Mineral and Trace Element Contents in Growing Stages of Microgreens

Bc. Juraj Markovič, MBA

Master's thesis
2023

Tomas Bata University in Zlín
Faculty of Technology

Univerzita Tomáše Bati ve Zlíně
Fakulta technologická
Ústav technologie potravin

Akademický rok: 2022/2023

ZADÁNÍ DIPLOMOVÉ PRÁCE

(projektu, uměleckého díla, uměleckého výkonu)

Jméno a příjmení:	**Bc. Juraj Markovič, MBA**
Osobní číslo:	**T22330**
Studijní program:	**N0721A210004 Technologie potravin**
Forma studia:	**Kombinovaná**
Téma práce:	**Obsah minerálních a stopových prvků v průběhu pěstování microgreens**

Zásady pro vypracování

I.Teoretická část
Charakterizovat technologii pěstování microgreens spolu s plodinami, které lze využít pro tento způsob pěstování. Shrnout nutriční benefity, popř. antinutriční látky, pro pěstované microgreens s důrazem na obsah minerálních a stopových prvků.
II.Experimentální část
Provést praktický pokus pěstování microgreens na vybraných semenech plodin a monitorovat v průběhu růstu obsah minerálních a stopových prvků. Využít analýzu pomocí ICP-MS.

Forma zpracování diplomové práce: **tištěná/elektronická**
Jazyk zpracování: **Angličtina**

Seznam doporučené literatury:

[1] Niroula, A et al. (2021). Pigments, ascorbic acid, total polyphenols and antioxidant capacities in deetiolated barely (Hordeum vulgare) and wheat (Triticum aestivum) microgreens. Food Chemistry 354, 129491
[2] Zhang, Y et al. (2021). Nutritional quality and health benefits of microgreens, a crop of modern agriculture. Journal of Future Foods 1-1, 58-66
[3] Sharma, S. et al. (2022). Vegetable microgreens: The gleam of next generation super Foods, their genetic enhancement, health benefits and processing approaches. Food Research International 155, 111038

Vedoucí diplomové práce: **doc. Ing. Daniela Sumczynski, Ph.D.**
Ústav analýzy a chemie potravin

Datum zadání diplomové práce: **31. prosince 2022**
Termín odevzdání diplomové práce: **12. května 2023**

L.S.

prof. Ing. Roman Čermák, Ph.D.
děkan

Ing. Robert Gál, Ph.D.
ředitel ústavu

Ve Zlíně dne 20. února 2023

PROHLÁŠENÍ AUTORA
DIPLOMOVÉ PRÁCE

Beru na vědomí, že:

- diplomová práce bude uložena v elektronické podobě v univerzitním informačním systému a dostupná k nahlédnutí;
- na moji diplomovou práci se plně vztahuje zákon č. 121/2000 Sb. o právu autorském, o právech souvisejících s právem autorským a o změně některých zákonů (autorský zákon) ve znění pozdějších právních předpisů, zejm. § 35 odst. 3;
- podle § 60 odst. 1 autorského zákona má Univerzita Tomáše Bati ve Zlíně právo na uzavření licenční smlouvy o užití školního díla v rozsahu § 12 odst. 4 autorského zákona;
- podle § 60 odst. 2 a 3 autorského zákona mohu užít své dílo – diplomovou práci nebo poskytnout licenci k jejímu využití jen s předchozím písemným souhlasem Univerzity Tomáše Bati ve Zlíně, která je oprávněna v takovém případě ode mne požadovat přiměřený příspěvek na úhradu nákladů, které byly Univerzitou Tomáše Bati ve Zlíně na vytvoření díla vynaloženy (až do jejich skutečné výše);
- pokud bylo k vypracování diplomové práce využito softwaru poskytnutého Univerzitou Tomáše Bati ve Zlíně nebo jinými subjekty pouze ke studijním a výzkumným účelům (tj. k nekomerčnímu využití), nelze výsledky diplomové práce využít ke komerčním účelům;
- pokud je výstupem diplomové práce jakýkoliv softwarový produkt, považují se za součást práce rovněž i zdrojové kódy, popř. soubory, ze kterých se projekt skládá. Neodevzdání této součásti může být důvodem k neobhájení práce.

Prohlašuji,

- že jsem diplomové práci pracoval samostatně a použitou literaturu jsem citoval. V případě publikace výsledků budu uveden jako spoluautor.
- že odevzdaná verze diplomové práce a verze elektronická nahraná do IS/STAG jsou obsahově totožné.

Ve Zlíně dne:

Jméno a příjmení studenta:

……………………………..
podpis studenta

ABSTRAKT

Teoretická část je věnována listové zelenině a semenům pro pěstování microgreens s důrazem na jejich nutriční benefit. V experimentální části byly pěstovány semena řeřichy a ředkvičky na univerzálním zahradnickém substrátu a vatě. U microgreens byly v šestém až devátém dnu růstu odebírány vzorky pro přípravu mineralizátu s následným měřením prvkového složení pomocí ICP-MS. Trendy v akumulaci jednotlivých prvků pro dané růstové medium a plodinu jsou zaneseny v grafech. Řeřicha měly nejvyšší koncentrace K, Mg, P, Mn, Zn, S, Fe, a ředkvička Mg, K, P, Mn, Fe, Zn, S. Ve vzorcích byly zaznamenány nejnižší koncentrace Cs, Ce, Y, Tl a nekteré prvky nebyly vůbec detekovány. Konzumace 100 g porce microgreens (řeřichy a ředkvičky) přispívá u žen 55 % k hodnotě RDA pro Mn, 48% pro Cu a 43% pro Mg. U mužů byly hodnoty podobné, s nižším příspěvkem. Co se týká toxických prvků pro osobu vážící 70 kg, příspěvek 100 g microgreens k hodnotě PTMI pro prvek Cd je 65 %. Pro ostatní težké kovy byly tyto hodnoty výrazne nižší. Microgreens tvoří bohatou zásobu minerálních a stopových prvků, přičemž jejich množství během procesu růstu roste a u některých prvků kolísá. Tahle práce prokázala, že microgreens pěstované na půdě jsou bohatší na obsah minerálních a stopových prvků. Pečlivý výběr růstového média a druhu microgreens, stejně jako načasování sklizně, může mít značný vliv na obsah prvků v microgreens.

Klíčová slova: microgreens, listová zelenina, ředkvička, řeřicha, minerální prvek, stopový prvek, toxický prvek, příjem živin, RDA, AI, PTWI, PTMI, ICP-MS.

ABSTRACT

The theoretical part is devoted to leafy vegetables and seeds for growing microgreens with an emphasis on their nutritional benefit. In the experimental part, garden cress and radish seeds were grown on a universal horticultural substrate and cotton wool. In the case of microgreens, samples were taken on the sixth to ninth day of growth for the preparation of the mineral with subsequent measurement of the elemental composition using ICP-MS. Trends in the accumulation of individual elements for a given growth medium and crop are plotted in graphs. Garden cress had the highest concentrations of K, Mg, P, Mn, Zn, S, Fe, and radish Mg, K, P, Mn, Fe, Zn, S. The lowest concentrations of elements were recorded in the samples Cs, Ce, Y, Tl, and some elements were not detected at all. Consumption of a 100 g portion of microgreens (garden cress and radishes) by women, contributes 55% to their RDA value for Mn, 48% for Cu and 43% for Mg. For men, the values were similar, with a lower contribution. Regarding toxic elements for a person weighing 70 kg, the contribution of 100 g of microgreens to the PTMI value for the element Cd is 65%. The values for other heavy metals were significantly lower. Microgreens are a rich source of minerals and trace elements, with their amount increasing during the growth process, for some elements fluctuating. This work proved that microgreens grown on soil are richer in mineral and trace element content. Careful selection of the growth medium and type of microgreens, as well as the timing of harvest, can have a significant effect on the elemental content of the microgreens.

Keywords: microgreens, leafy vegetables, radish, garden cress, mineral, trace element, heavy metal, dietary intake, RDA, AI, PTWI, PTMI, ICP-MS.

I would like to express my gratitude to my thesis supervisor, Dr. Ing. Daniela Sumczynski, Ph.D., for her exceptional guidance, professional mentorship, and assistance throughout the development of this work. Additionally, I would like to thank Ing. Jana Fojtíková for her help with sample processing in the laboratory and Dr. Ing. Miroslav Fišer, Csc. for his assistance with measuring samples on the ICP-MS.

I hereby declare that the print version of my Master's thesis and the electronic version of my thesis deposited in the IS/STAG system are identical.

CONTENTS

INTRODUCTION

The attractive look, distinctive flavors, and excellent nutritional content of microgreens have made them increasingly popular to grow in recent years. Due to their high vitamin, mineral, and antioxidant content, microgreens are preferred by consumers concerned about their health (Yang et al., 2020). However, the amount of minerals and trace elements in microgreens at various stages of growth is a subject on which little information is currently accessible. Therefore, this study aims to quantify essential minerals and trace elements in microgreens at various growth stages.

To better understand the nutritional value of microgreens and to help create improved growing techniques for the generation of high-quality microgreens, we examine the nutrient content of microgreens. The findings of this study will help farmers improve the cultivation of microgreens and nutrient management techniques, improving the quality and safety of the produce (Bian et al., 2017).

Furthermore, for microgreens to meet consumer nutritional demands and be suitable for human ingestion, it is crucial to know their mineral and trace element composition. Anemia, osteoporosis, and neurological illnesses can all result from the excess or deficit of specific minerals and trace elements in the human diet (Kader, 2002). To ensure that microgreens are safe and nutritious to eat, it is essential to keep an eye on their nutrient content.

To fully understand the nutritional value of microgreens and help create improved growing techniques to produce high-quality microgreens, it is crucial to investigate the levels of minerals and trace elements in microgreens in various stages of development. When making decisions on microgreen cultivation, processing, and consumption of microgreens, producers, food processors, and consumers will find the information from this study helpful (Wu et al., 2020). Scientific understanding of the nutritional value of microgreens will also benefit from this information, which will support and guide more research in the area (Miyashita et al., 2018).

I. THEORY

1 LEAFY VEGETABLES

A broad set of plants known as leaf vegetables, commonly called salad greens or potherbs, are primarily consumed fresh, uncooked as salads, or cooked as ingredients in various recipes (Paine et al., 2012). They are distinguished by their rapid growth and high rates of photosynthetic activity, as well as their edible leaves, which are generally thin and flat (Bian et al., 2020). Due to their excellent nutritional value and numerous health advantages, leaf vegetables are widely consumed worldwide (Rajput et al., 2018). The capacity of leafy vegetables to efficiently collect and utilize light energy through photosynthesis is a defining characteristic of their physiology. These plants have chloroplasts in their leaves, which are organelles that house chlorophyll, the pigment necessary for photosynthesis (Bian et al., 2020). As a result of their high photosynthetic rate and generally high leaf area index, which measures the total leaf surface area per unit of ground area, leaf vegetables can effectively create organic matter from carbon dioxide and water (Paine et al., 2012). The form, texture, and color of the leaves can be used to categorize leaf vegetables. For instance, although some leaf vegetables have smooth or waxy leaves, others have curly or frilly leaves. Some are green, while others have purple or crimson leaves (Bian et al., 2020). Leaf vegetables may also have a wide range of flavors and aromas, with some having a light and delicate flavor and others more pungent or unpleasant (Paine et al., 2012). These factors can be used to classify leaf vegetables.

1.1 Types of leafy vegetables

According to the Agricultural Research Service, Department of Agriculture in the U.S., leaf vegetables can be broadly classified into four classes:

- cooking greens,
- salad greens,
- herbs,
- microgreens.

As the name implies, **cooking greens** are leafy vegetables that are usually prepared before eating. Its harsher flavor, ranging from mildly bitter to delightfully earthy, and rougher texture are frequently used to describe them. Kale, collard greens, and Swiss chard are a few varieties of cooking greens (USDA, 2021).

A common kind of leafy vegetables that are often eaten uncooked in salads or sandwiches are **salad greens**. They are frequently distinguished by their delicate texture and mild taste. Salad greens include species such as lettuce, arugula, and spinach (USDA, 2021).

Herbs are a special type of leafy vegetable that is typically used in cooking as flavorings. They are frequently distinguished by their potent flavor and pungent scent. Herbs such as basil, cilantro, and parsley are examples (USDA, 2021).

A relatively new kind of leafy vegetables that has recently grown in popularity are **microgreens**. They are frequently used as a garnish or added to salads for more taste and nutrients. They are typically gathered when the plants are still young, only a few inches tall. Sprouts, arugula, and radish greens are examples of microgreens (USDA, 2021).

1.2 Importance of leafy vegetables in human nutrition

Leafy vegetables are an essential component of a healthy diet because they offer a variety of nutrients, including fiber, vitamins, and minerals, that are necessary to maintain good health (Zhou et al., 2020). According to Rajput et al. (2018), they have high concentrations of vitamins C, and K, as well as minerals, including iron, calcium, potassium, magnesium, zinc, and copper, which are necessary for various body processes. For instance, magnesium is essential for energy metabolism, neuron and muscle function, and bone health (Volpe, 2013). Zinc is vital for the development of red blood cells and connective tissue, while copper is required for immune system health, wound healing, and DNA synthesis (Wessells & Brown, 2012). These nutritional properties have been recognized to offer several other health advantages that support general well-being, such as reducing the likelihood of developing chronic conditions, including diabetes, certain malignancies, and cardiovascular disorders (Zhang et al., 2021; Sharma et al., 2022). A lower incidence of stroke and age-related macular degeneration has also been linked to eating green vegetables (Zhou et al., 2020). To maintain good health, consuming enough minerals is crucial, and leafy greens are a fantastic nutritional supply of several necessary elements (Rajput et al., 2018). A varied intake of the minerals and other components required for optimum health can be achieved by including leafy greens in the diet. They do improve not only our health but also the environment by encouraging sustainable farming methods. Leafy vegetables are an environmentally beneficial food source since they take less water and resources to grow than cattle (Karačić et al., 2020). In addition, green vegetables are essential for managing soil health. They may serve as soil coverings, preventing soil erosion and boosting soil organic matter (Rajput et al., 2018). In this way, they could indirectly affect the growth and farming of the other crops, providing them with better soil and therefore producing higher-quality products. Although they are an excellent source of vital nutrients, green vegetables can also contain potentially dangerous trace elements, including cadmium, lead, and arsenic (Hossain et al., 2017). These substances are found in the environment and can be absorbed by plants as they develop (Zhao, 2012). Many health problems, including an increased risk of cancer, kidney damage, and cardiovascular disease, have been associated with exposure to these elements (Satarug et al., 2010). These dangerous trace element concentrations in leafy vegetables could vary according to the soil, irrigation water, and industrial contamination (Hossain et al., 2017). As an illustration, green vegetables grown in regions with high industrial pollution levels are more likely to contain dangerous trace elements (Satarug et al., 2010). Hence, while evaluating the possible risk of leafy vegetables to human health, it is crucial to consider the source and the growing circumstances. Other than harmful trace elements, they can also contain dangerous germs, such as *E. coli*, *Salmonella*, and *Listeria*, which, if swallowed, can cause life-threatening sickness (Zhou et al., 2020). Due to this, proper food safety procedures should be applied when handling and cooking leafy greens to reduce the risk of contracting a foodborne disease. Consuming leafy greens grown in locations with low levels of industrial pollution and washing the vegetables well before eating can help consumers reduce their exposure to dangerous trace elements and pathogens (Liu et al., 2013).

Moreover, food processing methods such as boiling and blanching can reduce the amounts of hazardous trace elements and pathogens in leafy greens (Hossain et al., 2017). Using good agricultural practices (GAPs) and food safety management practices (FSMPs) is crucial to maximizing the advantages of eating leafy greens while reduces the risk of contracting a foodborne disease (Karačić et al., 2020). At every point in the food supply chain, from manufacturing to consumption, these procedures seek to reduce the risk of microbial and heavy metal contamination. The risk of foodborne disease can also be reduced by instructing customers on proper handling and preparing leafy greens.

In conclusion, a healthy diet must include leafy greens, which have many health benefits and support organic farming. If correct food safety procedures are not followed, they can potentially increase the risk of contracting a foodborne illness. Hence, to ensure leafy greens' safety and nutritional content, it is crucial to employ excellent agricultural practices and food safety management techniques (Rajput et al., 2018).

1.3 Common minerals in leafy vegetables

As mentioned above, leafy greens are a crucial source of nutrients and trace elements. The content and concentration of minerals vary widely from species to species and depend on the plants growing substrate. Generally speaking, between 15-20 different minerals can be found in leafy greens. Those nutritionally interesting are calcium, iron, magnesium, potassium, zinc, selenium, copper, and manganese, essential for numerous physiological processes (García-Casal et al. 2017). However, leafy vegetables can also contain toxic elements like lead, cadmium, arsenic, and mercury. These belong to a group of trace elements called heavy metals. Heavy metals are elements with a high atomic weight and density. These metals can collect in the soil and water where plants are growing and be absorbed by the roots (Maity et al., 2021)

1.3.1 Calcium

Calcium is a necessary element for the formation and maintenance of healthy bones and teeth. Moreover, it is essential for normal nerve and muscle communication (Zhang et al., 2021). Calcium-rich leafy foods include kale, collard greens, and bok choy (Bates et al., 2018). The presence of vitamin D, which is also present in some green vegetables, could improve calcium absorption from plant sources (Bates et al., 2018).

1.3.2 Iron

Iron is another essential element to create hemoglobin, the protein responsible for oxygen transport throughout the body. A common dietary problem, iron deficiency, can cause anemia and poor cognitive function (Stabler & Allen, 2004). Leafy vegetables are excellent sources of iron, including spinach, Swiss chard, and beet greens (Bates et al., 2018). Nevertheless, some variables, such as phytates and other inhibitors, could affect the amount of iron bioavailable from plant sources (Hurrell & Egli, 2010).

1.3.3 Magnesium

Magnesium is essential for several enzymatic events in the body, including energy metabolism, protein synthesis, and muscular function (Guerrero-Romero & Rodrguez-Morán, 2011). Leafy vegetables are excellent sources of magnesium, including spinach, Swiss chard, and collard greens (Bates et al., 2018).

1.3.4 Potassium

Another essential element for healthy muscle and nerve function is potassium. In addition, it supports the maintenance of healthy heart and blood pressure levels. Leafy vegetables are good sources of potassium, including spinach, Swiss chard, and beet greens (Bates et al., 2018).

1.3.5 Zinc

The zinc trace element is necessary for healthy immune response, wound healing, and taste perception (Wessells & Brown, 2012). Zinc can be found in leafy plants, including spinach, kale, and collard greens (Bates et al., 2018).

1.3.6 Selenium

Another trace element, selenium, is crucial for the thyroid gland and immune system to operate properly (Rayman, 2012). Leafy vegetables are excellent sources of selenium, including spinach, collard greens, and turnip greens (Bates et al., 2018).

1.3.7 Copper

Copper is another vital trace element for human health, as it is involved in the development of red blood cells and connective tissues, as well as the function of the immune and neurological systems (Turnlund, 1998). Leafy plants such as Swiss chard, spinach, and kale are excellent copper suppliers (Larson et al., 2000).

1.3.8 Manganese

Another significant trace element present in green leaves is manganese, which plays a role in antioxidant activity, metabolism, and bone health (Aschner & Aschner, 2005). The best sources of manganese include Swiss chard, collard greens, and spinach (Tucker et al., 1999).

In particular, the concentration of minerals and trace elements in leafy greens could vary depending on factors including soil type, climate, and farming techniques (Husted et al., 2017). Nonetheless, eating various green vegetables can help ensure an appropriate intake of these crucial elements. Generally, leafy greens are a good source of minerals and trace elements. Incorporating these nutrient-dense foods into one's diet can improve overall health and well-being.

1.4 Factors influencing the uptake of minerals by plants

Plants absorb minerals through a complicated process regulated by several variables, including soil pH, root absorption, and leaf uptake. Many minerals have ideal pH ranges for absorption; therefore, soil pH, for instance, can substantially influence the availability of minerals to plant roots (Kabata-Pendias & Mukherjee, 2007). One of the examples is iron. Although a crucial plant element, it is often scarce in alkaline soils. Iron may become insoluble and unavailable for plant absorption in certain soils. Yet, certain plants have created unique systems that improve their iron intake in these circumstances. For instance, even in alkaline soils, certain grasses produce substances called phytosiderophores that can chelate iron and increase its availability for absorption (Römheld & Marschner, 1986). This mechanism emphasizes the importance of maintaining the ideal soil pH for healthy plant development and mineral absorption. A general scheme of the nutrition uptake is explained in Figure 1.

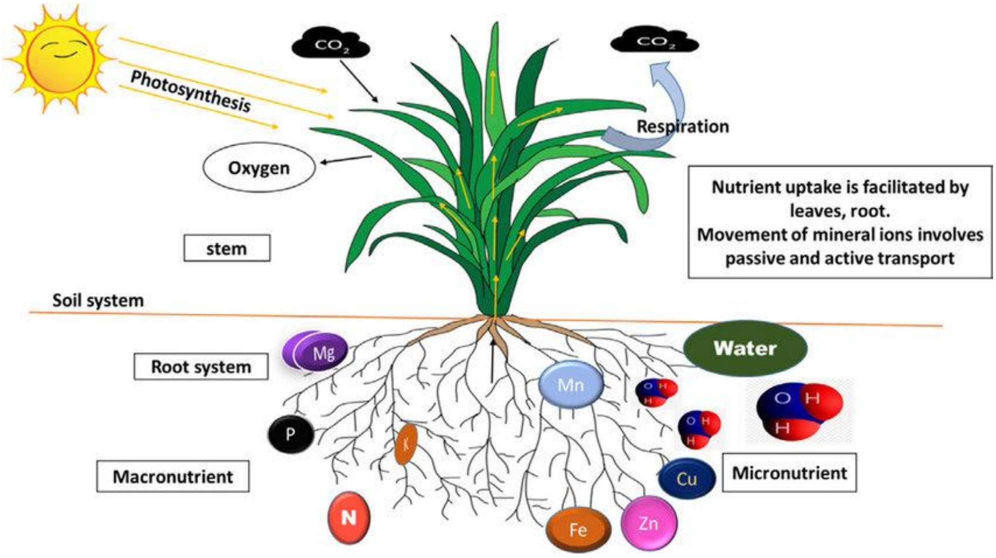

Figure 1: Root mineral uptake scheme (Kumari et al., 2021).

As it depends on the growth and development of the roots and the availability of minerals in the soil, the absorption of minerals by roots is another important consideration (Kabata-Pendias & Mukherjee, 2007). The intake of minerals is also greatly influenced by a plant's root system, with some plants having unique root structures that make this possible (Kabata-Pendias & Mukherjee, 2007). For instance, nitrogen is a crucial ingredient for plant development that is often in short supply in soils. Through their roots, plants absorb nitrogen as nitrate (NO_3^-) or ammonium (NH_4^+) ions. However, soil pH affects the intake of these ions, with ammonium more easily accessible in acid soils and nitrate accessible in alkaline soils (Lambers et al., 2012). A better understanding of these unique root systems and how they interact with various minerals will aid in maximizing plant development and growth.

Another critical mechanism, besides root uptake of nutrients, is the foliar feeding of leaves (Kabata-Pendias & Mukherjee, 2007). For plants grown in nutrient-poor soils or with small root systems, foliar feeding can be an efficient way of mineral absorption. An example is calcium. It is a crucial plant mineral and is often absorbed through the roots. However, certain plants may also take calcium through their leaves, particularly during periods of fast development or when the calcium supply in the soil is low. To prevent blossom-end rot, tomato plants, for instance, may absorb calcium through their leaves (White & Broadley, 2005).

Besides beneficial minerals and trace elements, a plant from the soil or the air can absorb other toxic elements, such as cadmium, lead, arsenic, or mercury. These are called heavy metals, which can cause serious health problems in high concentrations. A study conducted by Wang et al. in 2021 examined the amounts of heavy metal pollution in leafy vegetables from Beijing's rural and urban districts and the related health hazards. It has been discovered that heavy metal levels in vegetables were often more significant in urban regions than in rural areas. Cadmium (Cd) and lead (Pb) concentrations were more significant in vegetables from urban areas, whereas zinc (Zn), copper (Cu), and manganese (Mn) concentrations were higher in vegetables from rural areas. The study also discovered that heavy metal intake by the roots was the primary channel by which the metals were transported to the edible sections of the plant (Wang et al., 2021).

The complicated interactions between soil pH and mineral absorption by roots and leaves impact plant mineral intake. More study is required to fully understand these interactions and how they might be tuned for better plant nutrition and growth.

2 MICROGREENS

In recent years, crops such as microgreens have experienced remarkable growth in appeal (Miyashita et al., 2018). These young shoots of different vegetables and herbs are usually harvested 7 to 14 days after germination (Kader, 2002). To encourage healthy growth, these immature plants are often cultivated in trays, either in soil or hydroponically, under regulated lighting, temperature, and humidity conditions (Miyashita et al., 2018).

2.1.1 Nutritional value and health benefits

The nutritional value of microgreens varies per species, but it is typically regarded as a rich source of vitamins, minerals, and antioxidants (Lu et al., 2019; Sharma et al., 2022). Microgreens can contain up to 40 times more nutrients than mature plants of the same species, according to studies (Miyashita et al., 2018; Zhang et al., 2021). For instance, compared to mature red cabbage plants, red cabbage microgreens had much more significant amounts of vitamins C, E, and β-carotene (Zhang et al., 2021). Niroula et al. (2021) conducted a study to assess the pigments, ascorbic acid, total polyphenols, and antioxidant capacities of deetiolated barley and wheat microgreens. Both barley and wheat microgreens were found to be high in pigments, ascorbic acid, and total polyphenols, therefore having a high antioxidant capacity. Due to their high nutritional content and antioxidant characteristics, these microgreens have the potential to be functional foods (Niroula et al., 2021).

In addition to having a high vitamin and antioxidant content, they are also a great source of minerals, including iron, copper, and zinc (Lu et al., 2019; Sharma et al., 2022). According to studies, the mineral content of microgreens can be up to several times higher than that of mature plants, probably due to their rapid growth and nutrient intake in the early stages of development (Cohen et al., 2015). The mineral composition of microgreens can differ depending on the species and the growing environment, but it is generally considered a significant nutritional source of essential minerals (Wu et al., 2019). Several valuable nutrients can be provided by including microgreens in the diet, which can improve general health and well-being.

Some of these health benefits are reviewed in the study by Sharma et al. (2022), focusing on enhancing cardiovascular health. Nitrate levels in microgreens are high and associated with decreased blood pressure and improved vascular function. Moreover, microgreens include bioactive substances, including phenolic acids and flavonoids, which have antioxidant characteristics and can help reduce the risk of cardiovascular disease (Sharma et al., 2022).

In addition to cardiovascular benefits, microgreens have shown promise in supporting digestive health. According to research, eating microgreens can increase the number of good bacteria in the stomach, such as *Lactobacillus* and *Bifidobacterium*, improving gut health and immunological function. Furthermore, the dietary fiber in microgreens can assist digestion and control intestinal movements (Sharma et al., 2022).

2.1.2 Microgreens in gastronomy

Microgreens are a flexible and healthy ingredient in various meals, such as salads, sandwiches, soups, and stews. Furthermore, the variety of taste profiles and textures that microgreens provide can enhance foods' visual and gastronomic value (Miyashita et al., 2018). Arugula, kale, Radish, and cilantro are some regularly cultivated microgreens. Due to their tiny size and robust flavor, they make an excellent garnish or accent, and their high vitamin content makes them a favorite option among health-conscious customers (Wu et al., 2019).

2.1.2.1 Salads

Salads are one of the most common applications for microgreens. Microgreens can transform a plain salad into a visually beautiful and nutrient-dense dish by adding texture, flavor, and color. Due to their peppery flavor and crunch, microgreens such as arugula, Radish, and mustard greens are especially popular in salads (Gupta et al., 2017). Microgreens can also be added to sandwiches or wraps to add taste and texture.

2.1.2.2 Flavor enhancers

Microgreens can also be added as a garnish or topping to some foods. Their delicate size and strong flavor complement everything from soups and stews to pizzas and pasta dishes. Microgreens such as cilantro, basil, and chives frequently garnish ethnic meals, including tacos, pho, and pad thai (Wu et al., 2019). They can also be used as a key component in recipes such as omelets, frittatas, stir-fries, and garnish or accent. Microgreens can add flavor and nutrition to egg dishes, and their delicate texture makes them an excellent complement to stir-fries and sautés (Gupta et al., 2017).

2.1.2.3 Smoothies

Microgreens can also be utilized in the preparation of nutrient-dense smoothies and drinks. Adding microgreens to a smoothie or drink, such as kale, spinach, or wheatgrass, can increase its nutritional content and give various health benefits (Wu et al., 2019). Cocktails and mocktails can also benefit from adding microgreens for taste and nutrition.

2.1.2.4 Sauces, dips, and spreads

Microgreens may also be used to produce a variety of sauces, dips, and spreads. Pesto is often made using microgreens such as parsley, basil, and cilantro, although spinach and kale can be added to form a nutrient-dense dip or spread (Gupta et al., 2017).

2.1.3 Environmental benefits

Microgreens may be beneficial to the environment, nutritious, and tasty. They are a sustainable alternative to conventional agriculture, growing year-round in limited locations and requiring less water and resources (Chandrasekaran et al., 2020). In general terms, microgreens' health, gastronomic and environmental benefits can be credited to their growing popularity. Therefore, they offer a potentially new class of crops that may help create a healthier and more sustainable food system.

2.2 Growing substrates

Growth substrates are critical to the success of microgreen production.

These substrates nourish the growing plants while allowing proper water and nutrient uptake. Various growth substrates are frequently used for microgreens, each with its own benefits and drawbacks (Marvasi et al., 2016).

2.2.1 Soil-based substrates

Soil-based substrates are the most common and conventional growth mediums for microgreens. The soil offers a stable, nutrient-rich habitat for developing plants, resulting in healthy, strong development (Stamets, 2005). Soil also acts as a natural pH buffer and may retain moisture, reducing the need for frequent watering (Nair and Ngouajio, 2018). Soil also promotes forming a healthy root system, increasing nutrient uptake and overall plant health (Nair and Ngouajio, 2018). However, soil-based substrates have several downsides. Dirt may be heavy, making transportation and handling difficult, and it can also be prone to pests and illnesses (Stamets, 2005).

Moreover, soil nitrogen levels can be uneven, requiring extra fertilization to maintain optimal development (Nair and Ngouajio, 2018). Compost or compost-based blends are an alternative to soil-based substrates. Compost is a natural growth medium rich in nutrients that are formed from organic materials such as food scraps, yard waste, and animal dung (Stamets, 2005). Compost-based mixtures can be a cost-effective and ecological alternative to traditional soil-based substrates and a natural supply of beneficial bacteria that can boost plant health (Nair and Ngouajio, 2018). An example of a soil-based farm is displayed in Figure 2.

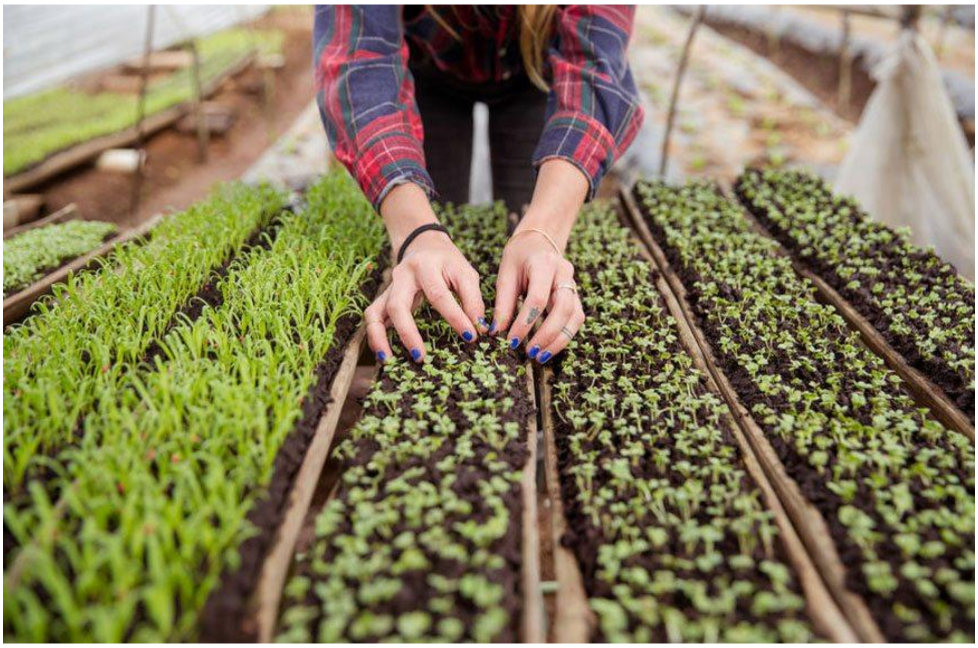

Figure 2: Soil-based farming of Microgreens (Morton, 2018).

2.2.2 Hydroponic substrates

Another option for addressing problems associated with soil-based substrates is to use a soilless mix, commonly composed of peat moss, coconut coir, vermiculite, and perlite. Soilless mixtures are light and can be sterilized to decrease the risk of pests and diseases. They also provide more regular fertilizer levels and pH, which can help plants develop faster. These hydroponic substrates are widely used among hobby growers and large enterprises

(Nair and Ngouajio, 2018). A simplified setup of hydroponic farming can be found in Figure 3.

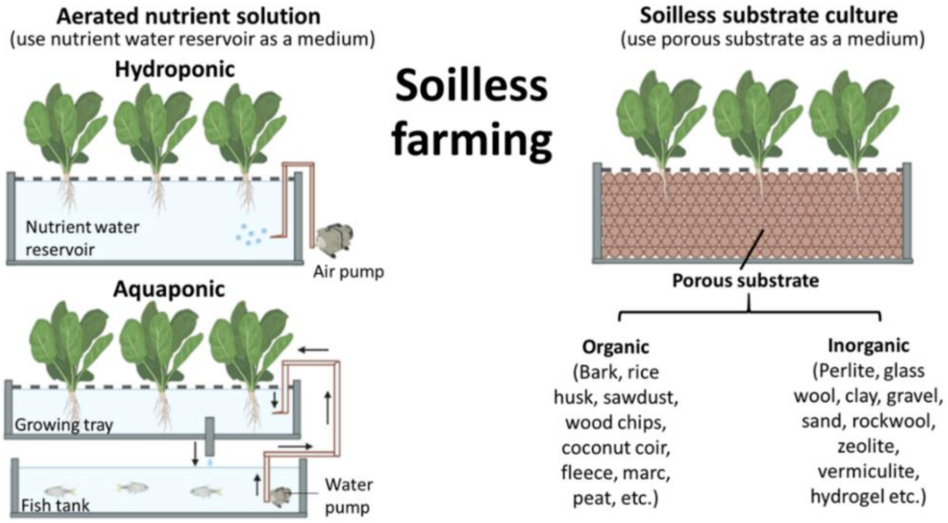

Figure 3: Hydroponic and Aquaponic system setup (Maluin et al., 2021)

Peat moss is a nutrient-rich and absorbent substrate often used in hydroponic systems. It has a high water retention capacity, which helps keep the growth medium wet and offers a stable environment for the plants. On the other hand, it is not a sustainable resource, and its exploitation could have significant environmental consequences (Graamans et al., 2017).

Coconut coir is another common growth medium for microgreens. It is a renewable and ecologically friendly solution created from the fibers of coconut husks. This substrate can be a good choice for hydroponic systems because of its outstanding water retention and drainage capabilities. Coconut coir is also resistant to pests and diseases and can be reused several times (Miles and Brown, 2019).

Perlite and vermiculite are lightweight, highly absorbent substrates often used in hydroponic systems. They provide a controlled growth environment and resist pests and diseases (Graamans et al., 2017). However, they may require additional nutritional supplementation due to their poor nutrient content (Nair and Ngouajio, 2018).

Cotton is another material that may be utilized as a microgreen's growth substrate. Its pads or balls can be used as seed germination and growth media in trays. Cotton is a cheap and commonly accessible material that is also biodegradable and compostable, making it an environmentally benign choice for microgreen manufacturing. On the other hand, it dries out rapidly and may need more frequent watering than other growth substrates, which can be labor-intensive. In addition, cotton may not support heavier microgreen types, resulting in lanky or spindly growth (Miles and Brown, 2019).

In summary, hydroponic and soil-based substrates can be used to cultivate microgreens, although they have important distinctions. Hydroponic growth surfaces provide a sterile environment, precise nutrition, and water distribution, leading to faster growth and larger harvests. However, they can be more expensive and require specialized equipment and knowledge (Miles and Brown, 2019; Marvasi et al., 2016). On the other hand, soil-based systems are comparatively inexpensive to install and maintain because they require basic gardening equipment and ingredients. However, they can be more challenging to manage as the soil can house pests and diseases that can harm the health of the plants. Moreover, soil-based systems require more space and resources to yield the same yield as the hydroponic system (Nair and Ngouajio, 2018; Marvasi et al., 2016). An example of a Hydroponic microgreens farm is shown in Figure 4.

Figure 4: Hydroponic farm (Worthmann, 2022).

2.3 Process of sprouting

The sprouting process is critical for the growth of microgreens. Sprouting begins by soaking seeds in water for a few hours, causing the germination process to begin (Hodges, 2014). After being soaked, the seeds are drained and evenly distributed in a tray or container filled with a growth medium such as soil or coconut coir. The seeds are then coated with a thin coating of the same growth medium and kept wet by regularly watering them (Dimitri, 2019). When seeds sprout, they need to be exposed to light. As a result, they are moved to an environment with enough light exposure, such as a greenhouse or under artificial light (Hodges, 2014). The optimal temperature and humidity levels for microgreens vary by species, but they typically require warm and humid conditions to flourish. Temperatures of 15–21°C and humidity levels of 70–80% are usually ideal (Dimitri, 2019). Microgreens require constant watering during the growth stage to keep the growing medium moist. Overwatering can promote mold growth, while underwatering can stifle growth (Hodges, 2014). The time it takes for microgreens to mature varies based on species and growth circumstances, but it usually takes 7–14 days (Dimitri, 2019).

As the microgreens reach maturity, they are harvested by cutting the stems slightly above the soil or growth medium at the base of the plant. The gathered microgreens can be cleaned and refrigerated in sealed containers for several days (Hodges, 2014). Soaking seeds, spreading them in a growth medium, exposing them to light, maintaining optimal temperature and humidity levels, frequent watering, and harvesting once the plants reach maturity are all steps in the sprouting process. High healthful and nutritious microgreen yields can be obtained with proper growth practices. The method of sprouting is displayed in Figure 5.

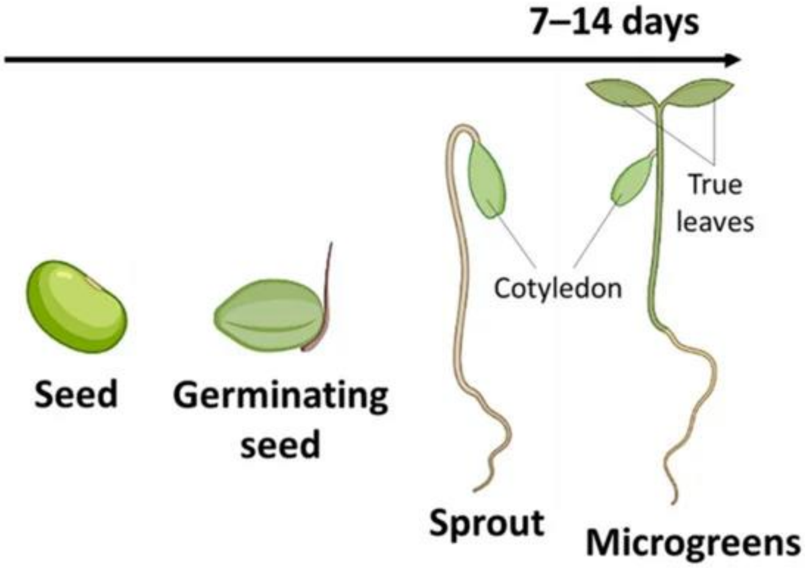

Figure 5: Process of Sprouting (Maluin et al., 2021).

2.4 Production technology

Several production methods, including soil-based and hydroponic systems, can be used to develop microgreens. Microgreens are grown in trays or containers filled with a growth medium, such as peat moss or coconut coir, in soil-based manufacturing. In addition to holding moisture and nutrients for plants, the growth medium supports the structure of the plants. Small-scale producers can use soil-based production techniques, as they are straightforward and inexpensive (Chandrasekaran et al., 2020).

Another technique used to cultivate microgreens is hydroponics. Without soil, plants are grown in this method in a nutrient-rich water solution. Many techniques, such as the nutritional film technique (NFT), deep water culture (DWC), or aeroponics, can be used for hydroponic production. Compared to soil-based systems, hydroponic systems provide several benefits, including faster growth rates, larger yields, and more effective use of fertilizers and water (Marvasi et al., 2016).

The best growth conditions are necessary for effective microgreen production regardless of the production technique. The right amount of warmth, light, humidity, and nourishment are among these requirements. Microgreens need high-quality light for effective photosynthesis and development, which is best delivered by full-spectrum LED lights. Depending on the type of microgreens that are grown, different temperatures and humidity levels are ideal, but generally speaking, a temperature range of 18–24 °C and a humidity level of 50–60% is advised (Chandrasekaran et al., 2020).

After harvesting, various processing methods, including cooling, drying, freezing, fermentation, and extraction, can be used to increase shelf-life and retain the nutritional content of microgreens for consumption.

For instance, drying is a popular approach to extending shelf life. This process involves removing moisture from the product using hot air or other means. In contrast, freeze-drying is a more expensive but successful procedure, including freezing microgreens and extracting the moisture using a vacuum. This technique produces a longer shelf-life product and greater nutritional and taste retention (Sharma et al., 2022).

In conclusion, the resources and preferences of the farmer can influence the technique used to produce microgreens. The most popular methods for growing microgreens are soil-based and hydroponic systems, each of which has benefits and drawbacks. Providing ideal growing conditions is essential to ensure the effective development of healthy and nutrient-dense microgreens regardless of the production system. Choosing a suitable processing method to ensure nutrition and taste retention is also crucial.

II. ANALYSIS

3 THE AIM OF THE STUDY

This study aims to determine the element composition of microgreens throughout their early development stage. The focus will be put on the concentration of each macro element essential for human nutrition and each toxic element that can pose some hazard. The goals of the research are listed below:

- Review the available literature on this topic,
- determine the element composition of microgreens,
- visualize the trend during the four days of the plant's development,
- calculate the RDA and AI values for the essential macro and micro elements,
- calculate the PWTI and PTMI values for the most toxic heavy metals,
- provide an objective view and prepare the ground for future research.

4 METHODOLOGY

In this part of the research, the methodology will be described. All the equipment and chemicals used in the experiment are listed below, including the characteristic and preparation of the samples and the methods used to determine the dry matter, ash contents, minerals, and trace elements.

4.1 Chemicals

The following chemicals were used for individual determinations:

- Analpure HNO_3 67% (Analytik Jena, Jena Germany),
- Ultrapure H_2O_2 30% (Ing. Petr Lukeš, Uherský Brod, Czech Republic),
- HF Analpure 48% (Analytik Jena, Jena Germany)
- ultrapure pure water (Purelab Classic Elga water system, London, UK),

4.2 Equipment

The following devices and tools were used for individual determinations:

- analytical balance (AFA 210 LC, Schoeller, Czech Republic),
- dryer (Venticell 111 Comfort, BTM a.s., Czech Republic),
- muffle furnace LM 112 10 ML W Elektro (VEBF, Germany),
- microwave system Milestone Ethos One (Sorisole, Italy),
- ICP-MS Scientific iCAP Q (Thermo Scientific, Waltham, MA, USA),
- adjustable micropipettes,
- desiccator,
- growth medium: 100% cotton pads (Jiva CZ, s.r.o., Czech Republic), soil (universal gardening substrate).
- Laboratory utensils: beakers, flasks, funnels, pipettes, aluminum bowls, porcelain cups, glass containers, teflon measuring cups, and spoons.

Plastic dishes were always used for mineral and trace element analysis.

4.3 Sample characteristics

Regarding research, two different kinds of microgreens seeds were used: Wolfberry organic garden cress (Czech Republic, 10 g) and Wolfberry organic radish mix (Czech Republic, 10 g). Seeds were planted on two different growth mediums; one of them was soil, and another one was pure cotton. The samples were stored in the laboratory at a room temperature of 21±2 °C with great access to daylight for a maximum of 9 days. They were irrigated daily. Samples were photo-documented and attached below.

Figure 6 Garden cress on soil/cotton fibers

Figure 7 Radish on soil/cotton fibers

4.4 Determination of moisture content

Aluminum dishes were placed in a preheated oven at 105±2 °C for about 1 hour. After cooling in the desiccator, the empty dishes were weighed, and then 1 g of sample was weighed into them with an accuracy of 0.0001 g. The dishes with the weighed samples were put back into a preheated oven at 105±2 °C for 2 hours. After drying, they were let to cool down in a desiccator and reweighed to the nearest 0.0001 g. The result is the average of two measurements.

Calculation of moisture content [%]:

$$W = \frac{m2 - m3}{m2 - m1} \,.100$$

(1)

where: m1 – the weight of the empty dish [g],

m2 – the weight of the dish with the sample before drying [g],

m3 – the mass of the dish with the sample after drying [g].

Calculation of dry matter content [%]:

$$Sdm = 100 - V$$

(2)

where: Sdm – dry matter content [%],

V – water content [%].

4.5 Determination of ash

The empty porcelain cups were first annealed at 550 ± 25°C for 1 hour. They were cooled in a desiccator and weighed to the nearest 0.0001 g. One gram of the sample was weighed to the nearest 0.1 mg in ceramic cups and placed in a muffle furnace. There the samples were burned at a temperature of 550 ± 25 °C for 6 hours. After burning, the samples were placed in a desiccator. After cooling, they were weighed again to the nearest 0.0001 g. The result is the average of two measurements.

Calculation of ash content [%]:

$$P = \frac{m1 - m2}{m3 - m2} \,.100$$

(3)

where: m1 – the weight of porcelain cup with ash [g],

m2 – the weight of an empty porcelain cup [g],

m3 – the weight of the porcelain cup with sample weight [g].

4.6 ICP-MS method

ICP-MS, Inductively Coupled Plasma Mass Spectrometry, is mass spectrometry with inductively coupled plasma. It is among the analytical methods used to determine the content of minerals and trace elements in a sample.

4.6.1 Mineralization of samples

First, 20 mg of the sample was weighed to the nearest 0.0001 g into Teflon containers. Then, 7 mL of 67% ultrapure HNO_3, 1 mL of 30% ultrapure H_2O_2, and 0,5ml of 48% HF analpure were added to each sample container. The samples were decomposed using a Milestone Ethos One microwave device. The parameters for the decomposition of the samples were as follows: 10 minutes, 150 °C, 500 W ramp-up; 20 minutes, 180 °C, 1 500 W endurance, and 10 minutes of cooling. Finally, the mineralized samples were made up to a volume of 25 ml with ultrapure redistilled water into small plastic vials and stored for no more than seven days in the fridge at 8±2 °C. Finally, the samples were injected into the ICP-MS (Sumczynski et al., 2018).

4.6.2 Determination of elemental composition using ICP-MS

Two sets of calibration standards were prepared as follows: ^{9}Be, ^{66}Zn, ^{63}Cu, ^{60}Ni, ^{27}Al, ^{24}Mg, ^{59}Co, ^{7}Li, ^{45}Sc, ^{107}Ag, ^{55}Mn, ^{88}Sr, ^{137}Ba, ^{205}TI, ^{209}Bi, ^{140}Ce, ^{133}Cs, ^{165}Ho, ^{181}Ta, ^{159}Tb, ^{238}U, ^{89}Y in a concentration of 3–35 µg/l. The second lower concentration range was ^{75}As, ^{44}Ca, ^{111}Cd, ^{52}Cr, ^{57}Fe, ^{202}Hg, ^{39}K, ^{31}P, ^{23}Na, ^{208}Pb, ^{77}Se, ^{118}Sn and ^{41}Ti in a concentration of 0.5–1.0 µg/l. Tune 7 and 8 tuning solution (Analytika Jena) was used. Reference-certified material was not used.

Mass spectrometry with inductively coupled plasma ICP-MS ThermoScientific iCAP Q based on a quadrupole analyzer (ThermoScientific, USA) with QCell technology (CCT – Collision Cell Technology) was used for the determination. In this technology, Helium is used as a collision gas that enables the disintegration of molecular associates. The operating parameters were set as follows: power 1550 W, sampling depth 5 mm, cooling gas flow 14.0 l.min-3, auxiliary gas flow 0.8 l/min, fogging gas flow 1.015 l/min, flow He rates 4.1 ml/min, nebulizer speed 40.00 rpm^{-1} and temperature inside the chamber 2.7 °C (Sumczynski et al., 2018).

4.7 The contribution of microgreens to the RDA, AI, PTWI, and PTMI values for appropriate elements

Daily levels of dietary intake for minerals and trace elements from microgreens samples were determined and compared to the Institute of Medicine's (IOM) values, such as RDA (recommended daily allowance) or AI* (adequate intake) recommendations. The RDA and AI were calculated for young adults (aged 19‒30). Toxic element intakes were also evaluated and compared to FAO/WHO values for PTWI (provisional acceptable weekly intake) or PTMI (provisional tolerable monthly intake) (FAO/WHO, 2006a, 2011a, 2011b, 2013). In terms of toxic element dietary intakes, appropriate nutritional consumption values for men and women with average weights of 70 and 90 kg were calculated. Figure 13 lists the recommended dosages for RDA, AI*, PTWI, and PTMI* values for specific minerals and trace elements. Because there is no guideline for the daily consumption of microgreens, the daily serving size for each life stage group was set at 100 g.

4.8 Statistical analysis

The results were expressed as mean ± standard deviation on a dry weight basis and were statistically evaluated using a one-way analysis of variance (ANOVA). Subsequently, Tukey´s test identified differences between the mean values with the level of significance set to 5% (p<0.05).

5 RESULTS AND DISCUSSIONS

5.1 Determination of dry matter and ash contents

We can describe dry matter as the remaining weight that is left after drying a sample at a certain temperature. Depending on the particular crop and other factors like growth circumstances and maturity, leafy vegetables' average water content might vary. A study by Koubala et al. (2006) examining the water content of several green Mediterranean diet vegetables discovered that the contents ranged from 94.4% in lettuce to 85.4% in arugula. According to another study by Gyoneche (2015), the moisture content in mature radish leaves is around 89.5%, meaning the remaining 10.5% is dry matter. Our measured dry matter values ranged from 7 to 15%, with the highest values being found in the radish grown on the cotton and the lowest in the garden cress grown on the soil. Overall, the samples grown on the soil had lower dry matter levels, and the garden cress had lower values than radish. Usually, the higher the dry matter is in the sample, the more nutrients it contains. As it is shown in Figure 8, the amount of dry matter declined throughout the growth.

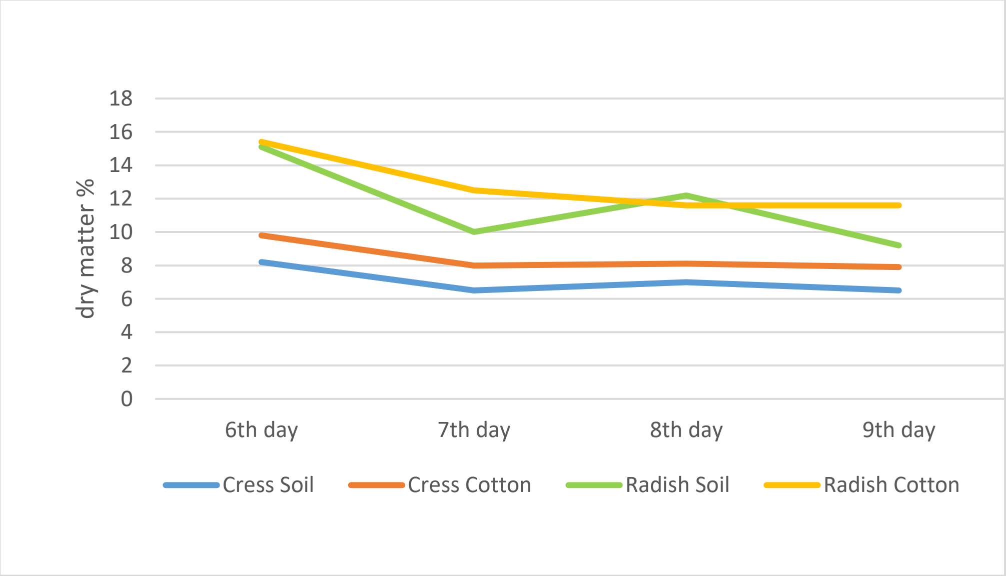

Figure 8: Dry matter contents

According to ISO 11287 (2011), the remaining mineral substances after burning the sample are called ash. According to Table 1, our values ranged from 3.84 to 9.85%, with the lowest value being measured in the radish sample grown on the cotton and the highest in the garden cress sample grown on the soil. Both soil and cotton-grown radish samples had the same amount of ash content on the 6th day; however, on the 9th day, the soil sample's ash content had increased significantly. The garden cress has also shown an increase throughout its growth. Due to their mineral-rich growth medium, the soil-grown samples had more ash content than their cotton-grown counterparts.

Table 1: The ash content

	Garden Cress		**Radish**	
	% per dry matter			
Growth medium	**Soil**	**Cotton**	**Soil**	**Cotton**
6th day	$7.42 \pm 0.24^{a,A}$	$4.56 \pm 0.13^{a,B}$	$3.94 \pm 0.07^{a,A}$	$3.84 \pm 0.36^{a,A}$
9th day	$9.85 \pm 0.62^{b,A}$	$7.23 \pm 0.36^{b,B}$	$8.82 \pm 0.34^{b,A}$	$6.06 \pm 0.20^{b,B}$

All results are presented on dry matter basis as means ± SD, n=3 (the mean of three measurements). Means within a column with at least one identical small superscript (soil or cotton separately for each sample) do not differ significantly ($p \geq 0.05$), means within a line with at least one identical capitalized superscript (for soil and cotton together for each sample) do not differ significantly ($p \geq 0.05$).

5.2 Determination of mineral and trace element composition using ICP-MS analysis

Data gathered from ICP-MS were averaged and converted into dry matter content. Forty-three minerals and trace elements were left to analyze. Due to the extensive number of elements, the rest of the chapter will focus mainly on essential macro and micro elements and toxic heavy metals.

5.2.1 Mineral and trace element content of soil and water

Before discussing the results, analyzing the mineral content in the growth medium (soil) and the water used for daily irrigation is appropriate. The type of soil used in the experiment was the garden substrate (universal), and tap water was used for regular watering. Since cotton has a negligible amount of ash, only 1.2%, compared to the soil, which is more than 60%, it is safe to assume that the cotton-grown samples absorbed most of their elements from the water (Ogundare, 2013). The analysis results are sorted from the highest concentration to the lowest one. The concentrations of elements in the soil are interpreted in μg/g; meanwhile, their concentrations in the water are in ng/g.

Marked red are the toxic elements (Table 2), with aluminum taking the first place in the table of results from the soil analysis. This is no surprise, as aluminum is one of the most abundant minerals in the Earth's crust. Based on their concentration levels, macro elements are highlighted in bold letters, of which the most common are magnesium, iron, potassium, and manganese. Interestingly, such essential minerals for plants as calcium and phosphorus are scarce in this sample.

According to Szeląg-Sikora et al. (2019), the soil's average calcium content was 29.5 µg/g and phosphorus 256 µg/g. Although, that research measured mineral content on the farming soil, which is usually fertilized and supplied by these elements. However, the values in our soil sample were significantly lower, with only 0.80 µg/g of Ca and 9.02 µg/g of P (Table 2). The significant difference in mineral content and composition between these two types of soils is further explained in the book by Kabata-Pendias (2007). It mentions different types of soils and includes thorough information on each type's macro-, micro-, and trace element content. The geographical location, geological history, and other environmental conditions can significantly impact the element content (Kabata-Pendias, 2007).

Table 2: Concentrations of elements

ELEMENT	*SOIL (µg/g)*			*WATER (ng/g)*		
Al	2204	±	53.2^{a}	21.8	±	0.66^{b}
Mg	386	±	50.5^{a}	2969	±	33.1^{b}
Fe	270	±	4.35^{a}	55.4	±	0.37^{b}
K	262	±	5.88^{a}	230	±	1.59^{b}
Mn	190	±	4.40^{a}	0.80	±	0.04^{b}
TI	168	±	9.91^{a}	99.2	±	1.72^{b}
Na	60.6	±	6.02^{a}	738	±	6.86^{b}
SC	9.43	±	1.44^{a}	16.8	±	0.39^{b}
P	9.02	±	0.32^{a}	0.48	±	0.03^{b}
SR	8.65	±	0.23^{a}	160	±	2.65^{b}
V	7.85	±	0.21^{a}	0.39	±	0.00^{b}
BA	6.30	±	0.78^{a}	4.10	±	0.05^{b}
ZR	6.02	±	0.02^{a}	<0.01	±	0.01^{b}
LI	5.67	±	0.15^{a}	8.91	±	0.10^{b}
CR	5.40	±	0.04^{a}	0.37	±	0.01^{b}
Zn	5.33	±	0.51^{a}	95.6	±	0.19^{b}
Ni	3.86	±	0.25^{a}	7.77	±	0.16^{b}
Cu	2.78	±	0.86^{a}	265	±	3.70^{b}
Ta	2.61	±	0.12^{a}	0.01	±	0.00^{b}
Pb	2.57	±	0.33^{a}	4.06	±	0.01^{b}
S	2.20	±	0.12^{a}	17.1	±	0.52^{b}
CE	1.36	±	0.19^{a}	0.00	±	0.00^{b}
CO	1.30	±	0.08^{a}	0.93	±	0.03^{b}
GA	1.07	±	0.02^{a}	0.02	±	0.00^{b}
B	1.01	±	0.06^{a}	14.1	±	0.12^{b}
Ca	0.80	±	0.03^{a}	7.49	±	0.00^{b}
Y	0.50	±	0.06^{a}	0.01	±	0.00^{b}
GE	0.47	±	0.01^{a}	<0.01	±	0.00^{b}
CS	0.40	±	0.01^{a}	<0.01	±	0.00^{b}
SN	0.31	±	0.01^{a}	<0.01	±	0.00^{b}
U	0.25	±	0.00^{a}	0.11	±	0.00^{b}
Hg	0.20	±	0.03^{a}	0.04	±	0.01^{b}
As	0.15	±	0.03^{a}	0.14	±	0.01^{b}
BE	0.14	±	0.00^{a}	<0.01	±	0.00^{b}
Cd	0.11	±	0.00^{a}	0.02	±	0.00^{b}
TL	0.08	±	0.02^{a}	<0.01	±	0.00^{b}
AG	0.07	±	0.02^{a}	0.04	±	0.00^{b}
Sb	0.07	±	0.00^{a}	0.07	±	0.00^{b}
MO	0.06	±	0.00^{a}	0.04	±	0.00^{b}
SE	0.04	±	0.01^{a}	0.12	±	0.03^{b}
HO	0.03	±	0.01^{a}	<0.01	±	0.00^{b}
TB	0.03	±	0.01^{a}	0.00	±	0.00^{b}
BI	0.02	±	0.00^{a}	<0.01	±	0.00^{b}

All results are presented as means ± SD, n=5 (the mean of five measurements). This means that within a line (for each elements) with at least one identical small superscript do not differ significantly ($p \geq 0.05$).

5.2.2 Essential minerals

The macro elements essential for human nutrition discussed further are magnesium (Mg), sodium (Na), calcium (Ca), potassium (K), sulfur (S), and phosphorus (P). Microelements like zinc (Zn) and iron (Fe), together with trace elements such as copper (Cu) and manganese (Mn), will also be discussed in this chapter. These minerals are essential nutrients for the human diet; therefore, this chapter focuses on this group of elements.

Throughout the four days of growth, there are changes in the concentrations of macro components in all samples. Overall, we can observe an uptrend of most of the previously mentioned elements, some of which increase significantly and some modestly. This is consistent with the fact that as plants age, they require more nutrients to maintain their growth and development. However, there are some differences among the examined samples. The results are visualized in Figure 9 and interpreted as changes in concentration (in µg/g) of examined elements during the 4-day growth period. The elements marked with * are data gathered from the cotton-grown samples.

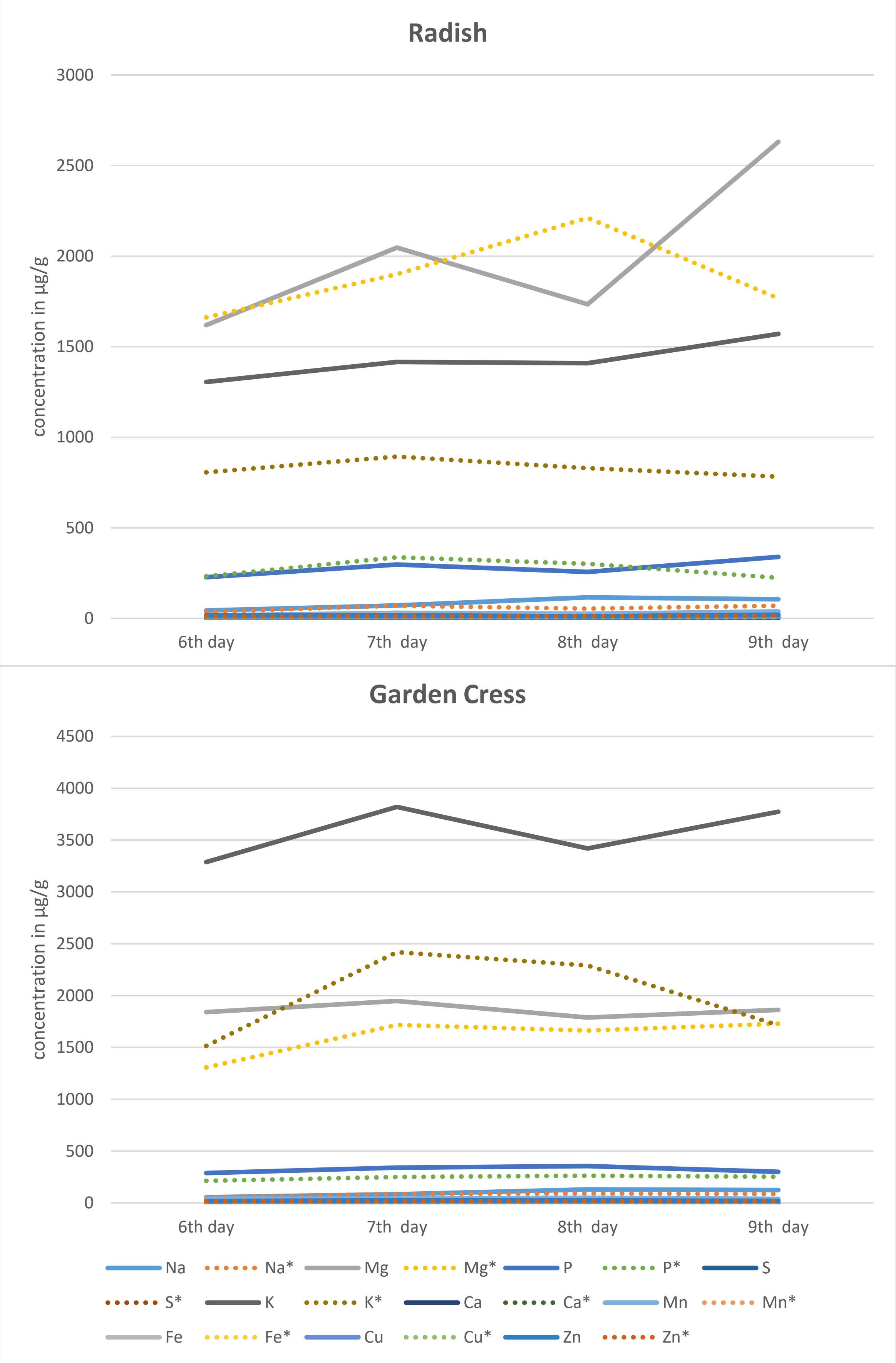

Figure 9: Concentration trends of essential elements throughout the growth

From first sight, it is noticeable that most macro element concentrations are lower in samples cultivated on the cotton medium than in those grown on a soil medium. This outcome was expected since soil as a growth medium contains way more minerals than pure cotton. Another observable difference is between the trends of the macro element content.

The content of elements in soil-grown samples maintains a positive trend throughout the four days of growth. However, cotton-grown samples seem to turn a negative trend after the 7th or 8th day. One of the main factors causing this could be the exhaustion of nutrients in the growth medium and the different retention times of elements by plants under other conditions, such as different pH levels (Kabata-Pendias & Mukherjee, 2007).

Another important fact based on the data is that the content of minerals varies not only by the growth medium used but also from plant to plant. Therefore, not every microgreen plant is the same. For example, the potassium content in the garden cress is significantly higher than in the radish samples, both grown in soil and cotton. In addition, the magnesium and phosphorus levels in both radish samples fluctuate more than the stable increase of the garden cress samples. This might be caused by different root uptake of minerals between those two plants (Kabata-Pendias & Mukherjee, 2007).

Sodium levels are usually low in leafy vegetables. However, a steep increase in both plants grown on the soil in the first three days of the experiment is noteworthy. Plants use sodium to maintain proper water balance and support their nutrient uptake. They need higher sodium levels as they grow due to their increasing height (Craig Plett, 2010).

The content of other minerals, such as calcium, iron, and manganese, increased moderately throughout the four days of the experiment, with slightly varying results between the two plants.

These findings imply that the growing medium influences macro element concentrations in plants, with soil typically offering a more conducive environment for nutrient absorption than cotton. Plants have varying nutritional needs and absorbing processes, resulting in variances in macro element concentrations.

When comparing radish and garden cress on soil, it is evident that they had significantly higher macro-element concentrations. This might be because garden cress is a robust feeder that quickly depletes soil nutrients, necessitating more fertilizer. When comparing radish and garden cress grown on cotton, it is noteworthy that the concentrations of macro-elements in both plants are typically lower. This might be because cotton is not a highly nutrient-rich substrate and cannot support as much growth or nutrient uptake as soil.

To compare the mineral composition of microgreens to their mature counterparts is best to refer to the study by Gyoneche et al. (2015). The researchers used an atomic absorption spectrophotometer to determine the mineral composition of mature radish. Based on their results, some of the elements can be found in higher concentrations in microgreens. For example, the concentration of Mg in the study by Gyoneche et al. was 149 μg/g; meanwhile, in our microgreen radish, it was 2630 μg/g. Fe concentration levels in mature radish was 1.50 μg/g; however, in our sample, it was 21.0 μg/g. Zinc in mature radish was measured at 2.40 μg/g, yet in microgreens, 19.7 μg/g. The concentration levels of manganese and copper were also significantly higher in microgreens than in mature vegetables. On the other hand, the concentration of elements like calcium, sodium, and potassium was considerably lower in microgreens compared to the mature radish. Microgreens had only 2.16 μg/g, while mature radish had 1480 μg/g of Ca. Sodium levels in microgreens were 116 μg/g, but in mature vegetables it was 1050 μg/g. The levels of K in microgreens were 1570 μg/g; however, in mature radish, it was 3800 μg/g (Gyoneche et al., 2015). This comparison proves that essential minerals like these can be found in various concentrations throughout the plant's development; therefore, knowing the right time for harvest and consumption is great.

Another study by Giandomenico et al. (2022) supports that microgreens are a rich source of these essential minerals. However, there are also differences between them. The results showed that K (22.2 μg/g) and Ca (12.6 μg/g), among the macroelements, and Fe (145 ng/g) and Zn (10.4 ng/g), among the microelements, are particularly abundant in hemp microgreens, but still significantly lower than in radish or garden cress (Giandomenico et al., 2022).

5.2.3 Toxic elements

Among common heavy metals found in the produce of modern agriculture are arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb), but some studies also include nickel (Ni), Aluminum (Al), and antimony (Sb). These elements can have various harmful effects on the human body. Long-term exposure to arsenic can cause skin lesions, cardiovascular disease, diabetes, and cancer. Cadmium poisoning can cause kidney damage, lung cancer, prostate cancer, and a decrease in bone density.

Lead poisoning causes developmental delays, brain impairment, anemia, and hypertension. Mercury is connected to brain damage, decreased cognitive function, visual and hearing loss, and developmental abnormalities. Therefore, limiting exposure to these components is essential by avoiding contact with polluted soil, water, and air and consuming contaminated food and products (WHO, 2010–2022).

The findings of our experiment reveal the existence of those hazardous substances in all samples. The trend of their fluctuation throughout the growth of the samples is shown in Figures 10 and 11.

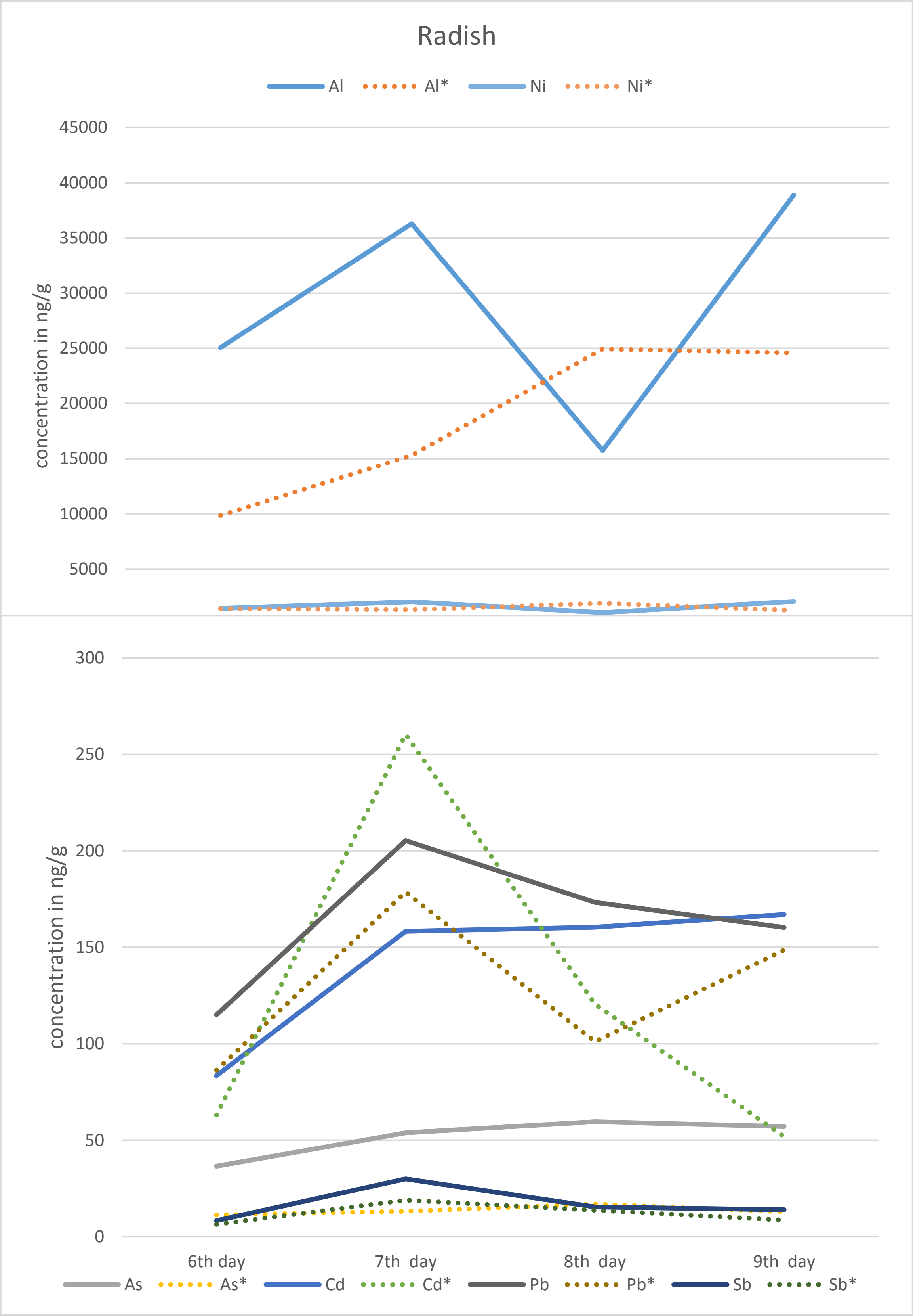

Figure 10: Concentration trends of toxic elements throughout the growth (radish)

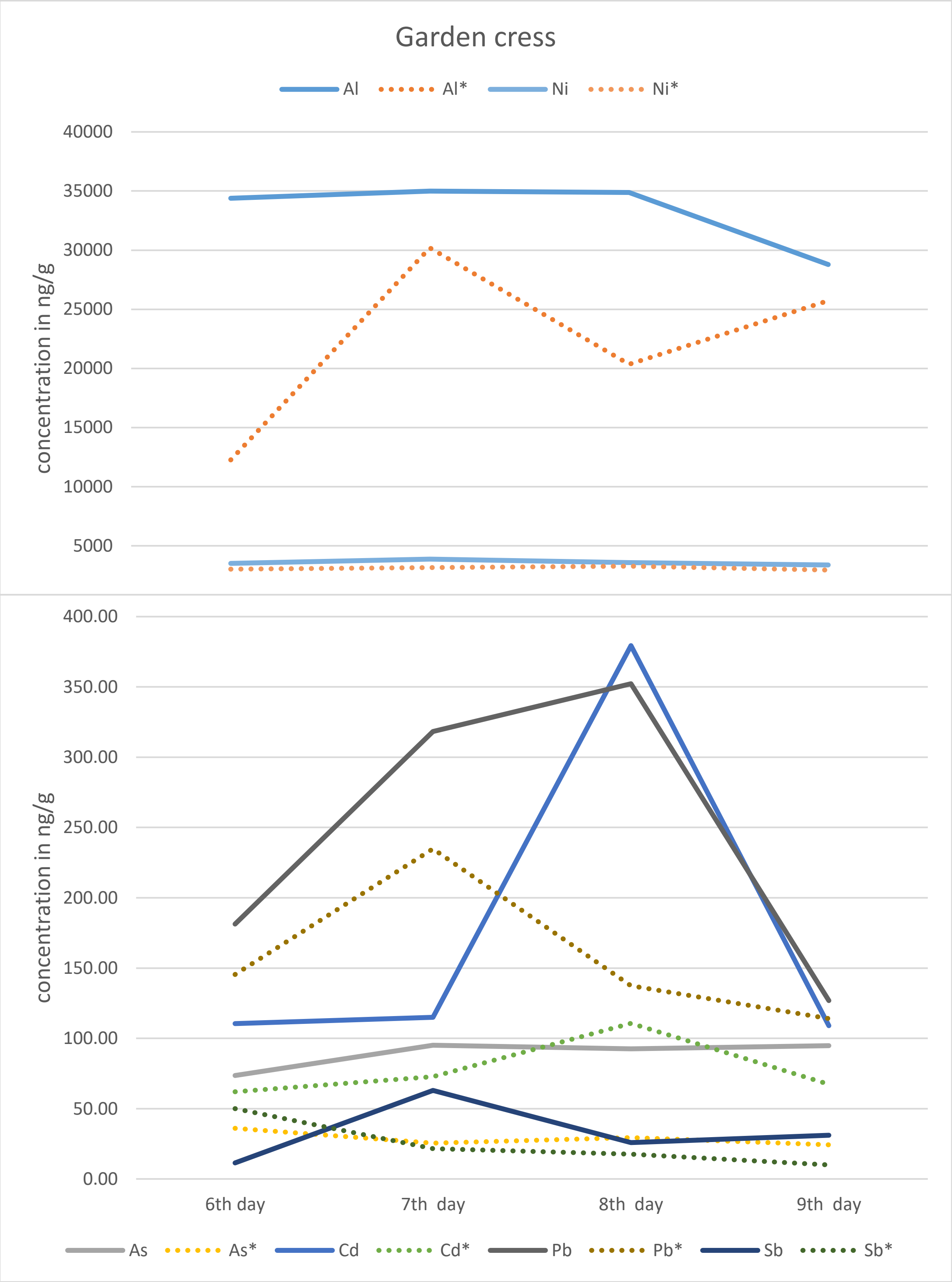

Figure 11: Concentration trends of toxic elements throughout the growth (garden cress)

Figure 11 shows that a certain element's concentration levels are much higher than others, with its lowest values of 9.85 µg/g in cotton-grown to its highest of almost 39.0 µg/g in soil-grown radish.

Aluminum can be toxic and beneficial for plants, with varying concentrations depending on the growing conditions and species. Green plants could accumulate high amounts of aluminum in their early stage of development due to its highly stimulating effect on growth and the promotion of nutrient uptake. However, too much aluminum could also harm plants as it can inhibit water uptake and cell division (Bojórquez-Quintal et al., 2017).

In general, the concentrations of other hazardous components in soil-grown radishes fluctuate during the growth period, with some elements exhibiting increasing trends and others showing decreasing trends.

However, concentrations of certain elements, such as mercury, increase significantly on days 8 and 9, indicating possible health hazards associated with eating these plants. The maximum permissible level for total mercury in leafy greens (excluding spinach) is 0.05 micrograms per gram (µg/g) of fresh weight, according to European Commission Regulation (EC) No 1881/2006. After a conversion to match the same unit with the results, it is safe to say that the limit is somewhere around 500 nanograms per gram (ng/g) of dry matter. The levels of mercury in all samples were more than 100 times higher than the limit on day eight and doubled on day nine. These values are abnormally high, probably caused by the ionic association after sample digestion on the eight day. Therefore, data concerning mercury has been excluded from this experiment.

The maximum allowable limit for arsenic is one µg/g converted to dry matter; meanwhile, values in all samples are steadily below 100 ng/g.

The lead limit is slightly higher, three µg/g after conversion to dry matter, while the highest concentration of 352 ng/g was found on the eight day in the garden cress grown on the soil.

It should be mentioned that the concentrations of most hazardous components in cotton-grown radish are lower than in soil-grown radish. There are notable outliers, such as cadmium, that exhibit a significant increase in concentration from 63 to 260 ng/g on the seventh day of development. Interestingly, it dropped to 52 ng/g on day 9. Almost the same trend was observed in the case of cotton-grown garden cress. On the other hand, cadmium levels in soil-grown radish doubled from 83.5 to 167 ng/g throughout the four days of growth. However, it is still safe for human consumption; according to European Commission Regulation (EC) No 1881/2006, the limits for Cd after conversion to dry matter are around 2000 ng/g.

In general, it appears that the cultivation of microgreens on cotton can reduce harmful element concentrations, although some oscillations could be observed. It should be noted that the concentrations of toxic components in garden cress on soil are often higher than those of radish on the soil or on cotton, which might be attributed to the various absorbing capacities of the plants. Most element concentrations fluctuate over time, with occasional peaks and falls. Arsenic follows a similar pattern as in the case of radish but with even higher concentrations.

Lead and cadmium soared in the middle of the experiment, reaching more than 350 ng/g, then dropping back to their original values of around 120 ng/g on the last day. However, aluminum levels decreased when comparing the first and final days of the experiment. Nickel levels are also noticeably higher than radish samples, reaching up to 3.60 µg/g on day 7. However, they are not even close to the limit, which is 50 µg/g in dry matter, according to the European Commission Regulation (EC) No 1881/2006. Generally, the evidence shows that the concentration of hazardous components in plants changes depending on the type of plant, the substrate, and the development phase. It also emphasizes the importance of monitoring harmful element concentrations in plants to maintain food safety.

In the study by Giandomenico et al. (2022), the researchers analyzed the mineral profile of hemp microgreens with ICP-OES (Inductively Coupled Plasma - Optical Emission spectroscopy). The values of heavy metal concentrations contrast with values from our research. For example, the concentration of Pb in hemp microgreens was lower (3.15 ng/g) compared to our lowest measured value in the radish grown on cotton fibers (86.3 ng/g). The same applies to Cd, where in hemp, the concentration was also lower (1.20 ng/g) than in radish (51.9 ng/g). Aluminum in hemp was only 44.3 ng/g, while in cotton-grown radish, it was significantly higher (9850 ng/g). According to this study, hemp microgreens tend to accumulate toxic heavy metals less than radish or garden cress. However, the final concentrations of toxic elements depend mainly on growth conditions and the growth medium. In the research by Giandomenico et al. (2022), plants grew in a closed environmental chamber with precisely monitored conditions and supplied minerals.

5.2.4 Trace elements

However, trace elements have the highest number of elements, reaching the lowest concentrations in the samples. Despite being in tiny amounts, trace elements are necessary for the growth and development of plants and animals. These components are essential in various physiological activities, including enzyme activity, hormone control, and cell division. Microelement deficiencies can cause stunted development, poorer yields, and less resilience to illness and stress. As a result, keeping an appropriate supply of microelements in the soil, water, and feed is critical to maintaining the health and production of plants and animals. Therefore, they are affecting human health both directly and indirectly.

In the figures below, fluctuation of microelements and trace elements can be observed in all samples.

The group is divided based on concentration into two figures for a better presentation. Figure 12 interprets the elements in micrograms per gram due to their higher concentrations. These are lithium (Li), boron (B), scandium (Sc), titanium (Ti), strontium (Sr), and molybdenum (Mo). In the second part (Figure 13), elements such as vanadium (V), chromium (Cr), cobalt (Co), gallium (Ga), germanium (Ge), selenium (Se), yttrium (Y), zirconium (Zr), silver (Ag), tin (Sn), cesium (Cs), barium (Ba), cerium (Ce), tantalum (Ta), thallium (Tl) are interpreted in nanograms per gram. Once again, the concentration of elements in the soil-grown samples is noticeably higher than those grown on cotton. When comparing the two plants, the radish appears to follow a steady upward trend during the four days of development, unlike the garden cress, where the concentrations of some elements even dropped.

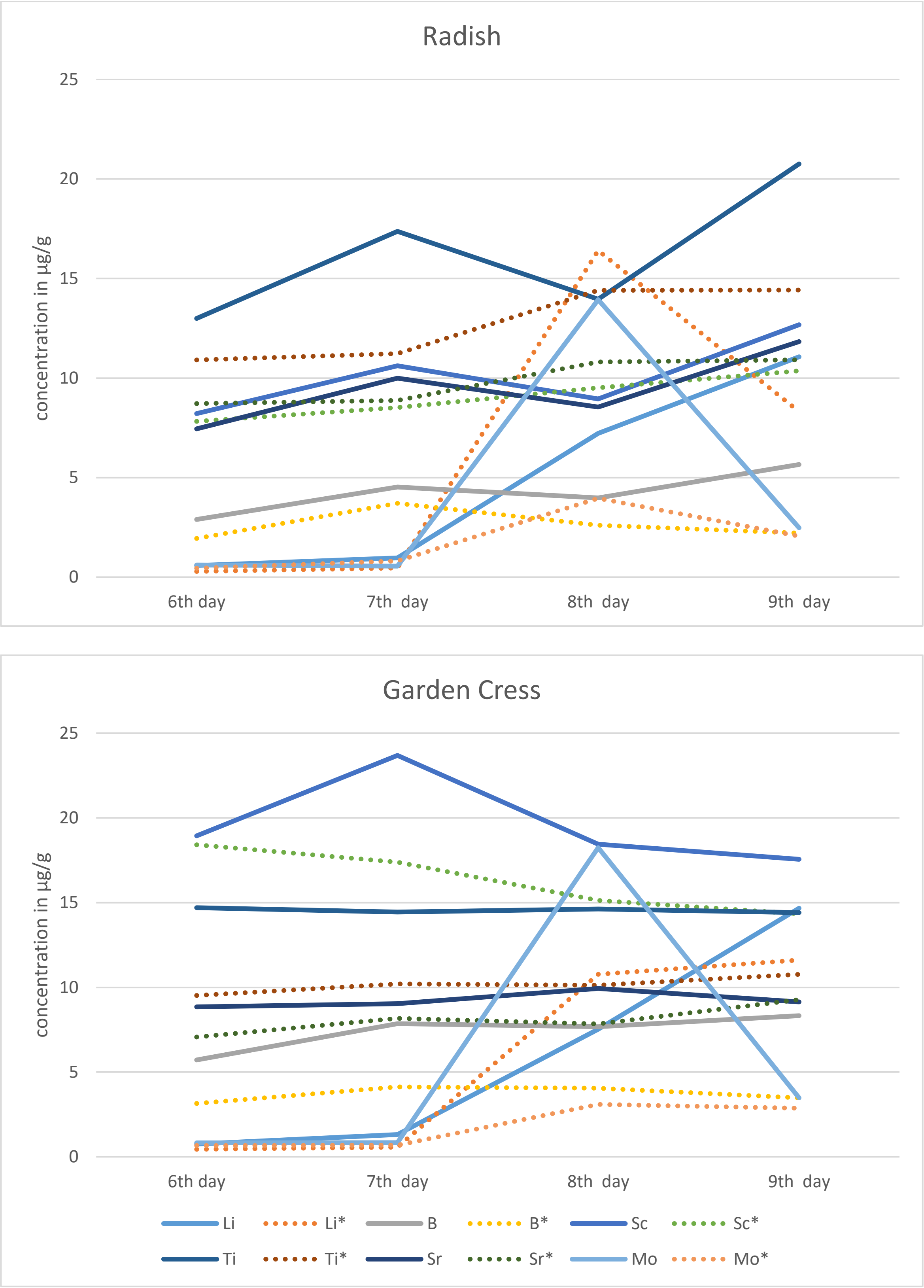

Figure 12: Concentration trends of elements throughout the growth (µg/g)

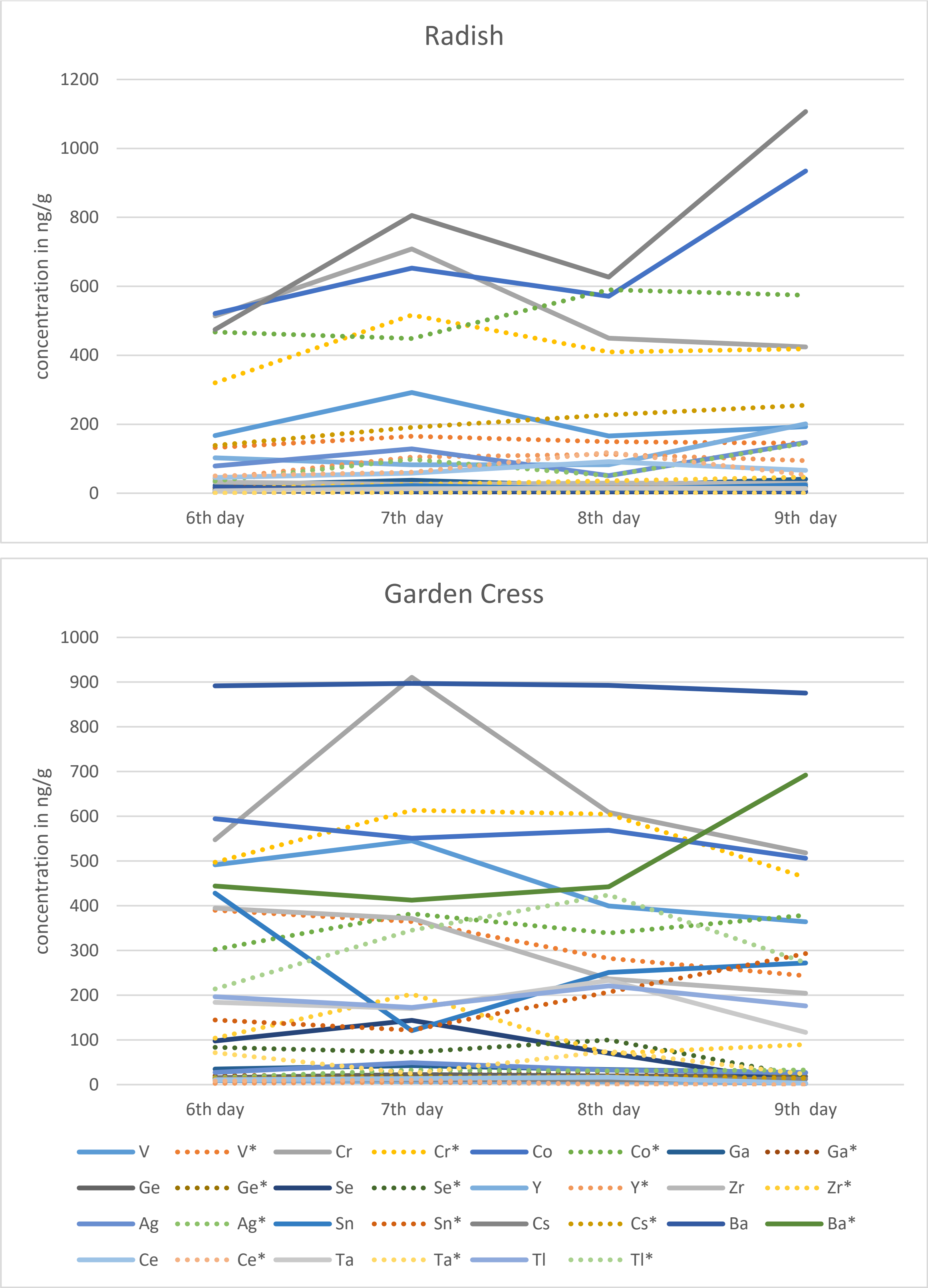

Figure 13: Concentration trends of elements throughout the growth (ng/g)

5.3 Estimated dietary intake of elements

5.3.1 Estimation of essential elements in the diet

Mineral and trace element shortages affect more than 2 billion people worldwide, with children under five and pregnant women at the highest risk. According to Affonfere et al. (2021), a diet deficient in minerals and trace elements or insufficient availability can cause anemia, osteoporosis, and other nutritional deficiencies that limit physical growth. These are just a few reasons it is critical to understand how food intake compares to the recommended nutritional intake guidelines. As a result, Table 3 lists the appropriate reference doses calculated as RDA or AI values for each stage group determined by the IOM (Institute of Medicine). The contribution of 100 g of microgreens to the RDA or AI values was calculated using the reference values for Ca, Cu, Fe, Mg, Mn, P, Zn, K, and Na for the specified life stage group, as well as the minimum and maximum values of the total concentrations of essential minerals and trace elements. The results are visualized in Figure 14 to compare the estimated amounts of essential minerals and trace elements in the diet of microgreens with the RDA or AI values for a group of young adults aged 19–30 years.

Table 3: RDA and AI values for males and females between 19–30 years old

Elements	Females	Males
*RDA or AI**	*mg/day*	*mg/day*
K*	4700	4700
Na*	1500	1500
Ca	1000	1000
P	700	700
Mg	310	400
Fe	18	8
Zn	8	11
Mn*	1.8	2.3
Cu	0.9	0.9

A food product may be considered as "significant source" of minerals under Regulation (EU) No 1169/2011 of the European Parliament and the Council on the provision of food information to consumers when it meets the requirement of 15% of the nutrient reference value of the nutrient supplied by 100 g. In this case, 100 g of microgreens was used in the RDA or AI calculations to determine if microgreens may be considered a "significant source" of a certain mineral or trace element.

Our findings show that microgreens had a non to negligible impact on the AI values of sodium (Na) and potassium (K) as well as RDA values of calcium (Ca), phosphorus (P), and zinc (Zn) for men and women aged 19 to 30 years. Sodium is a vital mineral for nerve and muscle function and is required to control blood pressure in the human body. The roles of Na are well established, including their involvement in regulating the volume and systemic distribution of total body water, facilitating the absorption of solutes by cells, and producing transmembrane electrochemical potentials through interactions with K (Affonfere et al., 2021). In addition to causing hypertension, this mineral taken in excess can have harmful effects on the body, including cirrhosis, renal disease, and congestive heart failure (Antoine et al., 2012).

The third most abundant mineral in the human body, potassium, governs the acid-base balance, osmotic pressure, and muscular contraction, notably in the heart muscle and the function of the cell membrane as an intracellular cation. It participates in energy transmission, hormone release, and protein and glycogen synthesis control as part of cell metabolism (Affonfere et al., 2021; Antoine et al., 2012). Except for breastfeeding women, for whom 5100 mg per day was recommended, the AI value for K was determined to be 4700 mg/day (IOM, 2005).

Ninety-nine percent of calcium is incorporated into the skeleton of the bones and teeth, while the remaining 1% percent is found in extracellular membranes. Additionally, it is necessary for nervous system function, blood clotting, hormone and enzyme secretion activation, and muscle contractions (Antoine et al., 2012; Affonfere et al., 2021). Depending on age and gender, humans need between 1000 and 1200 mg of calcium daily (IOM, 1997). Microgreens are not a reliable source of calcium and meet < 1% of the RDA for both genders in our age group. Calcium shortage can result in inadequate calcification and harm an adult's teeth. According to a published study, the excessive consumption of calcium supplements may contribute to decreased renal function among older generations (Affonfere et al., 2021).

Phosphorus is another mineral that, together with Ca salts, contributes to a healthy bone structure, among other benefits, such as transmitting cellular energy throughout the human body using adenosine triphosphate (Antoine et al., 2012;). Inadequate P nutrition has historically been linked to rickets and osteomalacia, which a lack of vitamin D and calcium can also cause. However, it does not seem that the usual adult diet lacks P; only 3% of the RDA for phosphorus is contributed by eating microgreens (Figure 14).

Microgreens seem to be an insignificant source of zinc. More specifically, microgreens supplied 13% of the RDA value for females and 9% of the RDA value of Zn for males. The micronutrients Zn and Fe are crucial for human health. Alkaline phosphatase, alcohol, glutamic, and lactate dehydrogenases, among other enzymes, require zinc as a cofactor. Affonfere et al. (2002) recognize it as crucial for gene expression and controlling cellular development and differentiation. According to estimates, 3 billion people worldwide suffer from various mineral deficiencies. For instance, deficiences in Zn, Cu, and Fe are common in developing nations (Fărcaș et al., 2022; Affonfere et al., 2021; EFSA, 2015). Zn insufficiency is explicitly regarded as an issue, with a risk of 33% for the general population (Fărcaș et al., 2022). This condition might be explained by factors such as insufficient Zn absorption caused by the inhibitory effects of phytate and polyphenols, commonly present in plant-based meals and are plentiful (Affonfere et al., 2021; EFSA, 2015).

The non-heme inorganic form of iron is the most prevalent dietary iron in food of plant origin. Most Fe is found in the human body as hemoglobin, the principal oxygen carrier from the lungs to the tissues. Anemia, decreased physical and cognitive function, and increased maternal and infant mortality are associated with its lack. When hemoglobin levels in women fall below 130 g/L, anemia is considered obvious, and the impact worsens with each increase in hemoglobin of 10 g/L. Furthermore, Fe deficiency, which primarily affects children and women in developing countries, has been reported to be the most common nutritional disorder globally, affecting more than 30% of the population (Rousseau et al., 2020). In plasma, Fe is also integrated into transferrin (Affonfere et al., 2021). This research revealed that males and females have drastically different RDAs for Fe. While young men can get up to 14% of their RDA from microgreens, young women can only get 6%. According to Antoine et al. (2012), several factors have been linked to Fe deficiencies. They are as follows: low bioavailability of Fe from foods, inability to meet Fe demand during growth spurts such as infancy and pregnancy, menstrual blood loss in women, blood losses from parasitic infections, and loss from diseases such as malaria.

Magnesium functions in the crystal structure of hydroxyapatite in bones, influencing bone health. Low magnesium levels are associated with an increased risk of type 2 diabetes. Additionally, it is a component of cofactor enzymes such as kinases. In simple terms, Mg is a crucial cofactor for enzymes involved in glucose metabolism. In addition, there are several other potential reasons for magnesium shortage, such as kidney and gastrointestinal problems, immune system issues, growth inhibition, muscular weakness, tetany, and a higher chance of developing chronic diseases (Affonfere et al., 2021; Antoine et al., 2012). Consuming 100 g of microgreens significantly increases the RDA value of magnesium and copper and the AI values of manganese (Figure 14). The average intake of Mg was calculated, representing 42% of RDA for females and 33% for males aged 19 to 30.

As a component of redox enzymes and hemocyanin, copper is necessary to the human body at trace levels for mitochondrial function, cell metabolism, connective tissue creation, and iron absorption and storage (Antoine et al., 2012). Since Cu is present in many foods and has a relatively low RDA value, deficiency is uncommon. Clinical conditions related to copper deficiency include heart failure, anemia, gastrointestinal problems, bone problems, and growth and reproduction problems. Adults have an acceptable upper intake threshold of 5 mg per day (EFSA, 2015). The ADI (acceptable daily intake) was recently set at 0.07 mg/kg bw by the EFSA Scientific Committee after it was determined that Cu retention is not anticipated to occur with ingestion of 5 mg/day (EFSA, 2023). Copper's calculated contribution to the diet's RDA value when consuming microgreens is the same for both genders (48%). Microgreens are a great source of copper.

Manganese is a necessary nutrient for humans and aids in forming bones, controlling protein and energy metabolism, and protecting cells against oxidative stress (Affonfere et al., 2021). Mn and Fe compete for the same absorption sites; therefore, diets rich in Mn may have limited bioavailability of Fe, which might lead to a shortage of Fe (Affonfere et al., 2021; EFSA, 2013a). The AI values of manganese for males and females aged 19 to 30 were set to 2.3 and 1.8 mg/day (IOM, 2001). The daily nutritional reference value (NRV) of manganese for adults is still 2.0 mg/g as per Regulation (EU) No 1169/2011 of the European Parliament and of the Council on the provision of food information to consumers (current consolidated version). The results show that microgreens are a great source of Mn for young adults. On average, 100 g of microgreens provide 55% (males) and 43% (females) of the AI value of manganese. In conclusion, it was discovered that both genders contributed differently to the proper RDA or AI values. According to this study, microgreens contribute to the RDA or AI values for specific minerals and trace elements, especially for Mg, Mn, and Cu.

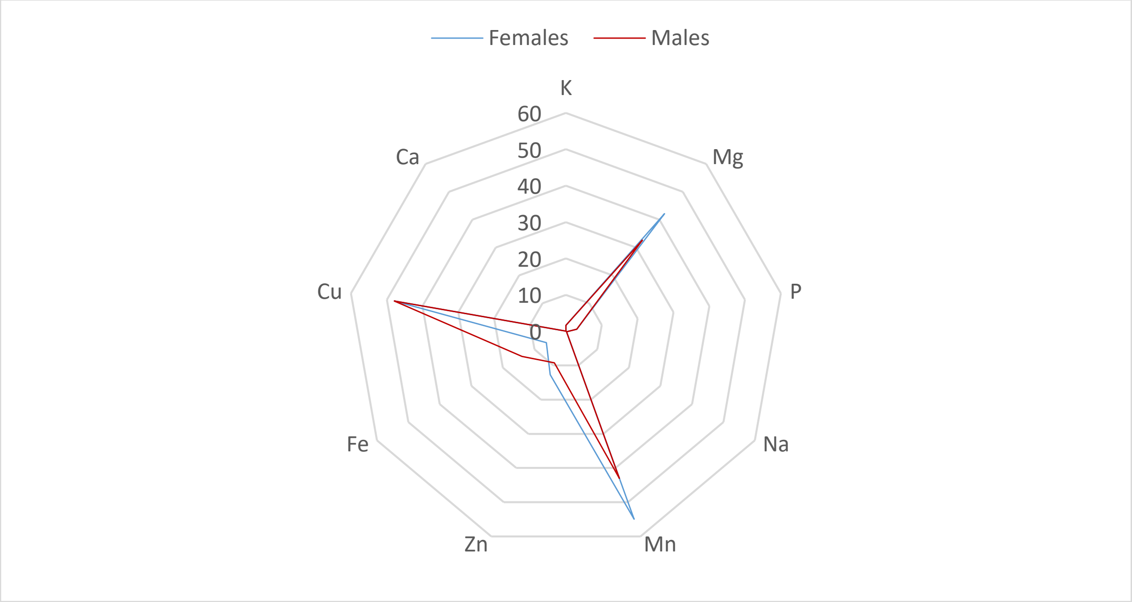

Figure 14: Dietary intake levels (%) of essential minerals and trace elements in the diet of microgreens for males and females aged 19–30

5.3.2 Estimation of toxic elements in the diet

Toxic substances could negatively impact any living organism. Given their propensity to accumulate over time, poor biodegradability, and lengthy biological half-lives, exceeding specific trace concentrations could result in various harmful health effects (Bielecka et al., 2020).

According to a combination of frequency, toxicity, and possible adverse effects on human health, arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), and tin (Sn) are among the top ten substances on the Substance Priority List (SPL) compiled by the Agency for Toxic Substances and Disease Registry (ATSDR, 2022). Aluminum (Al) has also been included due to its abundance in soil and green veggies. Due to the technical issue with Hg concentration determination in our samples, and its exclusion from the research, Hg will not be discussed further. The PTWI and PTMI values for selected toxic elements are listed in Table 4.

Table 4: PTWI and PTMI values of toxic elements

Toxic elements	PTWI, PTMI* *µg/kg*
Al	2000
As	15
Pb	25
Cd*	25
Sn	14000

After exposure through diet, humans absorb cadmium at a relatively low rate (3–5%). Despite this, it has a very long biological half-life of up to 30 years and is effectively retained in the liver and kidneys. Cd is predominantly harmful to the kidney, particularly the proximal tubular cells, where it can alter renal function over time due to accumulation. As a result, it is necessary to monitor such things (EFSA, 2009). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) determined the provisional tolerably monthly intake (PTMI) for Cd consumption (Table 4) as 25 g/kg bw (FAO/WHO, 2013). The EFSA (2011) report states that the recommended value of PTMI for Cd equates to a weekly consumption of 5.8 g of Cd/kg bw. As can be seen from the study's findings (Figure 15), for individuals weighing 70 and 90 kg, the estimated contribution of microgreens to the PTMI value for Cd has surpassed 50 and 65%. At first sight, these levels are disturbing; however, considering that the average serving size of microgreens is usually less than 10 g, the risk of Cd intoxication should be reduced.

The diet, with a wide range of differences in aluminum content between meals, is the primary way most of the population is exposed to aluminum. Cereals, cereal-based goods, and vegetables are the primary sources of Al in the diet. The exposure to drinking water is relatively low (EFSA, 2008). Aluminum has not been demonstrated to have any vital functions in people. However, it is neurotoxic in dialysis patients regularly exposed to high levels. Aluminum has also been associated with neurological illnesses in humans, such as Parkinson's disease, amyotrophic lateral sclerosis, and Alzheimer's disease (EFSA, 2008; Filippini et al., 2019). After a fluctuation of limits set by the authorities throughout time, Al's provisional tolerable weekly intake is set at 2 mg/kg bw (Table 5) (FAO/WHO, 2011b). As can be seen in our results (Figure 15), the dietary intake levels from microgreens to the PTWI value for Al could not be ignored, with estimates of more than 18 and 14% for adults weighted 70 and 90 kg.

Lead exposure has been associated with several adverse health issues, including neurological and behavioral ones, cardiovascular problems, decreased renal function, poor fertility, and unfavorable pregnancy outcomes. Adults in the EU were found to have a mean dietary exposure to Pb ranging from 0.36 to 1.24 g/kg bw (FAO/WHO, 2011c; EFSA, 2010; EFSA, 2012b). The PTWI value for Pb (25 µg/kg bw) was withdrawn in 2011, with the statement that it is not feasible to set a new PTWI that would be considered as protective for health even though Pb harms the neurological system and causes blood problems. The Committee emphasized that additional sources of lead exposure must also be taken into account as these estimates are based on dietary exposure (mostly food). However, the calculation was still done in this research because of its negative effects.

In addition, tap water, vegetables, and cereals were thought to be the main sources of Pb consumption for most Europeans (EFSA, 2012b). According to our results (Table 5), and based on the body weight, 11 to 14% of a PTWI can be obtained from a 100 g portion of microgreens.

The chemical form and solubility of arsenic affect how dangerous it is. Generally speaking, the As^{3+} form is more toxic than the As^{5+} form. Humans who consume inorganic arsenic over an extended period have been found to be more susceptible to developing cancer, skin lesions, cardiovascular disease, neurotoxicity, and diabetes. Arsenic, mainly found in foods as the non-toxic compounds arsenobetaine and arsenocholine is eliminated by the human tract (FAO/WHO, 2011; Antoine et al., 2012). According to FAO/WHO (2011), the average daily dietary exposure to inorganic As, including food and water intake, ranged from 100 to 3 000 mg/kg bw in the USA and several European and Asian nations. However, according to data from the EFSA, the average dietary intake for adults varied from 90 to 380 mg/kg bw, with grain-based processed items (apart from rice products) accounting for most of this exposure (EFSA, 2014a). The daily serving of microgreens (Figure 15) only contributes 5 to 6.3% of arsenic PTWI based on the body weight, which translates to < 0.01 mg/kg bw daily. However, the PTWI value for As was withdrawn by the committee in 2011, as it was no longer considered health protective (EFSA, 2011).

The acute harmful effects on humans following the consumption of foods with high tin concentrations were studied by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The previously defined provisional maximum tolerated daily intake (PMTDI) of 2 mg/kg of body weight was changed by the Committee to a provisional tolerable weekly intake (PTWI) of 14 mg/kg of bw (Table 5). Because of their limited absorption from the gastrointestinal tract, minimal accumulation in tissues, and rapid transit through the tract, inorganic Sn compounds were typically shown to have low systemic toxicity in animals (FAO/WHO, 2006). However, when consumed in a high amount, Sn can result in acute gastrointestinal system issues in people, including abdominal distension, discomfort, and vomiting. Food in unlacquered or partially lacquered Sn-plated cans is the primary dietary source of inorganic tin. Highly acidic meals have higher inorganic Sn migration from the tinplate to the food (FAO/WHO, 2006; Filippi et al., 2019). Microgreens did not affect the PTWI value of Sn in our investigation (Figure 15); contributions were less than 0.14% for any weight group.

To conclude, the contributions of toxic elements to PTWI and PTMI varies based on the type of element and, obviously, the body weight of individuals. A person weighing 70 kg could get two-thirds of a PTMI set for Cd by eating 100 g of microgreens daily. Meanwhile, a person weighing 90 kg would only get halfway. Based on our data, the contribution of the remaining toxic elements, such as Al, As, Pb, and Sn, to PWTI is not too disturbing.

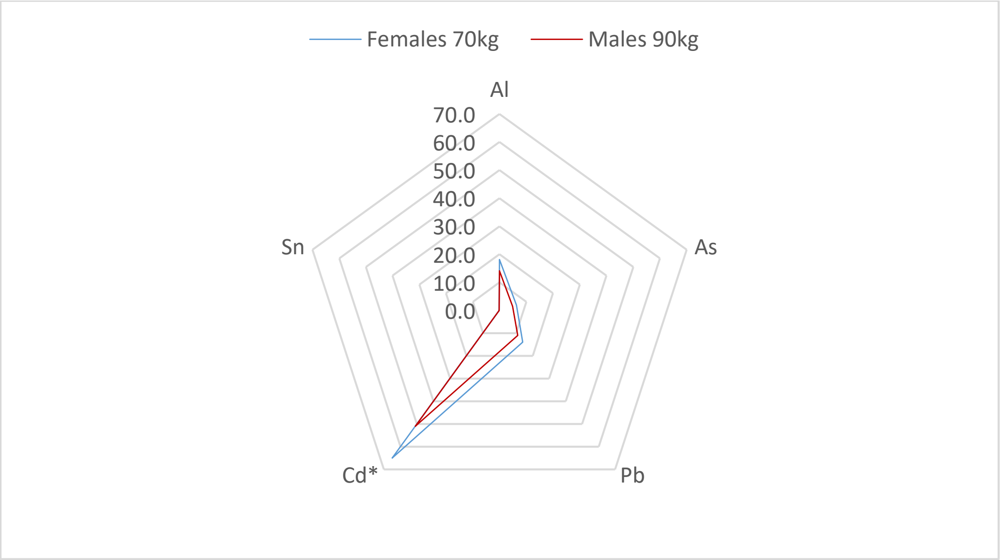

Figure 15: Dietary intake levels (%) of toxic trace elements from microgreens with respect to body weight

CONCLUSION

Based on available literature and an examination of microgreen element composition, it is clear that microgreens constitute a rich source of essential macro and microelements. The most abundant elements found in both samples were Mg and K.

The trend visualization throughout the four days of plant growth shows that the quantities of these nutrients change throughout the development process, with some minerals and trace elements soaring (Mg, K, Na), some slowly rising or fluctuating (Ca, P, Mn, Cu, Zn, Fe) with even some occasional and negligible decrease. Based on the results, most essential elements' peak concentration is on the 8th or 9th day of growth, which is the best time for harvesting microgreens. Overall, the growth stage at which microgreens are collected is critical since it can significantly influence their nutritional value.

Growing circumstances and the microgreens type can also impact the mineral and trace element content. The soil-grown microgreens have higher mineral content and grow faster into healthier plants than those grown on cotton fibers (Figures 6 and 7). There were also some differences in mineral content between the two types of microgreens, where garden cress seems richer in some minerals, especially K and Mn. With increasing concentrations of beneficial macro elements, there has been an observable uptrend in the toxic trace element concentrations, such as Pb, As, Al, Hg, and Sb. However, some of these dropped on the 9th day. Again the cotton-grown samples appeared to have lower concentrations of these heavy metals than the ones grown in soil. This corresponds with the mineral analysis of the soil (Table) we have used for increasing the microgreens.

Calculating RDA and AI values for critical macro and micro components demonstrates the nutritional benefits of routinely ingesting microgreens. Microgreens are a great source of Mg, Cu, and Mn, covering about half of the RDA in the 100g portion. On the other hand, calculating PWTI and PTMI values for the most poisonous heavy metals underscores the importance of carefully considering the growing circumstances and source of microgreens to minimize potential health hazards. These heavy metals were not found in significant amounts in microgreens; however, the Cd PTMI appeared to be disturbing, reaching more than 65% of tolerable monthly intake.

Overall, this study objectively assesses microgreens mineral and trace element content of microgreens while also laying the framework for future research in this area. Further research into the effects of growing conditions, variety selection, and harvesting timing on the nutrient content of microgreens might result in suggestions for optimizing nutritional advantages while reducing potential health hazards.

BIBLIOGRAPHY

1. Affonfere, M. et al. (2021). In-vitro digestibility methods and factors affecting minerals bioavailability: A review. Food Reviews International. https://doi.org/10.1080/87559129.2021.1928692
2. Antoine, J.M.R. et al. (2012). Dietary intake of minerals and trace elements in rice on the Jamaican market. Journal of Food Composition and Analysis, 26, 111–121. https://doi.org/10.1016/j.jfca.2012.01.003
3. Aschner, J. L., & Aschner, M. (2005). Nutritional aspects of manganese homeostasis. Molecular aspects of medicine, 26(4-5), 353-362.
4. ATSDR (2022). Agency for Toxic substances and Disease Registry. ATSDR´s Substance Priority List. https://www.atsdr.cdc.gov/spl/index.html#2022spl
5. Bates, B. et al. (2018). National Diet and Nutrition Survey Results from Years 7 and 8 (combined) of the Rolling Programme (2014/2015 to 2015/2016): U.K. https://www.gov.uk/government/statistics/ndns-results-from-years-7-and-8-combined
6. Bian, Z.-H. et al. (2017). Minerals and flavonoids in vegetables and their correlation. Food Chemistry 237: 25-31.
7. Bielecka, J. et al. (2020). Content of toxic elements in 12 group of rice products available on Polish market: Human health risk assessment. Foods, 9, 1906.
8. Bojórquez-Quintal E. et al. (2017). Aluminum, a Friend or Foe of Higher Plants in Acid Soils. Frontiers in Plant Science Journal, vol. 8, DOI=10.3389/fpls.2017.01767, https://www.frontiersin.org/articles/10.3389/fpls.2017.01767
9. Chandrasekaran, M. et al. (2020). Microgreens: A comprehensive review on nutritional, medicinal, and environmental aspects. Food Science and Human Wellness, 9 (1), 1-11.
10. Cohen, S. P. et al. (2015). Influence of seed maturity and harvest time on the content of glucosinolates, ascorbic acid and tocopherols in radish microgreens. Journal of Functional Foods, 16, 312-318.

11. Craig Plett, D., & Møller, I. S. (2010). Na(+) transport in glycophytic plants: what we know and would like to know. Plant, cell & environment, 33(4), 612–626. https://doi.org/10.1111/j.1365-3040.2009.02086.x
12. Dimitri, C. (2019). Microgreens Production: A Beginner's Guide. Cornell CooperativeExtension. https://s3.amazonaws.com/assets.cce.cornell.edu/attachments/38153/Microgreens_Production_A_Beginners_Guide.pdf?1551340362
13. EFSA (2008). Safety of aluminium from dietary intake. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact materials (AFC).
14. EFSA (2009). Scientific Opinion. Cadmium in food. Scientific opinion of the panel on Contaminants in the Food Chain. EFSA Journal, 980, 1–139. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2009.980
15. EFSA (2009a). Scientific Opinion. Scientific opinion on arsenic in Food. EFSA Journal, 7(10), 1351. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2009.1351
16. EFSA (2010). Scientific Opinion on Lead in Food. EFSA Journal, 8(4), 1570. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2010.1570
17. EFSA (2011). Scientific Opinion. Statement on tolerable weekly intake for cadmium. EFSA Journal, 9(2), 1975. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2011.1975
18. EFSA (2012). Cadmium dietary exposure in the European population. EFSA Journal, 10(1), 2551. https://www.efsa.europa.eu/en/efsajournal/pub/2551
19. EFSA (2012b). Scientific Report of EFSA. Lead dietary exposure in the European population. EFSA Journal, 10(7), 2831. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2012.2831
20. EFSA (2013a). Scientific Opinion on Dietary Reference Values for manganese. EFSA Journal, 11(11), 3419. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2013.3419

21. EFSA (2014a). Scientific Report of EFSA. Dietary exposure to inorganic arsenic in the European population. EFSA Journal, 12(3), 3597. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3597
22. EFSA (2015). Scientific Opinion on Dietary Reference Values for Copper. EFSA J. 2015, 13(10), 4253. DOI:10.2903/j. efsa.2015.4253.
23. EFSAJournal,754,1–34. https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2008.754
24. Fărcaș, A.C. et al. (2022). In vitro digestibility of minerals and B group vitamins from different brewers´ spent grains. Nutrients, 14, 3512. https://doi.org/10.3390/nu14173512
25. Filippini, T. et al. (2019). Aluminium and tin: Food contamination and dietary intake in an Italian population. Journal of Trace Elements in Medicine and Biology, 52, 293–301. https://doi.org/10.1016/j.jtemb.2019.01.012
26. García-Casal et al. (2017). Zinc, iron and calcium are major limiting nutrients in the production of three leafy vegetables and their multiple-harvest period. International Journal of Agriculture and Biology, 19(1), 1-8. doi: 10.17957/IJAB/15.0342
27. Giandomenico, C. et al (2022). Macro and trace element mineral composition of six hemp varieties grown as microgreens. Journal of Food Composition and Analysis. Volume 114, 104750, ISSN 0889-1575. https://doi.org/10.1016/j.jfca.2022.104750
28. Goyeneche, R. et al (2015). Chemical characterization and antioxidant capacity of red radish (Raphanus sativus L.) leaves and roots. Journal of Functional Foods. 16. 256-264. 10.1016/j.jff.2015.04.049.
29. Graamans, L. et al. (2017). The potential of hydroponic vegetable production in developing countries: A review. Agronomy for Sustainable Development, 37(4), 34.
30. Guerrero-Romero, F., & Rodríguez-Morán, M. (2011). Complementary therapies for diabetes: the case for chromium, magnesium, and antioxidants. Archives of medical research, 42(5), 347-355.
31. Gupta, D. et al. (2017). Dragon's blood: Botany, chemistry and therapeutic uses. Journal of ethnopharmacology, 202, 247-266.

32. Hodges, L. (2014). Growing Microgreens. University of Florida Institute of Food and Agricultural Sciences. https://edis.ifas.ufl.edu/pdffiles/HS/HS126500.pdf

33. Hossain, M. A. et al. (2012). Molecular Mechanisms of Heavy Metal Toxicity and Tolerance in Plants: Central Role of Glutathione in Detoxification of Reactive Oxygen Species and Methylglyoxal and in Heavy Metal Chelation. Journal of Botany, 2012, 37. https://doi.org/10.1155/2012/872875

34. Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. The American journal of clinical nutrition, 91(5), 1461S-1467S.

35. Husted, S. et al. (2017). Use of plant-based diets for improved mineral nutrition and human health. In Plant Macronutrient Use Efficiency (pp. 427-443). Academic Press.

36. IOM, 1997. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Institute of Medicine. National Academy of Sciences, the National Academy Press, Washington, DC.

37. IOM, 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Institute of Medicine. National Academy of Sciences, the National Academy Press, Washington, DC.

38. IOM, 2005. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Institute of Medicine. National Academy of Sciences, the National Academy Press, Washington, DC.

39. Kabata-Pendias, A., & Mukherjee, A. B. (2007). Trace elements from soil to human. Springer Science & Business Media.

40. Kader, A. A. (2002). Increasing Food Availability by Reducing Postharvest Losses of Fresh Produce. Acta Horticulturae 628: 155-166.

41. Karačić, A., Gaćina, M., & Pleadin, J. (2020). Leafy vegetables as a significant source of antioxidants. Current opinion in food science, 32, 1-7.

42. Koubala, B.B. et al. (2006). "Water content determination in some fresh vegetables using different drying techniques." Journal of Food Composition and Analysis, 19(1-2), 58-62.

43. Kumari et al. (2021). Physiological mechanisms and adaptation strategies of plants under nutrient deficiency and toxicity conditions. 10.1016/B978-0-323-85665-2.00012-1.
44. Lambers, H. et al. (2012). Plant nutrient-acquisition strategies change with soil age. Trends in ecology & evolution, 27(11), 625-632.
45. Larson, B. L., & Frontera, W. R. (2000). Micronutrients and bone health: calcium and beyond. The Journal of musculoskeletal and neuronal interactions, 1(4), 327-332.
46. Liu, H. Y. et al. (2013). Heavy metal accumulation and health risk assessment in leafy vegetables grown in uncontaminated soils of China. Environmental monitoring and assessment, 185(9), 7359-7365. https://doi.org/10.1007/s10661-013-3097-y
47. Lu, N. et al. (2019). Nutritional and phytochemical profiles of microgreens grown in hydroponics and soil. Journal of Food Composition and Analysis, 77, 27-33.
48. Maity, S. et al. (2021). Heavy metal contamination of soil and leafy vegetables in peri-urban agricultural lands in India: a systematic review. Journal of Environmental Management, 289, 112535. doi: 10.1016/j.jenvman.2021.112535
49. Maluin et al. (2021). Some Emerging Opportunities of Nanotechnology Development for Soilless and Microgreen Farming. Agronomy 11, no. 6: 1213. https://doi.org/10.3390/agronomy11061213
50. Marvasi, M. et al. (2016). Hydroponic systems for horticultural crop production: A review of nutrient management strategies. Scientia Horticulturae, 211, 220-234.
51. Miles, C., & Brown, A. (2019). Microgreens Production: A Guide for Growers in Indiana. Purdue Extension. https://mdc.itap.purdue.edu/item.asp?itemID=25118
52. Miyashita, M. et al. (2018). "Potential of microgreens as a novel crop in Japan." Acta Horticulturae 1201: 91-96.
53. Morton, K. (2018). Microgreens Are Your Ticket to Becoming the Barefoot Contessa of Plant Ladies. Online article. https://www.wellandgood.com/how-to-grow-microgreens/

54. Nair, A. and Ngouajio, M. (2018). Soilless Substrates for Commercial Production of Vegetable Crops: Review of Organic and Inorganic Materials. Horticulturae, 4(4), p.29.
55. Niroula, A et al. (2021). Pigments, ascorbic acid, total polyphenols and antioxidant capacities in deetiolated barely (Hordeum vulgare) and wheat (Triticum aestivum) microgreens. Food Chemistry 354, 129491
56. Ogundare, Margaret & Lajide, Labunmi. (2013). Physico-Chemical and mineral analysis of composts fortified with NPK fertilizer, Ammonium Chloride and Kaolin. Journal of Agricultural Chemistry and Environment. 02. 27-33. 10.4236/jacen.2013.22005.
57. Paine et al. (2012). How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. Methods in Ecology and Evolution, 3(2), 245-256.
58. Rajput, R. et al. (2018). Health benefits of leafy vegetables: A review. International Journal of Food Science & Technology, 55(1), 11-22.
59. Rayman, M. P. (2012). Selenium and human health. The Lancet, 379(9822), 1256-1268.
60. Römheld, V., & Marschner, H. (1986). Evidence for a specific uptake system for iron phytosiderophores in roots of grasses. Plant physiology, 80(1), 175-180.
61. Rousseau, S. et al. (2020). Barriers impairing mineral bioaccessibility and bioavailability in plant-based foods and the perspectives for food processing. Crit. Rev. Food Sci. Nutr. 2020, 60, 826–843. https://doi.org/10.1080/10408398.2018.1552243
62. Satarug, S. et al (2003). A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicology Letters, 137(1-2), 65-83. https://doi.org/10.1016/S0378-4274(02)00381-8
63. Sharma, S. et al. (2022). Vegetable microgreens: The gleam of next generationsuper Foods, their genetic enhancement, health benefits and processing approaches. Food Research International 155, 111038
64. Stabler, S. P., & Allen, R. H. (2004). Vitamin B12 deficiency as a worldwide problem. Annual review of nutrition, 24(1), 299-326.

65. Stamets, P. (2005). Growing Gourmet and Medicinal Mushrooms. Berkeley, Calif.: Ten Speed Press.
66. Szeląg-Sikora et al. (2019). Impact of Integrated and Conventional Plant Production on Selected Soil Parameters in Carrot Production. Sustainability. 11. 5612. 10.3390/su11205612.
67. Tucker, K. L. et al. (1999). Vitamin B-12 and bone mineral density in elderly men and women: the Framingham Osteoporosis Study. American journal of clinical nutrition, 69(4), 825-831.
68. Turnlund, J. R. (1998). Copper. In Present Knowledge in Nutrition (pp. 293-305). Academic Press.
69. U.S. Department of Agriculture. (2017). What are leafy vegetables? Retrieved from https://www.ers.usda.gov/topics/crops/vegetables-pulses/leafy-greens-background/
70. USDA Agricultural Research Service. (2021). Nutrient Data Laboratory. Retrieved from https://fdc.nal.usda.gov/
71. Volpe, S. L. (2013). Magnesium in disease prevention and overall health. Advances in Nutrition, 4(3), 378S-383S.
72. Wessells, K. R., & Brown, K. H. (2012). Estimating the global prevalence of zinc deficiency: results based on zinc availability in national food supplies and the prevalence of stunting. PloS one, 7(11), e50568.
73. White, P. J., & Broadley, M. R. (2005). Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytologist, 182(1), 49-84.
74. World Health Organization (2010). Lead Poisoning and Health. Retrieved from https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health.
75. World Health Organization (2010). Strontium and strontium compounds. Concise international chemical assessment document 77. WHO Press: Geneva. ISBN 978-92-4-153077-4. https://apps.who.int/iris/bitstream/handle/10665/44280/9789241530774_eng.pdf?sequence=1&isAllowed=y

76. World Health Organization (2017). Mercury and Health. Retrieved from https://www.who.int/news-room/fact-sheets/detail/mercury-and-health.
77. World Health Organization (2022). Chemical Safety and Health: Cadmium, Arsenic. Retrieved from https://www.who.int/news-room/fact-sheets/detail/arsenic
78. Worthmann, Ch. (2022). How To Grow Hydroponic Microgreens In 6 Steps. Online article. https://climatebiz.com/how-to-grow-hydroponic-microgreens/
79. Wu, D. et al. (2020). Effect of LED light on nutritional and functional quality of kale and radish microgreens. Journal of Food Science and Technology 57(3): 997-1005.
80. Wu, H. et al. (2019). Microgreens: A novel food in the functional food category. Food Science and Human Wellness, 8(2), 104-109.
81. Yang, T. et al. (2020). A Comprehensive Review on Nutritional Quality of Microgreens. Food Science and Human Wellness 9(2): 88-98.
82. Zhang, Y et al. (2021). Nutritional quality and health benefits of microgreens, a crop of modern agriculture. Journal of Future Foods 1-1, 58-66
83. Zhou, Y. et al. (2020). Dietary intake of vitamins, minerals, and fiber in relation to the risk of frailty: a systematic review and meta-analysis of observational studies. Nutrition journal, 19(1), 1-12.

LIST OF FIGURES

LIST OF TABLES