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Parameters of Drainage Waters Collected during Soilless Tomato Cultivation in Mineral and Organic Substrates

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Abstract: The aim was to determine the suitability of various substrates for application in a closed system of soilless tomato cultivation, based on the potential fitness of drainage waters from these substrates for recirculation. Four substrates were used: rockwool, coir substrate, lignite substrate (Carbomat) and biodegradable organic substrate (Biopot). Tomato plants grown in these substrates were fertilized with the same amount of nutrient solution, containing the same concentration of nutrients. The characteristics of drainage water from these substrates were analyzed during cultivation. The highest amount of drainage water was collected from the lignite substrate Carbomat. However, these leachates showed good properties for further recirculation: low electro conductivity and turbidity, high nutrient content, moderate microbial load with high population of *Trichoderma* fungi, and being beneficial for plant growth. Moreover, Carbomat produced the highest tomato yield compared to other substrates. This indicates that this organic substrate is an efficient alternative to rockwool and its drainage water may be reused in a recirculation system. On the contrary, the drainage water from the Biopot substrate showed the worst qualities: high pH and low EC, low concentration of nitrate nitrogen and phosphorus, very high turbidity and a high number of microorganisms. These parameters do not qualify Biopot drainage waters for reuse.

Keywords: soilless substrates; nutrient concentration; physical properties; microorganisms

1. Introduction

Soilless culture is a worldwide-used technique of plant production. Originally it was developed to reduce the risk of soilborne diseases [1]. The other advantage of such cultivation systems is the independence from soil quality and environmental conditions. In some countries (the Netherlands, Spain, US, Canada) soilless culture is one of the most popular techniques in vegetable production [2,3]. However, it was found that this system has also some negative impacts on the environment: the used growing substrate slabs' disposal and an excessive nutrient solution leaking from cultivation mats.

The most-used growing medium is mineral wool slabs [4]. This material has many advantages, such as substrate uniformity, low weight and ease of handling, and efficiency in plant production. However, there is a disposal problem with used mats [3], which are not biodegradable. The other popular organic material in soilless culture is peat, but peat resources are limited. Moreover, the devastation of peatlands by excessive excavation has a strong negative impact on the environment, as the peatlands are carbon and water reservoirs [5]. Therefore, the other substrates for soilless production, inorganic and organic, are used alternatively, but many of them are still in the test phase [3].

Fertilization in soilless cultivation technologies is carried out in open or closed system. In Poland, almost 100% of tomatoes (about 1500 ha) are grown using these methods in an open system [6]. Additionally, in the Mediterranean countries, crops with open fertilization systems prevail [7,8]. In this technology, about 70% of the nutrient solution supplied is used by the plants, while 30% is used to wash the mats in order to maintain proper physical and chemical parameters during cultivation. Leakage from cultivation mats, called drainage water, is discharged in an uncontrolled way deep into the ground of the greenhouse or into waste water. Mineral components presented in drainage waters, moving along with the so-called wetting front, get into the groundwater, and then into wells and rivers, causing environmental pollution [9]. The additional amount of medium preventing an excessive concentration depends on the plant species, water quality, the concentration of applied solutions and climatic conditions. The average amount of drainage water produced in the cultivation of tomato on rockwool is between 25 and 50% [10–12]. In the substrates with a high mechanical and physical sorption-surface and good retention properties, the concentration of minerals is higher. An example of such growing substrate is the most commonly used rockwool, in which mineral components are highly concentrated. In organic substrates, there is both biological and physico-chemical sorption, i.e., the exchange of ions between the sorption complex of the substrate and the solution or roots of plants.

In a closed system, the excess nutrient solution is collected and may be reused for fertilization. In farms with a small area of soilless culture, drainage water can be collected in tanks and then used to fertilize plants grown in the ground under cover or in an open field. In farms with a large area of soilless culture, where large quantities of drainage waters are produced, the best solution is to use fertilization systems with nutrient solution recirculation. Studies show that nutrient solution recirculation compared to open fertilization systems uses 30% less water and over 40% less fertilizer [13].

In Poland, closed systems with nutrient recirculation are used only in modern methods of vegetable and some ornamental plants production, e.g., seedling production on tables and flooded floors or hydroponic lettuce cultivation [14]. On a wider scale, this system is only used in the Netherlands [15]. However, due to increasing drought and water deficiency, such systems may become increasingly applicable. Nutrient solution recirculation should be more widely implemented to reduce water consumption and to save the environment from pollution by leachates. It is also necessary to develop processing techniques, in order to utilize drainage waters for rational water consumption in agriculture.

It was found that the solution leaking from the mats is more concentrated compared to the initial concentration of the nutrient solution applied for plants [6,8,16,17]. From one hectare of tomato cultivation, with 20% drainage, 5 tons of fertilizers, including microelements in chelated form, leak into the ground [18]. Malorgio et al. [19] reported that 2123 m^3/ha of fertilizer solution, containing 1477 kg of nitrogen, was discharged in an uncontrolled manner into the ground of the greenhouse, in an open soilless culture system. In the research of Dyśko [20], in the open system cultivation of tomato in rockwool, from the beginning of April to the end of September, the nutrient solution consumption was around 10,220 m^3/ha, while the drainage water volume was 3082 m^3/ha. The consumption of mineral components was 9.5 t/ha, of which 4.7 t/ha, including over 1 ton of nitrogen, was discharged into the ground with drainage water. In the area of Brest (Brittany, France), 500,000 m^3 of drainage waters are discharged into the sea from 140 ha of greenhouses [7], and they contain 560 tons of fertilizers. Although the issues concerning drainage water management are not entirely new, they are still not fully solved.

The other problem in hydroponic cultivation systems is the exposue of the plants to the risk of pathogens infection, causing root and vascular system diseases. Especially pathogenic fungi genera *Pythium* spp. and *Phytophtora* spp. are very easily spread in the aquatic environment [1]. They can be distributed with the nutrient solution throughout the whole growing system. There are differences in the pathogen distribution scale between the closed and open soilless culture systems. In a closed system, the intensive growth of microorganisms in the solution and growing mats may be a barrier to pathogen development. According to Tu et al. [21], the infection of tomato plants by *Pythium*, in the closed system of medium circulation, was significantly lower than in the open system, due to a higher bacterial count. Postma et al. [22] found that the level of root diseases was lower in the non-sterilized, reused rockwool mats than in the sterilized mats. However, to reduce the risk of yield loss related to infectious diseases, disinfection of nutrient solution is used in the recirculating soilless systems. The most common methods are thermal disinfection, UV radiation, ozone and slow filtration through the sand bed [23]. However, the efficacy of disinfection is often related to the microbial density in the solution, and turbidity.

Due to the increasing pressure to protect the environment from agricultural pollution, the excess nutrient solution leaking from cultivation mats, referred to as overflow or drainage water, should be well managed. Therefore, the aim of the study was the quantitative and qualitative evaluation of drainage waters from tomato soilless cultivation in the inert and various organic substrates. The parameters of the leachates, such as EC (electrical conductivity), nutrient concentration, turbidity and microbial load, may support the decision on the usability of these substrates in a closed system of the soilless production of tomato.

2. Materials and Methods

2.1. Greenhouse Experimental Design

The research was carried out in the Experimental Greenhouse of the Research Institute of Horticulture (Skierniewice, Poland) in the years 2014–2016. The greenhouse was equipped with suspended troughs, a three-circuit heating system, a computer system for controlling microclimate conditions and an Ami Completa fertilizer dispenser. Tomato plants cv. Altadena F1 were cultivated in a soilless system using slabs with mineral and organic growing media, as follows: (i) rockwool Grotop Master (Grodan), (ii) coir substrate (Coconut), (iii) organic growing mats made of lignite—2.5 to 5 mm fraction (Carbomat), (iv) mats containing a mixture of organic wastes (sawdust, sheep's wool, flax shives and crash brown coal) named Biopot. The detailed characteristics of Carbomat and Biopot substrates were described by Dyśko and Kaniszewski [24] and Dyśko et al. [25], and the basic physico-chemical properties of the used substrates are presented in Table 1.

Table 1. Physico-chemical characteristics of the substrates used in the soilless cultivation of tomato.

Substrate	pH	EC	Bulk Density kg m^{-3}	Water Capacity at pF 1.0% *v/v*	Air Capacity at pF 1.0% *v/v*
Rockwool	7.5	0.15	62.5	66.1	31.5
Carbomat	5.3	0.92	320.4	42.8	43.6
Biopot	6.7	0.46	130.6	55.8	36.5
Coir Substrate	6.4	0.94	108.4	68.6	24.6

Tomato seeds were sown in mid-February. Seedlings were produced in rockwool cubes (10 × 10 × 6.5 cm). The seedlings were transplanted into the rockwool slabs (mats) and into the mats containing other studied substrates when 50% of the flowers on the first bunch were developed (March 15–20). Tomatoes were grown in the prolonged cycle, extended to 10th November.

The experiment was performed in a system of random blocks in five replications. The one replication (one block) for each type of growing substrate included three cultivation mats with nine tomato plants. In all objects, for plant fertigation, the nutrient solution with standard nutrient concentration (mg dm^{-3}) was used: N 200–240, P 40–60, K 250–400, Mg 60–80, Ca 190–220, Fe 2.0–2.5, Mn 0.6–0.8, Cu 0.15, Zn 0.10, B 0.20, Mo 0.05. Tap water was used for the preparation of solution. The composition of the nutrients was changed during the vegetation period, depending on plant growth phase and weather conditions. The chemical analyses of solutions, sampled from the plant rhizosphere, were also performed to correct the composition of nutrients. The other agro-technical treatments were carried out according to the principles of integrated tomato cultivation under cover.

Tomato fruits were harvested once a week and sorted for the following fractions: (i) I_A—∅ fruits over 6 cm, healthy, shapely and smooth fruits; (ii) I_B—∅ fruits 4.5–6.0 cm, healthy, shapely and smooth fruits; (iii) II—∅ fruits 3.5–4.0 cm, healthy, notched, slightly shapely; (iv) healthy fruits clearly shapely; (v) ∅ fruits below 3.5 cm; (vi) cracked fruits; (vii) diseased fruits. Early yield was calculated from 1/3 of the harvest period.

2.2. Drainage Water Sampling and Analyses

For precise measurement of the amount of drainage water from the different types of the substrates, measuring stands have been developed. A single station consisted of a plastic container (140 × 25 × 8 cm), in which a cultivation mat was placed. A plastic tube was fixed to the container and was connected with a scaled cylinder (20 L) for collecting the leachate from the cultivation mat. The amount of drainage water in the cylinders was measured daily. The samples of drainage waters for physical and chemical analyses were taken at monthly intervals, in three replications.

Physical properties were determined by EC (specific electro conductivity), measuring the conductivity and turbidity of the drainage waters, expressed as nephelometric turbidity unit NTU, using the nephelometric method. The pH was evaluated with the potentiometric method. Mineral components were determined via a universal method [26]: (i) N-NO_3, P-PO_4—colorimetrically by means of the Sanplus Skolar flow auto-analyzer; (ii) K, Ca, Na, Mg, Mn, Zn, Cu and B—with the use of inductively coupled plasma (ICP) spectrometer, Optima 2000 DV of Perkin Elmer; (iii) Cl—potentiometric ion-selective electrode; (iv) SO_4—colorimetric; (v) C (organic carbon bound by microorganisms and inorganic carbon in the form of HCO_3-bicarbonates)—the Eltra model CS 530 analyzer.

2.3. Microbiological Analyses of Drainage Waters

In all experimental objects, analyses of the total number of bacteria and fungi, spore-forming bacteria and fluorescent pseudomonads were performed during the vegetative period. The occurrence and density of pathogenic fungi of the genera *Phythphthora*, *Pythium* and *Fusarium*, as well as coliforms, which are an indicator of water sanitary status, were also examined. Samples of the solution from the root zone of the plants and the samples of drainage water were taken three times: at the beginning of cultivation (May), at full yield (July) and at the end of cultivation (September). The samples (50 mL) were collected in triplicate with the use of sterile syringes, in sterile falcon-type tubes. The number of microorganisms in the drainage water and in the root zone solution was determined on selective media, recommended for the isolation of particular groups of microorganisms, using the dilution culture method. The total number of bacteria was assessed on tryptic soy agar (TSA, Difco), spore-forming bacteria were isolated on the same medium after previous heating of the sample at 80 °C for 10 min, fluorescent pseudomonads were determined on King B medium (Merck), and coliforms on VRBL medium (Violet Red Bile Lactose agar, Merck). The total number of fungi was enumerated on Martin's medium with Bengal rose and streptomycin [27]. *Fusarium* fungi were determined on Komada's medium [28]. Fungi of the genus *Pythium* were isolated on a medium with the following composition (g L^{-1}): corn flour agar 17 g, ampicillin 0.3 g, PCNB 0.1 g, nystatin 0.05 g, benomyl 0.02 g. *Phytophthora* fungi were isolated on a medium with the following composition (g L^{-1}): agar made of maize flour 17 g, nystatin 0.1 g, rifampicin 0.01 g, ampicillin 0.18 g, PCNB 0.03 g, benomyl 0.02 g, iprodione 0.2 g. Temperatures and incubation time: total bacteria, fluorescent pseudomonads, spore-forming bacteria—48–72 h at 28 °C, coliforms 48 h at 36 ± 2 °C, *Pythium* and *Phytophthora* fungi 24–48 h at 25 °C, filamentous fungi and *Fusarium* 7–10 days at 25 °C. The number of microorganisms was expressed as colony forming units per mL of the solution or drainage water. For statistical analysis the data were transformed to logarithms ($\log_{10}$).

2.4. Statistical Analysis

In order to determine the contents of nutrients and microorganisms in the drainage waters, and tomato yield, an analysis of variance was performed, comparing the averages using the Newman–Keuls test at the significance level $p = 0.05$. For the microorganisms, prior to analysis, normality was confirmed with the Shapiro–Wilk test and homogeneity of variance was verified with the Levene test. If assumptions were violated, as in the case of the microbiological analyses, the Kruskal–Wallis ANOVA and Dunn rank sums tests were performed. Statistical analyses of data in this study were performed using the STATISTICA v.13 software package (Dell Inc. 2016).

3. Results and Discussion

3.1. Quantity of Drainage Waters Produced during Tomato Cultivation in Various Substrates

The average nutrient solution consumption during the entire period of tomato cultivation in the years 2014–2016 was 1321 dm^3/m^2 (Table 2). The production of drainage waters differed slightly between years, but the trends were the same. These differences were related to external climatic conditions, mostly to sunlight and temperature. Significantly, the highest amount of the leachates was obtained from the lignite substrate Carbomat—on average, 553 dm^3/m^2 (Table 2). In the cultivation of tomatoes on Biopot, coir substrate and rockwool, the volume of drainage waters from the cultivation mats was at a similar level, which gave about 36% leachate.

Table 2. Effect of substrate type on the volume of drainage waters.

Substrates	Consumption of Nutrient Solution (dm^3/m^2)	Drainage Water (dm^3/m^2)	Drainage Water(%)
Biopot	1321	484 ^{b}	36.6 ^{b}
Rockwool	1321	475 ^{b}	36.0 ^{b}
Coir Substrate	1321	481 ^{b}	36.4 ^{b}
Carbomat	1321	553 ^{a}	41.8 ^{a}

Means followed by the same letter within columns are not significantly different according to Newman–Keuls test ($p = 0.05$).

In Poland, most authors give a lower percentage (20–30%) of leachate production from tomato cultivation mats [6,17,29,30]. In the other works, the level of the leachates was reported between 30% and 50% [10–12]. According to Thompson et al. [8], the amount of drainage waters depends primarily on the type of cultivation mats, climatic conditions, the quality of water used for nutrient solution preparation and on the growth phase of the plants.

3.2. Physical and Chemical Parameters of Drainage Waters

The type of substrate affected the physical and chemical parameters of the drainage waters. In a closed system of soilless cultivation, the drainage waters are reused by including them in the nutrient solution recirculation system. However, the application of drainage waters in the nutrient solution recirculation system depends on their quality, including such parameters as pH, mineral content, turbidity, microbial load and the presence of pathogens. The reuse of drainage waters for plant fertigation allows one to save water and mineral fertilizers, but only if the contamination of the leachates is not high (low turbidity and microorganisms density) and allows for their effective disinfection. Therefore, when choosing growing substrates for a closed system of soilless cultivation, attention should be paid to the characteristics of the leachates.

In our studies, the pH of the leachates from Carbomat was generally similar to the pH of the nutrient solution, which had an average level of pH 5.6 (Figure 1), except for the first two weeks of cultivation, where this value was about one unit higher.

The drainage waters from rockwool and coir substrate presented pH levels of 6.2 and 6.1, respectively, both values being higher than for Carbomat. Biopot substrate, at the beginning of tomato cultivation, strongly alkalized the medium (pH nearly 8.0) in the root zone. However, after one month, the pH of the leachates from Biopot gradually decreased, and was similar to the pH the leachates from the rockwool and coir substrate. Similar results were obtained by Breś and Ruprik [16] and Breś [29]: the pH of the drainage waters from tomato cultivation on rockwool and coir substrate increased by one unit in relation to the dosed medium. The low pH in the Carbomat substrate was also observed by Dyśko et al. [25] in their earlier experiments. The optimum pH in the root zone for most crops in soilless cultivation ranges from 5.5 to 6.5 [31]. However, according to Adams [32], pH values 5.0–5.5 and 6.5–7.0 still are not harmful for most plants, but pH >7 or <5 reduces their growth [33].

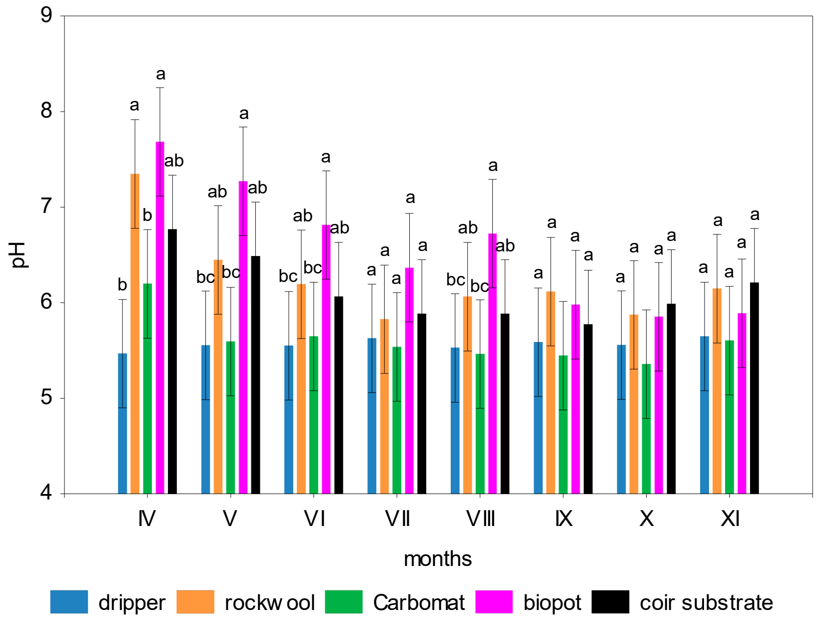

Figure 1. Influence of the type of substrate in soilless tomato cultivation on the pH (mean values ± standard error) of drainage waters during the experimental period 2014–2016. Means indicated by the same letter above bars, for each term of measurement, are not significantly different according to the Newman–Keuls test (p = 0.05).

The concentrations of minerals in drainage waters leaking from the cultivation mats, expressed as EC, were significantly higher than in the nutrient solution used for plant fertigation, during all vegetative seasons (Figure 2).

This was also reported by Moya et al. [34], who grew tomato plants in coconut mats. They had used nutrient solution with different EC values (EC 2.2, 3.5 and 4.5 mS.cm^{-1}), and in drainage waters the EC increased proportionally to the initial concentration of nutrients (EC 3.3, 5.6 and 7.7 mS.cm^{-1}). The higher EC of drainage waters is the result of nutrients concentration caused by increased transpiration during sunny weather in relation to their uptake by plants. In our studies, the highest content of elements was in the mats from the rockwool and coir substrate (EC 5.3 mS.cm^{-1} and EC 5.1 mS.cm^{-1}, respectively), while the lowest concentration of mineral components was characteristic of leachates from Carbomat and Biopot, due to the biological and chemical sorption of these substrates (EC 4.5 mS.cm^{-1} and EC 4.9 mS.cm^{-1}, respectively).

Hydroponic wastewater is particularly rich in nitrogen and phosphorus. The excess amount of runoff contains nitrate in quantities ranging 150–500 mg L^{-1} and phosphorus in quantities ranging 30–100 mg L^{-1} [35]. Such a high concentration of the nutrients in hydroponic effluents discharged into the environment is dangerous. In a natural water system, high levels of N and P contribute to algal bloom [36]. In the present studies, the content of N-NO_3 in the leachates from rockwool, Carbomat and coir substrate was at a similar level (Figure 3). The average concentration of N-NO_3 in these leachates ranged 312–347 mg L^{-1} (Table 3). A significantly lower content of nitrate nitrogen was found in the drainage water from Biopot. Its average concentration was about 40% lower than in the other leachates. The lowest content of nitrate nitrogen in Biopot drainage waters was detected at the beginning of tomato growth. According to Dyśko and Kaniszewski [24], Biotop is characterized by the biological sorption of nitrogen, especially in the initial period of cultivation.

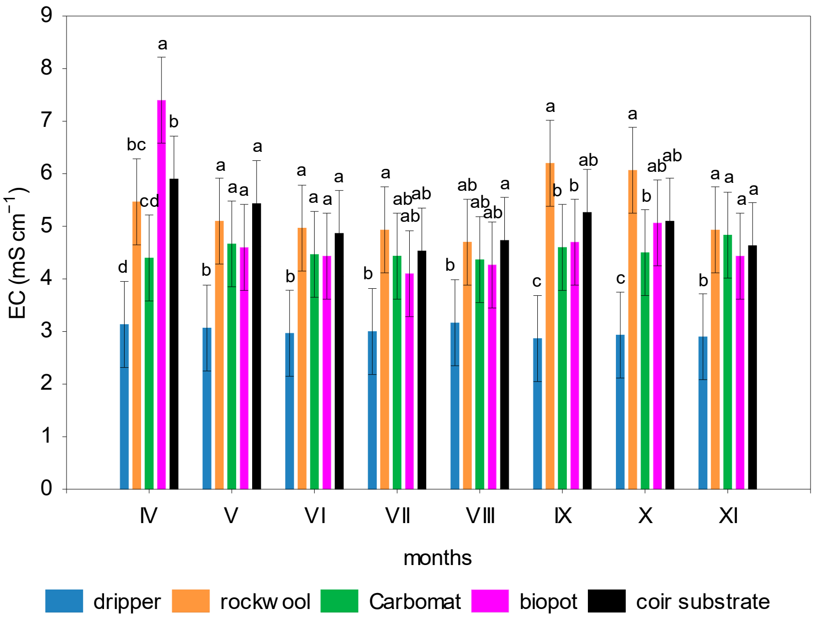

Figure 2. Influence of the type of substrate in soilless tomato cultivation on the electrical conductivity (EC) of drainage waters (mean values ± standard error) during the experimental period 2014–2016. Means indicated by the same letter above bars, for each term of measurement, are not significantly different according to Newman–Keuls test ($p = 0.05$).

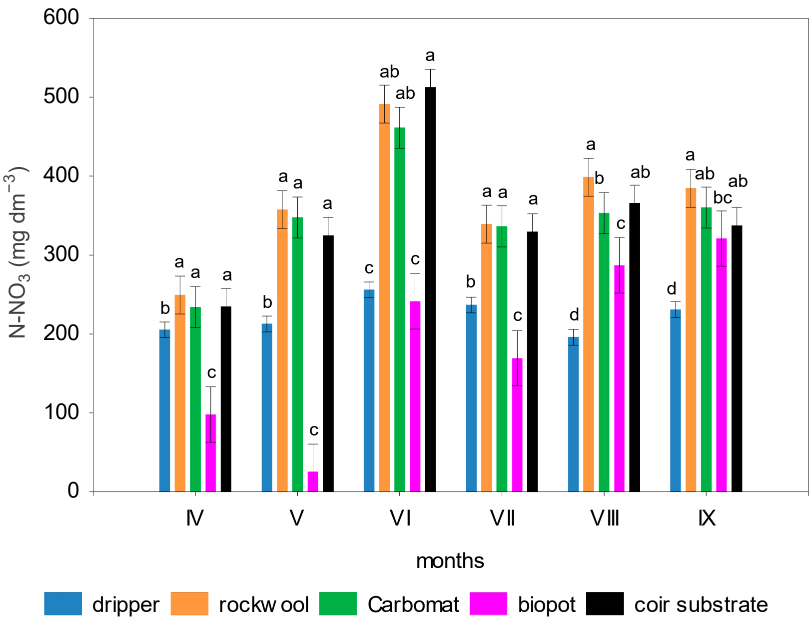

Figure 3. Influence of the type of substrate in soilless tomato cultivation on N-NO$_3$ concentration (mean values ± standard error) in drainage waters during the experimental period 2014–2016. Means indicated by the same letter above bars, for each term of measurement, are not significantly different according to Newman–Keuls test ($p = 0.05$).

The concentration of phosphorus was the highest for the leachates from the coir substrate and rockwool (Figure 4), with a mean values of 69 and 79 mg L^{-1}, respectively (Table 3). The concentration of this element in Carbomat drainage waters was close to nutrient solution. The lowest content of phosphorus was found in the drainage waters from Biopot (Figure 4). The average value of this nutrient in Biopot leachates was 18 mg L^{-1}, and it was significantly lower than in the drainage waters leaking from other substrates (Table 3).

Table 3. Influence of the substrate type in soilless tomato cultivation on the mean ± SE content of macronutrients (mg L^{-1}) in drainage waters.

Substrates	$N\text{-}NO_3$	$N\text{-}NH_4$	P	K	Ca	Mg
Rockwool	347 ± 22.6 ^{a}	11.8 ± 4.9 ^{a}	68.7 ± 11.2 ^{a}	546 ± 43.6 ^{a}	439 ± 33.8 ^{a}	205 ± 17.4 ^{a}
Carbomat	312 ± 19.3 ^{a}	10.7 ± 4.6 ^{a}	61.8 ± 14.5 ^{a}	461 ± 45.4 ^{a}	390 ± 28.6 ^{a}	148 ± 12.3 ^{b}
Biopot	191 ± 25.4 ^{b}	15.8 ± 6.7 ^{a}	17.9 ± 9.8 ^{b}	535 ± 81.8 ^{a}	375 ± 26.0 ^{a}	176 ± 11.6 ^{ab}
Coir Substrate	331 ± 18.7 ^{a}	11.5 ± 4.8 ^{a}	79.0 ± 8.8 ^{a}	658 ± 68.5 ^{a}	396 ± 38.1 ^{a}	174 ± 13.9 ^{ab}

Means followed by the same letter within columns are not significantly different according to Newman–Keuls test (p = 0.05).

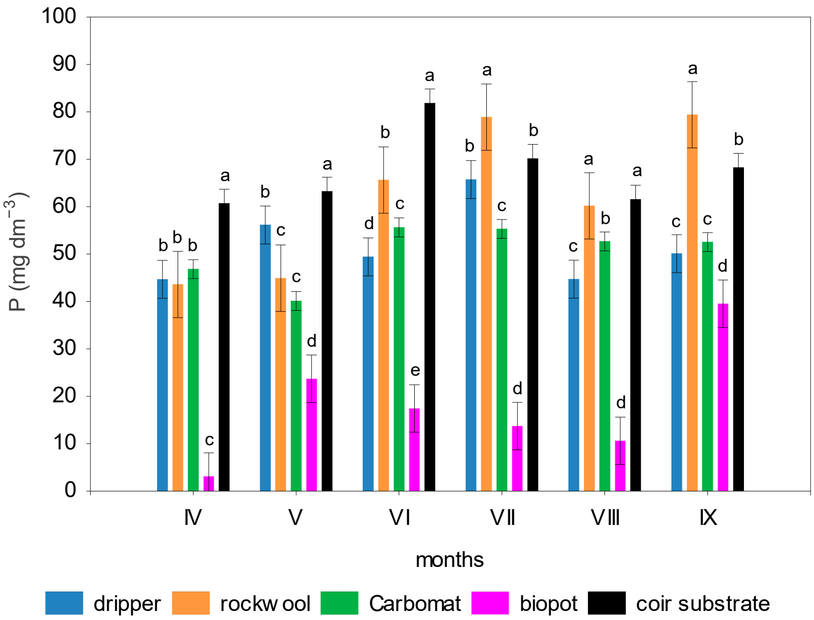

Figure 4. Influence of the type of substrate in soilless tomato cultivation on phosphate concentration (mean values ± standard error) in drainage waters during the experimental period. Means indicated by the same letter above bars, for each term of measurement, are not significantly different according to Newman–Keuls test (p = 0.05).

Drainage waters also showed an increased concentration of potassium compared to the dripper (Figure 5). However, the type of substrate only slightly differentiated K content in the initial period of tomato cultivation. During the first two months of tomato cultivation, the highest potassium concentration was found in the drainage waters from coir substrate, while the lowest was from Carbomat. Generally, the substrate type did not influence potassium content in the drainage waters (Table 3).

The concentration of calcium and magnesium was twice as high as in the standard nutrient solution used for fertigation. The highest content of these minerals was found in rockwool leachates, although the differences generally were not significant comparing to other substrates. The substrate type had no influence on the amount of $N\text{-}NH_4$.

As for micronutrients, their concentration increased in the drainage waters (Table 4), especially in the case of boron, which increased sevenfold compared to the nutrient solution (0.20 mg L^{-1} for nutrient solution and on average 1.3 mg L^{-1} for the leachates). Moreover, a high chlorine content was determined in the overflow from tomato cultivation on Biopot and coir substrate mats (308 mg L^{-1} and 163 mg L^{-1}, respectively). Kleiber [17] pointed out that less environmental contamination is generated in the case of cultivation on organic substrates. However, this has not been confirmed in these studies, comparing rockwool (mineral substrate) and other organic mats (coir substrate, Carbomat, Biopot), except for the lower N-NO_3 and P amounts in Biopot leachates.

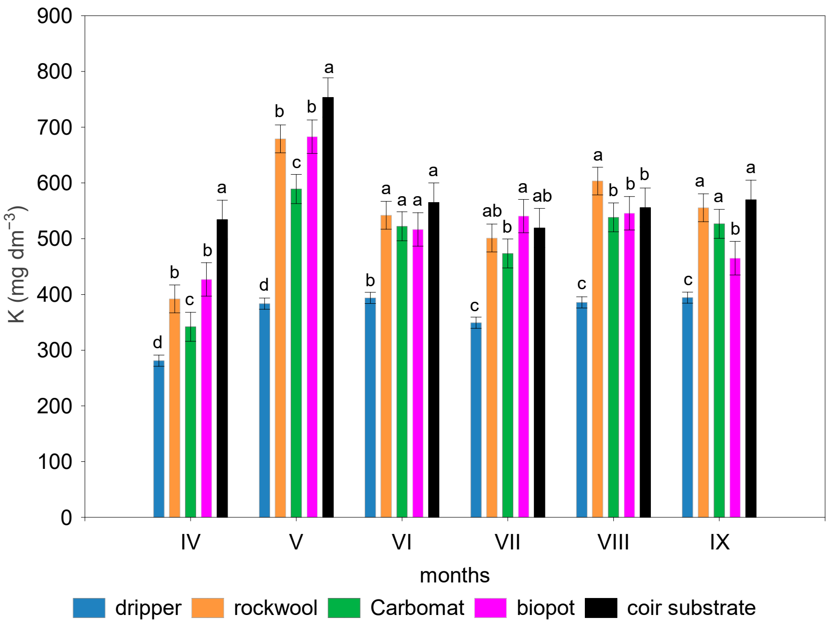

Figure 5. Influence of the type of substrate in soilless tomato cultivation on potassium content (mean values ± standard error) in drainage waters during the experimental period 2014–2016. Means indicated by the same letter above bars, for each term of measurement, are not significantly different according to Newman–Keuls test (p = 0.05).

Table 4. Influence of the substrate type on soilless tomato cultivation on the mean ± SE content of micronutrients (mg L^{-1}) in drainage waters.

Substrates	Fe	Mn	Zn	Cu	B	Cl
Rockwool	3.58 ± 0.48 ^{a}	0.74 ± 0.14 ^{a}	2.18 ± 0.40 ^{a}	0.51 ± 0.04 ^{a}	1.18 ± 0.10 ^{a}	71.6± 13.6 ^{b}
Carbomat	2.68 ± 0.49 ^{a}	1.29 ± 0.16 ^{a}	1.42 ± 0.33 ^{a}	0.17 ± 0.05 ^{b}	1.61 ± 0.55 ^{a}	61.5± 10.5 ^{b}
Biopot	4.55 ± 1.32 ^{a}	1.41 ± 0.51 ^{a}	1.51 ± 0.20 ^{a}	0.29 ± 0.09 ^{b}	1.31 ± 0.27 ^{a}	308.3± 91.8^{a}
coir substrate	1.86 ± 0.51 ^{a}	0.93 ± 0.18 ^{a}	2.05 ± 0.31 ^{a}	0.35 ± 0.05 ^{ab}	1.09 ± 0.13 ^{a}	162.5± 68.8^{b}

Means followed by the same letter are not significantly different according to Newman–Keuls test (p = 0.05). For the other micronutrients the differences were not significant.

Turbidity is one of the most important indicators of water quality. It is caused by suspended and colloidal matter, such as clay, silt, fine particles of organic and inorganic matter, plankton and microorganisms, as well as by colored organic and inorganic compounds [37]. Turbidity can be used as a measure of sediment amount [38], and in some cases may indicate the microbial contamination of water [37]. In soilless plant cultivation, high amounts of sediments may block fertigation equipment, and in the closed system with nutrient solution recirculation, the reuse of drainage waters may be restricted due to the low efficiency of disinfection. High turbidity caused by fine matter particles deteriorates the quality of disinfection by filtration or UV radiation [35,39].

In these studies, the most polluted drainage waters were found after the cultivation of tomato on Biopot (Figure 6). This was caused by the leaching of organic particles from this substrate. For five weeks the turbidity in the drainage waters from Biotop exceeded 350 NTU. The mean value of turbidity determined by the nephelometric method was 185 NTU. The microbial analyses of drainage waters, presented below, indicate that the high turbidity in Biopot leachates was not related to the multiplication of microorganisms. The microbial density in all types of drainage water was comparable (Tables 5 and 6). However, these results indicate that drainage waters from Biopot are not suitable for recirculation. The drainage waters from tomato cultivation on rockwool, Carbomat and coir substrate were characterized by low turbidity (10.4, 8.5 and 6.1 NTU, respectively), with the coir substrate leachate having a brown color for almost two months.

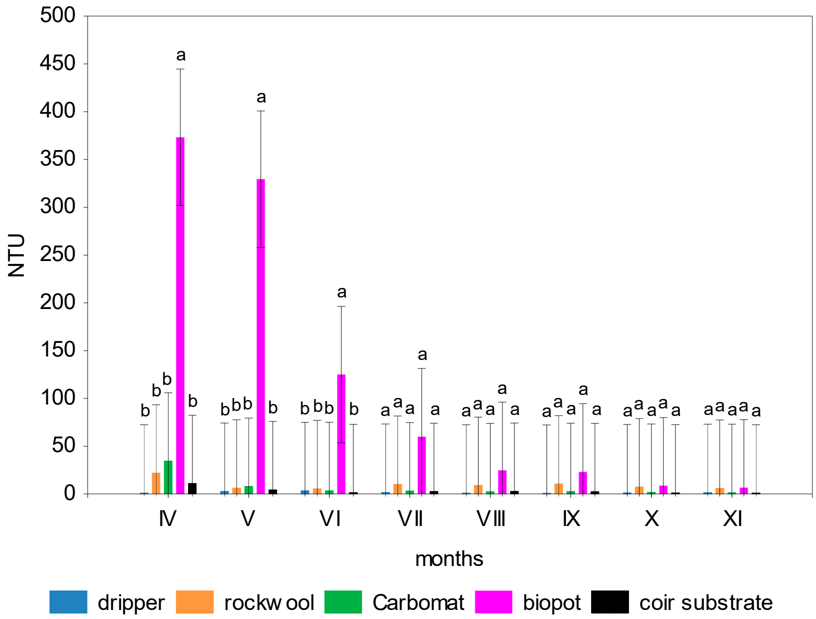

Figure 6. The effect of the substrate in tomato soilless culture on the turbidity of drainage waters (nephelometric turbidity unit - NTU) (mean values ± standard error) during the experimental period 2014–2016. Means indicated by the same letter above bars, for each term of measurement, are not significantly different according to Newman–Keuls test (p = 0.05).

Table 5. Influence of the substrate type on total bacterial count ($\log_{10}$ cfu mL^{-1} ± SE) in solution taken from the root zone and in drainage water, in soilless tomato cultivation (average values from 2014–2016).

Substrates	**Mesophilic Bacteria ($\log_{10}$ cfu mL^{-1})**		
	May	**July**	**September**
		Drainage Water	
Rockwool	5.81 ± 0.14 ^{bc}	5.26 ± 0.31 ^{b}	5.98 ± 0.14 ^{ab}
Carbomat	5.30 ± 0.16 ^{c}	5.13 ± 0.17 ^{b}	4.72 ± 0.22 ^{c}
Coir Substrate	5.70 ± 0.16 ^{bc}	5.19 ± 0.08 ^{b}	5.24 ± 0.15 ^{bc}
Biopot	6.15 ± 0.26 ^{a}	5.57 ± 0.24 ^{b}	5.94 ± 0.14 ^{ab}
		Root Zone	
Rockwool	6.37 ± 0.23 ^{ab}	5.71 ± 0.13 ^{b}	5.61 ± 0.13 ^{abc}
Carbomat	5.99 ± 0.36 ^{bc}	5.71 ± 0.15 ^{b}	5.62 ± 0.17 ^{abc}
Coir Substrate	6.36 ± 0.14 ^{a}	5.63 ± 0.18 ^{b}	5.53 ± 0.16 ^{abc}
Biopot	6.86 ± 0.14 ^{a}	6.56 ± 0.21 ^{a}	6.61 ± 0.21 ^{a}

Means followed by the same letter within column are not significantly different according to Newman–Keuls test (p = 0.05).

Table 6. Influence of the substrate type on the filamentous fungi number ($\log_{10}$ cfu mL^{-1} ± SE) in solution taken from the root zone and in drainage water, in soilless tomato cultivation (average values from 2014–2016).

Substrates	Filamentous fungi ($\log_{10}$ cfu mL^{-1})		
	May	July	September
	Drainage Water		
Rockwool	2.02 ± 0.22 ^{d}	3.02 ± 0.26 ^{a}	2.22 ± 0.64 ^{c}
Carbomat	3.21 ± 0.20 ^{c}	2.25 ± 0.57 ^{b}	2.16 ± 0.55 ^{c}
Coir Substrate	4.06 ± 0.31 ^{bc}	2.79 ± 0.36 ^{a}	2.32 ± 0.58 ^{bc}
Biopot	3.94 ± 0.30 ^{bc}	3.27 ± 0.20 ^{ab}	2.76 ± 0.38 ^{abc}

Table 6. *Cont.*

Substrates	Filamentous fungi ($\log_{10}$ cfu mL^{-1})		
	May	July	September
	Drainage Water		
	Root Zone		
Rockwool	3.86 ± 0.22 ^{bc}	2.28 ± 0.58 ^{b}	2.88 ± 0.56 ^{abc}
Carbomat	4.07 ± 0.20 ^{bc}	3.89 ± 0.22 ^{a}	4.04 ±0.58 ^{a}
Coir Substrate	5.10 ± 0.16 ^{a}	3.94 ± 0.33 ^{a}	4.18 ± 0.31 ^{a}
Biopot	4.77 ± 0.19 ^{ab}	3.58 ± 0.46 ^{a}	3.79 ± 0.51 ^{ab}

Means followed by the same letter within column are not significantly different according to Newman–Keuls test (p = 0.05).

3.3. Microbial Analyses of Drainage Waters

A higher number of total bacteria and fungi was determined in nutrient solutions taken from the root zone of tomato plants, grown in different types of substrates, compared to the drainage waters (Tables 5 and 6). However, the differences were not significant. These results are confirmed by the studies of Koohakan et al. [40], who were detecting more bacteria and fungi on roots than in nutrient solution, in various types of tomato soilless production systems. The higher number of microorganisms in the root zone is the result of the passive and active leakage of root exudates, which serve as nutrient source for these organisms [41,42].

Bacteria are the predominant microorganisms in soilless culture. The number of bacteria on tomato roots can reach 10 $\log_{10}$ cfu g^{-1} for fresh roots, while in nutrient solution this can be 5–6 $\log_{10}$ cfu mL^{-1} [1,43]. The fungal population on roots, estimated by Koohakan et al. [40], were 4–5.5 $\log_{10}$ cfu g^{-1}. In this study, the number of bacteria ranged from 4.7 to 6.2 $\log_{10}$ cfu mL^{-1} in drainage water and from about 5.5 to 6.9 $\log_{10}$ cfu mL^{-1} in the solution from the root zone (Table 5). For fungi, this was from 2.0 to 4.1 $\log_{10}$ cfu mL^{-1} in drainage water and from 2.3 to 5.1 $\log_{10}$ cfu mL^{-1} in the solution from the root zone (Table 6).

The size of the bacterial population was stabilized during cultivation season, in agreement with the observation of Koohakan et al. [40]. In turn, the number of fungi decreased in the middle of cultivation, and remained at a similar level until the end of the experiment (Table 6). This is contrary to the results reported by other authors, who have found that the number and diversity of fungi in the soilless cultivation of tomatoes grew over time [40,44]. However, it was related to the increase in the number of pathogenic fungi (*Pythium* spp., *Fusarium* spp.) at the end of the vegetation of the plants, whereas, in our studies, the numbers of fungi genera *Pythium* spp., *Phythophthora* spp. and *Fusarium* spp., evaluated on the selective media, were very low. In most studied samples these fungi were not detected. *Pythium* spp. And *Phythophthora* spp. fungi were not isolated from rockwool, Carbomat and coir substrate (drainage waters and root zone) in the whole experimental period. In the case of Biopot, these fungi were isolated only from the root zone, in average quantities (for three years) of 0.44 and

0.78 cfu mL^{-1} of the solution, respectively, for *Pythium* and *Phytophthora*. *Fusarium* spp. was isolated from all kinds of tested leachates (but not from all samples) during tomato vegetation. The numbers of these fungi were low—0.6, 1.7, 1.3, and 13.6 cfu mL^{-1} of the drainage waters from rockwool, Carbomat, coir substrate and Biopot, respectively. No tests have been carried out to recognize whether these were pathogenic or saprophytic strains. Symptoms of fusariosis have been observed on the plants.

The kind of growing medium in soilless system has an impact on the microorganisms that inhabit this medium. Gunert et al. [45] found that the type of medium makes a more significant contribution in the differentiation of microbial communities than time of sampling. Using high throughput sequencing analysis combined with molecular techniques, they found stable microbial community structures in the organic growing medium, while the mineral medium (rockwool) displayed high variability, increasing with plant growth. In these studies, Biopot (the mixture of recycled wool residues and organic wastes) contained the highest numbers of bacteria. In the three other substrates, the numbers of these microorganisms did not differ significantly (Table 5). The total number of filamentous fungi was the highest for coir substrate and Biopot (Table 6). Lower numbers of fungi were observed for Carbomat, but the differences were not always significant compared with coir substrate and Biopot. The lowest number of filamentous fungi was obtained in rockwool. Carbomat and Biopot were characterized by the presence of fungi genera *Trichoderma*. The highest number of these fungi was found in Carbomat, in both the root zone and in drainage water (Table 7). In this substrate, *Trichoderma* spp. represented on average as much as 89% of the fungal population. In Biopot, this average value was 56%. In both substrates the density of *Trichoderma* increased during plant growth. *Trichoderma* spp. are cosmopolitan fungi that occupy a variety of habitats, and are widely used for the biocontrol of numerous plant pathogens [46]. These fungi are known not only as biocontrol agents, but they also support plant growth and root development and induce plant defense mechanisms [47]. There are numerous reports in the literature about the beneficial effects of *Trichoderma* on tomato growth and yielding, for example Uddin et al. [48] or Herrera-Téllez et al. [49]. However, in the presented studies, no positive effect on the yield of tomato was found in the substrates highly colonized by these fungi (Table 10). Apparently, the strains of *Trichoderma* inhabiting Carbomat and Biopot did not have beneficial properties. However, these results suggest that both of these substrates may create an environment conducive to colonization by these fungi. This can be useful information when considering substrates for artificial inoculation with biocontrol *Trichoderma* preparations.

Table 7. Influence of the substrate type on the numbers of *Trichoderma* fungi ($\log_{10}$ cfu mL^{-1} ± SE) in solution taken from the root zone and in drainage water, in soilless tomato cultivation (average values from 2014–2016).

Substrates	*Trichoderma* spp. ($\log_{10}$ cfu mL^{-1})		
	May	**July**	**September**
		Drainage Water	
Rockwool	0.37 ± 0.37 ^{b}	0.00 ± 0.00 ^{b}	0.28 ± 0.27 ^{b}
Carbomat	1.91 ± 0.62 ^{a}	2.17 ± 0.55 ^{a}	2.68 ± 0.34 ^{ab}
Coir Substrate	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.28 ± 0.28 ^{b}
Biopot	1.04 ± 0.53 ^{a}	2.18 ± 0.57 ^{a}	0.93 ± 0.46 ^{b}
		Foot Zone	
Rockwool	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}	0.40 ± 0.40 ^{b}
Carbomat	2.79 ± 0.58 ^{a}	3.59 ± 0.20 ^{a}	4.29 ± 0.07 ^{a}
Coir Substrate	0.80 ± 0.53 ^{b}	0.00 ± 0.00 ^{b}	0.00 ± 0.00 ^{b}
Biopot	1.46 ± 0.74 ^{a}	2.24 ± 0.71 ^{a}	2.87 ± 0.72 ^{ab}

Means followed by the same letter within a column are not significantly different according to Dunn's test (p = 0.05).

The specific genera of bacteria, studied in this experiment, were fluorescent pseudomonads (Table 8). They are especially well adapted to grow and multiply in the plant rhizosphere, where these bacteria utilizing root exudates may produce a wide spectrum of bioactive metabolites and growth-promoting substances, and they compete successfully with other microorganisms [50,51]. *Pseudomonas* spp. are widely known for their plant growth-promoting properties, their induction of systemic resistance in plants and their suppression of plant pathogenic microorganisms [52]. Numerous studies indicate that *Pseudomonas* represents the core of plant growth-promoting rhizobacteria for many crops, including tomato [53,54]. The dynamic of the fluorescent pseudomonads' population in soilless cultures of tomato plants was investigated Koohakan et al. [40]. They found the highest population of these bacteria in tomato roots at the beginning of the experiment. Then, the population decreased and tended to stabilize at the same amount. Our studies confirm this trend. The highest number of fluorescent pseudomonads was observed in the samples analyzed in May, at the beginning of the vegetative season (Table 8). The number of bacteria ranged from 1.6 to 4.1 $\log_{10}$ cfu mL^{-1}; however, there were no significant differences in pseudomonads density between nutrient solutions taken from the root zone and the drainage waters. The type of substrate also had no effect on the concentration of these bacteria. In July, the number of pseudomonads decreased, with the exception of Biopot, where the amount of these bacteria was at a comparable level throughout the entire vegetation period. The mean number of fluorescent pseudomonads in the tomato root zone, after stabilization, was about 2 $\log_{10}$ cfu mL^{-1}. This represented about 30% of the total number of isolated bacteria. The higher number of these bacteria in the soilless cultivation represents the better growth and health of the plants. Déniel et al. [55] found that high populations of *Pseudomonas* in the filters in slow filtration systems increased the elimination of pathogenic fungus *Fusarium oxysporum*.

Table 8. Influence of the substrate type on the number of fluorescent pseudomonads ($\log_{10}$ cfu mL^{-1} ± SE) in solution taken from the root zone and in drainage water, in soilless tomato cultivation (average values from 2014–2016).

Substrates	Fluorescent Pseudomonads ($\log_{10}$ cfu mL^{-1})		
	May	July	September
		Drainage Water	
Rockwool	3.71 ± 0.40 ^{ab}	1.50 ± 0.40 ^{a}	1.15 ± 0.31 ^{a}
Carbomat	2.85 ± 0.11 ^{abc}	0.81 ± 0.40 ^{a}	1.00 ± 0.40 ^{a}
Coir Substrate	3.14 ± 0.19 ^{abc}	1.89 ± 0.38 ^{a}	1.61 ± 0.38 ^{a}
Biopot	1.75 ± 0.59 ^{bc}	2.24 ± 0.32 ^{a}	1.91 ± 0.49 ^{a}
		Root Zone	
Rockwool	2.98 ± 0.45 ^{abc}	1.49 ± 0.39 ^{a}	2.18 ± 0.34 ^{a}
Carbomat	1.63 ± 0.82 ^{c}	2.09 ± 0.44 ^{a}	1.63 ± 0.47 ^{a}
Coir Substrate	4.07 ± 0.20 ^{a}	1.99 ± 0.42 ^{a}	2.12 ± 0.44 ^{a}
Biopot	2.80 ± 0.58 ^{abc}	1.85 ± 0.51 ^{a}	2.46 ± 0.37 ^{a}

Means followed by the same letter within column are not significantly different according to Newman–Keuls test ($p = 0.05$).

The other specific genera of bacteria, studied in this experiment, were coliforms (Table 9). These bacteria may indicate the presence of fecal contamination in water [56]. The sanitary quality of vegetables is of great importance. Although soilless cultivation in a greenhouse ought to reduce the risk of human pathogen contamination of the produce, by eliminating contact with animals and manure fertilizers, there is a potential risk of pathogen transfer with water and nutrient solution [57,58]. The other risk in soilless production may be related to the applied organic substrates, alternatives to rockwool and peat, that may also potentially serve as transmitters of pathogenic microorganisms. The presence of pathogens in substrates or nutrient solution brings about the risk of cultured vegetable contamination, but also contamination of the leachates, which can get into the environment or can be recirculated. The limit to the number of coliforms for drinking water is defined. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption includes coliforms as an indicator parameter [59]. The Directive sets the parametric value for it at 0 cfu in 100 mL of water. However, for irrigation water, the parameters are not such strictly defined. The different guidelines generally set the limit at ≤10^3 coliforms in 100 of water or at ≤10^5 coliforms in 100 of water for restricted irrigation [60,61].

In the presented experiments, the mean number of coliforms in the nutrient solutions and in drainage waters was 1.4 $\log_{10}$ cfu mL^{-1}, and did not exceed ≤2.0 $\log_{10}$ cfu mL^{-1} for all studied substrates (Table 9). The density of this bacterial group was consistent throughout the vegetation period. This means that the amount of coli bacteria did not exceed the limit, and the type of substrate had no significant effect on the concentration of these bacteria.

Table 9. Influence of the substrate type on the coliforms count ($\log_{10}$ cfu mL^{-1}) in solution taken from the root zone and in drainage water, in soilless tomato cultivation (average values from 2014–2016).

Substrates	Coliforms ($\log_{10}$ cfu mL^{-1})		
	May	July	September
		Drainage Water	
Rockwool	1.36 ± 0.26 ^{a}	1.61 ± 0.19 ^{a}	1.38 ± 0.15 ^{a}
Carbomat	1.80 ± 0.19 ^{a}	1.54 ± 0.19 ^{a}	1.24 ± 0.29 ^{a}
Coir Substrate	1.30 ± 0.17 ^{a}	0.96 ± 0.21 ^{ab}	1.56 ± 0.21 ^{a}
Biopot	1.13 ± 0.35 ^{a}	0.92 ± 0.31 ^{ab}	1.47 ± 0.38 ^{a}
		Root Zone	
Rockwool	1.96 ± 0.41 ^{a}	1.48 ± 0.33 ^{ab}	1.27 ± 0.38 ^{a}
Carbomat	2.00 ± 0.30 ^{a}	0.86 ± 0.34 ^{ab}	1.56 ± 0.32 ^{a}
Coir Substrate	1.55 ± 0.43 ^{a}	0.46 ± 0.17 ^{b}	0.95 ± 0.17 ^{a}
Biopot	1.84 ± 0.49 ^{a}	1.63 ± 0.24 ^{a}	1.87 ± 0.37 ^{a}

Means followed by the same letter within the column are not significantly different according to Newman–Keuls test (p = 0.05).

3.4. Tomato Yield

In addition to the studies of the effects of tested substrate types on the quantity and quality of drainage waters, the influence of the used substrates on tomato yield was also investigated. The type of substrate did not have a significant effect on the yield of tomato cv. Altadena F1, although a tendency to decrease the yield on Biopot medium was observed (Table 10).

Table 10. Effect of substrate type on the yield of tomatoes cv. Altadena F_1 (kg.m^{-2} ± SE) (average values from 2014–2016).

Substrates	Yield		
	Early	Marketable	Total
Rockwool	12.1 ± 0.73 ^{a}	51.5 ± 2.90 ^{a}	52.4 ± 2.90 ^{a}
Carbomat	12.8 ± 1.13 ^{a}	52.1 ± 3.24 ^{a}	53.0 ± 3.22 ^{a}
Biopot	11.4 ± 1.19 ^{a}	45.4 ± 4.21 ^{a}	46.6 ± 3.93 ^{a}
Coir Substrate	12.3 ± 0.93 ^{a}	48.1 ± 2.83 ^{a}	48.9 ± 2.93 ^{a}

Means followed by the same letter within column are not significantly different according to Newman–Keuls test (p = 0.05).

Statistical analysis did not show any significant differences in total, marketable and early yield of tomato. A slightly higher total and marketable fruit yield was obtained from cultivation on Carbomat and rockwool (53.0 and 52.4, 52.1 and 51.5 kg m^{-2}, respectively). The soilless technology of tomato cultivation allows for precise nutrition control of the plants, and the function of the growing mats is to mechanically support the root system and to ensure proper air and water conditions. This gives great opportunities to use various materials as a substrate. Dyśko and Kaniszewski [24] have shown that in soilless cultivation, the type of substrate did not affect tomato yield. Similar observations were given by Lopez et al. [62] for greenhouse tomato cultured in rockwool, sand and zeolite, or Kraska et al. [63], who grew cucumbers and tomatoes on the different *Miscanthus* substrates, and obtained cumulative yields comparable to rockwool. However, in the studies of Kilic et al. [64], among tomato plants grown in rockwool, coir substrate or perlite, the highest yield was obtained for the perlite, but the best quality (test, aroma, acidity) was had by the fruits from coir substrate.

4. Conclusions

For a closed system of soilless growing, the quality of the drainage waters leaking from cultivation mats is a key factor, determining the possibility of their reuse in nutrient solution circulation systems. The studies showed that the kind of substrate used for soilless tomato culturing significantly affected the quality of drainage waters. In this case, substrates that have shown good effects in open system tomato cultivation may not be favorable in a closed system due to the low suitability of draining waters for recirculation (e.g., high turbidity and microbial load, increased number of pathogenic fungi). In these studies, the largest amount of drainage water was produced when tomatoes were grown on the lignite substrate—Carbomat. These leachates indicated the lowest pH and EC values compared to other substrates. The concentration of nutrients (N, P, K) was similar to rockwool and coir substrate leachates. The parameters of the drainage waters of the latter two were comparable, whereas the organic substrate Biopot, consisting of the mixture of organic components, exhibited the greatest differences in drainage water properties compared to other substrates. The leachates showed high pH and low EC, and the lowest concentration of nitrate nitrogen and phosphorus. They were also characterized by high turbidity and a high number of microorganisms. Moreover, in spite of the very low abundance of pathogens (*Phytophthora, Pythium, Fusarium*) in all of the drainage waters, those from Biopot indicated always the highest number of these microbes. Therefore, the drainage waters from Biopot should not be used for the re-fertilization of tomatoes in the recirculation system. Additionally, the yield of tomatoes grown on this substrate was the lowest. These results suggest that Biopot may not be a very good alternative to rockwool in soilless cultivation. The other substrates used in the experiments were comparable in their properties. However, Carbomat indicated several positive properties: the highest tomato yield, the low electro conductivity and turbidity of the drainage waters, and also a high population of *Trichoderma* fungi, beneficial for plant growth. This indicates that this substrate is efficient in cultivation and may be safe for the environment. The possibility of the reuse of both the substrate and its drainage water may be considered.

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