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WATERCRESS AS A MEDICINAL HERB GROWN IN A HYDROPONIC SYSTEM

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Abstract

In this research, synthetic bathing water was created treated by coagulation and mechanical filtration. The waters were qualified before and after treatment, so that the effectiveness of the treatment could be calculated. The greywater produced during the treatment was circulated in a hydroponic tower next to a tapwater control tower. The watercress grown was analyzed in the towers and their results were compared. The results show that the greywater treatment was effective and the watercresses were comfortable in the synthetic bathing water tower without nutrient solution. They grew larger than their counterparts grown in tapwater, and their average moisture content was also higher. Additionally, the spectrophotometric determination of indole-3-carbinol content was investigated in the plants. However, the obtained spectra were noisy, and the absorbance values were too small for reliable evaluation. Our further aim is to improve the determination of active ingredient using other methods (e.g. solid phase extraction).

Keywords*: greywater, watercress, hydropony, indole-3-carbinol.*

1. Introduction

Nowadays, the treatment and reuse of greywater from households is becoming increasingly common, as water is a limited resource. The reuse of greywater allows for a reduction in household water use, which contributes to reducing water consumption, and therefore it is important to raise awareness of the importance of reusing greywater in households. In addition, it is essential to protect our health, to which the consumption of medicinal herbs makes a major contribution. Watercress is a highly nutritious plant that has a number of beneficial effects on the human body, and indole-3-carbinol, which is thought to have cancer-preventive effects, is also found in it. With the right results, the sustainability of medicinal plant supply to the pharmaceutical and food industries can be enhanced through the cultivation of watercress in hydroponic towers.

2. Alternative water source: greywater

In households, two types of wastewater are distinguished based on quality criteria. These are blackwater and greywater. Blackwater is the more polluted fraction of wastewater, which is produced during the flushing of toilets and therefore contains contaminants that pose a serious health risk. Greywater (GW) is the less contaminated fraction of wastewater, which does not contain the blackwater from toilet flushing. It includes used water from bathroom activities, washing dishes and laundry. Greywater can be further subdivided into light and dark greywater fractions based on the degree of contamination. For many water-using activities, such as the self-irrigation of medicinal plants, greywater treatment is necessary, otherwise the plants would take up undesirable pollutants that would enter the food chain. In our research, synthetic bathing water was treated, which was based on tap water, using coagulation/flocculation chemical methods and mechanical filtration through sand [1, 2].

3. Hydroponic cultivation of watercress

Watercress (Nasturtium officinale) is a highly nutritious plant of the cruciferous family (Brassicaceae) that has many beneficial effects on the human body. It is full of vitamins and minerals such as vitamin C, vitamin A, vitamin K, as well as folic acid, calcium and iron. It also has antioxidants such as beta-carotene, lutein and quercetin. Furthermore, it is rich in a compound called glucosinolate, which is converted into indole-3-carbinol when plant cells are broken down (e.g. by chopping or chewing) [3].

By growing watercress in a treated greywater-based hydroponic system, the supply of medicinal plants can be made more sustainable to the pharmaceutical and food industries. By growing up to 600 plants on 7.5 m2 of land up to three times faster and 30% more efficient than conventional methods, and by recycling and reusing the water circulating in the system, 90% less water can be used up than conventional outdoor cultivation [4].

4. Material and methods

Based on the previous research results of the Department of Environmental Engineering [2],], a synthetic bathwater based on tap water was created, and this greywater fraction was treated by coagulation with ferric chloride ($FeCl_3$) (Figure 1) and mechanical filtration through sand. The greywater produced during the treatment was circulated in the hydroponic tower, which was assessed before and after treatment to calculate the effectiveness of the treatment. A tap water control tower was also operated for a better comparison. The irrigation water entering and leaving the towers was also assessed to monitor changes in water quality. Among the water quality parameters, pH, specific conductivity (EC), zeta potential (ZP), turbidity (TURB), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), and total organic carbon (TOC) were investigated.

For pH measurement, a SenTix 41 combined pH electrode was used, for specific electrical conductivity (EC) and zeta potential (ZP), a Zetasizer Nano Z device, for turbidity (TURB), a Turb 555-IR turbidity meter, for biochemical oxygen demand (BOD_5), OxiTop IS 12 devices, for chemical oxygen demand (COD), a NANOCOLOR Linus spectrophotometer, and for total organic carbon (TOC), a Shimadzu TOC-VCPN device were applied. Plants were pre-grown for 12 days and placed in hydrobaskets with rockwool cubes in Rotower hydroponic tubes, distributed by Green Drops Farm Ltd. as shown in Figure 2.

The watercresses spent 20 days in the hydroponic system. Each day, pH and EC parameters were measured in both towers. Different plants have different ideal pH requirements, but the optimal range varies between 6.5 and 7.5. The optimal level was maintained by the addition of 24.5% phosphoric acid. Water evaporated or consumed by the plants was replenished every 3 days (tap water was taken from the main water supply and treated greywater was prepared and treated in

Fig. 1. *Coagulation-flocculation chemical treatment.*

Fig. 2. *Watercress in the hydroponic system.*

advance and stored in a refrigerator). During the analysis of the watercresses, the average plant length, the maximum root length and the moisture content of the bushes in both towers were measured, and the average chlorophyll-a, chlorophyll-b and total carotenoid concentrations were also measured with a Nanocolor Vis spectrophotometer. The concentration of the active ingredient indole-3-carbinol (I3C) in the water sample was determined by UV/VIS spectrophotometry. The concentration of I3C was measured using an Agilent Cary 60 photometer and ethyl acetate and ultrapure water were used as solvents.

5. Results and conclusions

In this chapter, the results of the water and plant qualification and I3C determination studies are presented together with their evaluation.

5.1. Water quality parameters

As part of our research, synthetic bathwater was prepared and treated four times. Untreated greywater was assessed to evaluate the effectiveness of the treatment. In addition to using tap water as irrigation water in one of the hydroponic towers, it also served as the base for the synthetic greywater, so each time, tap water samples were taken and assessed, along with untreated and treated greywater. The quality parameters of the water types are presented, averaged and supplemented with standard deviations, using tables.

In **Table 1** it can be observed that tap water has the lowest average pH (7.67±0,1), which is basically higher than the typically neutral (7) value of tap water. This indicates that the tap water used in our study is slightly more alkaline. For the treatment of greywater, turbidity (TURB), BOD_5, and COD results were evaluated according to Regulation (EU) 2020/741 of the European Parliament and of the Council on minimum requirements for the reuse of water **[5]**, and Directive 91/271/EEC on urban wastewater treatment **[6]** respectively. Measurement of TURB is important in greywater treatment, as high turbidity affects filtration and disinfection processes, as well as reducing the aesthetic quality of the water. According to EU Directive 2020/741 **[5]** TURB should not exceed 5 NTU. The treated greywater had an average TURB value of 0.82±0.78 NTU, which is below the EU 2020/741 turbidity limit. The aim of greywater treatment is to minimize the amount of organic matter. According to EU Directive 2020/741 **[5]** the permitted value of BOD_5 for agricultural irrigation is ≤ 10 mg/l. The average BOD_5 value of the greywater treated was 4.13±3.64 mg/l which does not exceed this limit. The reduction of COD levels is a key factor in the treatment of greywater. The COD limit value under Directive 91/271/EEC **[6]** is 125 mg/l. The average value of the synthetic bathwater treated during the research was 14.1±4.5 mg/l, thus, this requirement was fulfilled with the applied treatment. Overall, the treated greywater fulfilled the TURB, BOD_5 and COD limits, and no significant differences were found compared to the drinking water, thus the treatment procedure can be considered effective. By comparing the parameters of untreated and treated synthetic bathing water, treatment efficiencies were also calculated, as shown in **Table 2.**

Based on the results of treatment efficiency, it can be said that the amount of pollutants was reduced by the treatment process. The treatment of greywater was mainly aimed at reducing TURB, BOD_5, COD and TOC, which was achieved by having a treatment efficiency of more than 90% for these parameters.

Table 1. *Summary table of results for water types*

Parameter	Unit of measurement	Tapwater	Untreated greywater	Treated greywater
pH	-	7.67±0.1	8.18±0.1	8.06±0.1
EC	μS/cm	7.67±38	917±23	851±203
ZP	mV	-	-32.57±1.70	-11.47±2.26
TURB	NTU	0.29±0.06	43.3±6.1	0.82±0.78
BOD_5	mg/l	1.38±0.52	137±61	4.13±3.64
KOD	mg/l	8.83±0.72	515±170	14.1±4.5
TOC	mg/l	2.74±0.27	57.7±5.4	3.86±0.94

Table 2. *The treatment efficiency of the water quality parameters*

Parameter	Unit of measurement	Treatement efficiency (%)
pH	-	decreased
EC	μS/cm	7,27
ZP	mV	64,78
TURB	NTU	98,11
BOD_5	mg/l	96,97
KOD	mg/l	97,27
TOC	mg/l	93,31

5.2. Analysis of the irrigation water before and after the hydroponic tower

Treated synthetic bathwater and tap water (used as a control) entering the hydroponic tower were certified. At the end of the trial period the water quality parameters of the tower water were measured again to monitor changes in water quality. Since water was added several times, the average of the water inputs was used as a basis.

In **Table 3,** it can be observed that the pH of the tap water decreased, which can be explained by the addition of phosphoric acid (which was added to maintain the correct pH). Smaller amounts of phosphoric acid were added, which did not cause a drastic drop in pH, so it had to be used more often to maintain the optimal pH. TURB also decreased, but BOD_5, COD and TOC increased due to plant activity.

Table 4 illustrates that the pH and ZP of the treated greywater also decreased, which is also due to the addition of phosphoric acid. More phosphoric acid was added at the beginning of the experiment than to the tap water, which resulted in a much lower pH than the tap water tower from the beginning of the experiment. The lower pH also affects the specific conductance and the nutrient uptake of the plants. The increase in EC is either due to the pH change or to the accumulation of possible organic or inorganic matter that the plants did not take up. The TURB value also decreased here, and BOD_5, COD and TOC also increased, again due to the activity of the plants. These suggest that the plants did not take up any residual organic and inorganic matter from the synthetic bathwater. In conclusion, the organic matter content (BOD_5, COD and TOC) of the tap water increased more than that of the treated greywater at the end of the experiment. However, turbidity was reduced more in the treated synthetic bathwater than in the tap water.

Table 3. *Tap water parameters before and after the hydroponic tower*

Parameter	Unit of measurement	Tapwater		Change
		Before	After	
pH	-	7.67±0.11	6.82±0.01	decreased
EC	µS/cm	767±38	580±11	decreased by 24.49%
TURB	NTU	0.29±0.06	0.12±0.03	decreased by 58.62%
BOD_5	mg/l	1.38±0.52	4±0	increased by 189.85%
KOD	mg/l	8.83±0.72	22±0	increased by 149%
TOC	mg/l	2.74±0.27	18.03±0.02	increased by 558%

Table 4. *Treated greywater parameters before and after the hydroponic tower*

Parameter	Unit of measurement	Treated greywater		Change
		Before	After	
pH	-	8.06±0.05	6.71±0.01	decreased
EC	µS/cm	851±203	1225±34	increased by 44.03%
ZP	mV	-11.47±2.26	−5.43±2.64	decreased by 52.66%
TURB	NTU	0.82±0.78	0.15±0.03	decreased by 81.71%
BOD_5	mg/l	4.13±3.64	4.50±0.71	increased by 8.96%
KOD	mg/l	14±4	17±1	increased by 20.74%
TOC	mg/l	3.86±0.94	6.50±0.06	increased by 40.62%

5.3. Analysis of watercresses

During the hydroponic plant cultivation, a total of 20 watercresses were analysed from each tower. In the analysis of the watercresses, the average plant length of each bush in each tower was measured using 3 parallel measurements and the maximum root length was measured using a tape measure. The results obtained were averaged separately for the parameters of the plants grown in the drinking water and in the treated greywater towers.

The average maximum root length of the watercresses grown in the tap water tower was 49±7 cm and that of the watercresses grown in the cultivated greywater tower was 35±8 cm, as illustrated in **Figure 3**. The difference in maximum root length is about 14 cm and the plants grown in drinking water have the longer roots.

The average plant length was 19±3 cm for the tap water plants and 19±6 cm for the treated greywater plants, as illustrated in **Figure 4**.

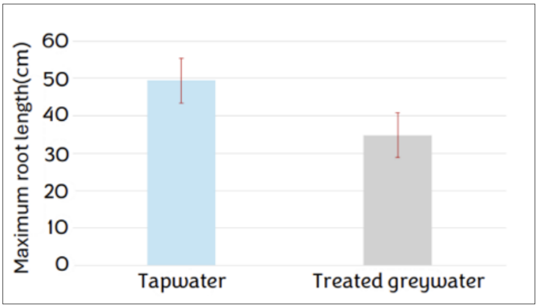

Fig. 3. *Comparison of the average maximum root length of the watercresses.*

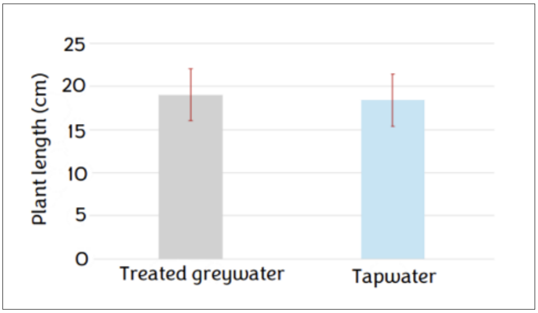

Fig. 4. *Comparison of the average plant length of the watercresses.*

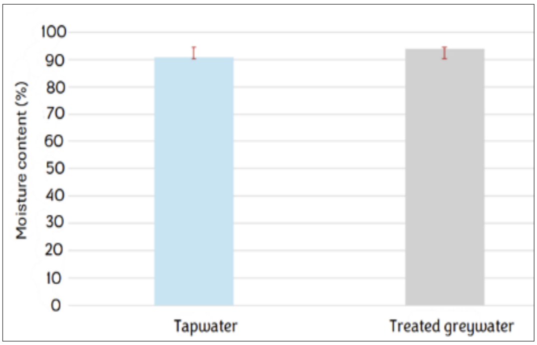

Fig. 5. *Average moisture content of the watercresses.*

Overall, there is no significant difference in plant length. The wet and dry biomass weights of the watercresses were measured to determine their average moisture content, which is compared in **Figure 5** .

In terms of average moisture content, it can be said that there is not much difference between the plants grown on tap water (90.94±0.02%) and treated greywater (93.84±0.01%), but the average moisture content of the watercresses in the treated synthetic water tower is slightly higher. However, our measurements support the claims in the literature **[7]**, regarding the high moisture content of watercress.

In order to determine the concentration of chlorophyll-a, chlorophyll-b and total carotenoids, pigments from the plants were firstly extracted and then determined spectrophotometrically. From these results, the concentrations of chlorophyll-a, chlorophyll-b and total carotenoids were calculated and averaged. **Figure 6** shows the average results of the watercresses grown on tap water and **Figure 7** shows the average results of the watercresses grown on treated greywater.

The average chlorophyll-a concentration of the tap water reared watercress was 1.50±0.25 mg/gSP, chlorophyll-b concentration 0.95±0.20 mg/gSP, and carotenoids concentration 0.43±0.08 mg/gSP.

The plants grown in the treated greywater had an average concentration of chlorophyll-a of 1.18±0.20 mg/gSP, chlorophyll-b of 0.71±0.12 mg/gSP and carotenoids of 0.32±0.60 mg/gSP. In comparison, the watercresses in the tap water hydroponic tower had a slightly higher average concentration of the pigments compared to the treated greywater tower.

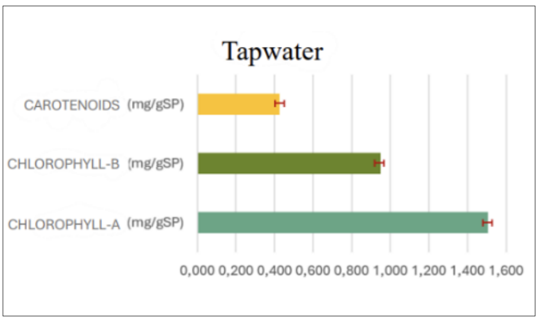

Fig. 6. *The average concentration of chlorophyll-a, chlorophyll-b, and carotenoids in the watercresses grown in tap water*

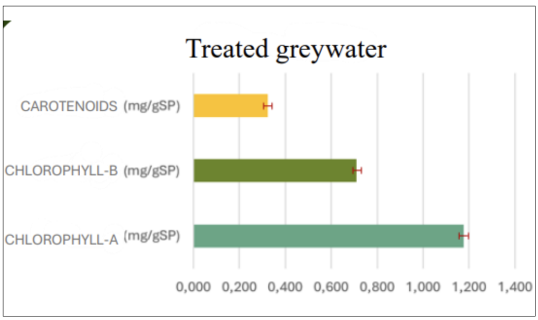

Fig. 7. *The average concentration of chlorophyll-a, chlorophyll-b, and carotenoids in the watercresses grown in treated greywater.*

5.4. Results of the I3C active ingredient determination analysis

As a preliminary experiment, the spectrum of ethyl acetate in aqueous solution was firstly determined. The absorbance of pure ethyl acetate was too intense, so aqueous solutions were analysed. Using this series of experiments, the molar spectrum (ε) of ethyl acetate in aqueous medium was determined in the wavelength range 190-290 nm, as illustrated in Figure 8.

The absorption maximum of ethyl acetate is between 200 nm and 210 nm, with low absorption at longer wavelengths. To determine the molar spectrum of I3C, I3C solid chemical was used. To prepare the calibration series, solid I3C was dissolved in ethyl acetate and diluted with ultrapure water. The molar spectrum of I3C between 225 and 300 nm is illustrated in Figure 9.

The calibration series showed that the absorption maximum of I3C was in the range of 250 nm to 300 nm. At these wavelengths, the interfering effect of ethyl acetate is negligible but can be corrected if necessary. For the I3C determination of watercress extracts, 0.5; 1; and 2 g of watercress slurries were weighted and extracted with ethyl acetate and diluted with ultrapure water. Representative spectra are shown in Figure 10.

The following conclusions were drawn from the spectra of extracts containing watercress pulp of different masses. The absorbance systematically increased with increasing volume of the extract in the cuvette, but unfortunately the fine structure of I3C (Figure 9) is difficult to discern. The measured spectra are noisy and the absorbances are too low for reliable evaluation. An additional method was also tried, but the same conclusions were drawn as for the spectra of watercress extracts. Experiments for the quantification of I3C are planned, both by further development of the spectrophotometric method and by the inclusion of other techniques. In the former case, increasing the cuvette concentration (and thus the absorbance) is a possible approach, but even then the matrix effect can be a problem. As an alternative method, for example, high-performance liquid chromatography (HPLC) is an obvious choice. In the coming period, we intend to further develop the spectrophotometric method for the determination of I3C using solid phase extraction (SPE).

It is important to note that these data were generated using synthetic greywater treated without nutrient solution during the cultivation of watercress in a hydroponic system.

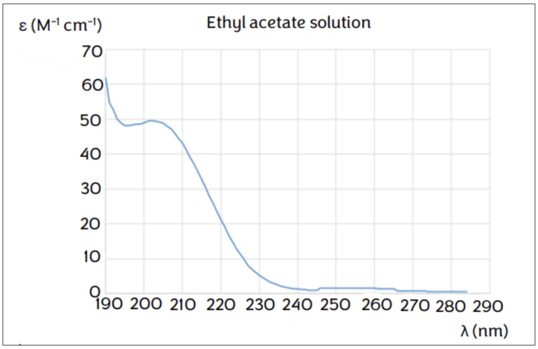

Fig. 8. *The spectrum of ethyl acetate.*

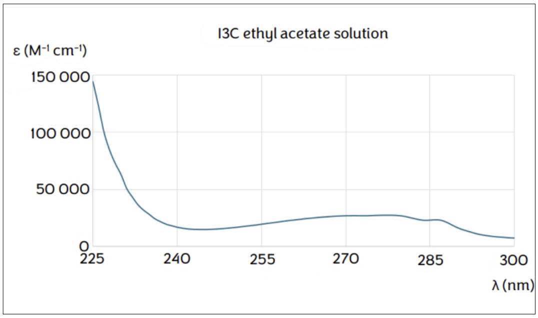

Fig. 9. *The spectrum of I3C ethyl acetate solution.*

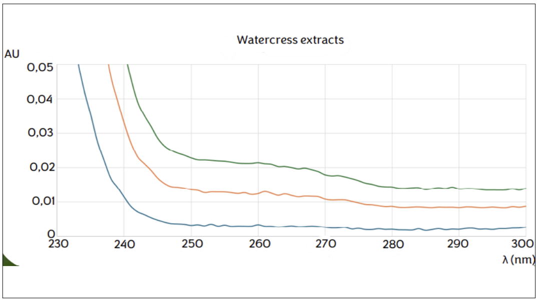

Fig. 10. The spectrum of the solution containing 1 g of watercress paste.

6. Conclusions

The management of greywater in households and the importance of its reuse were investigated, with a particular focus on the hydroponic cultivation of watercress (Nasturtium offi-cinale), which can make the supply of medicinal plants more sustainable by recycling greywater. Greywater is a type of household wastewater that does not contain black water (wastewater from toilet flushing), the proper treatment of which is a key to prevent plants from picking up unwanted contaminants. In this research, greywater was treated by the coagulation/floculation chemical method and mechanical filtration, and then applied to watercress cultivation in a hydroponic system. The quality parameters of the tap water and treated greywater were continuously measured and the results showed that the treatment of the greywater was successful as it fulfilled the water quality limits (turbidity, BOD_5, COD) set by the European Union **[5, 6]**. The growth and biological parameters of the watercresses were also monitored. There was no significant difference in plant length, root length and moisture content between plants grown on tap water and treated greywater, although tap water plants had slightly higher concentrations of chlorophyll-a, chlorophyll-b and carotenoids. The concentration of the active substance indole-3-carbinol (I3C) was determined by UV/VIS spectrometry. However, the measured spectra are noisy and the absorbances are too small for reliable evaluation. In conclusion, the results of this study showed that treated greywater can be effectively used to grow watercress in hydroponic systems, thereby reducing water logging in a sustainable manner, while not significantly affecting the quality parameters of the plants.

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