

Microbial Degradation of Phytotoxic Root Exudates in Portable Ebb-and-Flow Hydroponic Systems as a Factor of Environmental Sustainability in Urban Farming

Manuscript Received: 20/07/2026 Manuscript Accepted: 21/08/2026 Article Published: 23/08/2026

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ABSTRACT

This study evaluated the effectiveness of consortium-based microbial preparations (EM (Effective Microorganisms), EM-1, EM Agro and EM Bioactive) in degrading phenolic compounds and regenerating nutrient solution in portable ebb-and-flow hydroponic systems during sweet pepper (*Capsicum annuum* L.) cultivation under controlled conditions, using vertical portable hydroponic units. Microbial preparations were applied to the nutrient solution every 14 days. Total phenolic content, biometric parameters, chlorophyll content (SPAD index, Soil and Plant Analysis Development), nutrient solution parameters and phytotoxicity were assessed. Without regeneration, phenolic compounds accumulated to $458.6 \pm 31.4 \text{ mg L}^{-1}$ over 90 days, reducing plant productivity by 51.1 %. A strong significant negative correlation using Spearman's rank correlation coefficient with perfect rank-order reversal, $r_s = -1.0$, $p = 0.017$ was found between phenolic compound concentration and productivity. All microbial preparations effectively degraded phytotoxic exudates, with EM Bioactive showing the highest efficiency (89.4 %). This treatment increased plant productivity compared with complete nutrient solution replacement, while reducing water and fertilizer consumption and lowering solution phytotoxicity to a low-toxicity level. The findings indicate that microbial degradation of root exudates is an effective strategy for nutrient solution regeneration, improving environmental sustainability, and resource efficiency of closed portable hydroponic systems for sweet pepper cultivation. As the study was limited to a single crop and a single 90-day cultivation cycle, these conclusions regarding the ecological sustainability of urban farming and closed hydroponic systems more broadly should be regarded as preliminary, pending validation on other vegetable crops (e.g., tomato, cucumber, lettuce) and over multiple nutrient-solution reuse cycles.

Keywords: closed portable hydroponic systems, ebb-and-flow method, microbial preparations, nutrient solution regeneration, root exudates, sweet pepper

How to Cite: Kovalov, M., Sokolovska, I., and M, Y. (2026). Microbial Degradation of Phytotoxic Root Exudates in Portable Ebb-and-Flow Hydroponic Systems as a Factor of Environmental Sustainability in Urban Farming. *J. Agric. Sci. Tech.* 4(1), 1-17. <https://doi.org/10.36108/jast/6202.40.0110>

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Publisher: Lujosh Ventures Limited

INTRODUCTION

Rapid urbanisation and limited land resources are creating new challenges for the food security of modern cities. By 2050, 68 % of the world's population is expected to live in urban areas, which will require innovative approaches to food production under conditions of limited space and resources (Nugent and Steven, 2022). In this context, hydroponic systems, particularly portable ebb-and-flow systems, have gained considerable importance as an effective tool for urban farming, capable of delivering high productivity with minimal use of water and land resources (Asaduzzaman *et al.*, 2025; Nugent and Steven, 2022; Yusuf *et al.*, 2025).

Sweet pepper (*Capsicum annuum* L.) is an annual herbaceous crop of the family Solanaceae with a comparatively shallow, branched root system and high root metabolic activity, which drives intensive release of phenolic compounds and organic acids into the rhizosphere; this feature makes it a convenient model object for studying autotoxicity in closed hydroponic systems (Bharti *et al.*, 2026; Fratianni *et al.*, 2020).

Portable ebb-and-flow systems have gained particular importance as an effective tool for urban farming, capable of delivering high productivity with minimal use of water and land resources. Ebb-and-flow systems are characterised by a cyclical nutrient-solution delivery regime that provides an optimal combination of root-zone nutrition and aeration. (Chatterjee *et al.*, 2025; Campos *et al.*, 2025) However, closed recirculating systems face the critical problem of root-exudate accumulation, which leads to autotoxicity (Chatterjee *et al.*, 2025; Campos *et al.*, 2025; Hosseinzadeh *et al.*, 2019a; Hosseinzadeh *et al.*, 2017). Studies have shown that in closed hydroponic systems plant roots release a variety of organic compounds, including phenols, organic acids and other allelochemicals, whose concentration reaches a phytotoxic level over time (Hosseinzadeh, 2017; Wang *et al.*, 2024). Among the most toxic exudate components are benzoic acid, cinnamic acid derivatives and other phenolic compounds, which can substantially inhibit plant growth even at low concentrations (Pramanik *et al.*, 2020; Rizaludin *et al.*, 2021; Were *et al.*, 2022).

Traditional methods of removing phytotoxic compounds, such as adsorption onto activated carbon, membrane filtration or physico-chemical degradation, although

effective, are energy-intensive and economically unfeasible for scaling up, particularly in the context of portable urban-farming systems (Asao *et al.*, 2008; Gutiérrez *et al.*, 2021; Sabzehmeidani *et al.*, 2021). Microbial degradation, by contrast, is an environmentally safe and cost-effective alternative based on the natural biodegradation of organic compounds by rhizosphere microorganisms (Saeed *et al.*, 2021; Sun *et al.*, 2021).

Rhizosphere microorganisms play a key role in maintaining plant health and ecosystem functioning, demonstrating the ability to degrade a wide range of organic pollutants and phytotoxic compounds (Saeed *et al.*, 2021; Sun *et al.*, 2021). It has been shown that bacteria of the genera *Pseudomonas*, *Bacillus* and other plant-growth-promoting rhizobacteria (PGPR) are able to efficiently metabolise phenols and organic acids, converting them into non-toxic products (Saeed *et al.*, 2021). Particular attention has been paid to the role of root exudates in stimulating microbial activity: low-molecular-weight organic acids, sugars and amino acids released by roots serve as an energy source for rhizosphere microorganisms, creating favourable conditions for their colonisation and functioning (Yusuf *et al.*, 2025).

In the context of portable ebb-and-flow hydroponic systems, microbial degradation is of particular importance as a factor of environmental sustainability. The cyclical nature of flooding and draining creates unique conditions for microbial communities, providing periodic aeration and the supply of fresh nutrient solution, which sustains a high level of microbial activity (Thomas *et al.*, 2024). The recirculating nature of the system allows the nutrient solution to be reused, substantially reducing water and mineral fertiliser consumption, while requiring effective control of phytotoxic compound accumulation (Asaduzzaman *et al.*, 2025).

The environmental sustainability of urban farming depends directly on resource-use efficiency and the minimisation of negative environmental impact. Microbial degradation of phytotoxic exudates is a natural mechanism for maintaining balance in closed hydroponic systems, reducing the need for frequent nutrient solution replacement and the application of chemical purification methods (Fathidarehnejeh *et al.*, 2024; Rogowska-van der Molen *et al.*, 2023). This not only reduces economic costs but also contributes to the development of more resilient

and environmentally safer food-production systems under urban conditions.

Despite growing interest in the use of microbial communities in hydroponic systems, the question of optimising microbial degradation of phytotoxic exudates in portable ebb-and-flow systems remains insufficiently studied. A comprehensive understanding is needed of the interaction between system operating regimes, microbial community composition and the efficiency of allelochemical degradation. Furthermore, it is important to elucidate the mechanisms by which microbial degradation can be integrated into the design of portable hydroponic systems to maximise their environmental efficiency and productivity.

The Effective Microorganisms (EM) technology is based on a consortium of lactic acid bacteria, photosynthetic bacteria and yeasts acting synergistically: lactic acid bacteria suppress the development of pathogenic and putrefactive microflora, photosynthetic bacteria produce biologically active substances from root exudates and organic residues, and yeasts synthesise vitamins and amino acids that further stimulate the activity of other consortium members. In crop production, EM-based preparations are used to accelerate the decomposition of organic matter, suppress pathogens and improve nutrient uptake (Joshi *et al.*, 2019; Ncube, 2024).

Studies of the mechanisms of biofilm formation on root surfaces have shown that this process is regulated by a complex molecular communication system known as quorum sensing (QS). The QS system coordinates bacterial cell behaviour according to population density through the production and recognition of signalling molecules such as acyl-homoserine lactones (AHLs) in Gram-negative bacteria (Harjai and Sabharwal, 2017). It has been established that plant roots release up to 40 % of photosynthesis products in the form of various organic compounds, which serve not only as a nutrition source for microorganisms but also as regulators of biofilm formation and of the expression of genes responsible for the degradation of organic pollutants (Sun *et al.*, 2021; Yusuf *et al.*, 2025).

Considerable progress has been made in understanding the specific mechanisms of microbial degradation of phenolic compounds, which are the main components of

phytotoxic root exudates. Muñoz-Palazon *et al.* (2023), in a comprehensive review of biotechnological approaches to the treatment of high-polyphenol-content waters, summarised current knowledge of aerobic and anaerobic degradation pathways. In the metabolic pathways by which phenol is first converted to catechol by phenol hydroxylase and then cleaved by catechol-1,2-dioxygenase (ortho pathway) or catechol-2,3-dioxygenase (meta pathway), forming compounds able to enter the tricarboxylic acid (TCA) cycle. Representatives of the genera *Pseudomonas*, *Bacillus* and *Planococcus* play a particularly important role in these processes (Muñoz-Palazon *et al.*, 2023).

Despite considerable progress in understanding the mechanisms of microbial degradation of organic pollutants, gaps remain in the knowledge of their specific application in portable ebb-and-flow hydroponic systems. Most studies have focused on soil systems or conventional hydroponic units with continuous circulation, whereas the unique conditions of cyclical flooding and draining characteristic of ebb-and-flow systems create a distinctive environment for microbial communities that requires separate investigation. Furthermore, the mechanisms by which biofilm activity is maintained under conditions of periodic changes in water regime and oxygenation are insufficiently studied, which is critically important for optimising such systems in the context of urban farming.

The aim of this study was to determine the effectiveness of consortium-based microbiological preparations (EM-1, EM Agro, EM Bioactive) in degrading phytotoxic root exudates and regenerating the nutrient solution in closed portable ebb-and-flow hydroponic systems used for growing sweet pepper.

MATERIALS AND METHODS

The study was carried out at the "Hydroponic Vegetable Growing in a Dome Greenhouse" research laboratory of the Department of General Agriculture at the Central Ukrainian National Technical University.

Sweet pepper (*Capsicum annuum* L.) was used as the study object- a hybrid adapted to protected-cultivation conditions. The choice of crop was determined by the high level of phenolic-compound release by its root system (Camli-Saunders and Villouta, 2025).

Garden cress (*Lepidium sativum* L.) was selected as the bioassay species for the following reasons: it is one of the most widely used standard indicator species in phytotoxicity and ecotoxicity testing, recommended by international protocols (Organisation for Economic Co-operation and Development (OECD), 2016; Zucconi *et al.*, 1981); it is characterised by rapid and highly uniform germination (24–48 h), which minimises experimental variability and allows results to be reliably assessed over a short period; it shows high sensitivity to a broad spectrum of phytotoxic compounds, including phenolic acids, organic acids and heavy metals, making it particularly suitable for detecting residual toxicity of phenolic exudates and their degradation products; and the two-endpoint approach (proportion of germinated seed and root elongation) provides a comprehensive assessment of both acute toxicity and sub-lethal growth inhibition, as described by Bożym *et al.* (2020).

The three Effective Microorganisms (EM) preparations used in the study are commercial products based on the EM technology, originally developed by Professor Teruo Higa (University of the Ryukyus, Okinawa, Japan). All products are manufactured by EMRO EHG Germany GmbH and distributed in Ukraine by “EM Ukraine” (EM Ukraine LLC).

The EM-1 is the base preparation, containing the following microbial consortium (according to the Ukrainian certificate of state registration): *Lactobacillus plantarum* (4.7×10^5 CFU/mL), *Rhodopseudomonas palustris* (1×10^2 CFU/mL), *Saccharomyces cerevisiae* (2.9×10^5 CFU/mL) and *Azotobacter* sp. (4.0×10^4 CFU/mL). The EM Agro is a specialised formula developed for crop production. In addition to the base EM consortium, it contains enhanced strains that promote atmospheric nitrogen fixation, the conversion of unavailable nutrient forms into plant-available compounds, and accelerate the decomposition of plant biomass. The EM Bioactive was developed with enhanced enzymatic activity for the decomposition of organic matter. According to the manufacturer, this preparation acts as a primary decomposer of organic and inorganic compounds and a natural antagonist of putrefactive bacteria and pathogenic microflora. Batch numbers and expiry dates of all preparations used in the experiment were recorded; this information is available on request.

Plants were grown on an inert substrate (perlite or expanded clay with a particle size of 5–10 mm). The flooding regime supplied nutrient solution six times per day, for 15 minutes each. The total volume of nutrient solution in the system was 20 L per experimental group. Microbiological preparations were applied directly to the nutrient-solution reservoirs at a concentration of 1:1000 (10 mL of preparation per 10 L of solution). Reapplication was carried out every 14 days to maintain a stable microbial population density.

An individual plant ($n = 5$ per variant) served as the experimental unit for assessing biometric, physiological and morphometric plant parameters. For chemical composition and microbiological parameters of the nutrient solution, the reservoir served as the experimental unit ($n = 1$ per variant). Accordingly, data on nutrient solution dynamics are presented as descriptive statistics and were not used for statistical comparison between variants, in order to avoid pseudoreplication. All plants within a given variant were kept under identical microclimatic conditions.

Total phenolic content (total reducing capacity) of the nutrient solution was determined spectrophotometrically using the Folin–Ciocalteu reagent (Singleton *et al.*, 1999). Measurements were taken weekly, with results expressed as gallic acid equivalents (mg GAE L⁻¹). It should be noted that this method measures the total reducing capacity of the solution matrix, including both phenolic compounds and other endogenous reducing agents (e.g. ascorbic acid and reducing sugars present in root exudates).

At the end of the 90-day cycle, phytotoxicity bioassays were carried out using garden cress (*Lepidium sativum* L.) seed. Seeds ($n = 30$ in three replicates) were germinated on the spent nutrient solution for 72 h in darkness at 22 ± 1 °C; distilled water served as the control. Phytotoxicity was assessed using the germination (phytotoxicity) index (PI), which accounts for relative seed germination (RSG) and relative root elongation (RRE), following the method of Bożym *et al.* (2020):

$$RSG (\%) = \frac{G_{\text{sample}}}{G_{\text{control}}} \times 100;$$

$$RRE (\%) = \frac{L_{\text{sample}}}{L_{\text{control}}} \times 100;$$

$$PI (\%) = \frac{RSG \times RRE}{100}.$$

where $G(\text{sample})$ and $G(\text{control})$ are the number of germinated seeds in the variant and in the control (distilled

water); $L(\text{sample})$ and $L(\text{control})$ are the mean root length (mm).

It should be noted that testing was carried out on undiluted solution; the PI value therefore reflects the cumulative inhibition caused both by accumulated allelopathic metabolites and by the overall osmotic pressure of the solution.

Leaf chlorophyll content was assessed non-destructively using a portable LEAF CHL STD chlorophyll meter (FT Green LLC, USA) in SPAD units (Soil and Plant Analysis Development), reflecting the relative chlorophyll content of the assimilating tissue. Stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) and SPAD values were measured on 3–5 fully developed upper leaves of each plant at a fixed morning time (09:00–11:00).

Physiological measurements and harvesting were carried out according to a pre-defined protocol. Owing to technical constraints in servicing the hydroponic systems, measurements were taken under open conditions (without blinding as to variant identity). Observer bias was minimised through the use of calibrated digital instruments with automatic data logging and random selection of leaves for analysis.

The SPAD index values were automatically calculated by the instrument based on the difference in light absorption by chlorophyll at specific wavelengths. Gallic acid (GAE) was used as the analytical standard, determined by the colorimetric Folin–Ciocalteu method. Optical density was measured at a wavelength of 765 nm using a ULAB 101 spectrophotometer (ULAB, China). A calibration curve was constructed using gallic acid (purity $\geq 99\%$, BP/EP/USP grade; Fengchen Group Co., Ltd., Qingdao, China) as the standard, over a concentration range of 0 to 500 mg L^{-1} .

All physiological, morphometric and biometric plant measurements were carried out in five biological replicates ($n = 5$ plants per variant). The adequacy of this sample size was determined by a post-hoc statistical power analysis. At a significance level of $\alpha = 0.05$ and the observed large

effect size (Cohen's f), a sample of $n = 5$ provided a statistical power of approximately 80 % for the main growth and chlorophyll-content (SPAD) parameters.

Evaporation losses over the 90-day experiment were actively compensated by adding distilled water to maintain the solution volume at approximately 20 L per module. The reservoir level was checked every 3 days using a measuring vessel, with distilled water added as needed to restore the target volume.

The experimental component of the study was organised according to a completely randomised design (CRD) for a single-factor experiment, in which the main varied factor was the type of microbiological preparation. Five experimental variants were established for comparative analysis ($n = 5$ biological replicates/pepper plants per variant), placed in separate portable ebb-and-flow modules as indicated in Table 1. Initial spatial arrangement of modules and plants was determined using a random-number generator. To compensate for possible environmental and microclimatic gradients (uneven lighting, temperature regime, air movement), the position of the portable modules within the growing area was systematically changed (rearranged in random order) once a week throughout the entire 90-day experimental period.

All five pepper plants within a given variant were supplied from a single closed 20 L reservoir. Accordingly, the individual plant served as the experimental unit for assessing plant-level parameters (SPAD chlorophyll content, stomatal conductance g_s , biometric and yield parameters; $n = 5$ biological replicates per variant).

The reservoir, by contrast, served as the experimental unit for the chemical and microbiological parameters of the nutrient solution (pH, EC, phenolic compound concentration, bioassay toxicity index PI; $n = 1$ per variant). To avoid pseudoreplication, analysis of variance (ANOVA), F-tests and HSD calculations were not applied to the solution's chemical-composition parameters; these data are presented solely as descriptive statistics.

Table 1: Experimental design

Variant	Description
C–	Negative control: nutrient solution without microbiological preparations and without replacement throughout the growing cycle
C+	Positive control: complete nutrient solution replacement every 14 days
EM-1	EM-1 applied every 14 days
EM Agro	EM Agro applied every 14 days
EM Bioactive	EM Bioactive applied every 14 days

The experiment lasted 90 days – from the four-true-leaf stage to the completion of mass fruiting. To minimise error, stable microclimatic conditions were maintained for all variants: LED grow lights (16/8 h photoperiod, light intensity $250\text{--}300\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$); air temperature $24 \pm 2\ ^\circ\text{C}$ during the day and $18 \pm 2\ ^\circ\text{C}$ at night; nutrient-solution temperature $20 \pm 1\ ^\circ\text{C}$. Aeration was provided naturally during the nutrient-solution drainage phase.

The EC and pH were measured using an MP 521 laboratory pH/conductivity meter (ULAB) and an I-130 ion meter, while routine checks were carried out with a portable handheld device (model JQ008). All instruments were calibrated with standard buffer and conductivity solutions before each measurement session.

All statistical processing and graphical visualizations were executed in Python (version 3.10.11) using SciPy (v1.10.0), Matplotlib (v3.7.1), and Seaborn (v0.12.2), with post-hoc power analysis conducted in G*Power (version 3.1.9.7). One-way analysis of variance (ANOVA), and comparisons of mean values between variants were conducted using Tukey's honestly significant difference (HSD) test at a significance level of $P < 0.05$. All data in tables and figures are presented as $M \pm SD$ (mean $\pm$ standard deviation).

Statistically significant differences between variants are denoted by different Latin letters.

RESULTS AND DISCUSSION

Analysis of the dynamics of phenolic-compound accumulation (Table 2) revealed clear differences between variants. In the negative control without microbiological preparations (C–), the concentration of phytotoxic phenolic compounds increased from $12.3\ \text{mg GAE L}^{-1}$ to $458.6\ \text{mg GAE L}^{-1}$ over 90 days – a 37-fold increase. The initial phenolic-compound concentration on day 0 ($\sim 12.3\ \text{mg GAE L}^{-1}$) reflects the baseline reactivity of the Folin–Ciocalteu reagent with the nutrient-solution composition and minor leaching from the substrate (expanded clay), rather than substantial early root exudation, since at this stage the plants were at the four-true-leaf stage with minimal root mass. These results are consistent with the data of Wang *et al.* (2024), who reported similar rates of toxic exudate accumulation in closed hydroponic systems without solution regeneration. In the positive control with complete nutrient-solution replacement (C+), by contrast, phenol concentration remained close to the baseline level ($15.1\ \text{mg GAE L}^{-1}$), corresponding to an effective degradation rate of 96.7 %.

Table 2: Dynamics of phenolic-compound concentration in the nutrient solution (mg GAE L^{-1})

Variant	Day 0	Day 30	Day 60	Day 90
C–	12.3 ± 1.2	186.4 ± 15.3	342.8 ± 22.7	458.6 ± 31.4
C+	11.8 ± 1.1	14.2 ± 1.5	13.8 ± 1.3	15.1 ± 1.6
EM-1	12.5 ± 1.3	32.8 ± 3.2	48.6 ± 4.5	62.3 ± 5.8
EM Agro	12.1 ± 1.2	28.4 ± 2.8	42.7 ± 3.9	54.8 ± 4.7
EM Bioactive	11.9 ± 1.1	25.6 ± 2.4	38.2 ± 3.5	48.7 ± 4.2
HSD _{0.05}	–	6.91	8.97	13.63

Values are in mean $\pm$ standard deviation ($n = 5$); HSD- Tukey's honestly significant difference (HSD) test at $P < 0.05$

Application of microbiological preparations demonstrated high effectiveness in degrading phenolic compounds as indicated in Figs. 1 and 2. By day 90, as shown in Fig. 1, the

best result was obtained for EM Bioactive ($48.7\ \text{mg GAE L}^{-1}$) – substantially lower than for the base preparation EM-1 ($62.3\ \text{mg GAE L}^{-1}$), the difference ($13.6\ \text{mg GAE L}^{-1}$)

exceeding the threshold of statistical significance. EM Agro (54.8 mg GAE L⁻¹) showed an intermediate result, not statistically different from EM Bioactive but inferior to EM-

1. The degradation efficiency of EM Bioactive reached 89.4 %, only 7.3 % lower than that of the positive control with complete nutrient-solution replacement.

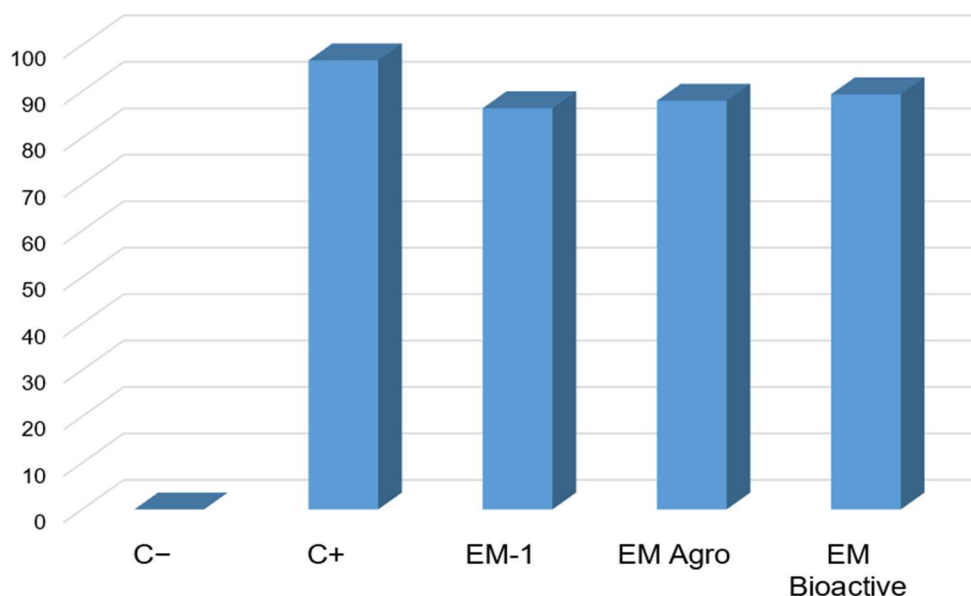


Fig. 1: Degradation of nutrient-solution phenolic compounds under the influence of EM preparations

By day 30 as shown in Fig. 2, all three EM preparations reduced phenol concentration compared with C⁻: EM Bioactive – 25.6 mg GAE L⁻¹, EM Agro – 28.4 mg GAE L⁻¹ and EM-1 – 32.8 mg GAE L⁻¹. These results are consistent with the findings of Tian *et al.* (2025), who reported that microbial consortia can effectively degrade phenolic

compounds at concentrations of up to 1500 mg GAE L⁻¹. The higher efficiency of EM Bioactive could be due to its enhanced enzymatic activity with the presence of phenol hydroxylases and catechol dioxygenases, which catalyse cleavage of the aromatic ring (Shebl *et al.*, 2024).

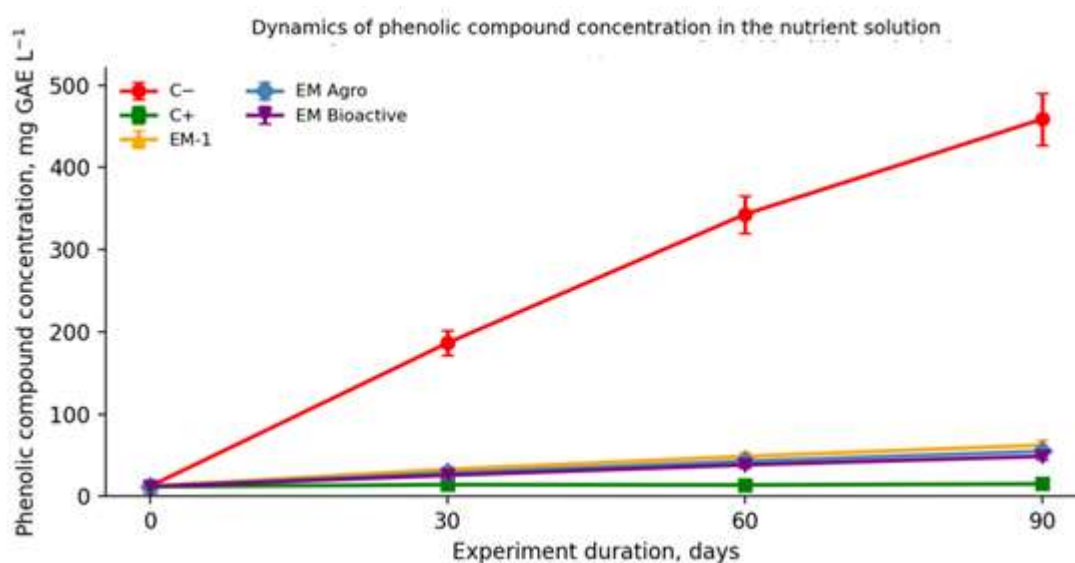


Fig. 2: Dynamics of phenolic-compound concentration in the nutrient solution over the 90-day experiment

Plant biometric parameters findings are indicated in Table 3. In the negative control (C–), substantial growth inhibition was observed: plant height was only 42.3 cm, while it was 68.7 cm in C+. The number of leaves was also lower, 18.2 compared with 32.4 in the positive control. The SPAD index, reflecting chlorophyll content, decreased to 31.4 compared with 48.6 in C+, indicating impaired photosynthetic activity under the influence of phenolic toxins. The most pronounced effect was observed for yield: fruit mass in C– was only 284.6 g per plant – lower than in C+ (582.3 g per plant).

Application of EM preparations substantially increased plant productivity in closed systems (Kovalov, 2025). EM Bioactive showed the best result among the biological treatments: plant height reached 66.4 cm (96.7 % of C+), not different from the positive control with solution replacement (difference of 2.3 cm). Similarly, the number of leaves (31.3) show no much difference from that of C+ (32.4). The SPAD index for EM Bioactive (47.1) also did not differ from C+.

Table 3: Biometric parameters of pepper plants at the end of the trial (day 90)

Variant	Plant height (cm)	Number of leaves	SPAD index	Fruit mass, g/plant
C–	42.3 ± 3.8	18.2 ± 2.1	31.4 ± 2.3	284.6 ± 28.5
C+	68.7 ± 4.2	32.4 ± 2.8	48.6 ± 3.1	582.3 ± 42.7
EM-1	61.2 ± 3.9	28.6 ± 2.4	44.2 ± 2.8	498.4 ± 38.2
EM Agro	64.8 ± 4.1	30.1 ± 2.6	46.3 ± 2.9	534.7 ± 40.3
EM Bioactive	66.4 ± 4.0	31.3 ± 2.7	47.1 ± 3.0	556.8 ± 41.5
HSD _{0.05}	5.22	3.30	3.69	52.87

Values are in mean ± standard deviation (n = 5); HSD- Tukey's honestly significant difference (HSD) test at $P < 0.05$

Regarding fruit mass as indicated in Fig. 3, EM Bioactive delivered 556.8 g per plant (95.6 % of C+), significantly more than EM-1 (498.4 g), by 58.4 g ($P < 0.05$; this difference exceeds the $HSD_{0.05} = 52.87$ for this parameter). The difference between EM Bioactive and C+ was only 25.5 g, which, although not statistically significant ($P > 0.05$), indicates a slight trend towards lower productivity. EM Agro showed intermediate values (534.7 g), not statistically different from EM Bioactive ($P > 0.05$), while the base preparation EM-1 reached 85.6 % of control productivity. These results confirm the conclusions of Muñoz-Palazon *et al.* (2023) that effective microbial degradation of phenolic compounds promotes optimal plant development.

Analysis of the nutrient solution's regenerative capacity as shown in Table 4 revealed substantial differences between

variants. In the negative control (C–), a critical decrease in pH from 6.0 to 4.8 was observed, associated with the accumulation of organic acids and plant metabolic products; this decrease is most likely explained by the accumulation of organic acids, although this was not directly confirmed in this study by organic-acid profiling (e.g. citrate, malate, oxalate). For reference, the target pH range for optimal pepper growth is approximately 5.5–6.5 (Fathidarehnejeh *et al.*, 2024). The decrease in electrical conductivity (EC) from 2.1 to 1.2 $\text{mS}\cdot\text{cm}^{-1}$ indicates nutrient depletion and disruption of the solution's ionic balance; the target EC range for optimal pepper growth is approximately 1.8–2.5 $\text{mS}\cdot\text{cm}^{-1}$, against which this decrease represents substantial nutrient depletion. Hosseinzadeh (2017) reported that such changes are typical of closed hydroponic systems with a high level of autotoxicity.

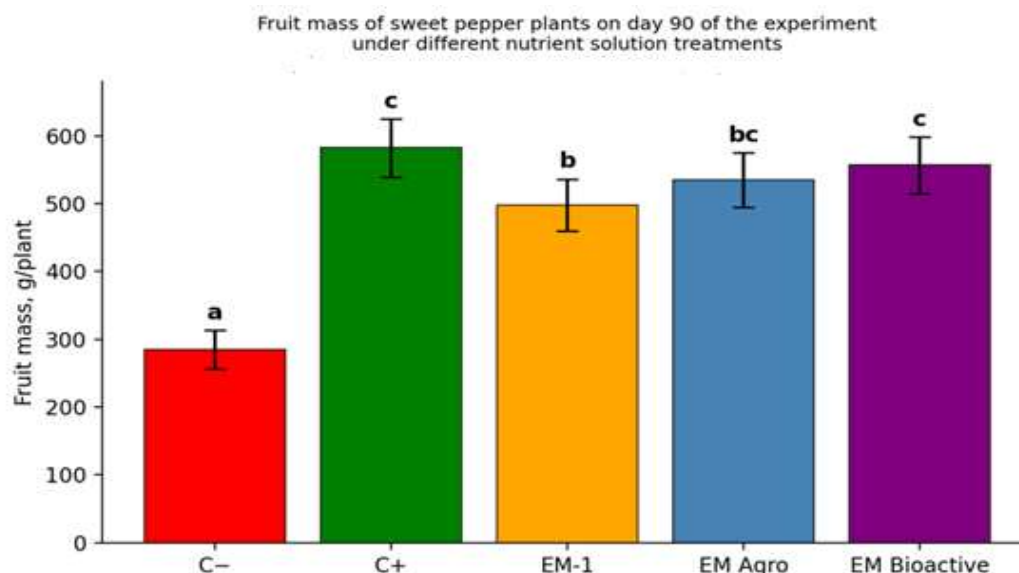


Fig. 3. Fruit mass of pepper plants on day 90 of the study under different nutrient-solution treatments. Identical letters indicate no significant difference, and different letters indicate a significant difference at $p < 0.05$

Table 4: Nutrient-solution regenerative-capacity parameters

Variant	pH (start)	pH (end)	EC, $\text{mS}\cdot\text{cm}^{-1}$ (start)	EC, $\text{mS}\cdot\text{cm}^{-1}$ (end)
C-	6.0 ± 0.1	4.8 ± 0.2	2.1 ± 0.1	1.2 ± 0.1
C+	6.0 ± 0.1	5.9 ± 0.1	2.1 ± 0.1	2.0 ± 0.1
EM-1	6.0 ± 0.1	5.6 ± 0.1	2.1 ± 0.1	1.8 ± 0.1
EM Agro	6.0 ± 0.1	5.7 ± 0.1	2.1 ± 0.1	1.9 ± 0.1
EM Bioactive	6.0 ± 0.1	5.8 ± 0.1	2.1 ± 0.1	1.9 ± 0.1

Values are in mean $\pm$ standard deviation ($n = 5$); reservoir-level data ($n = 1$ per variant), presented in descriptive form only

Application of EM preparations stabilised the physico-chemical parameters of the nutrient solution. EM Bioactive showed the best buffering capacity, maintaining pH at 5.8 (only 0.2 units below the initial value) and EC at $1.9 \text{ mS}\cdot\text{cm}^{-1}$ (90.5 % of the initial level); this moderate decrease in EC may partly reflect ion uptake by the expanded microbial biomass rather than solely improved plant nutrition. This effect can be explained by the ability of the microbial consortium not only to degrade phytotoxic compounds but also to convert organic acids into neutral products. EM Agro and EM-1 also maintained acceptable parameter stability, albeit somewhat less effectively than EM Bioactive.

Bioassays on garden cress (*Lepidium sativum* L.) confirmed the high phytotoxicity of the spent nutrient solution in the negative control (C-) (Table 5). The toxicity index was only 6.2 %, corresponding to the «highly toxic» category according to Bozym *et al.* (2020). Seed germination was suppressed to 34.2 %, and seedling length reached only 1.8 cm compared with 7.5 cm in the control (distilled water). These results are qualitatively consistent with the high phenolic-compound concentration (458.6 mg L^{-1}) found in this variant.

Table 5: Spent nutrient-solution bioassay results

Variant	Seed germination %	Seedling length (cm)	Toxicity index, %	Toxicity category
C–	34.2 ± 4.8	1.8 ± 0.3	6.2 ± 1.4	Highly toxic
C+	92.8 ± 3.2	7.6 ± 0.5	94.3 ± 2.8	Non-toxic
EM-1	84.6 ± 3.8	6.8 ± 0.4	76.8 ± 3.2	Low toxicity
EM Agro	88.2 ± 3.5	7.2 ± 0.5	84.9 ± 3.0	Low toxicity
EM Bioactive	90.4 ± 3.3	7.4 ± 0.5	89.4 ± 2.9	Low toxicity
Distilled water	94.0 ± 2.8	7.5 ± 0.4	100	Control
HSD _{0.05}	4.95	0.61	3.78	–

Values are in mean ± standard deviation (n = 5)

Application of EM preparations substantially reduced the toxicity of the spent nutrient solution. EM Bioactive showed the highest toxicity index among the biological treatments – 89.4 %, approaching the value for C+ (94.3 %) and classified as «low toxicity». Seed germination reached 90.4 %, and seedling length was 7.4 cm, corresponding to 98.7 % of the control. EM Agro showed a toxicity index of 84.9 %, while EM-1 showed 76.8 %. These results are consistent with the chemical analysis and confirm that microbial degradation of phenolic compounds not only reduces their concentration but also converts them into non-toxic or

low-toxicity metabolites, as reported by Ayilara *et al.* (2023).

Analysis of variance (ANOVA) shows the statistical significance of the effect of microbial preparations on all parameters studied (Table 6). The calculated F-values for the four parameters substantially significant. Phenol concentration had $F(4, 20) = 1459.87$, $P < 0.05$. Plant height had $F(4, 20) = 183.59$, $P < 0.05$. Fruit mass had $F(4, 20) = 172.39$, $P < 0.05$. Toxicity index had $F(4, 20) = 2537.45$, $P < 0.05$.

Table 6: Summary results of one-way analysis of variance (ANOVA) for the four parameters investigated at day 90

Parameter	F	df	P
Phenol concentration	1459.87	4, 20	< 0.05
Plant height	183.59	4, 20	< 0.05
Fruit mass	172.39	4, 20	< 0.05
Toxicity index	2537.45	4, 20	< 0.05

A strong negative relationship was found between phenolic-compound concentration on day 90 and fruit mass, calculated using Spearman's method on five pairs of variant mean values (day-90 phenol concentration and mean fruit mass for each of the five variants) as indicated in Table 7. Since correlations calculated from group means tend to systematically overestimate the magnitude of |r|

compared with correlations based on raw data (the aggregation/ecological-fallacy effect), this result should be regarded as a preliminary, illustrative estimate of the relationship, requiring confirmation at the individual-plant level (n = 25) in future studies with full system-level replication.

Table 7: Relationship between phenolic-compound concentration (day 90) and mean fruit mass by variant (n = 5 variant mean values)

Variant	Phenol concentration, mg GAE L ⁻¹	Fruit mass, g/plant
C–	458.6	284.6
EM-1	62.3	498.4
EM Agro	54.8	534.7
EM Bioactive	48.7	556.8
C+	15.1	582.3

Spearman's rank $r_s = -1.0$, $p = 0.0167$

Overall, the results obtained indicate that the use of consortium-based microbial preparations, in particular EM Bioactive, is an effective strategy for nutrient-solution regeneration in portable ebb-and-flow hydroponic systems. The EM Bioactive achieved 89–90 % degradation of phytotoxic exudates, maintaining plant productivity at 95–96 % of the control with complete nutrient-solution replacement. For most parameters (plant height, number of leaves, SPAD index), the differences between EM Bioactive and C+ were not much, which highlights the potential of biological nutrient-solution regeneration methods in closed hydroponic systems.

The results obtained confirm a fundamental problem of closed hydroponic systems – the accumulation of phytotoxic root exudates, leading to autotoxicity and reduced plant productivity. In our study, the phenolic-compound concentration in the C- variant reached a critical level of 458.6 mg GAE L⁻¹ after 90 days, causing a 51.1 % reduction in yield compared with the control with solution replacement. These data are consistent with the findings of Lee *et al.* (2006), who reported accumulation of phenolic acids of up to 500–600 mg GAE L⁻¹ in recirculating lettuce-growing systems, as well as with the study by Sharma *et al.* (2020), which documented similar rates of organic-acid accumulation in closed hydroponic units.

The mechanism of the phytotoxic action of phenolic compounds includes disruption of root cell membrane permeability, inhibition of H⁺-ATPase activity and inhibition of nutrient-uptake processes (Wang *et al.*, 2024). The strong negative correlation found in our study ($r_s = -1.00$; $p = 0.017$) between phenol concentration and fruit mass confirms the direct dependence of productivity on the level of phytotoxins in the solution. The decrease in the SPAD index from 48.6 to 31.4 in the C- variant indicates disruption of chlorophyll synthesis, consistent with the data of Camli-Saunders and Villouta (2025) on the inhibition of photosynthetic processes under the influence of allelopathic compounds in hydroponic systems.

The application of consortium-based microbial preparations in this study demonstrated high effectiveness in degrading phytotoxic exudates. EM Bioactive achieved 89.4 % degradation of phenolic compounds – only 7.3 % lower than treatment with complete solution replacement, while saving 100 % of water and nutrients. The efficiency of

microbial degradation in our study exceeds the results obtained by Hosseinzadeh *et al.* (2019a) for the physico-chemical UV/H₂O₂ method (70–85 % degradation), and is comparable with the results of Sonawane *et al.* (2022), who reported 85–92 % phenol degradation using immobilised microbial consortia. The higher efficiency of EM Bioactive compared with EM-1 and EM Agro can be linked to its enriched enzymatic profile. Based on the manufacturer's specifications regarding enhanced organic-matter decomposition and the known metabolic capabilities of the constituent microbial groups (Lactobacillus, Rhodopseudomonas, Saccharomyces, Azotobacter), it can be suggested that the consortium possesses phenol hydroxylase and catechol dioxygenase activity, which catalyse cleavage of the aromatic ring of phenolic compounds. However, confirmation of the specific degradation pathways and enzymatic activity would require metagenomic (16S rRNA, ITS) and metatranscriptomic analyses, which we identify as a priority for future molecular-metagenomic studies.

Similar functional results regarding phenol-degradation efficiency have been obtained for the specialised strain *Rhodococcus opacus* 3D, which degrades phenol via the catechol and protocatechuate branches of the β -ketoadipate pathway (Rehfuss *et al.*, 2005). Although similar enzymatic pathways may also operate in EM consortia, direct comparison is substantially limited by the fundamentally different nature of the biological systems – a pure culture versus a complex microbial consortium – and by the absence of molecular characterisation of the EM preparations in this study.

An important result of the study is confirmation of the nutrient solution's regenerative capacity under the influence of microbial preparations. Stabilisation of pH at 5.8 in the EM Bioactive variant (compared with the critical drop to 4.8 in C-) indicates that the microbial consortium is able not only to degrade phenolic compounds but also to neutralise organic acids accumulating as a result of plant activity. Hosseinzadeh (2017) emphasised that maintaining stable pH is a critically important factor for nutrient uptake in hydroponic systems. Preservation of electrical conductivity at 90.5 % of the initial value indicates minimal depletion of macro- and micronutrients, confirming the effectiveness of biological solution regeneration without the need for complete replacement.

Bioassay results on garden cress (*Lepidium sativum* L.) serve as an integral indicator of the residual toxicity of the spent solution. The toxicity index of 89.4 % for the EM Bioactive variant confirms near-complete detoxification of the solution and exceeds the results obtained by Bożym *et al.* (2020) for conventional wastewater-treatment methods (index of 75–80 %). Importantly, the bioassay cannot distinguish between complete detoxification and reduced but non-zero toxicity of intermediate degradation products; some microbial degradation pathways can form transient intermediate products that are more toxic than the parent compounds (Rogowska-van der Molen *et al.*, 2023). The high toxicity index in our EM-treated variants is consistent with predominantly non-toxic or low-toxicity degradation metabolites, although chemical analysis of the degradation products (e.g. by HPLC or GC-MS) is recommended in future work to directly confirm this.

When comparing the biological regeneration method with alternative technologies, its advantages and limitations should be noted. Activated carbon, widely used for adsorbing phytotoxins (Hosseinzadeh *et al.*, 2019a), requires regular replacement and regeneration, which increases operating costs. Electrodegradation and photocatalytic methods (Hosseinzadeh *et al.*, 2019b) are energy-intensive and require complex equipment. Microbial degradation, by contrast, is inexpensive, environmentally safe and requires no additional energy expenditure. However, this method has limitations regarding degradation rate at extremely high toxicant concentrations ($> 500 \text{ mg L}^{-1}$), where a combination of biological and physico-chemical methods may be required.

An economic analysis of the application of microbial preparations in portable hydroponic systems indicates their high cost-effectiveness. According to our calculations, the cost of EM Bioactive is approximately €0.8–1.2 per 20 L of nutrient solution per application every 14 days. Complete solution replacement every 14 days, by contrast, would cost €15–20 (depending on local fertiliser prices), additionally taking into account the cost of disposing of the spent solution; a detailed cost breakdown (preparation and fertiliser costs, together with the 2024 NBU exchange rate on which these figures are based) is provided as supplementary material. Over a 90-day growing cycle, the resulting savings amount to approximately 80–85 %, consistent with the findings of Ayilara *et al.* (2023) regarding the cost-effectiveness of biological treatment

methods. At the same time, it should be noted that this savings estimate assumes complete nutrient-solution replacement as the sole alternative and does not account for the cost of the EM preparation itself, labour costs for application and monitoring, or the somewhat lower yield of EM Bioactive compared with C+ (4.4 % lower fruit mass), which partly offsets the stated savings. The 80–85 % savings estimate is based solely on retail prices in Ukraine as of 2024 and a single UAH/EUR exchange rate, and should be regarded as illustrative and specific to this jurisdiction and period, rather than as a general economic conclusion, given the volatility of exchange rates and resource costs. An additional benefit is the reduced environmental impact, as the discharge of large volumes of nutrient-enriched effluent is avoided.

All cost calculations were based on retail market prices in Ukraine as of 2024. EM preparations were purchased directly from the manufacturer. Mineral fertiliser costs reflect average retail prices in Kirovohrad Oblast for 2024. All prices, originally given in Ukrainian hryvnia (UAH), were converted to euros at the average exchange rate of the National Bank of Ukraine for 2024 (1 EUR = 42.50 UAH).

The developed technology is particularly relevant for urban areas and small-scale operations. Portable ebb-and-flow hydroponic systems are gaining popularity in urban agriculture, where limited space and water resources make closed systems especially attractive. Yusuf *et al.* (2025) forecast an annual growth of the urban agriculture market of 8.2 % through 2030, underscoring the critical importance of effective nutrient-solution regeneration. Our results demonstrate that biological regeneration allows productivity to be maintained at 95.6 % of optimal with minimal maintenance costs, making the technology accessible to a wide range of users, including home gardeners and educational institutions.

Study Limitations

Since the results obtained relate to a single crop and a single growing cycle, this limitation directly affects how far the conclusions regarding the “environmental sustainability of urban farming” stated in the title of the article can be extended, and it is therefore emphasised here – in addition to the abstract and conclusions – rather than only below.

First, the experiment was conducted on a single crop (sweet pepper), whereas different plant species release

different spectra of root exudates. Further studies on other vegetable crops (tomato, cucumber, lettuce) are needed to confirm the generalisability of the method.

Second, the 90-day experiment covers one complete pepper growing cycle. Although regular reapplication of the microbiological preparations (every 14 days) provided stable biological control throughout this period, the long-term stability of microbial populations and their degradation capacity over several successive solution-reuse cycles remains an open question. For the practical operation of such systems, this underscores the importance of periodic reinoculation to prevent depletion of the beneficial microbiome.

Third, the study was conducted under controlled laboratory conditions; validation of the technology under real-world commercial greenhouse and vertical-farm conditions is needed, where results may be influenced by additional factors such as fluctuations in temperature, lighting and water quality.

Fourth, a single 20 L solution reservoir was used per experimental group ($n = 1$ at the system level). Although this allowed the individual plant response ($n = 5$) to microbiological treatment to be assessed, systematic evaluation of solution chemical composition requires further studies using 4–6 independent reservoir replicates per variant for full statistical generalisation.

Fifth, when assessing the phytotoxicity of the spent nutrient solution using the *Lepidium sativum* L. seed bioassay, exudate accumulation was assessed using the Folin–Ciocalteu reagent. Since this method responds to all reducing agents in the solution, the reported “total phenolic content” values may include non-phenolic metabolites, including sugars and ascorbic acid. Precise identification and mass-spectrometric profiling of the specific allelopathic phenolic acids (including benzoic and cinnamic acid derivatives) responsible for the phytotoxic effect would require chromatographic methods (HPLC or GC-MS) in future studies.

Sixth, the assessment of *Lepidium sativum* L. germination on undiluted spent solution showed a germination rate of 34.2 % in C–. Since the experiment did not include a dilution series (1:2, 1:4, 1:10) or a control with fresh nutrient solution of comparable composition, this inhibition cannot

be unambiguously attributed solely to phenolic toxicity. Part of the inhibitory effect is due to the high electrical conductivity (EC) and hypertonic osmotic potential of the solution. Serial-dilution experiments will be needed in future studies to clearly distinguish between osmotic and biochemical factors.

Seventh, the *Lepidium sativum* L. seed germination rate of 34.2 % in the C– variant requires cautious interpretation. This experiment used undiluted spent solution and did not include serial dilutions (1:2, 1:4, 1:10) or a control with fresh (unused) nutrient solution of comparable ionic composition. Since the hydroponic solution has high electrical conductivity ($EC = 1.8\text{--}2.5 \text{ dS}\cdot\text{m}^{-1}$), the observed inhibition of germination and root growth reflects the combined effect of biochemical (phenolic) toxicity and osmotic stress. To clearly distinguish between osmotic pressure and allelopathic inhibition, future studies should necessarily include both dilution gradients of the spent solution and a control with fresh nutrient solution.

Other methodological limitations of this design should also be noted for completeness. Independent verification of the microbial content, viability or strain identity of the EM preparations (e.g. by plate culturing or molecular identification) was not carried out in this study; this is acknowledged as a limitation, and the batch numbers and storage conditions of the preparations were recorded as a minimum level of documentation. The experimental design also did not include a heat-inactivated/autoclaved EM control or a control with individual strains, so the observed effects cannot be unambiguously separated from the possible contribution of organic matter, vitamins or pH-buffering compounds present in the EM preparations themselves; this is also noted as a limitation for future experimental designs. Furthermore, the positive control (C+, complete solution replacement) simultaneously removes phenolic compounds and replenishes nutrients, restoring pH and micronutrients; comparisons between EM treatments and C+ are therefore more accurately interpreted as “microbial treatment versus best-practice solution management” rather than as an isolated test of phenol removal, since a control with nutrient replenishment alone (without solution replacement) was not included. Tests of normality (e.g. the Shapiro–Wilk test) and homogeneity of variance (e.g. Levene’s test) were not carried out before ANOVA; given the small sample size ($n = 5$) and the very large F-values obtained, this is

acknowledged as a limitation, and non-parametric analysis (Kruskal–Wallis) is recommended as a robustness check in future work. Data on the number of fruits per plant, mean individual fruit mass, and marketable/non-marketable yield structure were not collected in this study; total fruit mass per plant was the only yield parameter assessed, which is also noted here as an additional limitation for future work.

Recommendations

An important direction for further research is a detailed study of nutrient-solution microbiota dynamics during the application of EM preparations. Metagenomic sequencing methods (16S rRNA and ITS) would allow the key microbial taxa responsible for degrading phenolic compounds to be identified, and changes in the microbial community to be tracked throughout the growing cycle. Mavrodi *et al.* (2011) emphasised the importance of understanding quorum-sensing mechanisms in microbial consortia for optimising their degradation activity. It would also be worthwhile to investigate the effect of different preparation concentrations and application frequencies on both degradation efficiency and microbial-community stability.

Prospects for further development of the technology include the creation of specialised microbial consortia adapted to the specific root-exudate spectra of individual crops. Liu *et al.* (2025) demonstrated the feasibility of targeted engineering of microbial communities with enhanced capacity to degrade specific phytotoxins. Integrating biological regeneration with automated nutrient-solution monitoring systems (pH, EC and ion-selective sensors) would allow the development of intelligent hydroponic systems with automatic dosing of microbial preparations depending on the level of toxicant accumulation. Combining microbial degradation with other environmentally safe technologies, such as phytoremediation and vermiculture, could enable a fully closed, zero-discharge production cycle.

CONCLUSION

This study established that in closed portable ebb-and-flow hydroponic systems, without the application of regeneration methods, critical accumulation of phytotoxic phenolic compounds occurs, reaching $458.6 \pm 31.4 \text{ mg L}^{-1}$ over a 90-day sweet pepper (*Capsicum annuum* L.) growing cycle, causing a 51.1 % reduction in plant productivity

compared with the control with systematic nutrient-solution replacement. Since this conclusion is based on a single crop and a single growing cycle, its applicability to closed hydroponic systems and urban farming in general should be confirmed on other vegetable crops and over several reuse cycles.

A strong negative correlation relationship using Spearman's rank correlation coefficient ($r_s = -1.00$; $p = 0.017$) was found between phenolic-compound concentration in the nutrient solution and plant productivity, indicating the direct phytotoxic effect of root exudates on growth processes, photosynthetic activity (the SPAD index decreased from 48.6 to 31.4) and the formation of generative organs.

High effectiveness of consortium-based microbial preparations in degrading phytotoxic exudates was demonstrated: EM Bioactive achieved 89.4 % degradation of phenolic compounds, EM Agro – 88.0 %, and EM-1 – 86.4 %. Taking into account overall performance (degradation rate, stabilisation of solution pH and electrical conductivity, toxicity index of 89.4 %), EM Bioactive was identified as the most effective preparation for nutrient-solution regeneration.

The feasibility of maintaining high pepper productivity in closed systems through microbial degradation of phytotoxins was confirmed: EM Bioactive treatment preserved 95.6 % of the productivity of the control with complete solution replacement (556.8 versus 582.3 g per plant) while saving 100 % of water and nutrients, confirming both the economic feasibility and the environmental safety of the technology.

The results of bioassays on garden cress (*Lepidium sativum* L.) showed that microbial degradation of phenolic compounds leads to the formation of non-toxic or low-toxicity metabolites: the toxicity index of the spent solution in the EM Bioactive variant was 89.4 %, corresponding to the "low toxicity" category and approaching the value for fresh solution (94.3 %).

AUTHOR'S CONTRIBUTION

KOVALOV, M., conceived and designed the study, conducted the experiments, collected and analyzed the data, and drafted the manuscript.

SOKOLOVSKA, I., contributed to the study design, interpreted the results, revised the manuscript critically for important intellectual content, and approved the final version.

MASHCHENKO, Y., contributed to the interpretation of the results, statistical evaluation, manuscript editing, and approved the final version.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to the staff of the Research Laboratory «Hydroponic Vegetable Production in a Dome Greenhouse» of the Department of General Agriculture, Central Ukrainian National Technical University, for providing the facilities and technical support necessary for conducting this research. The authors also appreciate the valuable comments and suggestions of the anonymous reviewers, which helped improve the quality of the manuscript.

FUNDING: This research received no external funding.

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

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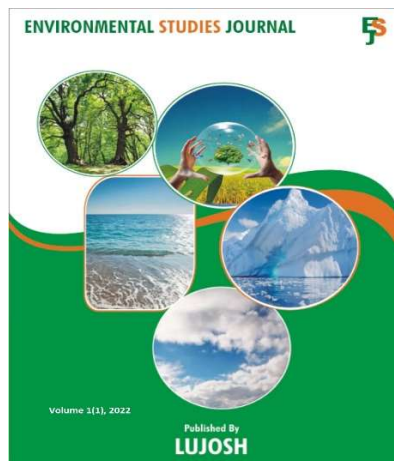
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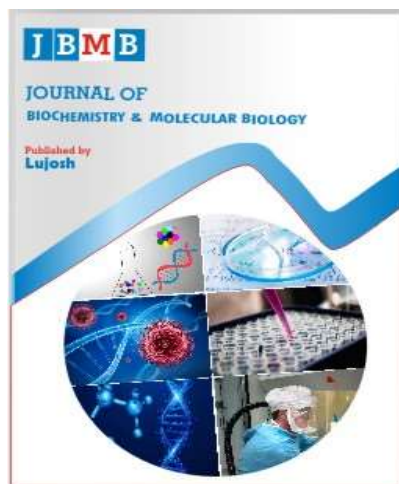
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