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HEALTH RISKS OF RED CABBAGE AND BROCCOLI AS MICROGREENS GROWN IN GREYWATER

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Abstract

In this research, we investigated the reuse of synthetically produced greywater in the cultivation of microgreens. Microgreens are young plant shoots that already have cotyledons. In the cotyledon, there is a higher concentration of vitamins, minerals, and antioxidants that are important for us compared to the mature plant. The use of greywater for food production is associated with food safety risks, so our aim is to investigate the effects of greywater on the micronutrient concentration and the yield in the microgreens. This was done by growing microgreens in clean water, untreated greywater and treated greywater that had undergone mechano-chemical treatment and observed the differences between the samples. The results showed that the edibility of the two crops was not drastically affected by the greywater, but the weight of the product was. The extent of this is plant specific. Further studies using real greywater samples are needed to establish the safe usability of greywater.

Keywords: *greywater, microgreens, edibility testing.*

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1. Introduction

As the global population grows, our clean freshwater supply is diminishing and our food problems are increasing. With water scarcity looming, we need to find alternative sources of water. Such alternative water sources could be greywater, which is the so-called "used water" produced by households. This water can be used for a variety of purposes, such as fire extinguishing, washing cars or flushing toilets, but in this research we have approached the potential use of greywater from an agricultural perspective, namely through the cultivation of microgreens.

1.1. A Greywater

Household greywater is defined as single-use water that is generated in households. The term greywater (GW) does not include blackwater from toilet flushing, as its microbial contamination is high and therefore harmful to human health. Since bathing water accounts for almost half of our average water consumption in Hungary, we used synthetic bathing water in our research, which is representative of the bathing water produced in households [1].

Using greywater can reduce our consumption of clean drinking water and promote a circular economy. There are areas where greywater can be used without treatment, such as flushing toilets, washing cars, or even extinguishing fires. However, in areas where it is important to have cleaner water, but it is not necessary to have drinking water purity, we can treat greywater to achieve the required clarity. In this research, we used tapwater, treated (TGW) and untreated (UTGW) synthetic bathing water. In the treatment process, coagulation and flocculation were combined with filtration on sand layer.

1.2. A Microgreens

Microgreens are young plants, usually vegetables or herbs, that are past the germination stage and have fully developed cotyledons. The vitamins important to us accumulate in the cotyledons, where they are stored in quantities many times greater than in the adult plant. While we may have to wait up to months to eat vegetables, microgreens generally mature in 7-10 days and many species can be consumed in as little as 4-5 days. In general, microgreens are high in vitamins and minerals and rich in phytochemicals, including carotenoids and phenolic compounds that act as antioxidants in the human body. These antioxidants bind carcinogenic substances in our body, thus preventing cancer [2].

For example, broccoli microgreens have been shown to contain four times more anti-cancer aliphatic glucosinolates than the adult plant. However, the benefits of microgreens are not limited to their anti-cancer effects. For example, red cabbage microgreens improve fat and cholesterol levels, reduce weight gain, triglycerides, liver cholesterol ester levels and inflammatory cytokines located in the liver in mice [3].

Since we used treated and untreated greywater alongside clean drinking water to grow microgreens, we needed to perform edibility testing to rule out health risks.

1.3. Edibility testing

Edibility testing is a comprehensive assessment system that allows us to determine with certainty whether a food, drink or any consumer product is harmful to the human body. Testing can involve a range of measurements and analyses, including the chemical, microbiological, or toxicological properties of the product's ingredients. The analysis includes testing for harmful substances in products, such as metals and heavy metals [4, 5].

The THQ (Target Hazard Quotient) is a scientifically accepted indicator that measures the health risk of exposure to a toxic substance. To calculate this value, a reference dose value is required, which has been established and published by the United States Environmental Protection Agency (USEPA). This is the maximum long-term exposure level for a given compound that is still considered safe for human consumption. A THQ below 1 is considered safe, but above 1 is considered a potential risk for consumption [5]. To calculate the value the (1.) formula is used:

$$THQ = \frac{EF \times ED \times IR \times Cm \times 10^{-3}}{RfD \times BW \times TA}, \qquad (1.)$$

where: *EF*: exposure frequency (days/year), *ED*: exposure duration (years), *IR*: ingestion rate of the plant (g), Cm: heavy metal concentration (mg/kg), *RfD*: reference dose (mg/kg/day), *BW*: average adult body weight (kg), *TA*: average exposure time (days) [5].

Another indicator is the *HI* (Hazard Index), which is calculated by assuming that we are exposed to several potentially toxic elements simultaneously when consuming a given food. For this reason, although the *THQ* value for a single item may be below the critical number, the cumulative effect of consuming a food can cause health damage. For *HI*, the critical value is also 1, below which it is safe to consume, but above it, consumption is not recommended [4]. To calculate the value of *HI* the (2) formula is used :

$$HI = \sum_{N=1}^{i} THQ_n. \qquad (2)$$

2. Material and methods

Three different types of water were used in the study, tapwater, treated greywater and untreated greywater. The tapwater was taken from the public drinking water network, while greywater was produced synthetically developed by the Department of Environmental Engineering in previous years [6], using a synthetic bathing water parameter based on tapwater with a constant and definite composition.

The composition of this water is typical of the greywater produced by domestic bathing.

Iron (III) chloride was used in the treatment of greywater during coagulation and flocculation. After sedimentation, mechanical filtration was applied using a sand filter [6].

For the cultivation of microgreens, fiber plates were used and placed on disinfected trays. These plates were soaked in the appropriate water (tapwater, untreated or treated greywater) and the seeds were placed on them. Three fiber plates per plant type were planted according to the three types of water. Our aim was to observe how the edibility and the productiveness of the microgreens changed when greywater was used to grow them, compared to drinking water. The productivity (yield) of microgreens was measured in two ways, by weight and by length. An analytical scale was used to measure weight. The microgreens were measured immediately after cutting (wet biomass weight) and after drying (dry biomass weight). The length was measured as an average. Randomly, 20-20 stems per sample were selected and measured with a ruler. To determine the health risk, a consumability test was performed using the THQ and HI values men-

Table 1. *Reference doses (RfD)* [7]

Elements	Reference dose (RfD)
Al	1
Cd	0.001
Co	0.003
Cu	0.04
Fe	0.7
Mn	0.14
Ni	0.02
Pb	0.0035
Zn	0.3

Table 2. *Values used to calculate THQ and HI*

Abbreviations	Parameters	Used values
EF	Exposure frequency (days/year)	365
ED	Exposure duration (years)	70
BW	Average adult body weight (kg)	70
IR	Ingestion rate of the plant (g)	10

Table 3. *Micronutrient content of waters*

		Al	Cu	Fe	Zn
Tap-water	Mean	7.5	48	<LoD	306
	SD	2.1	7.5	-	61
UTGW	Mean	13	66	12	264
	SD	2,5	16	-	54
TGW	Mean	15	12	<LoD	10
	SD	6	1	-	3

Fig. 1. *Developed red cabbage microgreens.*

tioned above. To calculate these, we need the reference dose (see **Table 1**). The reference dose is a calculated value published by the USEPA in the risk parameter list. [7].

In order to determine the presence of these elements in microgreens, an elemental analysis had to be carried out. During the research, the elemental analysis was performed by our collaborators at the Department of Inorganic and Analytical Chemistry, Faculty of Science and Technology, University of Debrecen, using an ICP-OES device.

In addition to the microgreens, the trace element content of water was also measured to obtain a more comprehensive picture. For our calculations, we aimed to determine the maximum consumable value and therefore used the values in **Table 2.** The ingestion rate (IR) refers to the number of grams consumed. The amount of microgreens consumed per day was estimated at 10 grams, based on literature calculations [8].

To calculate this, formula (1) and (2) were applied and the 10 g ingestion rate was gradually increased to obtain the maximum amount that could be consumed.

3. Results

When assessing the results, we should not only consider which water type poses the least health risk to the microgreens if consumed, but also consider whether the quantity of the product is affected by the use of greywater.

3.1. The micro-nutrient content of waters

In order to determine the health risks of microgreens grown in treated and untreated greywater, it is necessary to also consider the concentrations of toxic elements in the water. Of the nine elements (see **Table 1**) cadmium, cobalt, manganese, nickel and lead were below detection limits for all three water types. Iron was detectable on one occasion. The results are shown in **Table 3** in µg/l.

3.2. Parameters of red cabbage

Fig. 1. shows the matured red cabbage microgreens, from left to right grown on tapwater, untreated greywater (UTGW) and treated greywater (TGW). The data for red cabbage are presented in **Table 4**.

In both **Fig. 1** and **Table 4**, it is observed that the plant grown in the treated greywater grew taller, and significantly greater in biomass than its counterpart grown in tapwater or untreated greywater. In addition, its moisture content is also higher, while the other two samples have the same moisture content. In terms of average length, the samples grown in tapwater and untreated greywater gave almost similar results, but in terms of biomass, the sample grown in tapwater showed better growth.

Regarding the trace element content cadmium, cobalt and lead were below the detection limit for all microgreens grown on all three water types. Micronutrient content of red cabbage is presented in **Fig. 2**. Analyzing the results obtained it can be observed that there are small and large differences in the distribution of the different microelements. Although manganese and nickel were only present in the water at levels below the measurement limit (see **Table 3**), and iron was only detectable in the untreated greywater at 12 µg/l, they accumulated in the microalgae at detectable levels.

There was a greater variation in the distribution of manganese content. Most manganese was accumulated in the red cabbage grown in tapwater, while the least was accumulated in the sample grown in treated greywater. Strontium was increased in microgreens grown in treated grey water, while zinc was in a decreasing order, with most zinc being taken up by plants grown in tapwater and least by plants grown in treated grey water. These differences were further analyzed in the consumability study. The *THQ* and *HI* results for red cabbage are presented in **Table 4.**

As shown in **Table 5**, 10 grams of each type of water can be safely consumed of red cabbage microgreens. However, in order to determine the maximum amount that can be consumed, the critical THQ value for each plant grown in each water sample was determined, which is shown in **Fig. 3**.

Here it is observed that for red cabbage grown in all three water types the critical $THQ = 1$ was reached by manganese the fastest, but at different ingestion rates.

For the sample grown in tapwater (**Fig. 3/1**) $THQ = 1$ was reached at a consumption rate of 2.9 g/bwkg. Based on this, it can be determined that a person of average body weight can consume 203 g per day.

In the case of microgreens grown in untreated greywater (**Fig. 3/2**) the ingestion rate is 3.9 g/bwkg at $THQ = 1$. Therefore, the average person weighing 70 kg can consume 273 g per day, every day of the year.

For the treated greywater crop (**Fig. 3/3**)), the ingestion rate is 3.3 g/bwkg at the critical *THQ*. This means that an average person of average body weight can consume 231 g/kg in 365 days at maximum.

Table. 4. *Data on red cabbage*

	Tapwater	UTGW	TGW
Average length [cm]	2.44 ± 0.54	2.52 ± 0.62	3.01 ± 0.72
Wet biomass [g]	3.80	3.51	6.10
Dry biomass [g]	0.37	0.34	0.47
Moisture [%]	90	90	92

Table 5. *THQ and HI values of red cabbage at 10 g/356 days of consumption*

	Tapwater	UTGW	TGW
THQ_{Al}	$9.93\cdot10^{-4}$	$1.57\cdot10^{-3}$	$1.57\cdot10^{-3}$
THQ_{Cu}	$1.92\cdot10^{-2}$	$1.76\cdot10^{-2}$	$1.62\cdot10^{-2}$
THQ_{Fe}	$1.30\cdot10^{-2}$	$1.32\cdot10^{-2}$	$1.30\cdot10^{-2}$
THQ_{Mn}	$4.93\cdot10^{-2}$	$3.66\cdot10^{-2}$	$4.34\cdot10^{-2}$
THQ_{Ni}	$9.21\cdot10^{-3}$	$6.84\cdot10^{-3}$	$5.16\cdot10^{-3}$
THQ_{Zn}	$2.45\cdot10^{-2}$	$2.25\cdot10^{-2}$	$2.09\cdot10^{-2}$
HI	$1.16\cdot10^{-1}$	$9.84\cdot10^{-2}$	$1.00\cdot10^{-1}$

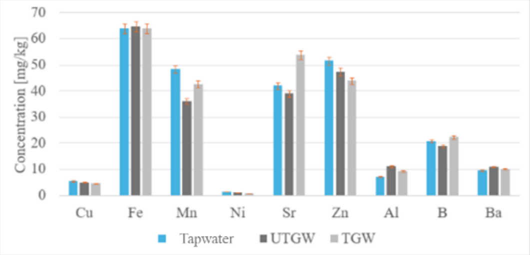

Fig. 2. *Micronutrient content of red cabbage.*

In addition to the *THQ* value, the *HI* value was also determined using formula (2), where the *THQ* values were summed. From this, we obtained **Fig. 4.** The figure shows the *HI* value, the hazard index. This indicates that the red cabbage with the lowest edible content was grown in tapwater. This critical value was reached at 1.23 g/bwkg, while the ingestion rate was 1.43 g/bwkg for cabbage grown in treated greywater and 1.45 g/bwkg for cabbage grown in untreated greywater. These results suggest that an average body weight person can consume 86 g of the sample grown in tapwater, 100 g of the sample grown in treated greywater and 102 g of the microgreens grown in untreated greywater every day of the year.

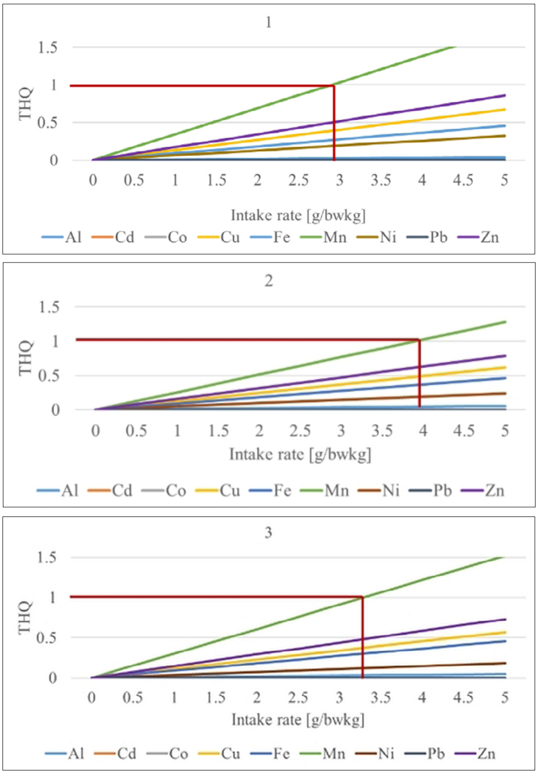

Fig. 3. *THQ values of red cabbage grown on tapwater (1), untreated greywater (2) and treated greywater (3).*

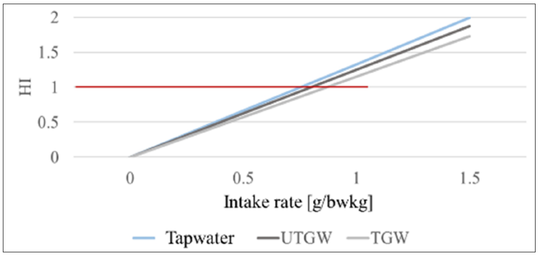

Fig. 4. *HI values of red cabbage grown in different waters.*

Fig. 5. *Mature broccoli microgreens.*

These data suggest that the consumption of red cabbage does not decrease but increases when grown in greywater. However, looking back at the biomass (see **Table 4**) although the best results in terms of edibility were achieved with microgreens grown in untreated greywater, their growth was limited. For this reason, the second-best sample in terms of edibility and the best sample in terms of biomass was the red cabbage microgreens grown in treated greywater.

3.3. Parameters of broccoli

Analyzing the data in **Fig. 5** and **Table 6**, , it can be seen that the microgreens grown in treated greywater have the longest average length, while the shortest is the one grown in tapwater, but for both wet and dry biomass, the sample grown in untreated greywater is the leader, followed by the one grown in treated greywater, and finally the one grown in tapwater. The same order is observed for moisture content.

The microelement content is shown in **Fig. 6**.

Regarding the trace elements, it was observed that although manganese and nickel were not found in detectable amounts in the water (**Table 3**) in any of the samples, they accumulated in the plants. It can also be seen that strontium levels increased in the microgreens grown in untreated greywater and then increased further in plants grown in treated greywater (see **Table 3**),), but the same trend is observed in the elemental analysis of the water itself. Iron is slightly lower than the ideal range (50-75 mg/kg) **[9]**. For broccoli, the

Table 6. *Data on broccoli*

	Tapwater	UTGW	TGW
Average length ± SD [cm]	4.43 ± 0.75	5.00 ± 1.13	5.96 ± 0.63
Wet biomass [g]	8.53	13.29	10.43
Dry biomass [g]	0.71	0.80	0.75
Moisture [%]	92	94	93

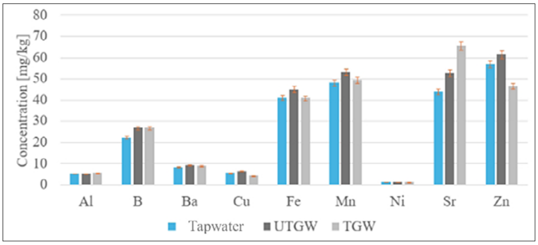

Fig. 6. *Microelement content of broccoli.*

Table 7. *THQ and HI of broccoli at 10 g/356 days of consumption*

	Tapwater	UTGW	TGW
THQ_{Al}	$7.33*10^{-4}$	$7.21*10^{-4}$	$7.60*10^{-4}$
THQ_{Cu}	$1.91*10^{-2}$	$2.22*10^{-2}$	$1.44*10^{-2}$
THQ_{Fe}	$8.37*10^{-3}$	$9.16*10^{-3}$	$8.31*10^{-3}$
THQ_{Mn}	$4.90*10^{-2}$	$5.42*10^{-2}$	$5.03*10^{-2}$
THQ_{Ni}	$8.43*10^{-3}$	$9.21*10^{-3}$	$7.50*10^{-3}$
THQ_{Zn}	$2.71*10^{-2}$	$2.92*10^{-2}$	$2.21*10^{-2}$
HI	$1.13*10^{-1}$	$1.25*10^{-1}$	$1.03*10^{-1}$

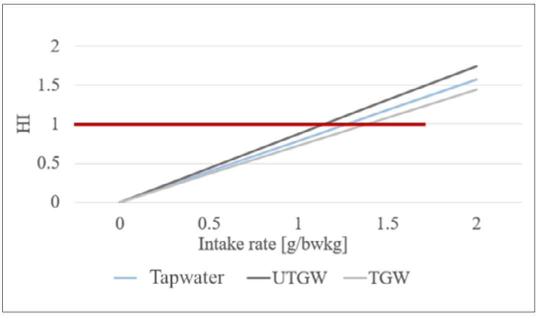

Fig. 8. *HI values of broccoli grown in different waters.*

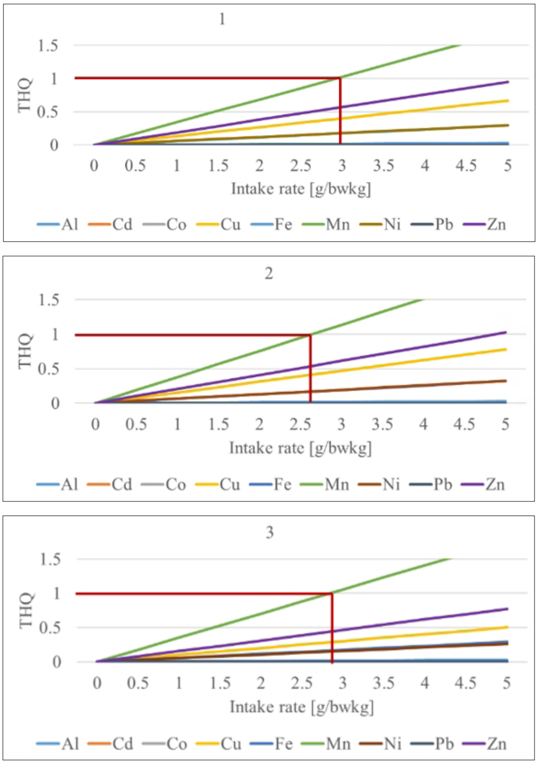

Fig. 7. *THQ values of broccoli grown on tapwater (1), untreated greywater (2) and treated greywater (3).*

levels of cadmium, cobalt, chromium and lead were below the detection limit.

The results of the consumability study based on a 10 g/365 day consumption are presented in **Table 7**: if 10 g are consumed every day of the year, the microgreens grown on all water types are safe to eat.

To determine the maximum amount that can be consumed, **Fig. 7.** and **8.** were used.

Firstly, the critical values of the *THQ*, the target hazard quotient, were determined. For all three water samples, the value of manganese determined the consumable quantity, as it reached the critical value the soonest. However, the ingestion rate is different in all three cases.

In the case of broccoli grown on drinking water (see **Fig. 7/1**), $THQ = 1$ was achieved at a consumption rate of 2,92 g/bwkg. This means that an average person (70 kg) can consume 204 g.

For broccoli grown in untreated greywater (see **Fig. 7/2**) the critical *THQ* was reached at the ingestion rate of 2.64 g/bwkg. Based on this, it can be calculated that a person with an average body weight of 70 kg can consume 185 g per day, every day of the year. This is a reduction compared to tapwater, but still more than 18 times of the amount we put into our bodies in 1 consumption.

The broccoli grown in treated greywater (see **Fig. 7/3**) reached the critical $THQ = 1$ at an ingestion rate of 2.84 g/bwkg. On this basis, the consumption rate of 199 g per day of the year was calculated.

The *HI* hazard index was also determined for the consumptive use test using **Fig. 8**. This value was obtained using formula (2), where THQ values were summed.

From the graph, it can be observed that although the THQ value was highest for the sample raised on drinking water, the HI value was highest for the treated grey water. The maximum daily intake of broccoli grown on treated greywater was 97 grams, compared to 89 grams for the broccoli grown in tapwater and 81 grams for the broccoli grown in untreated greywater, all for a person with an average body weight over the course of the entire year.

These values are all above the average daily consumption (10 grams), thus it can be stated that broccoli microgreens can be consumed both when grown in treated and untreated greywater and if the biomass weights are observed (see **Table 6**), the best method of growing broccoli is, based on the present results, to grow it on untreated greywater.

5. Conclusion

The research investigated the potential of reusing treated and untreated greywater for the cultivation of microgreens. Coagulation, flocculation and mechanical filtration were used as treatments to remove organic impurities and insoluble colloidal particles from the greywater.

In the case of microgreens, it can be stated that the use of greywater is highly plant specific.

The biomass mass of broccoli grown in untreated greywater was also the highest, being 56% higher than in the sample grown in tapwater. However, consumability decreased with the use of both types of greywater. Nevertheless, both treated and untreated greywater samples could still be consumed several times more than the daily average. Therefore, it can be concluded that although the consumability is reduced, it is still safe to consume broccoli grown in greywater.

In the case of red cabbage, while the biomass mass decreased by 8% using untreated greywater, the biomass mass increased by 60% using treated greywater. In terms of consumability, both HI and THQ values indicate that the sample grown in untreated greywater is the most consumable, followed by the sample grown in treated greywater. Overall, the most ideal growing method for red cabbage is to grow it in treated greywater.

For both broccoli and red cabbage, manganese was the critical element. It can be concluded that reducing manganese content would increase the consumption of microgreens. However, in several cases, the manganese content was highest in the microgreens grown in the tapwater sample. This suggests that the tapwater itself would need to be purified to increase the consumption of microgreens. Thus, it can be concluded that the element content of tapwater is the most important factor affecting the edibility of microgreens. While consumption varies slightly between different water samples, but there are no significant differences. Therefore, the present study concludes that cultivating microgreens using treated and untreated synthetic bathing water does not drastically affect their edibility but does impact the quantity of the product.

Since these data were obtained using synthetic bathing water, further investigation of microgreening using real bathing water samples is needed in the future

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