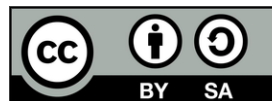




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Research Article

Relation of the irrigation water's metal content and geographical cultivation area of *Brassica rapa* subsp. *chinensis* growing in Northern Vietnam

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Abstract

Metal content in irrigation water and in edible part of pakchoi harvested from Chuong My, Dan Phuong and Me Linh districts, which are the main vegetable growing area for Ha Noi market, was investigated. Principal component analysis (PCA) was used to observe the metal content in the edible part of pakchoi and in the irrigation water as principal component (PC) 1 and 2. Zn, Mn, and Ba were discovered as the key metals contributing to the identification of geographical origin of pakchoi. Samples from Chuong My had high Zn content ($130.38 \pm 12.65 \text{ mg.kg}^{-1}$ DW for edible parts and $92.48 \pm 10.10 \text{ mg.L}^{-1}$ for irrigation water), samples from Dan Phuong were distinct for their high Mn content ($99.44 \pm 8.11 \text{ mg.kg}^{-1}$ DW for edible parts and $207.37 \pm 21.55 \text{ mg.L}^{-1}$ for irrigation water), and samples from Me Linh stood out for their high Ba content ($396.88 \pm 39.59 \text{ mg.kg}^{-1}$ DW for edible parts and $138.88 \pm 17.35 \text{ mg.L}^{-1}$ for irrigation water). The ratio of Ba: Mn of pakchoi's edible parts and irrigation water also played an important role in helping identify the geographical origin of pakchoi. They supported the fact that the fertilization method of Dan Phuong and Chuong My was different from that of Me Linh district.

Keywords: pakchoi, geographical origin, vegetable, edible parts, ICP - MS, Ha Noi market

Abbreviations

DW – dry weight; ICP - MS – inductively coupled plasma mass spectrometry; PC – principal component; PCA – principal component analysis; PE – polyethylene; PTFE – polytetrafluoroethylene.

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Introduction

Pakchoi (*Brassica rapa* subsp. *chinensis*) is a common vegetable grown and widely used in China and Northern Vietnam. Pakchoi is resistant to heat and humidity and is relatively resistant to soil-borne pests and diseases due to its short growing period, its yield is 10-20 tons/ha for small cultivars and 20-30 tons/ha for large cultivars (Li 1981). There are several varieties of pakchoi: Tai sai, White pakchoi, Green fortune (Kalisz et al. 2012), Dwarf carton white, Joi choi, Meiquin, etc., with different characteristics, habitats, and shapes. The pakchoi variety grown and used in Vietnam is mainly the Green Fortune due to its good disease resistance and heat tolerance (Fig. 1).



Figure.1. Green fortune pakchoi (*Brassica rapa chinensis*)

In pakchoi there are kinds of vitamins (A, B, C), minerals (Ca, Mn, K, Zn, Fe, Na, Mg, Se, P) and lots of flavonoids (carotenoids: 633 - 982 $\mu\text{g.g}^{-1}$; lutein: 17 - 28%; violaxanthin: 13%, neoxanthin made: 13%, β - carotene: 19 - 27%). Vitamin C or ascorbic acid is about 9.2% (dry season) to 13.8% (rainy season). Calcium content from 21.8 mg.g^{-1} to 36.9 mg.g^{-1} , iron from 534 $\mu\text{g.g}^{-1}$ to 644 $\mu\text{g.g}^{-1}$, zinc from 49 $\mu\text{g.g}^{-1}$ to 61 $\mu\text{g.g}^{-1}$, glucosinolate content from 12.2 to 21.0 $\mu\text{mol.g}^{-1}$ (Hanson et al. 2009).

Metal accumulation in plants is related to plant's characteristics and factors that transfer metals from soil to plants (Inyinbor et al. 2019; Rattan et al. 2005). Those factors may include atmospheric deposition from industrial emissions, vehicle emissions, mining waste, and agricultural waste such as fertilizers and pesticides (Smith 2009).

Metals basically cannot be biodegraded, so they last a long time in aquatic and terrestrial environments. They can be transported from soil to groundwater or absorbed by plants. The metal can then be transferred from the plant roots to the edible parts (Deng et al. 2024). Meanwhile, irrigation water often used for agriculture comes from domestic, industrial and commercial water sources (Singh et al. 2012). Therefore, metals in plants can be affected by metals in irrigation water sources, which potentially become a finger marker to support the geographical origin traceability of vegetable.

Vietnamese people consume about 30 - 50% of vegetables in their daily meals, of which pakchoi accounts for a significant portion. At the same time, pakchoi is also a popular vegetable and is easily imported from Southeast Asian countries, especially China, due to its direct border with Vietnam. As a result, vegetables and pakchoi of unknown origin have been threatening Vietnam's domestic vegetable market. This leads to the need to maintain the authenticity and ensure the quality of Vietnamese vegetable products in general and pakchoi in particular. Therefore, we conducted this study based on the urgent need to authenticate the origin of pakchoi.

Materials and Methods

Sampling and storing samples. Three districts Chuong My, Dan Phuong and Me Linh were chosen as they have agricultural cooperatives producing pakchoi to serve Hanoi's market. Pakchoi's edible parts (stems and leaves) and irrigation water were collected from Chuong My, Dan Phuong and Me Linh (Fig. 2, Table 1). Pakchoi was planted in January, February, April, September, October and December in the year 2023.

Edible parts of pakchoi (stems and leaves) were sampled in the 5th week of growth and dried at 70°C to 10 - 11% moisture content in a drying cabinet. All samples were then ground into powder (<400 μm particle size) with pulverize before storing in clean polyethylene zip bags. After that, samples' bags were stored in a dry place.

Irrigation water was collected from ponds, lakes or any water source which was used for irrigation depending on each cultivation method of each district.

Water was collected at the same time of pakchoi sampling (6 samples). Irrigation water was then stored in a PE bottle and kept in a dry place before being digested.

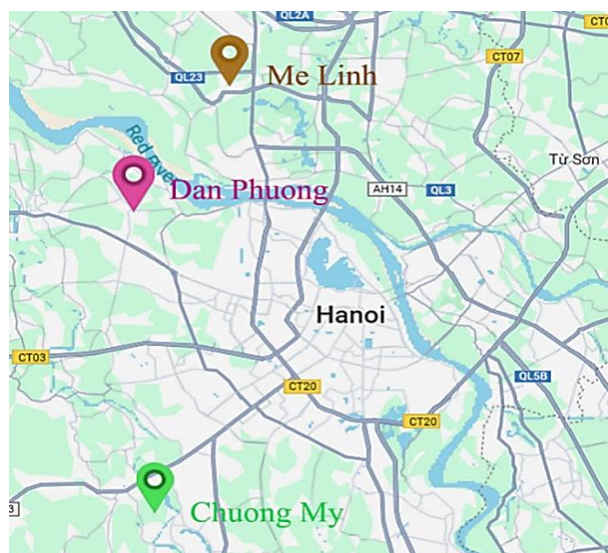


Figure 2. Pakchoi and water sample's location

Metal content has been determined in the edible part of pakchoi using the ICP - MS method, as described with following procedures.

Table 1. Locations of pakchoi sampling

	Pakchoi	Pakchoi's Irrigation water
Chuong My	20°51'45.6"N	20°51'45.7"N
	105°39'40.0"E	105°39'40.3"E
	20°51'55.3"N	20°51'53.8"N
	105°39'25.9"E	105°39'26.0"E
	20°52'02.0"N	20°52'01.7"N
Dan Phuong	105°39'40.0"E	105°39'40.6"E
	21°05'06.0"N	21°05'06.3"N
	105°42'22.3"E	105°42'22.2"E
	21°05'08.9"N	21°05'07.3"N
	105°42'25.4"E	105°42'26.1"E
Me Linh	21°05'11.8"N	21°05'12.2"N
	105°42'24.2"E	105°42'24.3"E
	21°10'19.7"N	21°10'19.7"N
	105°45'38.3"E	105°45'38.8"E
	21°10'27.6"N	21°10'27.5"N
	105°45'31.7"E	105°45'32.3"E
	21°10'36.4"N	21°10'37.6"N
	105°45'39.9"E	105°45'38.9"E

Digestion of samples. *Pakchoi's edible parts samples:* After measuring absolute moisture content, pakchoi's samples (0.1 g) were pre-digested for at least 24 h with 4 mL of HNO₃ (65%) and 2 mL of H₂O₂ (30%) before being digested with Microwave Digestion System – MARS 6. The digestion program of pakchoi's edible part is shown in Fig. 3.

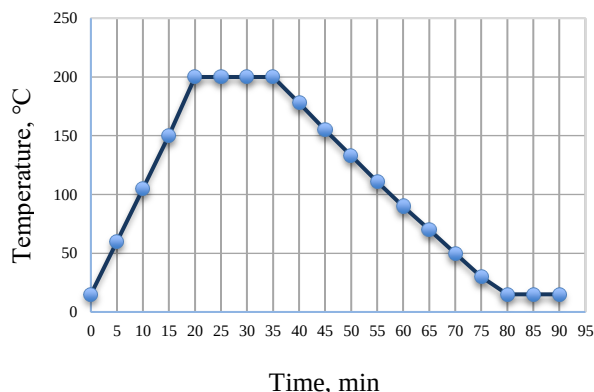


Figure 3. Heating program of Mars 6 Microwave

Irrigation water samples. 50 mL of samples was added in a PTFE vessel with 2 mL H₂O₂, 4 mL HNO₃ 65% and let pre-digested for at least 24 h in a laboratory hood. After 24 h, samples were filtered and made up to 50 mL in a volumetric flask. The metal contents of samples then can be analysed with ICP - MS.

Acid nitric 65% (HNO₃) and hydroperoxide 30% (H₂O₂) were from Merck, USA. Ultrapure deionized water (18.2 m.Ω.cm) was taken from the water filtration system Milli - Q plus (Millipore, Bedford, MA, USA).

Metal content analysis. ICP - MS is an analytical method that uses mass spectrometry combined with inductively coupled plasma to determine the quantitative and qualitative elements in a sample. This method is especially effective in analysing elements at trace (very small) concentrations. In the food industry, it is widely used to determine the content of elements, especially metal elements thanks to its accuracy at the micro-level, helping to assess food safety and ensure product quality (Bakardzhiyski 2023; Mazarakioti et al. 2022).

Metal contents of digested samples were determined using ICP - MS equipment. Standard set of ICP - MS analysis included Li, Bo, Mg, Al, Ti, V, Cr, Mn,

Fe, Co, Ni, Cu, Zn, As, Rb, Sr, Nb, Mo, Sb, Cs, Ba, Hg, Tl, Pb, Bi (TraceCert).

The instrumental detection limit in this study was calculated as follows:

$$IDL = \frac{3 \times SDb\text{blank} \times Cx}{Sx - S\text{blank}} \quad (1)$$

Where:

SDblank - is the drift titration of multiple blank samples (using 2% ultrapure nitric acid);

Cx - is the average signal used for the standard;

Sx - is the signal for Cx;

Sblank - is the signal for the blank.

The metal content of edible part sample was calculated as equation below:

$$\text{Con. (mg.kg}^{-1}) = \frac{C \times V}{W \times S} \quad (2)$$

Where:

C - Value obtained from the device (ppb or mg/L);

V - Volume of sample after digestion (L);

W - Wet sample weight (kg);

S - As % dry matter/100 % dry matter = (g absolute dry sample)/ (g sample) $\times$ 100.

The metal content of irrigation water was calculated by the following equation:

$$\text{Con. (}\mu\text{g.L}^{-1}) = \frac{C \times V_f \times DF}{V} \quad (3)$$

Where:

C: Value obtained from the device (ppp or $\mu\text{g.L}^{-1}$);

Vf: Sample volume after digestion (mL);

V: Initial quantitative sample (mL);

DF: Dilution factor.

Principal components analysis. Principal component analysis is a method of reducing the dimensionality of multivariate data while retaining most of the original data. In the food field, PCA is widely used in processing food sensory data (Ghosh & Chattopadhyay 2012; Ervina 2024), authenticating food origins (Rodionova et al. 2024), etc. It transforms the data into a new coordinate system such that the new set of variables, the principal components, are linear functions of the original variables (Dimitar 2018).

The data entered the PCA analysis were the metal concentrations from each month at each sampling location with coordinates shown in Table 1. Data

was processed using Principal Components Analysis (PCA) and Microsoft Excel 2023 upgraded with XLSTAT software version 2024.

Results and Discussion

Metal marker in the pakchoi's edible parts. The results of PCA analysis are presented in Fig. 4a, 4b, 4c. The Scree plot of the eigenvalues F1 to F17 can be seen in the Scree plot (Fig. 4a). The first two components account for a total of 76.10% and 22.63% of the sample variation, which is the most important information of the dataset with significant reliability.

The differences of the sample groups according to their locations can be seen in the Observation plot (Fig. 4b) and the Biplot plot of the PCA of the edible parts of pakchoi for 6 separate months in 2023 (Fig. 4c). The Biplot chart showed that the edible portion's metal content varied significantly depending on the growing district. The metals Mo, Zn, As, V, Al, Fe, Cs, and Sb are found in the edible parts of pakchoi from Chuong My district; Li, Co, Sr, Tl, and Mn are found in Dan Phuong's; and Ni, Ti, Ba, Cu, Rb, Nb, and Pb are found in Me Linh's. The average amount of metal described in the edible portions of pakchoi in the districts of Chuong My, Dan Phuong, and Me Linh in 2023 can be seen in Table 2.

According to Vietnam's technical regulation on the limits of heavy metal contamination in food, the contamination limit in cruciferous vegetables for As is 1.0 mg.kg⁻¹ DW (dry weight), for Cd is 2.5 mg.kg⁻¹ DW, and for Pb is 13 mg.kg⁻¹ DW. Thus, the heavy metal contents of As, Cd, and Pb of edible parts of pakchoi in the districts were all within the permissible threshold.

Table 2 shown that samples from Chuong My have more Fe, Zn, and Al with more prominent content than samples from other locations. The Fe results of Chuong My are quite similar to the results of author Hanson and colleagues, while the results of Zn are much lower (Hanson et al. 2009). These metals have long been believed to be easily absorbed from the soil to the leaves and stems during their growth.

On the contrary, the samples from Me Linh area have a high copper content. The copper content in this vegetable is similar to the average copper content of soil in the Me Linh area in the research

of Dang. (Dang et al. 2022). However, the content of other metals in the edible part and the soil is different. In particular, Pb has a fairly high content in the soil, which leads to a high content in the edible part of the vegetables grown here, but still within the permissible level.

As for Dan Phuong, Li, Mn, and Sr metals are found in abundance. These metals help Dan Phuong to lie separately from the other two regions on the 2 - dimensional plane constructed from PCA.

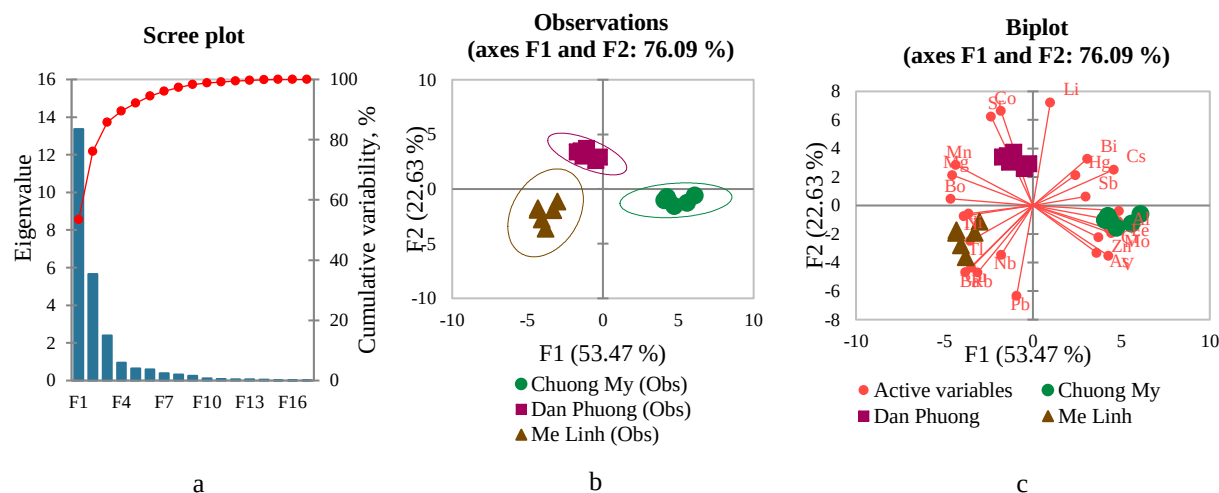


Figure 4. Scree plot chart (a), Observations chart (b), and Biplot chart (c) of PCA on average metal content of Pakchoi's edible parts in 2023

Table 2. Average content of characterized metal in pakchoi's edible parts in Chuong My, Dan Phuong and Me Linh districts, 2023 (average of 6 months in 2023, mg.kg⁻¹ DW)

Chuong My		Dan Phuong		Me Linh	
Element	Content	Element	Content	Element	Content
Mo	5.12±0.8	Li	1.07±0.06	Ni	5.14±1.25
Zn	130.38±12.65	Co	1.13±0.13	Ti	59.64±4.86
As	0.83±0.07	Sr	108.6±9.08	Ba	396.88±39.59
V	11.2±1.14	Tl	0.06±0.01	Cu	29.22±3.11
Al	4517.04±154.57	Mn	99.44±8.11	Rb	26.22±2.47
Fe	5115.08±330.91			Nb	1.72±0.14
Cs	0.41±0.03			Pb	9.26±1.64
Sb	1.09±0.11				

Metal marker in the irrigation water of pakchoi.

The metal content in the irrigation water is illustrated in Fig. 5a, 5b, 5c. As can be seen in the Scree Plot and Observations charts (Fig. 5a, Fig. 5b), the first 2 components accounted totally for more than 80.89% of the sample variation which were of the most important information of the dataset and the PCA was significantly reliable. The different of sample groups corresponding to their

locations are seen on the Observations (Fig. 5b) and Biplot plots of the PCA (Fig. 5c). Fig. 5c showed that the irrigation waters of Chuong My, Dan Phuong and Me Linh were characterized by the metal Pb, Cu, Zn; Tl, Sb, Mn, As, Bo, Pb, Cs, V, Ti, Fe, Rb; and Hg, Bi, Ba, respectively. Table 3 showed the average content of characterized metal in Pakchoi's irrigation water following Chuong My, Dan Phuong and Me Linh districts in 2023.

Comparing the irrigation water of only 3 areas, using PCA will give us a lot of metal choices that can help distinguish the 3 areas. In principle, PCA will find the 2 - dimensional plane that gives the most information about the metal of the areas and project the areas onto that plane. The more areas, the fewer characteristic metal choices we'll get to distinguish the areas.

In Table 3, some metals appear in high concentrations in irrigation water but not in the edible part of the plant. Meanwhile, pakchoi is a kind of plant with abundant metal content (Cao et al. 2024).

Table 3. Average content of characterized metal in pakchoi's irrigation water (Chuong My, Dan Phuong and Me Linh districts in 2023; average of 6 months in 2023, mg.L⁻¹)

Chuong My		Dan Phuong		Me Linh	
Element	Content	Element	Content	Element	Content
Pb	12.07±0.86	Tl	0.13±0.02	Hg	0.11±0.01
Cu	51.21±5.94	Fe	723.71±56.25	Bi	0.12±0.01
Zn	92.48±10.10	Rb	32.69±1.87	Ba	138.88±17.35
		Sb	0.93±0.19		
		Mn	207.37±21.55		
		As	4.89±0.58		
		Bo	74.65±13.48		
		Pb	7.93±0.66		
		Cs	0.52±0.03		
		V	7.63±0.29		
		Ti	107.73±15.63		

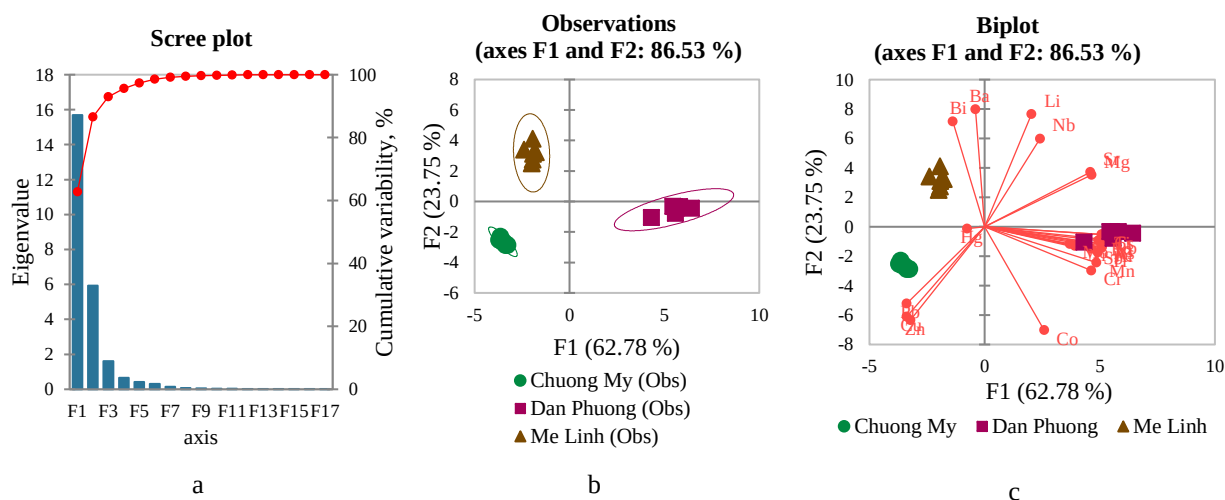


Figure 5. Scree plot chart (a), Observations chart (b), and Biplot chart (c) of PCA on average metal content of pakchoi's irrigation water by month.

Irrigation water sources do not show a metal-soil relationship when the characteristic metals of irrigation water in each area do not coincide with the characteristic metals of the edible part of the plant. This only happens when irrigation water is brought from another area or is supplied from the water plant through a pipe system that does not contact the soil in that area.

The relationship of metal content in irrigation water and in edible pakchoi plants. As shown in Table 2 and Table 3, there was 1 single characterized metal identified in both irrigation water and edible part of pakchoi for each district. These metals could play an important role in differentiating 3 districts. They are Ba, Mn, Tl and Zn representatives for Chuong My (Ba), Dan Phuong (Mn and Tl) and Me Linh (Zn) district (Table 4).

Table 4. Average content of Ba, Mn, Tl, Zn in pakchoi's edible part (mg.kg⁻¹ DW) and irrigation water in 2023 (Chuong My, Dan Phuong and Me Linh)

Sample	District	Ba	Mn	Tl	Zn	Ba: Mn
Edible parts, mg.kg⁻¹ DW	Chuong My*	20.36±0.77	44.81±3.17	0.05±0.01	130.38±12.65	1:2.22
	Dan Phuong*	49.13±5.61	99.44±8.11	0.06±0.01	103.55±6.15	1:2
	Me Linh**	396.88±39.59	94.29±9.94	0.08±0.01	103.8±11.86	--
Water, mg.L⁻¹	Chuong My*	23.02±1.59	31.76±2.36	0.01±0.01	92.48±10.10	1:1.4
	Dan Phuong*	55.83±11.19	207.37±21.55	0.13±0.02	0.09±0.02	1:3.7
	Me Linh**	20.36±0.77	44.81±3.17	0.05±0.01	130.38±12.65	--
Ratio of metal content in edible part and water	Chuong My*	1:1.13	1.41:1	--	--	--
	Dan Phuong*	1:1.13	1:2.1	--	--	--
	Me Linh**	--	--	--	--	--

* Fertilization in the soil in the 2nd week after seeding

** Fertilization on the leaves in the form of spray irrigation in the 2nd week after seeding

Thallium (Tl) accounted for an insignificant content compared to the remaining 3 metals (Table 4). Meanwhile, Ba, Mn, and Zn all were presented in the leaves (edible part) of pakchoi and irrigation water. The barium content in the irrigation water of Me Linh district was the highest (138.88±17.35 mg.L⁻¹), followed by that of Dan Phuong (55.83±11.19 mg.L⁻¹) and of Chuong My districts (23.02±1.59 mg.L⁻¹). As seen in Table 4, each of the metals Ba, Mn, Zn appeared in the highest concentrations in the irrigation water and edible part of pakchoi in each District Me Linh, Dan Phuong and Chuong My, respectively. (138.88±17.35 mg.L⁻¹ and 396.88±39.59 mg.kg⁻¹ DW; 207.37±21.55 mg.L⁻¹ and 99.44±8.11 mg.kg⁻¹ DW; 92.48±10.10 mg.L⁻¹ and 130.38±12.65 mg.kg⁻¹ DW).

Table 4 showed that Ba and Mn are two major elements in the pakchoi edible part and pakchoi irrigation water in all three districts. In Me Linh, pakchoi was fertilized on the leaves in the form of spray irrigation in the 2nd week after seeding. In Dan Phuong and Chuong My districts, pakchoi was planted in the same way, fertilization in the soil in

Acting as a cofactor for several enzymes, Mn is involved in essential plant processes (Khoshru et al. 2023; Thomine 2021). In the case of Zinc, Zn is an important micro nutrient for plants with the ability to participate in many important cellular functions such as metabolic and physiological processes, ion homeostasis and enzyme activation (Alsafran 2022). Zn participates in regular metabolic activities

the 2nd week after seeding. The fertilization method obviously affected the metal content of the pakchoi and the water as well. The ratio of Ba to Mn in edible part growing in Chuong My and Dan Phuong were similar which were 1:2.2 and 1:2; respectively and this ratio of irrigation water were 1:1.4 and 1:3.7. The ratio of Ba content in the edible part and water of that in Chuong My and Dan Phuong were the same which was of 1:1.13. Therefore, the ratio of Ba and Zn in the edible part can be used to define the growing area different from Chuong My and Dan Phuong. The ratio of Zn contents in the edible parts and water between Dan Phuong and Chuong My can be used to distinguish. Manganese is vital for plant growth and development and is thought to be involved in many physiological and biochemical processes (Alejandro et al. 2020).

Lacking of Mn can result in young leaves and branches appearing abnormally green (O'Sullivan 1997). In addition, Mn deficiency can cause lignin deficiency in plants and lead to the formation of a lethal form in the roots, reducing the ability to resist fungal attack (Rashed et al. 2021).

in the bodies of living organisms as a trace element (Sturikova et al. 2018). Besides, Zn is also an important nutrient for plants because of its ability to participate in a number of biological and chemical reactions (Noman et al. 2019).

Barium, on the other hand, is considered harmful to the growth of most plants (Monteiro et al. 2011). Ba in soil is mainly associated with soil constituents,

occurring in rock-forming minerals such as K-feldspar, micas, apatite and calcite (Madejón 2012). Barium also occurs in wastewater and agricultural fields can accumulate it over time, especially with the use of irrigation water stored in nearby ponds and lakes. In groundwater, sulphate plays an important role in the dissolution of Ba and is the dominant factor controlling Ba solubility and transport (Adams et al. 2024). Thus, all three metals Zn, Mn, and Ba, besides showing the characteristics of Pakchoi growing areas through irrigation water, have different effects on plant growth.

Conclusions

The PCA used for assessing metal content in water and in the edible part of pakchoi showed the ability to distinguish geographical regions between Chuong My, Dan Phuong, and Me Linh districts. Ba, Mn and Zn played key indicators of the difference of pakchoi's edible part and irrigation water from Chuong My, Dan Phuong and Me Linh District of Northern Vietnam.

This study also further clarified the relation of the metal composition, especially Ba, Mn and Zn as regional specific metal for Chuong My, Dan Phuong and Me Linh District in irrigation water and in pakchoi's edible parts.

Author Contributions

Conceptualization, M.T.V., T.N.M and N.T.M.T; methodology, M.T.V., T.N.M and N.T.M.T; formal analysis M.T.V., and N.T.M.T; investigation, M.T.V.; resources, M.T.V., T.N.M and N.T.M.T; data curation, M.T.V., and T.N.M; writing – original draft preparation, M.T.V., and N.T.M.T; review and editing, M.T.V., and N.T.M.T; supervision, N.T.M.T; All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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