

Bosiacki, M., Markiewicz, B., Misiak, K., Rogalski, J. and Frąszczak, B. (2026) 'The effect of increasing nickel concentrations and the use of re-used rockwool on the yield and chemical composition of lettuce (*Lactuca sativa* L.)', *Journal of Elementology*, 31(1), 117-136, available: <https://doi.org/10.5601/jelem.2025.30.3.3644>



RECEIVED: 4 September 2025

ACCEPTED: 11 December 2025

ORIGINAL PAPER

The effect of increasing nickel concentrations and the use of re-used rockwool on the yield and chemical composition of lettuce (*Lactuca sativa* L.)*

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Abstract

Rockwool is one of the most popular substrates in soilless cultivation. Mineral wool has good physicochemical properties, and it is an inert, sterile substrate free from pathogens, toxic and ballast substances. It is a substrate with optimal conditions for the development of the root system, maintaining an optimal air-water ratio. Due to the lack of exchange sorption, it is possible to precisely control the nutrition of plants. In most crops, rockwool is used in one cultivation cycle; therefore, the aim of the conducted research was to determine the possibility of reusing wool in the cultivation of lettuce (*Lactuca sativa* L.) Zeralda F1, the effect on its yield, and chemical composition. The influence of increasing concentrations of nickel in the hydroponic solution on the yield of lettuce and its chemical composition was also studied. The research factors were two types of rockwool (new and re-used) and increasing concentrations of nickel (0-control; 5 and 10 mg dm⁻³). The plants were grown in a greenhouse in a closed system without nutrient recirculation. It was found that rockwool could be reused for lettuce cultivation. No differences in lettuce yield were found between growing in new and re-used rockwool. The authors compared the content of individual components and heavy metals in lettuce leaves grown in two types of rockwool, to which no nickel was introduced. The higher contents of potassium, calcium, magnesium, zinc, manganese, nickel, lead and cadmium were found in lettuce grown in re-used rockwool. On the other hand, in lettuce grown in new rockwool, higher contents of phosphorus and sulfur were found. Nickel in concentrations of 5 and 10 mg dm⁻³ of the nutrient influenced a higher greenness index of lettuce leaves. Increasing concentrations of Ni did not significantly affect the content of nitrogen, sodium, and copper in lettuce leaves, while the content of other nutrients and heavy metals depended on their concentration.

Keywords: hydroponic, macronutrient, micronutrient, lead, cadmium

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* The research was financially supported by the Ministry of Education and Science. Poland.

INTRODUCTION

Soilless cultivation, which includes hydroponics, is a model of sustainable food production, providing soil protection and reducing water consumption compared to traditional cultivation (Savvas 2003, Molden 2013, Muller et al. 2017). Soilless cultivation offers the advantage of being implementable in areas without agricultural land or with poor quality soil and in urban areas (Despommier 2011, FAO 2023, Mielcarek et al. 2024), as well as enabling higher yields by optimizing the content of nutrients, water, and air (Caron, Nkongolo 2004, Assouline et al. 2012, Blok et al. 2017). In some countries (Poland, the Netherlands, Spain, the USA, Canada), inert substrates, mainly rockwool, expanded clay, and polyurethane foam, play an important role in intensive cultivation under cover (Kleiber 2014, Markiewicz et al. 2016, Gruda 2019, Markiewicz et al. 2019, Komosa et al. 2020). According to Jarosz and Dzida (2006), rockwool provides good and stable physical properties (3% solid phase, 45% air, 52% water), and as an inert substrate, it does not create favorable conditions for pathogen presence (Wohanka 1998). On the other hand, as much as 150 m³ of rockwool waste is generated per ha of covered cultivation each year (Dannehl et al. 2015). Thus, the main disadvantage of rockwool is its limited reusability after the first cultivation cycle. One option is to use spent rockwool as an additive to soil or substrate mixtures (Bussell and McKennie 2004). Addition of rockwool improves physical properties of the resulting mixture (Fonteno, Nelson 1990, Choi et al. 1999, 2000). To extend its life, a rockwool mat can be reused in the next cultivation cycle. According to Komosa et al. (2020), the reuse of rockwool is economically justified, and the yield obtained in annual mats is higher than that harvested from new mats. Unfavorable changes in physical properties occurring in reused rockwool, consisting of reduced air availability, do not affect yield (Łażny et al. 2021). The optimized chemical composition of nutrient solution in hydroponic lettuce cultivation on rockwool is crucial for achieving optimal yield quantity and quality. Nickel has been considered as an essential nutrient since 1986 (Brown 1987); however, concentrations of nickel in hydroponic nutrient solutions for growing vegetables and ornamental plants are still a subject of ongoing studies. Research on the nickel content has demonstrated that the distribution of this micro-element in plant organs is a species-specific feature (Gorlach, Mazur 2002, Strączyński 2003). According to Ociepa-Kubicka and Ociepa (2012), accumulation of nickel in plants consumed by humans can cause adverse changes in the human body, including chronic poisoning, damage to the nervous system, mutations, and even the development of cancer. In soil and organic substrates, nickel uptake by plants depends on pH and the content of organic substances with which nickel forms complex compounds. Ions antagonistic to nickel include calcium and magnesium (Kabata-Pendias 1993) as well as iron, zinc, and copper (Komosa 2012). Nickel is essential for the proper

course of metabolic and physiological processes in plants. However, nickel concentrations in the range of 10 to 100 ppm are considered toxic to plants (Kleszczewska, Kaczorowski 2000). Nickel is involved in nitrogen metabolism; therefore, maintaining an optimal concentration of this micronutrient in hydroponic systems can reduce nitrate accumulation in plants (Brown et al. 1990, Watanabe, Shimada 1990). Additionally, nickel is a component of urease, the enzyme responsible for urea hydrolysis (Hänsch and Mendel 2009; Komosa 2012). At toxic concentrations, nickel adversely affects photosynthetic activity by reducing pigment content in chloroplasts (Veeranjaneyulu, Das 1982, Bishnoi et al. 1993).

The aim of the model study was to determine the effect of increasing concentrations of nickel on the yield, chemical composition of hydroponically grown lettuce (*Lactuca sativa* L.) in new and re-used rockwool.

MATERIALS AND METHODS

Vegetation experiment

The vegetation experiments were conducted in 2021 and 2022, in 2 independent cycles, in an unheated greenhouse at the Experimental Station of the Departments of the Faculty of Agriculture, Horticulture and Biotechnology, Poznan University of Life Sciences. The first cycle was conducted each year at the following dates: planting lettuce seedlings in rockwool on April 26, lettuce harvest on May 31. The second cycle was conducted from June 1 (lettuce planting) to July 6 (lettuce harvest). In each cultivation cycle, lettuce was grown in rockwool for 5 weeks (36 days of vegetation). The effect of increasing nickel concentrations and reused substrate on the yield and chemical composition of the aerial parts of butterhead lettuce (*Lactuca sativa* L.) cv. Zeralda F1 was studied. The plants were grown in a closed system without nutrient recirculation. The experiment was conducted in 6 combinations, each consisting of 4 replicates. One replicate was one rockwool mat with 4 plants. Each combination had 16 lettuce plants. Seeds were sown 30 days before the beginning of a cultivation cycle. They were sown pointwise into rockwool sticks, which had been saturated with a nutrient solution 48 hours before sowing. Seedlings were planted into new or reused rockwool saturated with the appropriate nutrient solution and then placed in cultivation gutters. The solution was prepared using tap water of pH 7.00, EC – 0.735 mS cm⁻¹ and the following chemical composition (mg dm⁻³): N-NH₄⁺ – alkaline, N-NO₃⁻ – 3.7, P-PO₄³⁻ – 0.3, K – 1.8, Ca – 57.3, Mg – 13.4, S-SO₄²⁻ – 58.3, Na – 22.7, Cl – 42.2, Fe – 0.08, Mn – 0.06, Zn – 0.50, Cu – alkaline, B – 0.11, Mo – trace amounts, HCO₃⁻ – 277.5. For fertigation, a solution with pH 5.50, EC 2.80 mS cm⁻¹ and composition (mg dm⁻³) was used: N-NH₄⁺ – 2.1, N-NO₃⁻ – 173, P – 42, K – 310, Ca – 135, Mg – 60, S-SO₄²⁻ – 120, Na – 35, Fe – 0.32, Mn – 0.52, Zn – 0.51, Cu – 0.03.

Nickel aqueous solution was prepared and added individually to each buffer tank (V1000 dm³). Nickel sulphate solution (NiSO₄ 6H₂O) at 0 (control 0.03); 5.0; 10.0 mg dm⁻³ of nutrient solution was used. The nutrient solution was prepared using fertilizers intended for hydroponic cultivation. Potassium nitrate (13% N-NO₃, 38.2% K), calcium nitrate (14.5% N-NO₃, 19.6% Ca), magnesium nitrate (11.0% N-NO₃, 9.5% Mg), monopotassium phosphate (22.3% P, 28.2% K), potassium sulfate (44.8% K, 17.0% S), magnesium sulfate (9.5% Mg, 12.7% S), Librel FeDP7 iron chelate (7% Fe), manganese sulfate (MnSO₄ H₂O, 32.3% Mn), copper sulfate (CuSO₄ 5H₂O, 25.6% Cu) and sodium molybdate (Na₂MoO₄ 2H₂O, 39.6% Mo) were used. The nutrient solution was acidified with nitric acid (38%), and the dose of nitrogen introduced with the acid was accounted for in the assumed level of this element. Each year, the aerial parts were collected 36 days after the seedlings were planted.

Analysis of SPAD

Before harvest, measurements of light absorption by the leaf blade (Leaf Greenness Index) were taken in 25 replicates from each plant. The measurements were made using a SPAD 502 device (Konica Minolta, Warrington, United Kingdom), which was employed in the study to assess the chlorophyll content in leaves. The device is equipped with two light sources emitting different wavelengths of 650 nm (corresponding to the maximum absorption by chlorophyll a and b) and 940 nm (corresponding to far red, retained by the leaf tissue).

Chemical analyses of plants

To determine the content of macro- and micronutrients, the collected aerial parts of lettuce were dried at a temp. of 45-55°C and then ground. The ground material was dried at a temp. of 105°C. To determine the general forms of nitrogen, phosphorus, potassium, calcium, magnesium and sodium, the plant material was mineralized in concentrated sulfuric acid (96%, pure per analysis) and in a mixture of nitric and perchloric acids in a 3:1 ratio in order to determine the micronutrient and sulfur (Bosiacki, Roszyk 2010). Chemical analyses after mineralization of plant material were performed using the following methods: N – general – the Kjeldahl method on a Parnas-Wagner distillation apparatus; P - colorimetrically with ammonium molybdate; K, Ca, Mg, Fe, Mn, Zn, Cu, Ni, Pb and Cd – by atomic absorption spectrometry (FAAS, Zeiss-Jena 5 apparatus).

Analysis of dry matter (DM)

On the last day of each cycle (May 31 and July 6), the fresh weight of a lettuce head (g) was measured.

Statistical analysis

The data were analysed with the Statistica 13.3 software (StatSoft Inc., Tulsa, OK, USA). The results of lettuce fresh weight yield measurements and chemical analyses of plants were statistically analyzed using Duncan's test ($\alpha=0.05$). A two-factor analysis of variance was used for 6 combinations, and each combination consisted of 16 plants. The two-factor analysis of variance was performed for the following research traits: lettuce fresh weight yield, content of Ni, Fe, Zn, Mn, Cu, N, P, K, Ca, Mg, S, Na, Pb, Cd and SPAD in lettuce leaves.

RESULTS AND DISCUSSION

In most horticultural crops, rockwool is used in one cultivation cycle. Reusing rockwool for cultivation may cause a higher risk of rhizosphere diseases. In the conducted study on reusing wool and the effect of increasing nickel concentrations, the authors obtained the highest lettuce yields (401.63 g plant⁻¹) in re-used rockwool (a combination without added nickel), while the lowest lettuce yield (284.00 g plant⁻¹) was harvested from plants grown in new rockwool with a dose of 5 mg Ni dm⁻³ (Table 1). No effect of increasing Ni concentrations on lettuce yield was found.

Table 1

The influence of rockwool type and increasing Ni concentrations on the yield of lettuce fresh mass (g plant⁻¹)

Type of rockwool	Nickel concentration (mg dm ⁻³)			Mean
	0	5	10	
New rockwool	320.75 ^{ab*} ±98.11	284.00 ^a ±81.00	372.88 ^{ab} ±73.94	325.88 ^a
Re-used rockwool	401.63 ^b ±131.93	367.63 ^{ab} ±110.04	313.38 ^{ab} ±78.27	360.88 ^a
Mean	361.18 ^a	325.81 ^a	343.13 ^a	

* Values followed by the same letters do not differ significantly at $\alpha=0.05$

According to Łażny et al. (2021), the reuse of mineral wool mats in cucumber cultivation had no significant effect on the overall growth of plants, the size and area of leaves, and the length and diameter of shoots compared to cultivation in fresh substrate. In the case of plants grown on used substrate, the number of fruits and the total weight of the crop increased significantly. No significant changes were observed in the average weight of a single fruit. Physiological tests showed a significant increase in the content of lutein, β -carotene, while parameters such as dry weight and chlorophyll a and b content did not change. According to Komosa et al. (2020), tomato growing in re-used 1-year-old rockwool slabs was demonstrated as a feasible solution, and the marketable and total yields were by 10.0-7.5% higher (respectively)

than from tomatoes grown in new rockwool. According to Tzortzakis and Economakis (2008), the addition of organic matter to inert substrates (perlite and pumice) had a positive effect on growth, yield, acceleration of harvest, and improvement of biological properties of tomatoes. It is likely that the addition of organic matter in the inert substrate, due to microbiological activity, increases the temperature in the root environment, exerting a specific effect on plants.

Nickel is the latest element to have been established as an essential micronutrient needed by plants (Brown 1987). Research is being conducted worldwide to determine its optimal levels for the cultivation of many plant species. Amir Ahmady et al. (2022) determined that increasing concentrations of nickel in the nutrient solution had a negative effect on the growth of *Trifolium repens*. Seedling death was observed at a concentration of 100 μM and above. In the case of shallot cultivation in hydroponic conditions, nickel concentrations above 0.025 mg dm⁻³ showed a negative effect on the weight and height of plants, as well as the weight of the onion. These trends intensified with increasing nickel concentrations in the hydroponic nutrient solution (Kuse et al. 2024). Antonkiewicz et al. (2016) claim that the ability to accumulate nickel in plant tissues depends on the species and the part of the plant being tested.

Although the amounts of nickel in the root system were not analyzed in this study, it was observed that the content of Ni in lettuce leaves grown in new rockwool ranged from 4.31 mg kg⁻¹ in the control combination to 6.59 at the highest applied nickel concentration (10 mg dm⁻³) – Table 2. In turn, in leaves of lettuce grown in re-used rockwool, nickel was found from 6.66 mg kg⁻¹ in the control combination to 21.31 mg kg⁻¹ at the highest applied nickel concentration. In both substrates, increasing Ni concentrations resulted in an increase in the amount of nickel in lettuce leaves. A higher nickel content was also found in lettuce leaves grown in re-used rockwool compared to the content in leaves obtained when growing it in new rockwool. The authors assume that the reason for obtaining a higher amount of Ni in lettuce leaves grown in re-used rockwool may be the transfer of a larger amount of nickel into the solution, previously taken up by the root systems undergoing humification

Antonkiewicz et al. (2016) claim that in the case of lettuce, higher nickel contents accumulate in the roots. The authors obtained the following results: significantly the lowest nickel content in roots was in the control combination (0.03 mg Ni dm⁻³) 18.1 mg Ni kg⁻¹ d.m, while the highest nickel content was obtained in the combination with the highest nickel concentration in the nutrient solution (10.0 mg Ni dm⁻³) 2660.6 mg Ni kg⁻¹ d.m. On the other hand, in the aerial part of lettuce, the lowest nickel content was obtained in the control combination (4.8 mg Ni kg⁻¹ d.m.), being similar to the one obtained in the cited study. However, the significantly highest content was obtained in samples where the nickel content in the nutrient solution

Table 2

The influence of rockwool type and increasing Ni concentrations on the content of micronutrients (mg kg⁻¹ d.m.) in the lettuce leaves

Type of rockwool	Nickel concentration (mg dm ⁻³)			Mean
	0	5	10	
Ni				
New rockwool	4.31 ^{a*}	4.82 ^a	6.59 ^b	5.24 ^a
Re-used rockwool	6.66 ^b	19.86 ^c	21.31 ^d	15.94 ^b
Mean	5.48 ^a	12.34 ^b	13.95 ^c	
Fe				
New rockwool	101.11 ^a	135.80 ^b	110.43 ^a	115.78 ^a
Re-used rockwool	99.34 ^a	132.24 ^b	109.30 ^a	113.62 ^a
Mean	100.22 ^a	134.02	109.86 ^a	
Zn				
New rockwool	77.23 ^a	88.77 ^a	158.25 ^{bc}	108.08 ^a
Re-used rockwool	184.77 ^c	152.35 ^b	133.79 ^b	156.97 ^b
Mean	131.00 ^{ab}	120.56 ^a	146.02 ^b	
Mn				
New rockwool	59.86 ^a	99.02 ^b	141.06 ^{cd}	99.98 ^a
Re-used rockwool	157.31 ^d	139.77 ^{cd}	124.75 ^c	140.61 ^b
Mean	108.58 ^a	119.40 ^a	132.90 ^b	
Cu				
New rockwool	7.45 ^a	6.90 ^a	7.57 ^a	7.31 ^a
Re-used rockwool	6.90 ^a	7.09 ^a	7.46 ^a	7.15 ^a
Mean	7.17 ^a	7.00 ^a	7.51 ^a	

* Values followed by the same letters do not differ significantly at $\alpha=0.05$

was from 8.5 to 10 mg Ni dm⁻³ (221.7 mg Ni kg⁻¹ d.m.). The authors also found that the medium with nickel concentrations ranging from 0.5 to 9.0 mg dm⁻³ stimulated the development of lettuce compared to the control combination (0.03 mg Ni dm⁻³).

The nickel content limit given in Commission Regulation (EU) 2024/1987 of 30 July 2024 for leafy vegetables including lettuce is 0.5 mg kg⁻¹ of fresh mass. After calculating the recommended maximum limit in fresh mass, this content was converted to dry mass and compared with the amounts of nickel in lettuce obtained in the study (Figure 1). The maximum nickel level was found to be exceeded in lettuce grown in re-used rockwool to which 5 and 10 mg Ni dm⁻³ were introduced in the solution. The authors assume that the increase in Ni content may be caused by earlier accumulation of nickel in the remains of root systems, which overgrew the rockwool mat during the previous lettuce cultivation cycle, and were its source after humi-

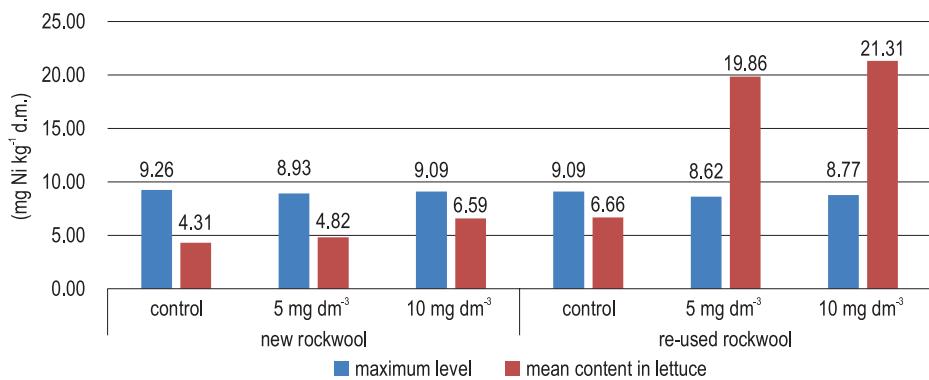


Fig. 1. Maximum content and Ni content in lettuce (in dry matter) depending on the type of rockwool and nickel concentration in the nutrient solution

fication for plants in the repeated cultivation in this substrate. The authors also assume that the increased nickel uptake by lettuce may have been caused by increased microbiological activity, which induced an increase in temperature in the root environment. In the remaining combinations, the Ni content in lettuce was not exceeded.

In the conducted study, the dry matter content of lettuce ranged from 5.4 to 5.8% (Figure 2). A higher dry matter content of lettuce was found when it was grown in re-used rockwool.

High nickel concentrations may reduce the translocation of micro-nutrients in the plant, especially Fe and Zn, because Ni has similar chemical properties (Myśliwa-Kurdziel et al. 2004, Ahmad et al. 2010). Nickel may also hinder the uptake of Cd and Pb due to ionic antagonism (Myśliwa-Kurdziel et al. 2004).

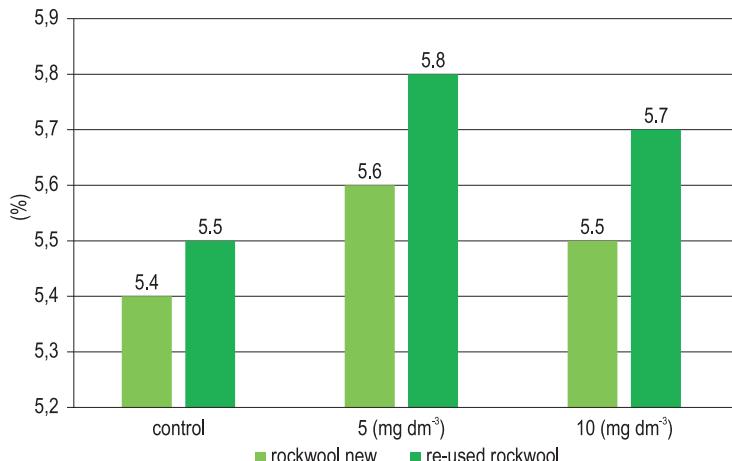


Fig. 2. The influence of rockwool type and increasing Ni concentrations on the average dry matter content of lettuce

In this study, the iron content ranged from 99.34 to 135.80 mg kg⁻¹ (Table 2). In new and re-used rockwool, the applied nickel concentration of 5 mg dm⁻³ significantly increased the iron content in lettuce.

The zinc content ranged from 77.23 to 184.77 mg kg⁻¹. The highest Zn content (184.77 mg kg⁻¹) was obtained in lettuce leaves growing in re-used rockwool without nickel. In this substrate, increasing doses of Ni resulted in a decrease in the amount of zinc in lettuce leaves. On the other hand, more nickel was found (158.25 mg kg⁻¹) in the new rockwool to which nickel was added in the amount of 10 mg dm⁻³ of the solution, compared to the other combinations within this substrate. Analyzing the average Zn content in lettuce, regardless of the nickel dose, the highest value of 156.97 mg kg⁻¹ was obtained in lettuce leaves grown in re-used rockwool.

The manganese content in lettuce leaves ranged from 59.86 to 157.31 mg kg⁻¹. The highest Mn content (157.31 mg kg⁻¹) was obtained in lettuce leaves growing in re-used rockwool without nickel addition. In lettuce leaves growing in new rockwool, increasing nickel concentrations significantly influenced the increase in manganese content. On the other hand, the concentration of 10 mg Ni dm⁻³ of the solution introduced to re-used rockwool influenced the obtaining of a lower manganese content in comparison to the Mn content obtained in lettuce leaves growing in the control combination. Analyzing the average Mn content in lettuce, regardless of the nickel dose, the highest 140.61 mg kg⁻¹ was obtained in lettuce leaves growing in re-used rockwool.

The copper content ranged from 6.90 to 7.57 mg kg⁻¹. There was no effect of increasing nickel concentrations or rockwool type on copper content in lettuce leaves.

In our study, we compared the content of individual micronutrients in lettuce leaves grown in two types of rockwool to which no nickel was added. Higher zinc, manganese and nickel content was found in lettuce grown in re-used rockwool (Table 2).

In studies on *Bornmuellera emarginata* grown hydroponically (Ly et al. 2024), at different nickel levels, a significant effect of increasing nickel concentrations was demonstrated on the decrease in the content of Fe (275 mg kg⁻¹ to 47.7 mg kg⁻¹), Mn (180 to 50.8 mg kg⁻¹), Cu (10.6 to 3.4 mg kg⁻¹). However, no effect of increasing nickel concentrations on the zinc content in tissues was demonstrated. However, in the studies of Amir Ahmady et al. (2022), increasing nickel concentrations in the range from 10 to 50 µM significantly influenced the decrease in the content of iron, zinc, and copper compared to the control combination.

Many scientific centers are conducting research on the optimization of lettuce cultivation in hydroponics. In this type of technology, it is important to obtain a good quality lettuce yield with the appropriate chemical composition. In the conducted studies, the content of obtained macroelements in lettuce was in the range (% d.m.): nitrogen content in lettuce leaves was in the range from 3.89 to 4.10, P 0.58-0.71, K 5.00-7.34, Ca 0.73-2.32,

Mg 0.31-0.65, S 0.42-0.79, and Na 0.16-0.53 (Table 3). On the other hand, the content of obtained microelements in lettuce was in the range (mg kg⁻¹ d.m.): Fe 99.34-135.80, Zn 77.23-184.77, Mn 59.85-157.31, Cu 6.90-7.57 and Ni 4.31-21.31 (Table 2).

In the study by Dylag et al. (2023) on the biofortification of lettuce with iodine, the following contents of micronutrients (mg kg⁻¹) was in the range: Fe 165.4-191.9, Cu 5.7-13.3, Zn 54.9-100.9, Mn 75.5-205.7, Mo 0.7-1.4, B 46.9-71.8. Dasgan et al. (2023) in their studies on the use of biofertilizers in hydroponic cultivation of lettuce found the range of micronutrient contents was (mg kg⁻¹): Fe 69.23-101.42, Mn 21.52-33.38, Zn 51.0-70.94, Cu 3.99-5.99.

According to many authors, the content of nutrients and sodium in the aboveground parts of lettuce is very diverse (Table 3).

Table 3

The content of macronutrient (% d.m.) in lettuce leaves according to other authors

Source	N	P	K	Ca	Mg
Dylag et al. (2023)	3.9-4.3	0.9-1.1	8.9-11.4	1.4-1.9	0.4-0.5
Dasgan et al. (2023)	5.24-6.46	0.20-0.25	7.46-9.70	0.74-1.06	1.0-1.28
Al-Karaki and Altuntas (2021)	2.82-3.46	0.42-0.50	5.89-6.59	1.3-1.48	0.25-0.49
Breš et al. (2022)	3.36	0.61	6.35	0.53	0.47
İkiz et al. (2024)	3.32-5.51	0.27-0.54	3.97-8.31	0.52-1.07	0.36-0.63

Many research centers are conducting studies on the effects of nickel on plant nutrient content. Toxic concentrations of nickel interfere with the uptake of macronutrients and micronutrients by plants and disrupt their translocation from roots to aerial parts of plants (Pandey, Sharma 2002). Yusuf et al. (2011), studying mung bean and chickpea, found that nickel at toxic concentrations reduced nitrogen content in the roots and leaves of these plants. An impact on nitrogen uptake from roots to shoots was also observed by Chen et al. (2009) and Ameen et al. (2019). The mechanism by which excessive nickel reduces nitrogen content in plants is by interfering with the functioning of enzymes involved in nitrogen metabolism. Nickel at toxic concentrations can disrupt the function of membrane transporters and enzymes responsible for the absorption of potassium, phosphorus, and calcium. Excess nickel can damage cell membranes or block binding sites on membrane transporters, hindering the penetration of these nutrients into plant cells. In the case of potassium, which is crucial for osmotic regulation and cellular function, excess nickel can reduce its uptake, impairing the plant's ability to maintain water balance and osmotic pressure. Similarly, phosphorus and calcium can have limited absorption because nickel can disrupt the function of membrane transporters or damage membrane structures, hindering their penetration into cells.

In this study, increasing nickel concentrations did not significantly affect the nitrogen content in lettuce leaves. There were also no significant changes

in the nitrogen content in lettuce grown in the tested substrates, in new and re-used rockwools (Table 4). The range of phosphorus content in lettuce leaves was from 0.58 to 0.71%. In new rockwool, the applied Ni concentrations significantly influenced the achievement of a lower phosphorus content in lettuce. The highest phosphorus content in new rockwool, amounting to 0.69%, was obtained in the combination without its addition. On the other hand, in re-used rockwool, increasing Ni concentrations influenced the increase in phosphorus content in lettuce leaves. In this substrate, the highest phosphorus content was obtained using 10 mg Ni dm^{-3} in the medium. Regardless of the Ni concentrations used, no significant differences were found in the phosphorus content in lettuce grown in two types of rockwool. When analyzing the average phosphorus content in lettuce under the influence of increasing Ni concentrations, regardless of the rockwool type, a significant effect of the highest nickel concentration in the solution, at which the highest phosphorus content of 0.67% was obtained, was found.

The potassium content ranged from 5.00% to 7.34%. In new rockwool, the applied nickel concentrations significantly increased the potassium content in lettuce, reaching its highest content of 6.68% when growing lettuce at a concentration of 5 mg Ni dm^{-3} . In re-used rockwool, however, the increase in nickel content in the solution resulted in a decrease in potassium content in lettuce leaves. Its highest content (7.34%) was obtained in the control combination. Regardless of the applied nickel doses in the cultivation solution, a higher potassium content (6.66%) was obtained in lettuce growing in re-used rockwool. Analyzing the average amount of potassium in lettuce, regardless of the type of rockwool, the highest K content was obtained in lettuce leaves under the influence of 5 mg Ni dm^{-3} and amounted to 6.67%. Calcium content ranged from 0.73% to 2.32% (Table 3). In new rockwool, the applied nickel concentrations significantly influenced the increase in calcium content in lettuce, obtaining its highest content of 1.85% when growing lettuce at a concentration of 5 mg Ni dm^{-3} . In re-used rockwool, however, the increase in nickel content in the solution influenced the decrease in calcium content in lettuce leaves. The highest content (2.32%) was obtained in the control combination. Regardless of the applied nickel doses in the cultivation solution, a higher Ca content (1.81%) was obtained in lettuce growing in re-used rockwool.

The magnesium content ranged from 0.31% to 0.65%. In new rockwool, the applied nickel concentrations significantly increased the magnesium content in lettuce, achieving its highest content of 0.56% when growing lettuce at a concentration of 5 mg Ni dm^{-3} . In re-used rockwool, however, the increase in nickel content in the medium resulted in a decrease in the magnesium content in lettuce leaves. Its highest content (0.65%) was obtained in the control combination. Regardless of the applied nickel doses in the cultivation solution, a higher magnesium content (0.54%) was obtained in lettuce growing in re-used rockwool. Analyzing the average amount of magnesium in lettuce, regardless of the type of rockwool, the highest Mg content was

obtained in lettuce leaves under the influence of 5 mg Ni dm⁻³, where it amounted to 0.53%.

The sulfur content ranged from 0.42% to 0.79%. In rockwool, the use of 10 mg Ni dm⁻³ significantly influenced the achievement of a lower sulfur content in lettuce, amounting to 0.49%. In re-used rockwool, however, the increase in nickel content in the nutrient increased the sulfur content in lettuce leaves. The highest sulfur content (0.79%) was obtained in the combination with a dose of 10 mg Ni dm⁻³. Analyzing the average amount of sulfur in lettuce, regardless of the type of rockwool, the highest sulfur content was obtained in lettuce leaves under the influence of 10 mg Ni dm⁻³ and amounted to 0.64%.

Sodium content ranged from 0.16% to 0.53%. No significant differences in sodium content were found under the influence of the factors studied.

The authors compared the content of individual macronutrients in lettuce leaves grown in two types of rockwool to which nickel was not introduced. Higher potassium, calcium and magnesium content in lettuce grown in re-used rockwool was found (Table 4). In turn, the content of phosphorus and sulfur was higher in lettuce grown in new rockwool.

Nickel can affect photosynthesis by disrupting the chloroplast structure and blocking chlorophyll synthesis (Ghazanfar et al. 2021). High nickel concentrations can hinder the availability and uptake of iron by plants by competing for the same transport mechanisms in cell membranes or for binding sites in enzymes and proteins (Myśliwa-Kurdziel et al. 2004, Ahmad et al. 2010). This, in turn, can lead to iron deficiency in the plant, manifesting itself, for example, by the yellowing of leaves (chlorosis). A decrease in the chlorophyll level under the influence of increasing nickel concentrations was shown by Dubey and Panday (2011), and Gurpreet et al. (2012). Opposite results were obtained by Rampazzo et al. (2022) in the cultivation of sugar cane (*Saccharum officinarum*), suggesting that the dose of 0.5 mg Ni kg⁻¹ d.m. had a beneficial effect on plant metabolism, increasing its yield. The addition of nickel caused an increase in chlorophyll content in rapeseed (Bybordi, Gheibi 2009). In a study of nickel application methods to optimize soybean growth, Rodak et al. (2024) found that all the Ni application methods resulted in a 1.1-fold increase in the SPAD index, a 1.2-fold increase in photosynthesis, a 1.4-fold increase in nitrogenase, and a 3.9-fold increase in urease activity. The chlorophyll content is measured through chemical analyses run in a laboratory setting (Zhang et al. 2014). A portable chlorophyll meter (SPAD meter) is based on the principle of Beer's Law and it uses two wavelengths, 650 nm and 940 nm, for simultaneous detection, which enables rapid measurement of the chlorophyll content in plant leaves. SPAD values are highly correlated with the chlorophyll content of plants (Lin et al. 2010). The significant correlations between SPAD values and chlorophyll concentrations were shown by Markwell et al. (1995), Uddling et al. (2007), and Li et al. (2024).

Table 4

The influence of rockwool type and increasing Ni concentrations on macronutrient and sodium content (% d.m.) in lettuce leaves

Type of rockwool	Nickel concentration (mg dm ⁻³)			Mean
	0	5	10	
N				
New rockwool	3.96 ^{a*}	3.89 ^a	3.92 ^a	3.92 ^a
Re-used rockwool	3.99 ^a	4.10 ^a	4.10 ^a	4.06 ^a
Mean	3.97 ^a	3.99 ^a	4.01 ^a	
P				
New rockwool	0.69 ^d	0.61 ^{ab}	0.64 ^{bc}	0.65 ^a
Re-used rockwool	0.58 ^a	0.67 ^{cd}	0.71 ^d	0.65 ^a
Mean	0.63 ^a	0.64 ^a	0.67 ^b	
K				
New rockwool	5.00 ^a	6.68 ^c	6.11 ^{bc}	5.93 ^a
Re-used rockwool	7.34 ^d	6.67 ^c	5.98 ^b	6.66 ^b
Mean	6.17 ^a	6.67 ^b	6.04 ^a	
Ca				
New rockwool	0.73 ^a	1.85 ^c	1.62 ^{bc}	1.40 ^a
Re-used rockwool	2.32 ^d	1.53 ^b	1.60 ^{bc}	1.81 ^b
Mean	1.52 ^a	1.69 ^a	1.61 ^a	
Mg				
New rockwool	0.31 ^a	0.56 ^c	0.48 ^b	0.45 ^a
Re-used rockwool	0.65 ^d	0.49 ^b	0.49 ^b	0.54 ^b
Mean	0.48 ^a	0.53 ^b	0.49 ^a	
S				
New rockwool	0.59 ^c	0.60 ^c	0.49 ^b	0.56 ^a
Re-used rockwool	0.42 ^a	0.53 ^{bc}	0.79 ^d	0.58 ^a
Mean	0.50 ^a	0.56 ^b	0.64 ^c	
Na				
New rockwool	0.53 ^a	0.18 ^a	0.16 ^a	0.29 ^a
Re-used rockwool	0.23 ^a	0.16 ^a	0.18 ^a	0.19 ^a
Mean	0.38 ^a	0.17 ^a	0.17 ^a	

* Values followed by the same letters do not differ significantly at $\alpha=0.05$ for individual parameters

In this study, the absorption of light by the leaf blade (Leaf Greenness Index) was measured using the SPAD 502 apparatus in order to assess the chlorophyll content in lettuce leaves (Table 5). The concentrations of nickel of 5 and 10 mg dm⁻³ in the solution in new rockwool resulted in a higher leaf greenness index. However, the same concentration of nickel in the solution

Table 5

The influence of rockwool type and increasing Ni concentrations on the SPAD leaf greenness index

Type of rockwool	Nickel concentration (mg dm ⁻³)			Mean
	0	5	10	
New rockwool	12.88 ^{a*}	15.25 ^b	15.05 ^b	14.39 ^a
Re-used rockwool	12.63 ^a	14.68 ^{ab}	13.95 ^{ab}	13.75 ^a
Mean	12.75 ^a	14.96 ^b	14.50 ^b	

* Values followed by the same letters do not differ significantly at $\alpha=0.05$

in re-used rockwool had no significant effect on the leaf greenness index (SPAD). While analyzing the average SPAD content of lettuce leaves, regardless of the rockwool type, it was found that the applied doses of Ni resulted in a higher greenness index of lettuce leaves.

The Pb content in lettuce leaves ranged from 2.33 to 4.67 mg kg⁻¹, while Cd from 1.24 to 1.78 mg kg⁻¹ (Table 6). In the control combination without Ni, higher Pb and Cd content in lettuce was found when it was grown

Table 6

The influence of rockwool type and increasing Ni concentrations on Pb and Cd content (mg kg⁻¹ d.m.) in the lettuce leaves

Type of rockwool	Nickel concentration (mg dm ⁻³)			Mean
	0	5	10	
Pb				
New rockwool	2.33 ^{a*}	3.54 ^{ab}	3.35 ^{ab}	3.07 ^a
Re-used rockwool	4.67 ^b	2.65 ^a	3.02 ^a	3.44 ^a
Mean	3.50 ^a	3.09 ^a	3.18 ^a	
Cd				
New rockwool	1.24 ^a	1.46 ^b	1.70 ^c	1.46 ^a
Re-used rockwool	1.78 ^c	1.59 ^{bc}	1.58 ^{bc}	1.65 ^b
Mean	1.51 ^a	1.53 ^a	1.64 ^a	

* Values followed by the same letters do not differ significantly at $\alpha=0.05$

in re-used rockwool. In lettuce leaves grown in new rockwool, increasing nickel concentrations did not affect the Pb content, but significantly increased the Cd content in lettuce. Increasing Ni concentrations in re-used rockwool significantly reduced the Pb content in lettuce leaves, but no effect of Ni in re-used rockwool solution on the Cd content in lettuce was found.

The content limit in Commission Regulation (EU) 2023/915 of 25 April 2023, for leafy vegetables (EU 2023), including lettuce in fresh mass is for Pb (0.30 mg kg⁻¹ and Cd (0.10 mg kg⁻¹). After converting the recommended maximum limit in fresh mass to the content in dry mass, it was compared with the amounts obtained in lettuce in the study (Figures 3 and 4).

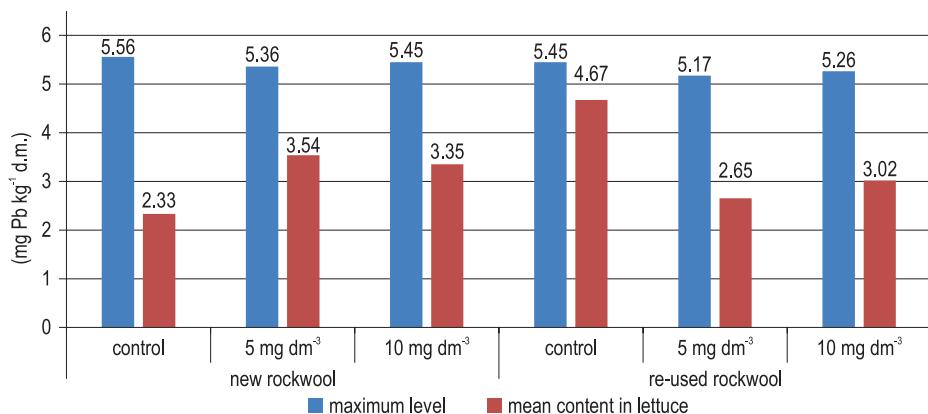


Fig. 3. Maximum content and mean Pb content in lettuce (in dry matter) depending on the type of rockwool and nickel concentration in the nutrient solution

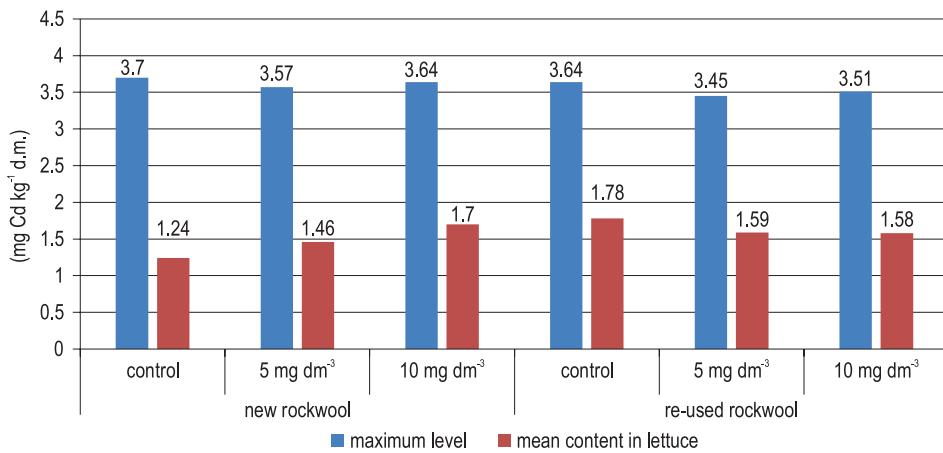


Fig. 4. Maximum content and mean Cd content in lettuce (in dry matter) depending on the type of rockwool and nickel concentration in the nutrient solution

The permissible maximum limit of Pb and Cd content in lettuce leaves was not exceeded under the influence of the tested factors.

CONCLUSIONS

It was found that mineral wool can be reused for growing lettuce, which reduces the amount of waste and costs. No differences in lettuce yield were found between growing in new and re-used rockwool. The maximum nickel level was exceeded in lettuce grown in re-used rockwool to which 5 and 10 mg Ni dm⁻³ was introduced in the solution. The maximum permissible limit of Pb and Cd content in lettuce leaves was not exceeded under

the influence of increasing nickel concentrations and the type of rockwool. The authors compared the content of individual components and heavy metals in lettuce leaves grown in two types of rockwool to which no nickel was introduced. A higher content of potassium, calcium, magnesium, zinc, manganese, nickel and lead, cadmium was found in lettuce growing in re-used rockwool. On the other hand, lettuce growing in new rockwool without nickel addition had higher phosphorus and sulfur content. Nickel in concentrations of 5 and 10 mg dm⁻³ of the solution causes a higher greenness index of lettuce leaves. Increasing concentrations of Ni did not significantly influence the content of nitrogen, sodium and copper in lettuce leaves, while the content of other nutrients and heavy metals depended on its concentration.

Author contributions

Conceptualization – M.B., B.M. K.M., B.F., methodology – M.B., B.M., K.M. and B.F., material preparation, data collection and analysis were performed – K.M., M.B., B.M. and J.R.; chemical analysis, B.F., K.M. and J.R.; data curation, M.B., B.M. and K.M., writing – original draft preparation, M.B. B.M. and B.F., and writing – review and editing, M.B. and B.F. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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