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Recycled water use with added microbial consortia in the cultivation of aromatic plants

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Abstract

Research objective: This research aims to evaluate the potential of biologically purified water with added beneficial microorganisms for soil and plants for possible use in agriculture. In such a way that this type of water, which is not always accepted by growers, can instead be positively perceived by technicians in the sector and by those who often have no other water resources to irrigate their plants.

Materials and Methods: The experiments, which began in April 2023, were conducted to the Abbey of S. Antimo in Piombino (LI) on different types of aromatic plants (rosemary, sage, mint, thyme). The experimental groups were: control; aqueduct water; aqueduct water with added microorganisms; recycled water; recycled water with added microorganisms. On 17 May 2023, plant height, number of leaves, vegetative weight, volume and length of roots, number of microorganisms in the substrate, number of dead plants and pH of the substrate were determined.

Results and Discussion: The experiment showed that it is possible to use recycled water supplemented with microorganisms to significantly improve plant height, leaf number, and vegetative and root biomass, reducing plant mortality on aromatic species such as mint, rosemary, thyme and sage. In general, a significant increase in plant height and number of leaves as well as vegetative and root biomass was observed in plants treated with microorganisms

The trial significantly showed how water inoculated with plant-stimulating microorganisms can significantly increase the vegetative and root biomass of various aromatic plant species. This aspect was also found in other horticultural species.

Conclusions: The possible use of these waters in agriculture requires a careful study of the microbial communities present in them, which are often not suitable for plant growth. This experiment aimed to assess whether the use of plant-specific micro-organisms added to previously biologically treated water could improve the adaptation and growth performance of certain aromatic species. The results were significant and certainly deserve further studies on other horticultural species.

Keywords: Microorganisms; Recycled water; Aromatic plants; Biological purification; Rhizosphere

1. Introduction

Most organisms involved in biological purification systems are too small to be seen with the naked eye. In the field of biological purification, bacteria are undoubtedly the most important micro-organisms; they are followed by algae,

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viruses, protozoa, rotifers, fungi and moulds [1,2]. The qualitative and quantitative presence of living species in biological purification systems is, however, intimately linked to two factors; firstly, the food relationships existing in the particular microbial system, defined by physical, chemical and biological characteristics, and secondly, the inter- and intra-specific interactions due to the biological phenomena shown in Table 1. In biological purification plants, it is evident that every factor that can influence microbial growth has an effect on the effects of this growth, namely purification. It is therefore important to know what environmental conditions are suitable for micro-organisms and also what factors can influence them negatively [3,4]. These factors can be summarised as: temperature, pH, light, dissolved oxygen, organic load, micronutrients, toxic substances. The concomitant variability of these factors constitutes the physical-chemical environment where the biological phenomenon of microbial growth must take place [5]. This is influenced both in its physical-biological characteristics, especially its good sedimentability and bioflocculatory capacity, and in the qualitative composition of the bacterial population: it is therefore evident that it is the environment itself that will select the bacterial species [6,7]. In biological purification systems, where the substrates are generally heterogeneous and complex, it can be said that a particular biological population situation is established for each plant, due to the fact that the set of factors that determined it are never exactly repeatable in another system [8]. Very often, the effects of environmental phenomena that have an unfavourable influence on bacterial growth, if they remain constant and within a certain range, diminish over time, due to the adaptation and predominance of new bacterial species increasingly selected by the environment. This fact is of particular importance when starting up a new plant, especially one operating on industrial effluents [9].

Table 1 Microorganism interaction phenomena

Competition	similar organisms compete for space and food
Predation	one species feeds on another
Parasitism	one organism lives behind another's back
Commensalism	two organisms live together while being independent
Synergism	two organisms possessing a cooperative metabolism
Mutualism	each organism benefiting from the growth of the other
Antibiosis	one organism produces excretions harmful to another

1.1. Use of selected bacterial cultures

The purification efficiency of a system depends on the sufficient number of specialised bacteria to metabolise the pollutant substrates present [10]. The bacterial species that grow in systems are not single or a few selected species, but a heterogeneous mixture of dozens and perhaps hundreds of species. It is possible to isolate and cultivate in the laboratory bacterial strains that are particularly active in the degradation of molecules considered 'difficult', such as celluloses, phenols, animal fats, and certain solvents [11]. However, not all of these strains are usable in practice as they require very particular and constant chemical-physical habitats and co-substrates, which are not guaranteed in purification system. By emphasising the objective of water quality and all the factors that can affect it positively or negatively, it is possible to define an ecosystem whose parameters gravitate around the fundamental objective under consideration [12]. These parameters do not have unidirectional influences but, depending on environmental conditions, may evolve mainly in one direction or the other, increasing or decreasing water purity [13]. The main factor that can intervene in favour of quality is saprophytic, i.e. destructive, bacterial activity, which through fermentation not only removes but also recirculates materials through their partial gasification and mineralisation [14]. In addition to the primary and immediate objective of restoring water, there is thus the more important ecological objective of redistributing the removed materials in the same way as in nature. This last objective is secondary in terms of the immediate cost-benefit ratio and is hardly ever taken into serious consideration when it comes to solving environmental problems, which are thus dealt with half-heartedly [15,16].

1.2. Applicability and limitations of the use of purified/recycled water in agriculture

In accordance with the ecological significance of biological water purification plants, the ultimate use can only be agricultural use, the only form of recycling and reuse of the substances contained therein [17]. The practice of agricultural use of recycled water has been widely applied in the USA and in many European countries for a long time, but since the 1960s it has been seriously questioned, especially because of aspects related to the dangers of secondary pollution [18]. The problem is the subject of studies and research in many countries around the world, but the conclusions are not always unanimous. On the other hand, there is unanimous agreement that the practice of disposing of purified water in agriculture cannot be liberalised, but requires careful controls, if not legal regulations [19].

1.3. Research Objectives

This research aims to evaluate the potential of biologically purified water with added beneficial microorganisms for soil and plants for possible use in agriculture. In such a way that this type of water, which is not always accepted by growers, can instead be positively perceived by technicians in the sector and by those who often have no other water resources to irrigate their plants.

Figure 1 Phases of the experimental test at the Abbey of S. Antimo, Piombino (LI)

2. Material and methods

The experiments, which began in April 2023, were conducted to the Abbey of S. Antimo in Piombino (LI) on different types of aromatic plants (rosemary, sage, mint, thyme).

The plants were placed in ø 12 cm pots, 10 plants per experiment for each aromatic species. All plants were fertilised with a controlled-release fertiliser (1 kg m-3 Osmocote Pro®, 9-12 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before sowing. The experimental groups were:

- Control group (POT) (peat 80%+ pumice 20%), irrigated with aqueduct water (50 ml per plant, three times a week) and previously fertilised substrate;
- Group with water from the aqueduct with added microorganisms (50 ml per plant, three times a week) (POTMICR) (peat 80% + pumice 20%) and fertilised substrate;
- Group with recycled water (50 ml per plant, three times a week) (ASA) (peat 80% + pumice 20%) and fertilised substrate
- Group with recycled water with added microorganisms (50 ml per plant, three times a week) (ASAMICR) (peat 80% + pumice 20%) and fertilised substrate

The micro-organisms used are species selected by the CREA in Pescia (PT), which have proved useful in the biostimulation of various plant species. They were added to 50-litre tanks, containing different types of water, ten days before the start of the experiment.

On 17 May 2023, plant height, number of leaves, vegetative weight, volume and length of roots, number of microorganisms in the substrate, number of dead plants and pH of the substrate were determined.

2.1. Analysis methods

- pH: For pH measurement, 1 kg of the substrate was taken from each plant, and 50 g of the mixture was placed in a beaker containing 100 ml of distilled water. After 2 hours, the water was filtered and analyzed [20];
- Microbial count: directly determining total microbial count by microscopy cells contained in a known sample volume using counting chambers (Thoma chamber). The surface of the slide is etched with a grid of squares, with the area of each square known. Determination of viable microbial load after serial decimal dilutions, spatula seeding (1 ml) and plate counting after incubation [21];

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analyzed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by the LSD multiple-range tests ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results

The experiment showed that it is possible to use recycled water supplemented with microorganisms to significantly improve plant height, leaf number, and vegetative and root biomass, reducing plant mortality on aromatic species such as mint, rosemary, thyme and sage (Table 2-3-4-5).

In general, a significant increase in plant height and number of leaves as well as vegetative and root biomass was observed in plants treated with microorganisms (Figure 2-3-4-5); in particular, the POTMICR and ASAMICR theses were consistently better than their respective control in all plant species tested. The POTMICR treatment was by far the best, also showing an improvement in root elongation and root hairs. The ASAMICR thesis in mint, rosemary and thyme showed a higher microbial content of the substrate than the other theses, an aspect also highlighted in POTMICR. This was probably the aspect that most influenced the vegetative and root growth of the plants; the microorganisms probably utilise the metabolites contained in these plants as a nutrient substrate for their multiplication. Growing plants consequently benefit from an increase in microbial biomass in the rhizosphere, because plant-soil interactions are significantly increased, with an increased supply of nutrients. In general, there were no significant differences in substrate pH and plant mortality.

Table 2 Evaluation of the use of recycled water on vegetative and root biomass of mint

Groups	Plant height (cm)	Leaves number (n°)	Substrate total bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm³)	Roots length (cm)	Plants dead number (n°)	pH substrate
POT	12.42 b	23.65 c	2.23 d	36.45 c	21.03 c	5.07 c	0.20 a	6.72 a
POTMICR	14.80 a	31.21 a	4.33 b	39.69 a	24.56 a	6.32 a	0.20 a	6.74 a
ASA	10.61 c	18.21 d	2.60 c	33.10 d	19.50 d	4.55 d	0.20 a	6.82 a
ASAMICR	14.75 a	27.24 b	4.65 a	38.99 b	23.83 b	6.02 b	0.00 a	6.74 a
ANOVA	***	***	***	***	***	***	ns	ns

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05). Legend: POT: aqueduct water; POTMICR: aqueduct water + microorganisms; ASA: recycled water; ASAMICR: recycled water + microorganisms

Table 3 Evaluation of the use of recycled water on vegetative and root biomass of rosemary

Groups	Plant height (cm)	Leaves number (n°)	Substrate total bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm³)	Roots length (cm)	Plants dead number (n°)	pH substrate
POT	14.39 c	8.00 c	2.24 c	31.62 b	20.68 c	5.41 c	0.20 a	6.72 a
POTMICR	19.09 a	20.41 a	4.54 b	34.84 a	24.78 a	7.94 a	0.20 a	6.74 a
ASA	13.27 d	8.24 c	2.42 c	28.18 c	19.77 d	4.38 d	0.00 a	6.82 a
ASAMICR	18.64 b	15.23 b	4.81 a	34.57 a	23.62 b	6.94 b	0.00 a	6.74 a
ANOVA	***	***	***	***	***	***	ns	ns

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05). Legend: POT: aqueduct water; POTMICR: aqueduct water + microorganisms; ASA: recycled water; ASAMICR: recycled water + microorganisms

Table 4 Evaluation of the use of recycled water on vegetative and root biomass of thyme

Groups	Plant height (cm)	Leaves number (n°)	Substrate total bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm³)	Roots length (cm)	Plants dead number (n°)	pH substrate
POT	11.31 b	21.00 c	2.21 c	33.85 b	19.77 b	4.94 b	0.00 a	6.72 a
POTMICR	13.55 a	28.21 a	4.81 a	35.51 a	21.71 a	5.78 a	0.00 a	6.74 a
ASA	9.98 c	16.44 d	3.44 b	30.58 c	18.04 c	3.95 c	0.00 a	6.82 a
ASAMICR	13.53 a	23.84 b	4.92 a	34.04 ab	21.57 a	4.94 b	0.00 a	6.74 a
ANOVA	***	***	***	***	***	***	ns	ns

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05). Legend: POT: aqueduct water; POTMICR: aqueduct water + microorganisms; ASA: recycled water; ASAMICR: recycled water + microorganisms

Table 5 Evaluation of the use of recycled water on vegetative and root biomass of sage

Groups	Plant height (cm)	Leaves number (n°)	Substrate total bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm³)	Roots length (cm)	Plants dead number (n°)	pH substrate
POT	20.51 c	23.21 b	3.32 d	34.70 c	13.32 d	5.07 c	0.00 a	6.72 a
POTMICR	24.23 a	27.61 a	4.76 a	39.40 a	16.94 a	7.58 a	0.00 a	6.74 a
ASA	19.65 d	23.63 b	3.59 c	31.27 d	14.62 c	4.81 c	0.00 a	6.82 a
ASAMICR	22.07 b	24.65 ab	4.18 b	39.61 b	15.00 b	6.13 b	0.00 a	6.74 a
ANOVA	***	ns	***	***	***	***	ns	ns

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05). Legend: POT: aqueduct water; POTMICR: aqueduct water + microorganisms; ASA: recycled water; ASAMICR: recycled water + microorganisms

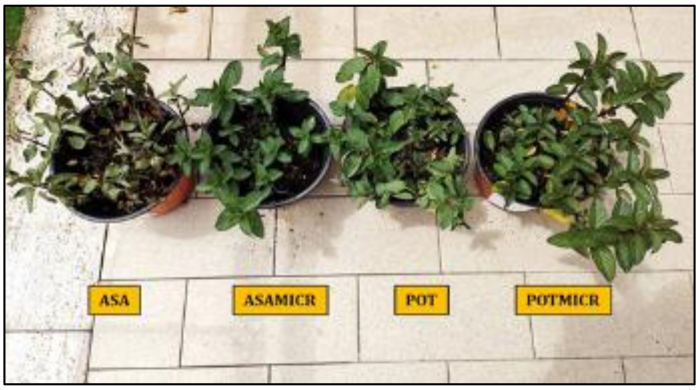

Figure 2 Comparison of different experimental theses (plant development and diameter) in the plant species mint**.** Legend: POT: aqueduct water; POTMICR: aqueduct water + microorganisms; ASA: recycled water; ASAMICR: recycled water + microorganisms

Figure 3 Comparison of different experimental theses (plant development and diameter) in the plant species rosemary. Legend: POT: aqueduct water; POTMICR: aqueduct water + microorganisms; ASA: recycled water; ASAMICR: recycled water + microorganisms

Figure 4 Comparison of different experimental theses (plant development and diameter) in the plant species thyme. Legend: POT: aqueduct water; POTMICR: aqueduct water + microorganisms; ASA: recycled water; ASAMICR: recycled water + microorganisms

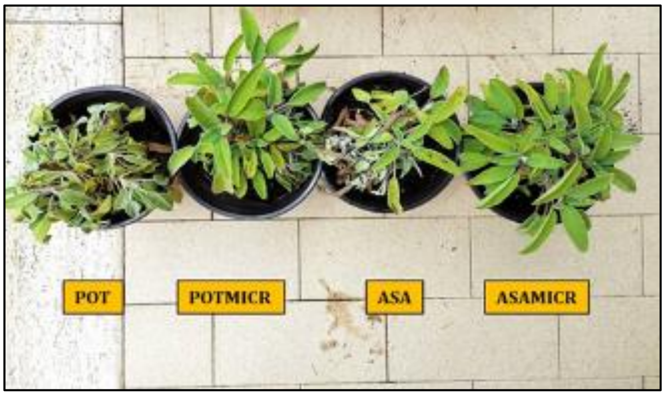

Figure 5 Comparison of different experimental theses (plant development and diameter) in the plant species sage. Legend: POT: aqueduct water; POTMICR: aqueduct water + microorganisms; ASA: recycled water; ASAMICR: recycled water + microorganisms

4. Discussion

One of the main characteristics of wastewater is its biodegradability, which makes it possible to purify it through biological treatment [22,23]. Phenomena biological phenomena are therefore at the basis of the purification processes used for water of waste water of organic origin, always for domestic ones, whenever possible for industrial or mixed ones [24]. Water purification realised with biological processes artificially reproduces the natural process already present in watercourses [25]. Purification plants, in fact, can be considered as artificial ecosystems under extreme conditions. The phenomenon natural phenomenon exploited is a mixed microbial fermentation, both in terms of the substrate to be removed, which often consists of a very heterogeneous mixture very heterogeneous mixture of compounds, and for the microorganisms responsible for the process, which are mainly mixed colonies of saprophytic bacteria, i.e. breakers of dead organic matter of prevalent faecal origin. In general, the biomass that develops, in the basin, is made up of 95% bacteria and for the remaining part by more complex organisms (Protozoa and Metazoa) [26,27,28,29]. The role of this biomass in the purification process is twofold: metabolising the organic substance and organic matter and to form sludge flocs [30]. In the metabolisation of organic matter, a fundamental role is played by bacteria, the other species present, while having a secondary role, are also also necessary because they help to contain the growth of bacteria by by limiting their overgrowth and thus determining the maintenance the balance of the ecosystem [31]. At the level of biochemical reactions, there is aerobic degradation, based on bacterial development that uses organic pollution as a food substrate [32,33,34]. These bacteria grow aggregated with the organic particles on which they feed, forming sludge flakes, which can be separated, by gravity, from the purified water in the final settler [35]. Thus, while a part of the removed substrate is mineralised with the formation of gaseous products of bacterial catabolism that are released into the atmosphere, a second part goes to form a solid-liquid, strongly putrescible residue that must still be treated and disposed of in a hygienically and ecologically correct manner [36]. Even in this case, biological treatments can be used, which have the function of reducing the volume, the putrescibility, odour and enterobacterial load of the water and prepare it for their final use on agricultural land, which constitutes their destiny ecological vocation. Generally, in the natural environment, microorganisms tend not to remain single isolated cells, but to organise themselves into true communities (films, flocculates and biological sludge) ubiquitously distributed in both terrestrial and aquatic environments. These aggregates grow and develop by colonising any available surface, both natural and artificial, adapting to even extreme environmental conditions [37]. The term biofilm is therefore commonly used to refer to all these types of microbial association regardless biological structure and the environment in which they form [38]. The aim of this experiment was to evaluate the addition of micro-organisms useful in agriculture to water previously treated with biological techniques, in order to make it more suitable for use in plant cultivation [39]. The trial significantly showed how water inoculated with plant-stimulating microorganisms can significantly increase the vegetative and root biomass of various aromatic plant species. This aspect was also found in other horticultural species [40,41].

5. Conclusion

The biological water treatment process consists of the combined action of microbial communities that utilise the substances and certain components of the discharge for their own metabolic needs, giving rise to new microorganisms and catabolism products. The main types of processes used in the field of biological water treatment take place in the presence of oxygen and in its absence, which has considerable application implications. The possible use of these waters in agriculture requires a careful study of the microbial communities present in them, which are often not suitable for plant growth. This experiment aimed to assess whether the use of plant-specific micro-organisms added to previously biologically treated water could improve the adaptation and growth performance of certain aromatic species. The results were significant and certainly deserve further studies on other horticultural species.

Compliance with ethical standards

Acknowledgments

The research is part of the project **AQUAFLOR:** Use of recycled water treated with microorganisms for irrigating ornamental plants

Disclosure of conflict of interest

The author declares no conflict of interest.

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