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Microbial processing of tannery waste for compost production in the growth and quality improvement of ornamental plants

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Abstract

Research goal: The article presents research results that aim to highlight microbial activity in the composting process of waste from tanning industries. The various steps of the compost production process with microbial inocula and the resulting stable material for the production of growing substrates for ornamental plants are emphasised.

Materials and Methods: The experimentation was divided into two phases, a first part of the transformation of tannery waste by microbial activity took place at the company Sirp spa (VR), and the second part of the evaluation of the material for plant cultivation was carried out at the CREA-OF greenhouses in Pescia (PT). The experimental theses involved using peat and biological sludge, respectively, treated with microorganisms in liquid form, supplemented with a solid matrix colonised by microorganisms, and added with sugar cane molasses.

Results and Discussion: The test showed that it was possible to cultivate *Delosperma cooperi* plants on biological sludge treated with microorganisms, showing an improvement in the agronomic characteristics of the plants and substrates in which the microorganisms were inoculated during the composting phase. In general, a significantly more significant improvement in the theses treated with liquid microorganism solution than when a solid organic matrix was used as a substrate for inoculating the microorganisms. Furthermore, the biological sludge treated with microorganisms was better than the peat controls and those treated with sugar cane molasses alone. Furthermore, the untreated biological sludge thesis was the worst of all the theses tested. Finally, the trial also showed how the use of microorganisms could positively and significantly influence the reduction of some essential industrial contaminants in biological sludge after only six months of treatment.

Conclusions: This research may interest growers who want to reduce synthetic chemicals in agriculture and are concerned about sustainability, but also those companies whose primary goal is to produce new substrates as an alternative to peat and who are looking for new matrices. Also, for those industries that are environmentally friendly and want to transform the waste produced by their industrial processes and reuse it, perhaps to grow ornamental plants.

Keywords: Composting; Microorganisms; Sustainable agriculture; Tannery waste; Rhizosphere

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1. Introduction

Composting is an exergonic process (a process that releases thermal energy and consequently induces a temperature rise) of partial decomposition of fresh organic material in the presence of oxygen, one of the objectives of which is to obtain stabilised organic products with an acceptable macronutrient content (nitrogen, phosphorus, potassium) and good microbial biodiversity (bacteria, fungi, algae) [1]. The term composting defines the process of controlled biological maturation, in an aerobic environment, of the organic substance of animal and plant residues, through which there is the production of more complex molecular chain materials, more stable, sanitised, rich in humic compounds, helpful in fertilising crops and restoring the organic substance of soils [2,3]. The process is carried out by various microorganisms operating in an aerobic environment: bacteria, fungi, actinomycetes, algae and protozoa, naturally present in organic biomass or possibly inoculated. Compost is the material resulting from the composting process of organic waste: it is an odourless and stable humus-like material rich in carbohydrates, proteins and other organic molecules. Amendants are materials added to the soil to preserve or improve physical or chemical characteristics or biological activity, either separately or solely [4]. Composted soil improvers are of three types, differentiated according to the method of preparation and the essential components: green composted, mixed and peat composted soil improvers. There are several functions that the organic matter supply of compost can perform for agro-systems to contribute to the increase and stability of the soil in the medium and long term; to guarantee a supply of slow-release macro- and micronutrients, which thus remain available to the crops and do not undergo the processes of removal by leaching or insolubilisation; to exert an activity of control of pathogenic microbial forms for the crops; to supply substances with pseudo-hormonal physiological activities for the crops, capable of performing a growth stimulating action both towards the root systems and towards the epigeal organs [5,6]. In the composting plant, the treatment cycle is divided into a series of operations, all of which can be traced back to four phases: i) pre-treatment for the preparation of the mixture; ii) biological degradation phase; iii) maturation phase; iv) refining and ennobling of the product. The choice of materials for the preparation of the mixture and how it is mixed are fundamental to obtaining a homogenous mass that allows air to penetrate so as not to promote anaerobic degradative reactions, resulting in the production of bad smells and leachate [7,8].

When preparing the substrate mixture for composting, the materials used are classified into nutrients, structuring agents and correctives or additives. Among the most important criteria for good composting is achieving a carbon/nitrogen ratio of between 20 and 40 units in the composted mass. The structuring agent often performs an essential function in correcting excess water by having a high dehydrating capacity. Correctives or additives are constituents that intervene in minute quantities by correcting pH, balancing nutritional deficiencies, or biodynamic composting techniques, stimulating microbial activity or attracting earthworms [9,10,11].

1.1. Parameters and indices of the composting process

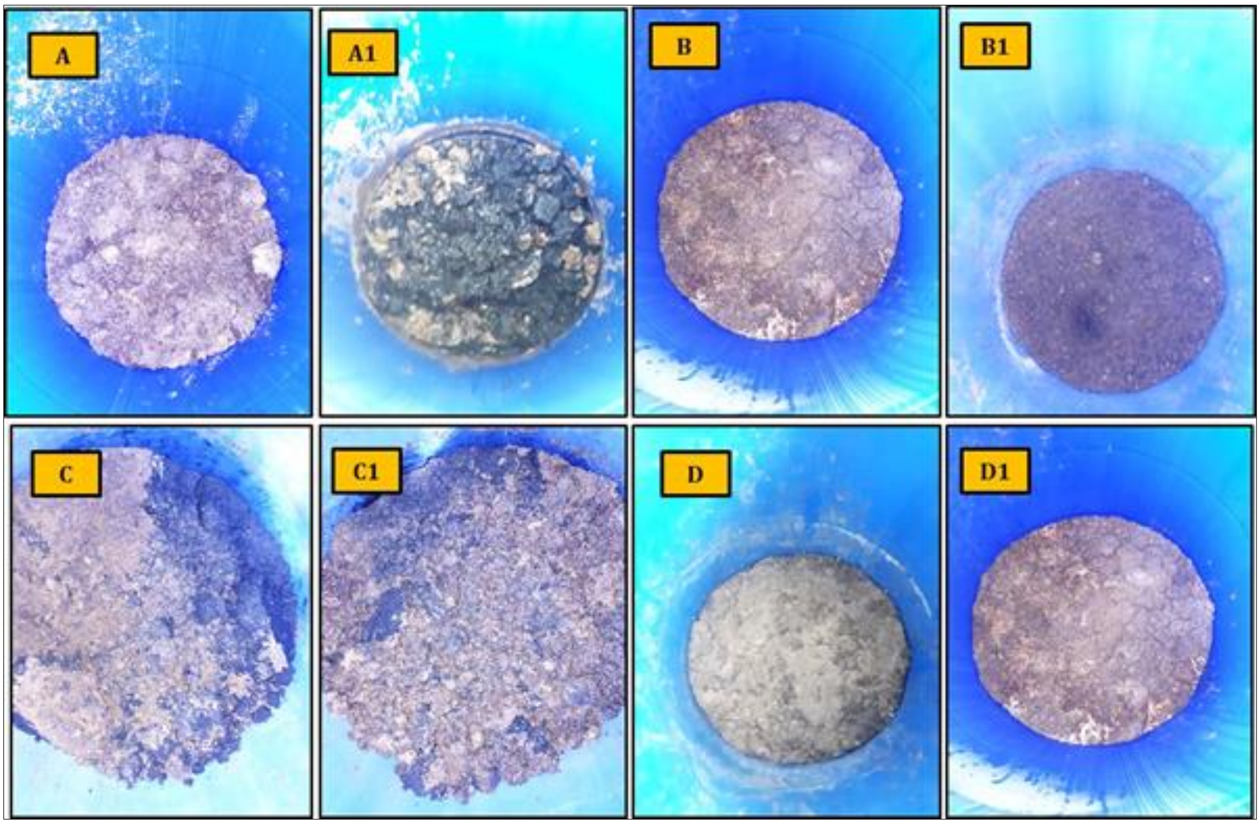
The composting process is mainly conducted by different populations of aerobic microorganisms that decompose organic material to grow and reproduce. Different microbial populations establish themselves and predominate at the various composting stages. *Composting* is a dynamic process carried out by microbial populations in rapid succession. The main groups involved are bacteria, including actinomycetes and fungi [12,13]. Although the total number of microorganisms does not change significantly during composting, the microbial diversity can vary during the different phases. Microorganisms play a crucial role in the decomposition of organic matter, and there is a clear relationship between their activity and the evolution of the composting process. In other words, the progress and speed of the process are closely dependent on the factors that influence the optimal conditions for the life of the microorganisms operating in the different process steps [14]. The different microorganisms operate at defined temperature regimes, and their activity is influenced by the process temperatures so that they can be divided into three classes: psychrophilic, mesophilic and thermophilic. As a result of the variation in temperature of the biomass during the composting process, the active microbial populations vary. Subsequently, there is a substantial selection among the microbial populations to the advantage of thermophilic species [15]. Microorganisms are distinguished into aerobic and anaerobic microorganisms. Composting is, by definition, an aerobic process characterised by the presence of only aerobic microorganisms. Sometimes, however, even when process operations appear to be optimal, anaerobic fermentations are formed in the biomass in areas where oxygen deficiency leads to the initiation of anaerobic fermentations [16]. The gaseous products of these fermentations, including short-chain organic acids such as acetic, butyric and propionic acids, form mixtures with a characteristic unpleasant odour. During the entire composting process, the evolution of the heap temperature is an essential indicator of microbial activity. Its daily measurement, which can be carried out with various devices, makes it possible to assess any deviations in the process from the ordinary course [17].

1.2. Tannery waste management using microbes

Currently, research has focused on biological methods for removing contamination from materials that could be used in composting. Principles for removing pollutants with the help of biological technologies that are generally cost-effective and environmentally harmless can be used at the contaminated site. Bioremediation is an excellent strategy for detoxifying water, soil and materials contaminated with heavy metals with the metabolic aid of microorganisms, which can clean up xenobiotic pollutants [18]. The significant aspect of bioremediation technology is the use of a microbiological approach, which involves various techniques such as biostimulation (stimulation of nutrient supplements), bioaugmentation (introduction of a viable indigenous microbial population), bioaccumulation (accumulation of tanning pollutants such as chromium by microbes) and bioabsorption (adsorption of chromium using live or dead microbial biomass). Compared to other remediation techniques, green technologies, such as those using microbes for detoxification, have many advantages. There are several categories of green technologies [19,20].

1.3. Research Objective

The article presents research results that aim to highlight microbial activity in the composting process of waste from tanning industries. The various steps of the compost production process with microbial inocula and the resulting stable material for the production of growing substrates for ornamental plants are emphasised.



Legend: (A) organic sludge 96% + bokashi 4%; (A1) organic sludge 96% + bokashi 4% + Ema 20%; (B) organic sludge 100% + Ema 20%; (B1) organic sludge 100% + Ema 30%; (C) organic sludge 100% + molasses 10%; (C1) organic sludge 100% + molasses 50%; (D) chemical-physical sludge 100% + Ema 50% (2 litres); (D1) chemical-physical sludge 100% + Ema 50% (4 litres)

Figure 1 Detail of biological sludge during the transformation process with inoculated microorganisms

2. Material and methods

The experimentation was divided into two phases. The first part of the transformation of tannery waste by microbial activity occurred at the company Sirp spa (Verona) (from December to April 2022). 90 kg of material per thesis (8 theses) were placed inside bins that were subsequently closed. The outside temperature during the transformation process was kept at 20°C in a particular boiler room of the company. The internal temperatures of the material inside the bins fluctuated between 16°C and 22°C (Figure 1).

The second part of the material evaluation for plant cultivation took place at the CREA-OF greenhouses in Pescia (PT) on *Delosperma cooperi* plants starting in May and ending in July 2022. The plants were placed in plateaus (12 plateaus per experimental thesis) and fertilized with a slow-release fertilizer (2 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 transplanting (Figure 2).

The experimental groups were:

- Group control (CTRL) (peat 100%), irrigated with water and substrate previously fertilized.
- Group control1 (CTRL1) (peat 96%) + (Bokashi 4%), irrigated with water and substrate previously fertilized.
- Group control2 (CTRL2) (peat 100%) + (Ema 20%), irrigated with water and substrate previously fertilized.
- Group organic sludge (CM) (organic sludge 100%), irrigated with water and substrate previously fertilized.
- Group organic sludge (A) (organic sludge 96%) + (Bokashi 4%), irrigated with water and substrate previously fertilized.
- Group organic sludge (A1) (organic sludge 96%) + (Bokashi 4%) + (Ema 20%), irrigated with water and substrate previously fertilized.
- Group organic sludge (B) (organic sludge 100%) + (Ema 20% in ceramic water), irrigated with water and substrate previously fertilized.
- Group organic sludge (B1) (organic sludge 100%) + (Ema 30% in ceramic water), irrigated with water and substrate previously fertilized.
- Group organic sludge (C) (organic sludge 100%) + (molasses 10%), irrigated with water and substrate previously fertilized.
- Group organic sludge (C1) (organic sludge 100%) + (molasses 50%), irrigated with water and substrate previously fertilized.
- Group chemical-physical sludge (D) (100%) + (Ema 50%, 2 litres), irrigated with water and substrate previously fertilized.
- Group chemical-physical sludge (D1) (100%) + (Ema 50%, 4 litres), irrigated with water and substrate previously fertilized.

The plants were watered two times a week and grown for three months. The plants were irrigated with drip irrigation. The irrigation was activated by a timer whose program was adjusted weekly according to climatic conditions and the leaching fraction. On July 14, 2022, plant height, vegetative weight, roots weight, flower number, flower time, substrate microbial count, and substrate pH were analyzed.

Figure 2 Preparation stage of the plateau with biological mud inoculated with microorganisms where the *Delosperma cooperi* seedlings were placed

2.1. Analysis methods

2.1.1. PH

For the ph measurement, 1 kg of the substrate was taken from each thesis, and 50 g of the mixture was placed inside a beaker with 100 ml of distilled water. After 2 hours, the water was filtered and analysed [21].

2.1.2. Microbial count

Direct determination of total microbic charge by microscopy of cells contained in a known sample volume through counting chambers (Thoma chamber). The surface of the slide is etched with a grid of squares of which the area of each square is known. Determination of viable microbial load following serial decimal dilutions, spatula seeding (1 ml) and plate counts after incubation [21].

2.1.3. Analysis equipment

IP67 PH meter HI99 series – Hanna instruments; combined test kit for soil analysis - HI3896 - Hanna instruments; microbial diversity of culturable cells [21].

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analysed 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 LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results

The test showed that it was possible to cultivate *Delosperma cooperi* plants on biological sludge treated with microorganisms, showing an improvement in the agronomic characteristics of the plants and substrates in which the microorganisms were inoculated during the composting phase. In general, a significantly more significant improvement in the theses treated with liquid microorganism solution than when a solid organic matrix was used as a substrate for inoculating the microorganisms. Furthermore, the biological sludge treated with microorganisms was better than the peat controls and those treated with sugar cane molasses alone. Furthermore, the untreated biological sludge thesis was the worst of all the theses tested.

Table 1 Evaluation of biological sludge treated with micro-organisms on the agronomic characters of *Delosperma cooperi*

Groups	Plant height (cm)	Leaves number(n°)	Vegetative weight (g)	Flowers Number (n°)	Flowers Time (days)
CTRL	5.61 ^{e}	4.2 ^{g}	8.28 ^{f}	4.8 ^{de}	3.2 ^{d}
CTRL1	5.91 ^{d}	5.4 ^{e}	8.48 ^{d}	5.4 ^{cd}	4.4 ^{c}
CTRL2	5.97 ^{cd}	6.2 ^{d}	8.71 ^{c}	5.8 ^{bc}	4.6 ^{bc}
CM	5.04 ^{h}	3.4 ^{h}	7.79 ^{g}	3.6 ^{f}	2.0 ^{e}
A	5.92 ^{d}	6.2 ^{d}	8.49 ^{d}	5.8 ^{bc}	4.8 ^{abc}
A1	6.09 ^{c}	7.0 ^{c}	8.74 c	6.4 ^{b}	4.6 ^{bc}
B	6.29 ^{b}	9.2 ^{b}	10.24 ^{b}	8.2 ^{a}	5.2 ^{ab}
B1	6.50 ^{a}	11.2 ^{a}	10.49 ^{a}	8.0 ^{a}	5.4 ^{a}
C	5.24 ^{g}	8.6 ^{b}	8.36 ^{e}	6.4 ^{b}	4.4 ^{c}
C1	5.35 ^{fg}	9.0 ^{b}	8.50 ^{d}	6.4 ^{b}	4.8 ^{abc}
D	5.39 ^{f}	4.6 ^{fg}	8.37 ^{e}	4.6 ^{e}	4.4 ^{c}
D1	5.48 ^{ef}	5.2 ^{ef}	8.45 ^{d}	5.4 ^{cd}	4.2 ^{c}
ANOVA	***	***	***	***	***

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: (CTRL) peat 100%; (CTRL1) peat 96% + bokashi 4% ; (CTRL2) peat 100% + Ema 20%; (CM) organic sludge 100%; (A) organic sludge 96% + bokashi 4%; (A1) organic sludge 96% + bokashi 4% + Ema 20%; (B) organic sludge 100% + Ema 20%; (B1) organic sludge 100% + Ema 30%; (C) organic sludge 100% + molasses 10%; (C1) organic sludge 100% + molasses 50%; (D) chemical-physical sludge 100% + Ema 50% (2 litres); (D1) chemical-physical sludge 100% + Ema 50% (4 litres)

In the microbiological analyses of the substrates, it can be seen that the level of microorganisms was higher in the organic sludge than in the peat controls. Furthermore, in the theses in which the pre-composting phase with inoculated microorganisms was carried out, the microorganism count was higher than in those in which no bacterial strains were inoculated. The B and B1 theses (Table 1) treated with microorganisms in liquid form were the significantly better theses for most of the agronomic parameters analysed (plant height, number of leaves, vegetative and root weight, number of flowers and flower duration, root length). In addition, a significantly higher microbial level in the substrate (Table 2) was found than in the other theses, which probably improved plant growth (Figure 3).

Regarding the pH of the substrates, it should be noted that the use of microorganisms can influence this parameter to some extent; in the theses with microorganisms, the pH was one point lower than in the untreated theses. On the other hand, no significant differences are shown for seedling mortality in all substrates, although plant mortality appears to be lower in the theses treated with microorganisms.

The organic sludge treated with microorganisms also showed a significant increase in flowers and flowering time; on average, two flowers were produced and one day of life longer than the untreated theses (Figure 4). However, the physical-chemical sludge treated with microorganisms generally performed worse on plant growth than the treated biological sludge, probably because the material's structure does not guarantee good root growth. During the pre-composting phase of the materials, analyses have shown that the use of microorganisms can positively and significantly influence the reduction of some essential industrial contaminants, already after only six months of treatment (data not shown). It is therefore evident how using microorganisms can improve plants' growth performance in a conventional peat-based substrate. The results, however, show how the use of microorganisms also enables plant growth in biological sludge of industrial origin, which is usually unsuitable for this use.

Table 2 Evaluation of biological sludge treated with micro-organisms on substrate characteristics and plant mortality of *Delosperma cooperi*

Groups	pH	Microbial Count (cfu/g)	Roots weight (g)	Roots lenght (cm)	Dead plants number (n°)
CTRL	6.50 d	1.1×10^2 h	6.25 h	15.37 g	0.2 c
CTRL1	6.48 d	1.13×10^3 e	6.33 g	15.93 e	0.4 c
CTRL2	6.44 d	1.57×10^3 d	6.43 e	16.65 b	0.2 c
CM	7.46 b	5.5×10^2 g	5.23 j	14.47 h	1.6 a
A	6.80 c	1.57×10^3 d	6.46 d	15.76 f	1.2 ab
A1	6.80 c	1.58×10^3 d	6.53 c	15.93 e	1.2 ab
B	6.48 d	1.84×10^3 b	7.25 b	16.71 ab	0.2 c
B1	6.48 d	1.86×10^3 a	7.33 a	16.75 a	0.2 c
C	7.48 ab	5.8×10^2 f	6.34 fg	16.24 d	0.6 bc
C1	7.58 a	5.4×10^2 g	6.41 e	16.48 c	0.4 c
D	6.74 c	1.62×10^3 c	6.21 i	15.74 f	0.4 c
D1	6.76 c	1.63×10^3 c	6.36 f	15.83 f	0.6 bc
ANOVA	***	***	***	***	***

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: (CTRL) peat 100%; (CTRL1) peat 96% + bokashi 4% ;(CTRL2) peat 100% + Ema 20%; (CM) organic sludge 100%; (A) organic sludge 96% + bokashi 4%; (A1) organic sludge 96% + bokashi 4% + Ema 20%; (B) organic sludge 100% + Ema 20%; (B1) organic sludge 100% + Ema 30%; (C) organic sludge 100% + molasses 10%; (C1) organic sludge 100% + molasses 50%; (D) chemical-physical sludge 100% + Ema 50% (2 litres); (D1) chemical-physical sludge 100% + Ema 50% (4 litres).

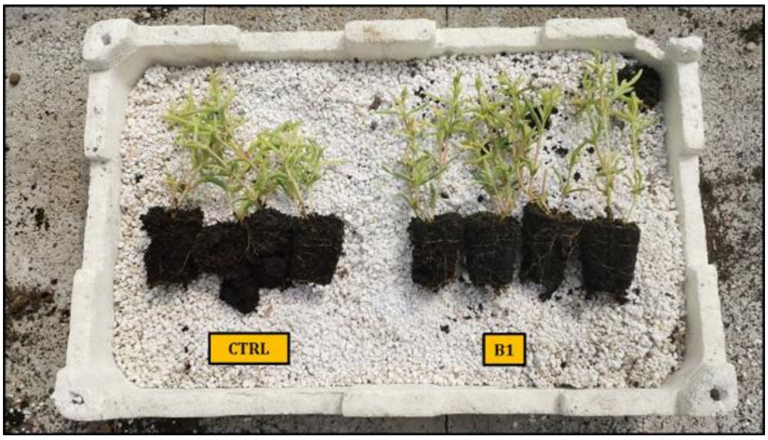


Figure 3 Comparison of *Delosperma cooperi* seedlings treated with (B1) organic sludge 100% + Ema 30% versus control in 100% peat (CTRL)

Figure 4 Effect of biological sludge inoculated with microorganisms on flowering of *Delosperma cooperi*

4. Discussion

The use of quality compost can ensure an essential contribution of organic matter to the soil and growing media [22]. Compost can lend itself to replacing manure, organic fertilisers and chemical fertilisers in all their functions and can fully fulfil the concept of restitution of mineral elements removed with production [23,24]. In some experiments, the fertilising effect of compost applied under cover on arboreal crops (vines, apple trees, citrus fruits) has resulted in a marked improvement in the nutritional state of the plants, compared to the exclusive use of chemical fertilisers, with an apparent attenuation of the symptoms of certain physiopathology due to temporary unfavourable edaphic conditions (rachis dryness, ferric chlorosis, early phylloptosis) [25]. The addition of fully stabilised organic materials, such as compost, entails covering the nutritional needs of plants and improving the soil's physical and hydrological conditions and, consequently, reducing the plants' susceptibility to nutritional imbalances. The use of compost also enriches soils and substrates with other nutrients that can increase the effectiveness of mineral fertilisation. During some experimental field trials, the use of compost on crops such as wheat and tomato caused both an increase in fresh biomass and dry matter. The improvement in soil fertility following compost was evidenced by the increased content of organic matter and nutrients such as total nitrogen, phosphorus and assimilable potassium, aspects also found in this trial [26]. The organic sludge treated with microorganisms probably had greater availability of nutrients, which were efficiently utilised by the plants and resulted in more significant growth and flower production. For every increase in organic carbon, compost results in a reduction in bulk soil density and an increase in water infiltration, root-level gas exchange, soil permeability and water retention capacity, related to improved soil particle aggregation and macroporosity. Compost contributes to slow-release carbon sequestration, with positive effects on restoring soil fertility and positively contributing to the CO_2 balance [27]. The soil amendment action of compost results in a significant increase in the organic matter of soils and consequently in the improvement of their physical and chemical properties. In this trial, this action could be seen as an improvement in plant growth in vegetative and root biomass and increased flowering. An increased presence of organic matter in the soil or substrate is then reflected in an increase in the content of microbial biodiversity interacting with plants, as also demonstrated in this research. The continuous use of compost can positively affect the phytosanitary state of the composted crops by significantly reducing the incidence and intensity of diseases caused by phytopathogenic fungi or bacteria [28]. This ability is more generally termed suppressiveness and refers to the ability of composts to create unfavourable conditions for the occurrence and development of various plant diseases, particularly for composts resulting from a substantial input of organic matrices. Suppressive composts, therefore, possess considerable potential for use, aimed at favouring the rationalisation of phytosanitary management of crops in a sustainable key, as they can allow the appreciable decrease in the use of chemical means of control, as well as improve the quality of production [29,30]. On *Delosperma cooperi*, the addition of biological sludge inoculated with microorganisms led to a reduction in plant mortality and, as far as product quality is concerned, an increase in flowering days, which is a fascinating aspect on a commercial level. The microbiological component of composts, widely diversified into phylogenetic and functional groups, is believed to be the main responsible for suppressiveness. The antagonistic microorganisms brought in with the compost act through the classic mechanisms of biological control: parasitism, antibiosis, competition for space and infection sites, and competition for nutrients, fungi stasis, and induction of plant resistance [31]. The abiotic part of the compost, i.e. the chemical part present in the complex humified organic matrix, which is rich in active molecules, can also make a substantial contribution to suppressiveness, either through direct antifungal action or by modifying the physical-chemical characteristics of the environment, or even by improving the nutritional status of the plant, which is thus better able to defend itself. The organic substance of the compost incorporated into the soil and substrate also stimulates the microbial community residing in the soil, thus feeding any microorganisms that are useful to the plants [21].

5. Conclusion

The test on *Delosperma cooperi* showed that it is possible to use biological sludge obtained from tannery waste, previously transformed through microbial activity, for the composition of substrates useful for cultivating ornamental plants. In particular, the microbial composition in the transformed biological sludge is vital in guaranteeing the interactions between plant and soil and the related exchange of water and nutrients. Furthermore, since the plant has more minerals, it is more resistant to biotic and abiotic stresses, photosynthesises better and grows more, producing more flowers that have a longer life. All these aspects can be of interest to growers who want to reduce synthetic chemicals in agriculture and are concerned about sustainability, but also to those companies whose primary goal is to produce new substrates as an alternative to peat and who are looking for new matrices. In addition, this research can be fascinating for those environmentally friendly industries that want to transform the waste produced by their industrial processes and reuse it, perhaps as in this case, to grow plants. Further experiments are underway to flank the microbial transformation with earthworms to obtain an increasingly high-performance final compost.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declares no conflict of interest.

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