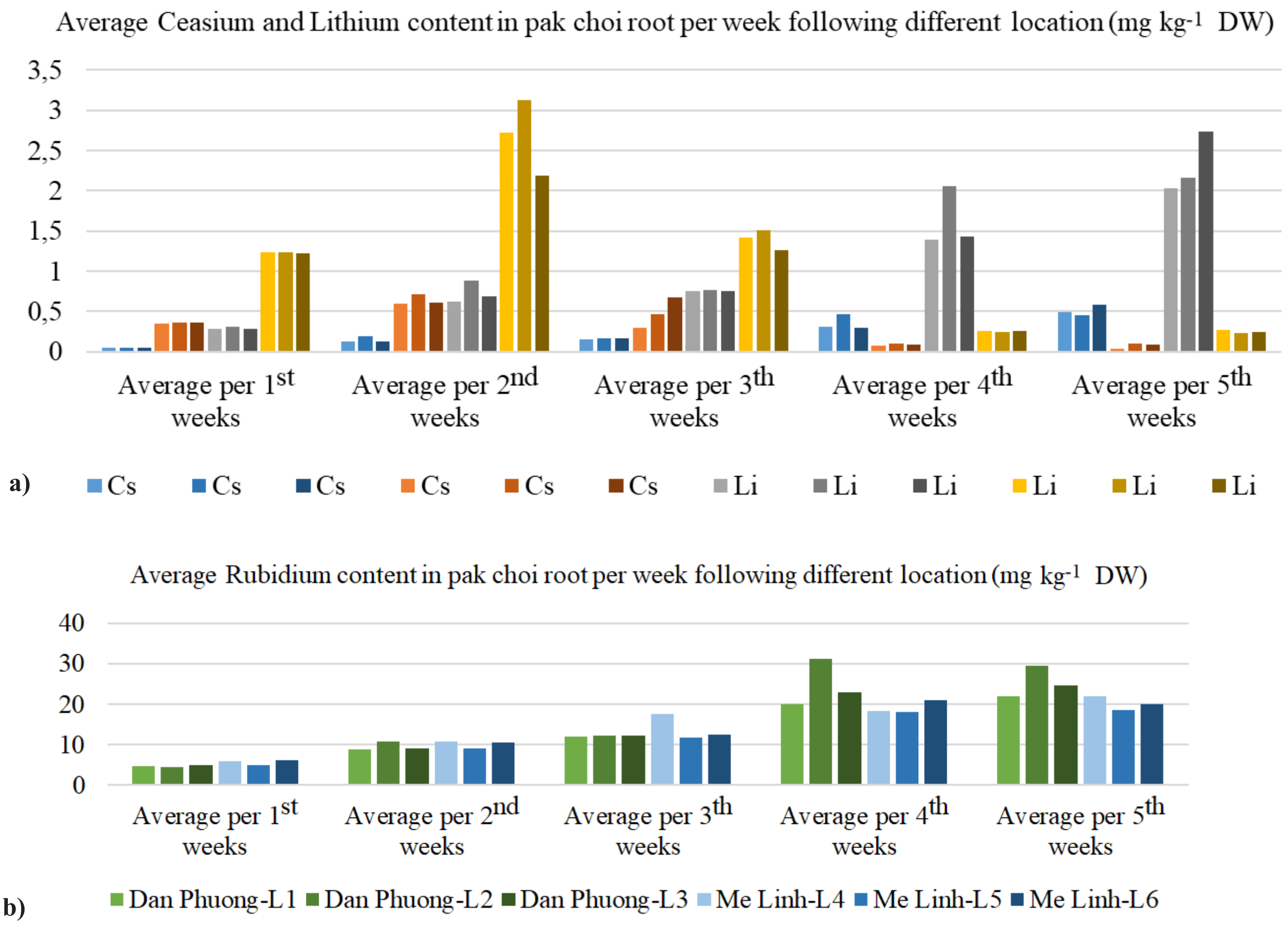


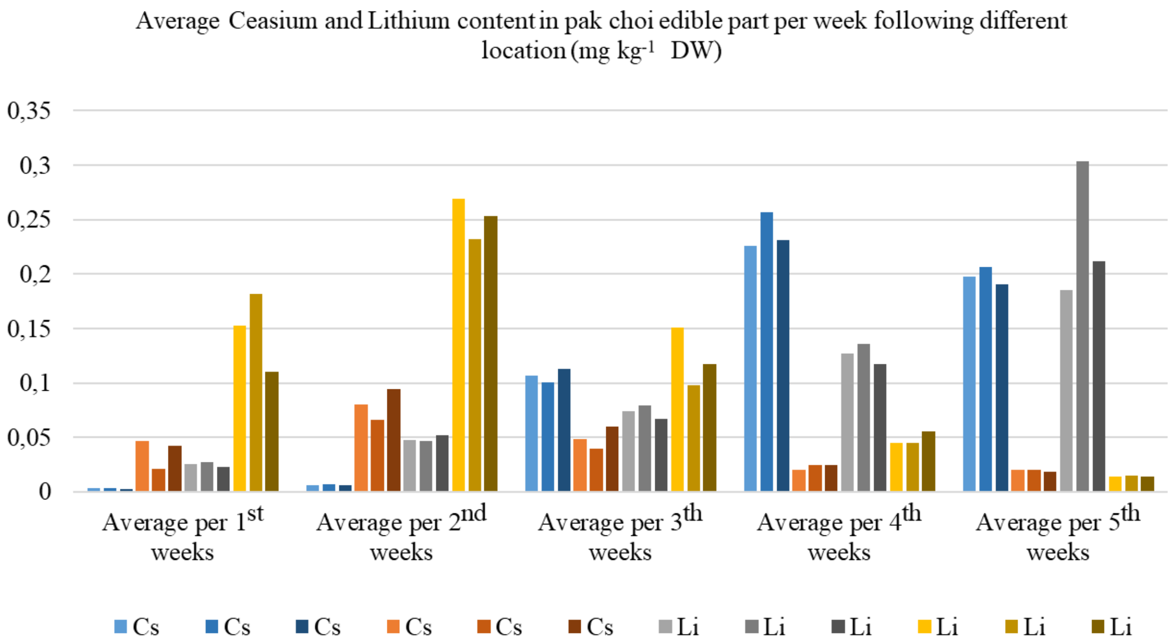
Fig. 10. a) Average Cs and Li content in pak choi root per week following different location (mg kg^{-1} DW); b) Average Rb content in pak choi root per week following different location (mg kg^{-1} DW)
Source: Authors' own elaboration

Dynamic of UTAM – Rb accumulation during pak choi growing

Figure 12 shows the dynamics of Rb content in pak choi parts and soil from Dan Phuong and Me Linh districts over five growth weeks. In Dan Phuong, the average Rb content in pak choi edible parts increased from 10.32 mg kg^{-1} DW to 47.11 mg kg^{-1} DW during the first four weeks and then decreased to 30.20 mg kg^{-1} DW in the final week. In Me Linh, the average Rb content in edible parts increased from 9.01 mg kg^{-1} DW to 19.35 mg kg^{-1} DW, likely due to foliar fertilization, then slightly decreased to 16.89 mg kg^{-1} DW in the third week before stabilizing at 20.38 mg kg^{-1} DW.

The average Rb content in pak choi roots increased over time in both districts, from 4.72 mg kg^{-1} DW to 25.26 mg kg^{-1} DW in Dan Phuong and from 5.66 mg kg^{-1} DW to 20.18 mg kg^{-1} DW in Me Linh. In Me Linh, after foliar fertilization during the second growth week, the average Rb content in edible part decreased while it increased in root. In contrast, in Dan Phuong, the average Rb content of every pak choi part showed a steady increase. This pattern indicates the translocation of Rb from edible parts to roots in pak choi.

Rb was absorbed from the soil into the roots and subsequently translocated to the edible parts in both districts. In the first week, the average Rb content in Dan Phuong soils (102 mg kg^{-1} DW) was approximately twice that in Me Linh soils (45.93 mg kg^{-1} DW). Soil plowing after harvest contributed to the stability of Rb content during the



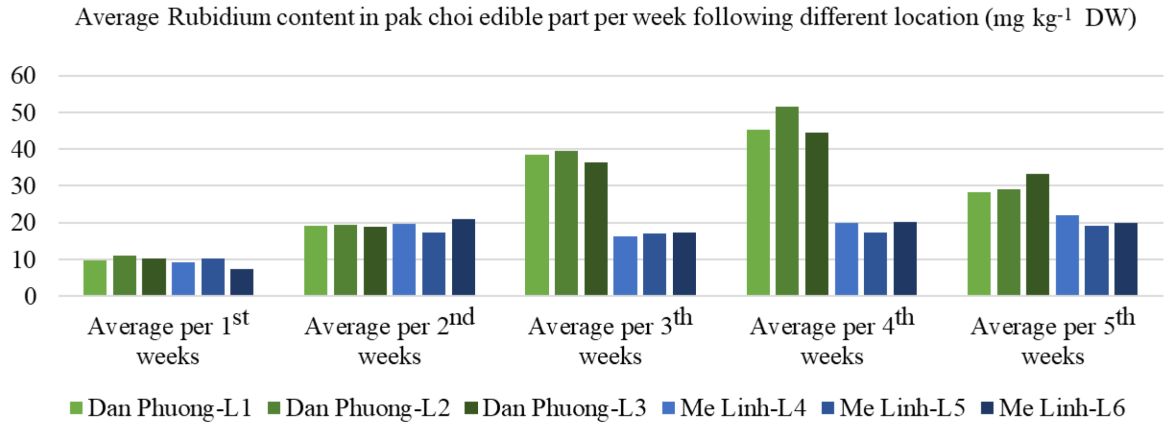


Fig. 11. a) Average Cs and Li content in pak choi edible part per week following different location (mg kg^{-1} DW); b) Average Rb content in pak choi edible part per week following different location (mg kg^{-1} DW)
Source: Authors' own elaboration

first week. Rubidium is known to be absorbed by plants in a manner similar to potassium, utilizing the same transport mechanisms (Baligar and Barber, 1978). According to Hara et al. (1977), Rubidium and Sodium had a good influence on the *Brassica oleracea*'s growth under hydroponic conditions (Hara et al., 1977). The average Rb content in pak choi parts during the first week was similar between the two districts, with approximately 10 mg kg^{-1} DW in edible parts and 5 mg kg^{-1} DW in roots. However, in the final growth week, the average Rb content in pak choi parts and soil from Dan Phuong was higher than that from Me Linh. This difference can be attributed to variations in soil metal composition, environmental conditions, and farming practices. Due to the distinct Rb content in the soil of each district, pak choi exhibited stable accumulation patterns in both edible parts and roots during the final growth stage. These findings support the potential use Rb as a biomarker for the geographical authentication of pak choi. A. M. El-

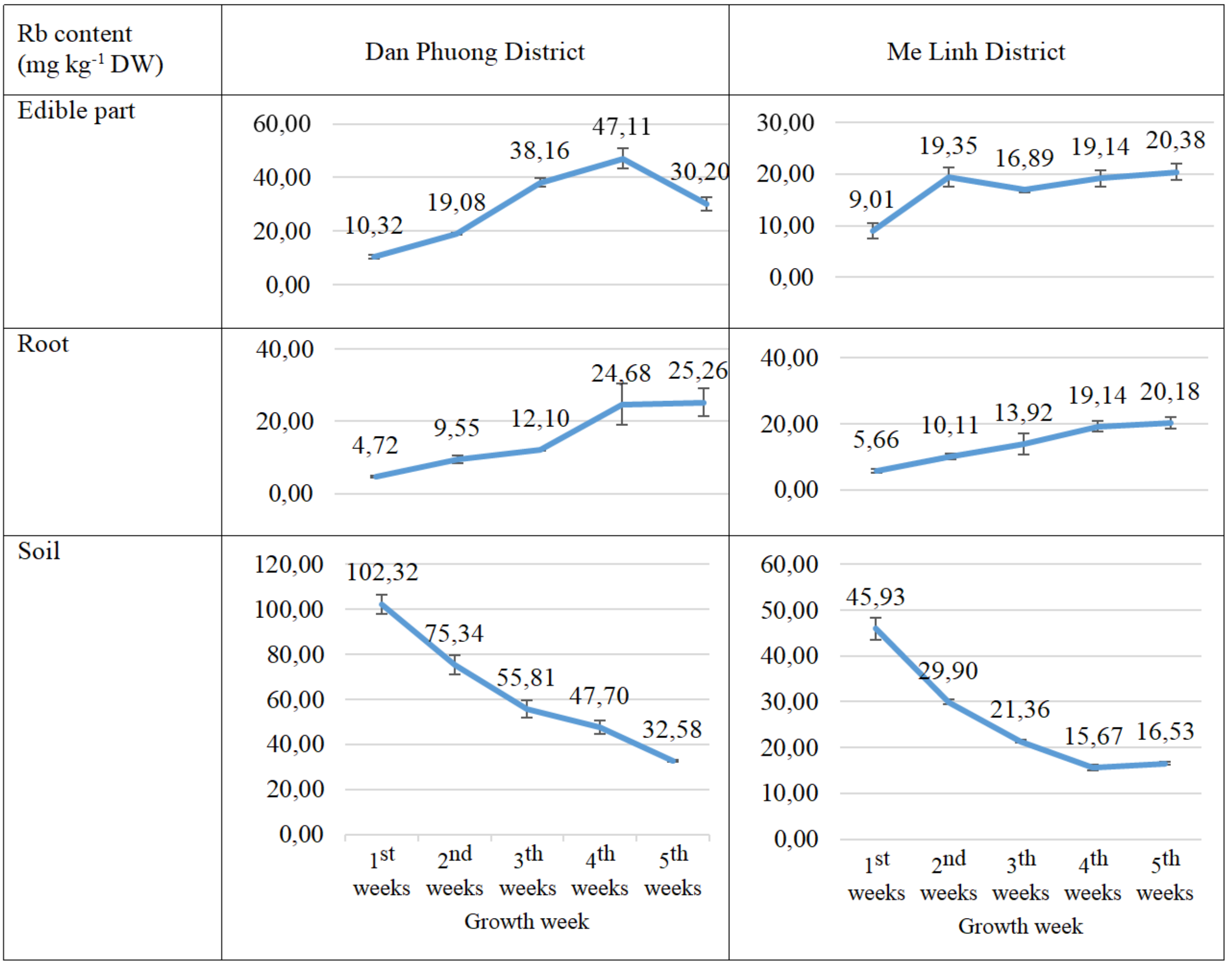


Fig. 12. Rb accumulation in pak choi parts and soil during 5 weeks of growing in Dan Phuong and Me Linh districts
Source: Authors' own elaboration

Sheikh also reported that rubidium plays an important role in plant growth and can serve as a biomarker when assessing the origin of agricultural products (El-Sheikh, 1967).

Conclusion

Considering the UTAM content in pak choi roots, edible parts, and soil over five growth weeks across three consecutive crops at six cultivation locations in two districts (Dan Phuong and Me Linh), a clear similarity was observed in the average metal content by week within the same district. Among the three ultra-trace alkali metals (Cs, Li, and Rb), the average Rb content in pak choi parts and soils remained relatively stable during each growth week. In the first week, the average Rb content in edible parts and roots was similar in both districts, approximately 10 mg kg^{-1} DW and 5 mg kg^{-1} DW, respectively. By the final growth week, the average Rb content in Dan Phuong reached 30.20 mg kg^{-1} DW in edible parts and 25.26 mg kg^{-1} DW in roots, whereas in Me Linh it was 20.38 mg kg^{-1} DW in edible parts and 20.18 mg kg^{-1} DW in roots. The average Rb content in soil during the first week in Dan Phuong (102 mg kg^{-1} DW) was approximately wice that in Me Linh (45.93 mg kg^{-1} DW). These results indicate that Rb, as an ultra-trace alkali metal, could serve as a reliable biomarker for the geographical authentication of pak choi.

Acknowledgments

This research was supported by the Center for High Technology Research and Development, Vietnam Academy of Science and Technology, and Department of Food Engineering, Hanoi University of Science and Technology.

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Received: **March, 06, 2024**; *Approved*: **February, 04, 2025**; *Published*: **June, 2026**