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TECHNICAL GUIDELINES



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Forewords

This booklet originated from the results of the demonstration trial carried out during SUBSED LIFE+ project LIFE17 ENV/IT/00347 ‘Sustainable substrates for agriculture from dredged remediated marine sediments: from ports to pots’ (www.lifesubsed.com), in which the phytoremediated marine sediment has been tested as a possible substitute for commercial standard matrixes such as peat, coconut fiber, and wood fiber, in the cultivation of potted plants. These guidelines are aimed at describing the demonstration activity and the application possibilities of sediment-based substrates for soilless ornamental and food crop production.

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The dredged sediments and their remediation

The sediments used in the SUBSED project were partially decontaminated in a previous European project (AGRIPORT Agricultural Reuse of Polluted dredged Sediments, No. ECO/08/239065/S12.532262), using plants (phyto-treatment) and an organic amendment (such as compost) at pilot scale.

About 80 m³ of contaminated sediments mixed with 24 m³ of soil were placed in a sealed basin (20 m length, 5 m width, and 1.3 m depth) located in Leghorn port (Fig. 1). Organic matter (4 kg m² of compost) was applied on the top of the soil-sediment mixture and the following plant species were planted: *P. vaginatum*, *S. junceum* and *T. gallica*.

After about two years of treatment, the reduction in organic (about 50%) and inorganic (about 20%) contaminants and the chemical and biochemical properties reached by the sediments at the end of the bioremediation, proved the effectiveness of the process in remediating and recovering the sediments from an agronomical and functional point of view.

Remediation process: landfarming

In SUBSED project, with the aim to make the sediment a suitable substrate for plant growth, a landfarming process has been planned as refining treatment after a previous phytoremediation treatment. In order to avoid transportation cost, it is advisable to carry out the landfarming process in the same demonstration basin of phytoremediation. So, in the SUBSED project, the landfarming was carried out in the demonstration basin of the previous AGRIPORT project (AGRIPORT No. ECO/08/239065/S12.532262), in Livorno port (43°33'31.78"N, 10°18'29.32"E) (Fig. 1).

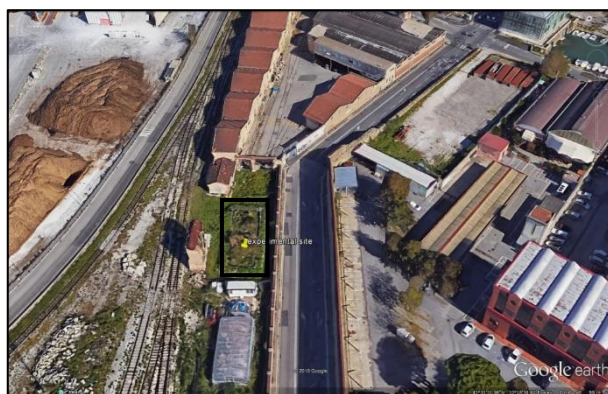
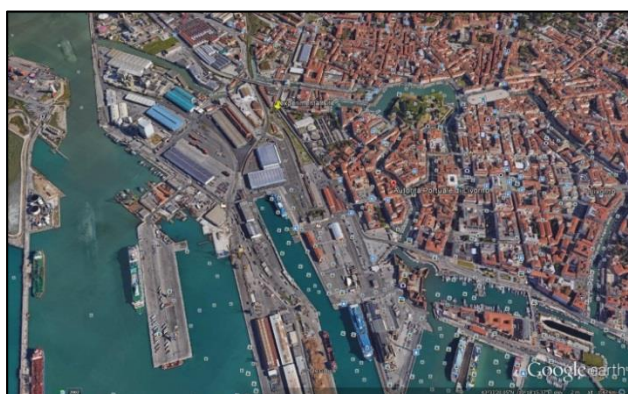


Figure 1. Port of Livorno and the landfarming basin

The main objectives of the landfarming technology were: i) homogenize the substrate, ii) increase the biological activities and, as a consequence, iii) further reduce the organic contamination in order to obtain a suitable substrate for plant growth.

With the goal of evaluating the technical specification (number of mixes, nutrient and organic matter addition) of the landfarming treatment, the sediments should be characterized in advance, from a physical, chemical, biochemical and toxicological point of view.

In SUBSED project, the characterization of partially phytoremediated sediments showed a good nutrient content and biological activity, quite low contamination level and absence of phytotoxicity. For this reason, a 3 months landfarming process, consisting only in the periodical sediment aeration by mechanical moving, was deemed sufficient to achieve the plan objectives. It is advisable to mix and aerate the sediments at least once a week and repair them from the rain to avoid an excessive water content that could cause anoxic conditions (negative for pollutant microbial degradation) and difficulties in the workability (sediment mixing).

In SUBSED project, every week, for 3 months, the sediments have been mixed and aerated until 60 cm through a little excavator and during rainy days, they have been covered by plastic sheet (Fig 2).



Figure 2. Landfarming process with little excavator

In case of fresh sediments (just dredged) with great amount in easily and quickly degradable organic matter and high enzymatic activity and higher contamination, a longer landfarming period is recommended from 4 to 8 months. Obviously, the initial characterization will be decisive for the choice of the treatment time.

Monitoring of landfarming process

Periodical sampling campaigns for sediment physical, chemical, biochemical and toxicological characterization are advisable to monitor the development of the landfarming process. The number of sampling and sampling times depend on the duration of the process and the quantity and type of material (fresh ore stabilized sediments) under landfarming.

For sediments that have already been partially phytoremediated, such as that of SUBSED project, with stable organic matter, low microbial activities and contamination, in which degradation of contaminants and organic matter transformation will occur more slowly and with less intensity, 1 sampling every 1 or 2 months is suggested. Instead, in the case of just dredged sediment, where more fast and intense process are expected, a more frequently sampling, every 15/30 day, is recommended. As for the number of samples, we recommend at least 1 every 10/15 m².

In the SUBSED project, the sediments were collected at the start of the landfarming process (end of November 2018), at the middle (middle of January 2019) and at the end of the landfarming process (end of February 2019). Three sampling points were selected in the landfarming basin area (about 40 m²). In an area of about 1 m³ around each sampling point, 10 sub samples of sediment were collected by a shovel, mixed, collected in plastic bags and transported to the CNR-IRET laboratory for the analyses (Fig. 3). The sediment samples were sieved (2 mm) and stored dried at room temperature until physical, chemical, biochemical, toxicological and hydrological analysis. The sediment samples for organic contaminant determination were, instead, stored as such at 4°C.

In detail, the analyses carried out were the following:

- physical analysis: texture and bulk density;
- chemical analysis: pH, electrical conductivity, nutrient in total and available forms, cation exchange capacity;
- biochemical analysis: hydrolytic and oxidoreductase enzyme activities;
- toxicological analysis: total heavy metals and available heavy metals, C>12, PAH, other organic pollutants, ecotoxicological test (phytotest);
- hydraulic analysis: water retention curve (not planned but it gives very important information on the agronomic substrate structural quality).



Figure 3. Sediment sampling

Results of landfarming process

Due to the aeration induced by the landfarming process, which resulted in an oxidative and very favorable environmental conditions for microbial pollutant biodegradation, a sharp reduction in organic contaminants, such as C>12 (heavy hydrocarbons), was observed (Table 1). At the end of the landfarming process, in fact, the C>12 resulted lower than the quantification limits (100 mg/kg). Instead, a significant variation was not recorded at the end of the process for the more recalcitrant PAHs (poly-aromatic hydrocarbons) (Table 1), still higher than the legal limit for the civil reuse, even if much lower than the limit for industrial reuse (D.lgs 152/2006) (Table 1).

Table 1. Organic contaminants (C<12, C>12 and PAHs) in the sediments at the start and end of landfarming process compared to the Italian regulation (D.lgs 152/2006)

Parameter	Started Sediments mg/kg	Sediments at the end of landfarming mg/kg	D.lgs 152/2006 Table A civil use mg/kg	D.lgs 152/2006 Table B industrial use mg/kg
C>12	747 ± 97	<100	50	750
C<12	< L.Q	< L.Q	10	250
PAHs	43.0± 12	51.6± 5.1	10	100

Comparing the sediment characteristics at the end of three months of landfarming process with that required by Italian regulation for agronomic substrates (D.lgs. 75/2010), it is possible to conclude that the sediment properties are in accordance with possible re-use of this matrix as a cultural substrate in horticulture, with the only exception of organic carbon and bulk density (Table 2) which are out of the limits. The bulk density was, in fact, slightly higher than the maximum limit, while the organic carbon was lower.

The major problem concerning the reuse of this sediment is related to the residual contamination still present, as concentrations of poly-aromatic hydrocarbons, which were still higher than the legal limit for the civil reuse (Table A), even if much lower than the limit for industrial reuse (Table B) (D.lgs 152/2006) (Tables 1). However, the slight persistent contamination should not compromise the reuse of the phytoremediated sediments in horticulture since the toxicological tests showed no phytotoxicity of the sediment at the end of the landfarming process. Indeed, the germination index of about 130-140% indicated the presence of nutrients able to stimulate seed germination.

Table 2. Physical and chemical characteristics of sediments at the end of the landfarming process in comparison with the Italian regulation for agronomic substrates (D.lgs. 75/2010)

Parameter	Sediments at the end of landfarming in the SUBSED Project	D. lgs. 75/2010
Bulk density (g/cm ³)	1.19 ±0.05	<0.95
pH	7.4±0.2	4.5-8.5
Electrical conductivity (dS/m)	0.13 ±0.01	<1
TOC %	1.38 ±0.08	>4
TN %	0.11±0.01	<2.5
P ₂ O ₅ %	0.17 ±0.01	<1.5
Cd (mg/kg)	< LOD	1.5
Cu (mg/kg)	48.6 ±1.7	230
Hg (mg/kg)	0.070 ±0.001	1.5
Ni(mg/kg)	37.7 ±0.7	100
Pb(mg/kg)	37.2 ±6.4	100
Zn (mg/kg)	145 ±4	500

Recommendations for good landfarming process

- Three months of landfarming process can be effective in homogenizing the substrate, further reducing organic contamination, and reaching physical and chemical characteristics comparable with those of an agronomic substrate. However, based on initial sediment properties a longer landfarming process could be planned to reach the required characteristics.
- For further reduction of the organic contamination, some adjustments to the landfarmig process could be: i) extension of landfarming process (e.g. 6 months instead of 3); ii) favor the microbial community in the degradation process through the addition of oxygen, water and nutrients readily available for microorganisms (such as nitrogen and phosphorus); iii) inoculate specific microbial consortia or fungi capable of quickly degrading hydrocarbons.
- To reach the C concentration required by Italian regulation, the sediments can be mixed with a source of organic matter rich in Carbon, such as peat, sludge, coconut.
- The addition of light organic matter will also contribute to the decrease in bulk density of sediment, permitting the reaching the characteristics required by Italian regulation for growing media (D The addition of light organic matter will also contribute to the decrease in bulk density of sediment,

permitting the reaching the characteristics required by Italian regulation for growing media (D.lgs 75/2010).

The use of treated sediments in ornamental and flower crop production

Italy is one of the largest European producers of flower and ornamental plants, with Tuscany representing the heartbeat of the Italian nursery stock production. The production facilities of plant nurseries are highly organized. Plants are grown in containers or in the open field. Plants are often propagated by seed, cuttings or rhizome in pots where they remain until the sale, otherwise they are transplanted into the ground or transferred in larger containers. This sector is characterized by highly intensive processes, and the contribution to CO₂ in container production depends mainly on two specific factors: the peat component used in the preparation of growing media and the considerable amount of plastic, essentially pots and irrigation pipes



Figure 4. Cherry laurel

The phytoremediated marine sediment is a relatively inert matrix with a lower carbon footprint that can be incorporated to reduce peat content in growing media recipes, representing at the same time a smart solution to the problem of waste disposal. However, little is currently known on the compatibility and combinability of the phytoremediated sediment with other standard matrixes for ornamental and flower production. Thus, the LIFE SUBSED project focused on the best practices for sustainable production of commercially important ornamental and flower plant species on sediment enriched substrates. *Prunus laurocerasus* cv ‘Novita’, *Zantedeschia aethiopica* and *Protea cynaroides* cv ‘Little Prince’ were chosen as representative case studies.



Figure 5. Calla lily



Figure 6. Protea

Producing *Prunus laurocerasus* L. (cherry laurel) cv. ‘Novità’ rooted cuttings

Prunus laurocerasus cv ‘Novità’, a typical and very diffuse evergreen ornamental with very fast growth and plant development, was chosen as representative Tuscan outdoor woody ornamental species. Nowadays, *P. laurocerasus* is recognised as one of the most commercially important ornamental barrier plant species for the Italian nursery sector. The species grows well in the Mediterranean climates and adapts to all types of soil, tolerating light, medium and heavy clay soil. Different proportions (0, 25, 50% v/v) of the treated sediment (TS) were mixed with three types of standard substrates, commonly used for ornamental and flowering crop cultivation in Tuscany in order to obtain seven growing media, as described in Table 1. Standard substrates were based on peat (Pe), coconut fiber (CF) and wood fiber (WF), each one containing 40% of inert pumice. Growing media prepared using commercial peat-based substrate only (LMix 1) was considered as the control treatment. The different mixtures were prepared using defined volumes of previously homogenized pure sediment and standard substrates which were carefully mixed in a concrete mixer before being used for pot filling. Experiments were set up and performed under greenhouse conditions at Franceschini facility (Montecarlo, LU, Italy) during the season June 2020 - March 2021. Two different water regimes (WR) were applied in order to evaluate their effect on plant growth and productivity in relation to the substrate mixtures tested: normal = 250 cc average annual water supply per pot and per day (WR1); reduced by 20% = 200 cc average annual water supply per pot and per day (WR2). In total 336 cherry laurel semi-hardwood cuttings were planted in 8.5-L pots. Each substrate/water regime combination was replicated in three blocks, each containing four pots, each one consisting of two cuttings (totalling 8 cuttings x 14 treatments x 3 blocks = 336 cuttings).

Table 3. Composition of tested growing media

Growing medium	Matrixes (%) v/v				
	Peat	Pumice	Coconut fiber	Wood fiber	Treated sediment
LMix 1 = TS0	60	40			0
LMix 2 = Pe:TS25	45	30			25
LMix 3 = Pe:TS50	30	20			50
LMix 4 = CF:TS25		30	45		25
LMix 5 = CF:TS50		20	30		50
LMix 6 = WF:TS25		30		45	25
LMix 7 = WF:TS50		20		30	50

Except for LMix 3, sediment-based media mixtures showed physicochemical parameters and heavy metal content in line with the Italian regulation for mixed growing media (L.D. 75/2010). Although sediment-based substrates were rich in Zn, foliar component analysis showed that this element was found at very low concentrations in plants. Peat, used as stand-alone growth media component, showed lower EC and pH values with respect to all the mixtures examined; however, findings regarding nutritional elements (P, Ca, Mg and K) highlighted the potential suitability of the treated sediment as a component of an agronomic substrate. Substrate mixtures had a clear effect on all final plant aerial parameters. Growth media containing WF and TS were the most limiting for *P. laurocerasus*. Reduced plant development was likely to be primarily associated with substrate pH which affects mineral mobility and, thereby, nutrient availability. Results showed that the properties of the substrate components by themselves are not as important as the properties achieved after blending various standard substrates together with the TS into the final mix. In fact, the phytoremediated sediment was successfully used as a growth medium constituent in all the media other than peat and coconut in the proportion varying between 25-50%, though in combinations with WF it reduced cherry laurel growth and aboveground biomass.



Figure 7. Cherry laurel cultivation

Soilless cultivation of *Prunus laurocerasus* L. (cherry laurel) cv. ‘Novita’

One-year-old rooted *P. laurocerasus* plants from the previous trial on cutting propagation were used. The rooted cuttings were transplanted in 10-L drip-irrigated pots. To better understand the effect of water retention caused by the incorporation of the sediment into the substrate, plants were supplied with an average daily water volume per pot of 250 cc (WR1) and 200 cc (WR2). Recirculating drip irrigation was used to control water supply and all plants were irrigated from the same reservoir. The pH of irrigation water was maintained between 5.6 and 6.0 (EC at 2.0 ds m⁻¹).

A randomized complete block design was used with seven growing media (GM = TS0, Pe:TS25, Pe:TS50, CF:TS25, CF:TS50, WF:TS25, WF:TS50) and two irrigation daily volumes (WR1; WR2). The growth trial started in April 2021 with three replications of each treatment, and three cherry laurel plants in each replication. Thus, a total of fourteen treatments were evaluated, with nine cherry laurel plants grown per treatment (n = 9). All experimental GM were supplied with Nitrophoska Gold®

(NPK 15:9:15) at the dose of 7 g per pot. The trial was finished in March 2022 with the physical and chemical characterization of the growing media and the destructive analyses of the plant material.

Growth parameters (base stem diameter, maximum plant height, number of vegetative sprouts, length of vegetative sprouts, number of fully expanded leaves on vegetative sprouts), and leaf blade colour were recorded on June, September, and December 2021. Measurements of leaf gas exchanges were conducted during full vegetative activity in June. Plant biomass production, pigment (chlorophylls and carotenoids), and ion's analysis were determined at the end of the trial in March 2022.

Plant grown on WF:TS mixes had a more compact shape and significantly lower DW biomass (areal + roots) compared to the control plants. The lowest biomass production observed was mainly attributed to the reduction in both stem weight and total leaf area. It is possible that the initial exposure of the plants to high pH levels in WF caused reduced leaf areas, thereby preventing future growth by lowering water and mineral uptake and rates of photosynthesis. Interestingly, the effect of water deficit appeared more evident immediately after the extremely hot-dry summer growth period on plants grown on Pe alone and Pe:TS25. In this regard, it should be noted that the higher water holding capacity of TS might have compensate for the greater hydrophobicity in peat-based mixes under restrictive irrigation.

Recommendations

In the case of outdoor ornamental plants, human health risks due to the presence of toxic residues are minimized. Moreover, cherry laurel is not used as pot plant for green interiors, but as barrier (hedge) and urban roadside plant in open environments. There is therefore no danger that residues of polluted substrate can reach domestic windowsills or homes and this practice could be successfully applied to other outdoor ornamental tree or shrub species grown in containers. Main disadvantages of the TS were its high bulk density, high EC and pH, high Ca and metal content; however, its higher water-holding capacity than perlite, is seen as a major advantage of this medium component, especially under reduced water availability. Because of growth restriction, a proper scheduling of irrigation and technology management for the optimization of plant growth is however needed, especially when fast-growing species are cultivated on mixes containing WF and TS. Moreover, the treated sediment must be carefully refined and sieve before using it as a substrate component. Mixtures are considered in volume; therefore, it is advisable to use a pot with marks for each fourth, fill the desired fourth, and then proceed with complete filling. Suitable substrate blends are LMix 2 (Pe:TS25), LMix 3 (Pe:TS50), LMix 4 (CF:TS25) and LMix 5 (CF:TS50). On the other hand, LMix 6 (WF:TS25) and LMix 7 (WF:TS50) have be used more cautiously, especially if the cultivation cycle is very short. In fact, over time the initial growth deficits are recovered.

Soilless cultivation of *Zantedeschia aethiopica* (L.) Spreng (calla lily)

Calla lily [*Zantedeschia aethiopica* (L.) Spreng.] is a perennial rhizomatous species, native to South Africa. Thanks to the beauty of its flowers (from the Greek kalos = beautiful), this species is of major economic importance worldwide and was chosen as species for cut flower production. In Italy, calla is grown as outdoor garden and potted plant, but also largely raised for cut flower production under greenhouse conditions. Furthermore, calla has been widely used for the treatment of wastewater or contaminated wetland as it is considered moderately tolerant to heavy metals. The tubers can be grown in practically many types of soil, but very important factors are an air-permeable structure and the capability to hold sufficient moisture. Therefore, substrate selection is one of the most important factors affecting calla development and flowering in the greenhouse.



Figure 8. Calla lily flower



Figure 9. Calla lily harvested flowers

Calla rhizomes were planted at Salvestrini facility (Pescia, PT, Italy) in greenhouse in late summer (September 12th, 2020), as usually done for this species. Three different growing media (GM), combining different proportions of the treated sediment (TS) with a standard peat-based substrate (peat 60%, 40% pumice v/v), were tested: i) 100% Pe, control (CMix 1 = TS0); ii) 75% Pe and 25% TS (CMix 2 = Pe:TS25); iii) 50% Pe and 50% TS (CMix 3 = Pe:TS50). Rhizomes were planted in 30-L containers placed on three raised benches served by different water regimes (WR) by a drip irrigation system: i) WR1, high water regime=WR2+30% (1220 mL/day of water per pot on average); ii) WR2, normal water regime (930 mL/day of water per pot on average); iii) WR3, low water regime=WR2-30% (650 mL/day of water per pot on average). Containers were

arranged in three blocks, each consisting of 7 pots per Sm*Wr treatment, for a total of 189 pots each holding 2 rhizomes (7 containers x 3 substrate mixtures x 3 water regimes x 3 blocks). Before planting, rhizomes were soaked in a solution of copper oxychloride 0.5 % in order to prevent *Erwinia carotovorum* attacks. Plants were fed with a nutrient solution commonly adopted for the cultivation

of soilless calla. The total amount of nutrients per pot was: 245 g of Peters Excel CalMag Finisher (13-5-20 + 7 CaO + 2 MgO by ICL) and 8 g of Farben H50 (EDDHA Iron chelate 6% by Du Pont).

Calla vegetative growth and productivity in terms of length of leaf stem and leaf blade, number of flowers and flower length, were monitored during plant life cycle. The colour of flower spathes, flower senescence, fluorescence emission, leaf chlorophyll and heavy metals concentrations in leaves and oxidative stress were determined.



Figure 10. Fluorescence analysis on calla leaves

Both peat-based and sediment-based substrate mixtures allowed the cultivation and blooming of calla lily. The number of flowers per plant exhibited the highest value in the presence of 50% of treated sediment (Fig. 1A). A 30% water reduction determined a highly significant decrease in flower production (Fig. 1B). A similar trend was found when the relative sale price of the flowers during the whole harvest season was considered. The lower plant development and flower production obtained when calla was cultivated on peat-based substrate might be ascribed to a lower nutrient uptake and also to a lower water retention capacity of the peat fraction compared to the sediment.

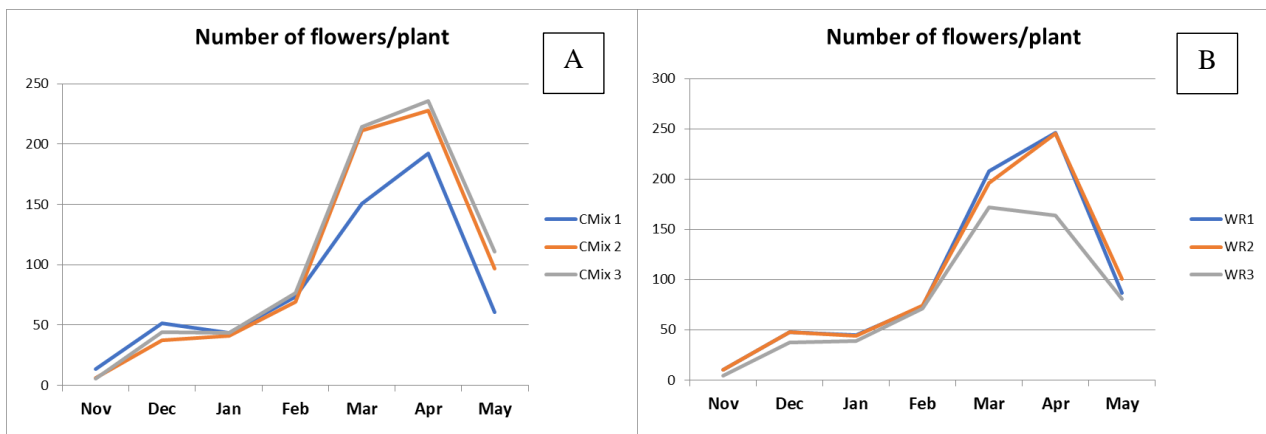


Figure 11. A) Flower production of calla lily grown on CMix 1, CMix 2 and CMix 3 substrate mixtures; CMix 1 = 100% Pe; CMix 2 = Pe:TS25; CMix 3 = Pe:TS50. B) Flower production of calla lily grown with different water regimes; WR1=WR2+30%; WR2=normal water regime; WR3=WR2-30%. Values are means of 9 replicates

Recommendations

Calla lily is considered a marginal aquatic plant, that grows in moist soil along riverbanks, ponds or streams. Moreover, calla lilies in pots dry out much more quickly than calla planted in the ground. Therefore, soilless cultivation of calla lily needs frequent watering and, even more important, a relatively constant rate of substrate humidity over time. In this context, the sediment-based substrates exerted a positive effect on plant development, allowing a more prolonged contact between the roots and the nutrient solution.

Suitable substrate blends are both CMix 2 (Pe:TS25) and CMix 3 (Pe:TS50). Higher the concentration of TS, higher the GM water retention; thus, mixes of TS \approx 50 % are recommended when water is less available. Increasing the sediment percentage in the substrate ($>$ 25%) could allow a greater flower production and quality compared to those obtained using a common peat based commercial substrate.

Soilless cultivation of *Protea cynaroides* L. cv ‘Little Prince’



Figure 12. *Protea* cultivation

National Flower of South Africa, protea is commercially relevant for the flower industry and in Italy it is extensively cultivated as flowering pot and exported all over the world. This perennial flowering species is adapted to nutrient-poor soils, with a pH 4-6, and a clay content of less than 20% with low levels of P, K and Na. Water requirements are high when grown under soilless conditions. Hot, humid conditions are not well tolerated by protea and

sufficient air movement is required for healthy growth.

Different proportions (0, 25, 50% v/v) of the treated sediment (TS) were mixed with different proportion of peat (Pe), coir fiber and dust (CF_a and b) in order to obtain seven growing media, as described in Table 2. Totally 630 protea plantlets were transferred in 2-L pots: 10 pots for each combination of ‘substrate x water regime’ (each consisting in 1 plantlet), replicated 3 times. Plantlets were planted at Simoncini facility (Pescia, PT, Italy) on June 2020 under greenhouse conditions. Plants were supplied with 3 different daily water volumes: WR1 = normal; WR2 = low (reduced by 30%); WR3: very low = reduced by 50%.

Growth parameters, flower production and quality, and sensitive indicators of the cellular metabolic state (leaf blade colour, membrane lipid peroxidation, elemental analyses, chlorophyll and carotenoids contents, fluorescence and leaf gas exchange measurements) were measured.

Table 4. Composition of tested growing media

Growing medium	Matrixes (%) v/v				
	Peat	Pumice	Coir fiber	Coir dust	Treated sediment
PMix 1	60	40			0
PMix 2	45	30			25
PMix 3	30	20			50
PMix 4		18	34.2	22.8	25
PMix 5		12	22.8	15.2	50
PMix 6		18	17.1	39.9	25
PMix 7		12	11.4	16.6	50

In general, plants raised under reduced water availability (WR3) showed a high mortality. The incorporation of 50% v/v TS in the growing media significantly reduced plant growth and flower production in all tested mixes. On the other hand, protea grown on TS25 mixes had a more compact shape, but developed a good number of flowers, except for PMix 7 containing a higher percentage of coir dust. In this regard, it should be noted that a compact behaviour might represent a valuable feature for this species, when cultivated as a flowering pot. Suggested factors limiting plant development and flower production could be related to high pH values, high clay and nutrient content, especially phosphorus, inherited from the sediment matrix. Plants have radical proteoids on the roots, which increase the effectiveness of nutrient absorption that might negatively affect plant response.



Figure 13. Protea plants grown on PMix 1 (1), PMix 2 (2), PMix 3 (3), PMix 4 (4), PMix 5 (5), PMix 6 (6), and PMix 7 (7)

Recommendations

Proteas prefer a nice sandy loam or open soil. Some proteas are less fussy and are quite happy in a heavier soil although they will not survive in heavy clay soils. Moreover, proteas prefer to grow in acidic soils as this reflects the conditions where they originate in South Africa and Australia. This species develops proteoid roots for increased nutrient absorption, being therefore adapted to poor soils of low nutritional status, particularly phosphorus. The incorporation of treated sediment in a dose greater than 25% is not recommended for protea cultivation and mixes containing coir dust should be avoided.

Suitable substrate blends are those based on peat and coir fiber in combination with 25% TS (PMix 2 = Pe:TS25; PMix 4 = CF_a:TS25). It is advisable to check the pH value of the mixture before planting and make any corrections. Moreover, phosphorus fertiliser must be limited, while irrigation must be constant but not excessive in order to avoid waterlogging.

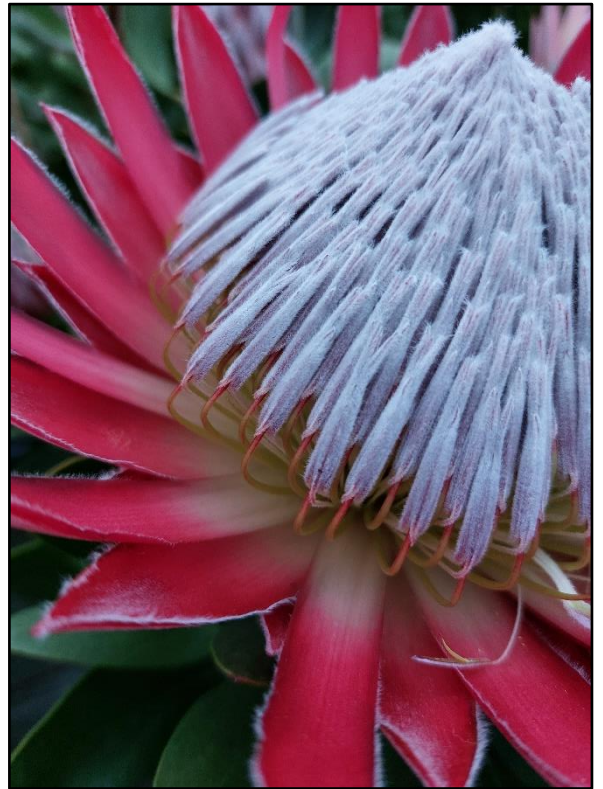


Figure 14. Protea flower at the maximum opening

The use of treated sediments in food crop production

The fruit and vegetable sector must comply with very strict rules for all segments and stages and, to be sustainable, must also respond to the consumers demand for high quality fruits and vegetables and environmentally low impact production. Fruits are generally very rich in nutraceutical substances having beneficial effects on human life, and consumers perceive vegetables and fruits as useful food by paying particular attention to their quality in terms of healthiness.

The LIFE SUBSED project aimed to evaluate whether the remediated sediments could be used as growing media for grafted fruit tree production (olive and lemon) and soilless cultivation of fruit and aromatic crops (lemon, wild strawberry, blueberry, and basil). The quality of the whole plants and their edible parts (leaves/fruits) were evaluated by taking into account morphological and physiological traits as well as nutraceutical properties, sensorial attributes and also food safety. Indeed, the use of the phytoremediated sediment can cause potential criticality to human health, due to the nature of the sediment itself. To be sure that the products met all statutory safety

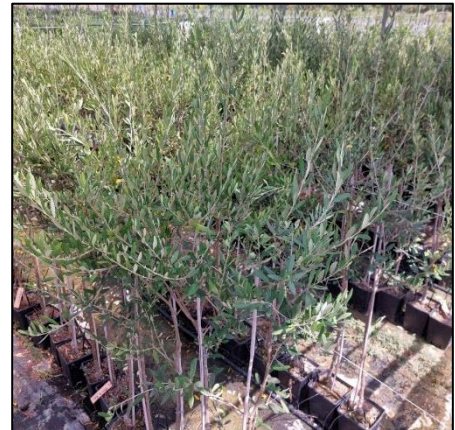


Figure 15. Olive



Figure 16. Lemon

requirements under European or national law, the concentration of organic and inorganic contaminants in strawberry fruits, basil leaves and different part of blueberry plants were analyzed. The official and standardized analytical methods applied in the determination of contaminants in edible parts covered a wide range of elements and chemical compounds in order to obtain transparent and reliable results. Results were compared with safe limits found in European and Italian legislation and health risk assessment was investigated.



Figure 17. Wild strawberry



Figure 18. Blueberry



Figure 19. Basil

Producing grafted *Olea europaea* L. (olive) plants cv. 'Frantoio' using sediment-based substrate

Olive growing is a very important agricultural activity in Italy, accounting for 15%-18% of the world production (second after Spain). Moreover, the Italian olive oil sector is a leading player in the global competitive scenario, both in terms of production and export.

The olive tree is an evergreen and broad-leaved tree, whose vegetative activity is almost continuous, with attenuation in the winter period. It has slow growth and is very long-lived: in favorable climatic conditions it can become millennial and reach heights of 15-20 meters. Olive trees prefer calcareous and rocky soils.

Certified grafted plants of olive cv. 'Frantoio' were used as plant material. Plants were grown on five growing media composed of different proportion (0, 25, 50, 75 and 100% v/v) of treated sediment (TS) in combination with

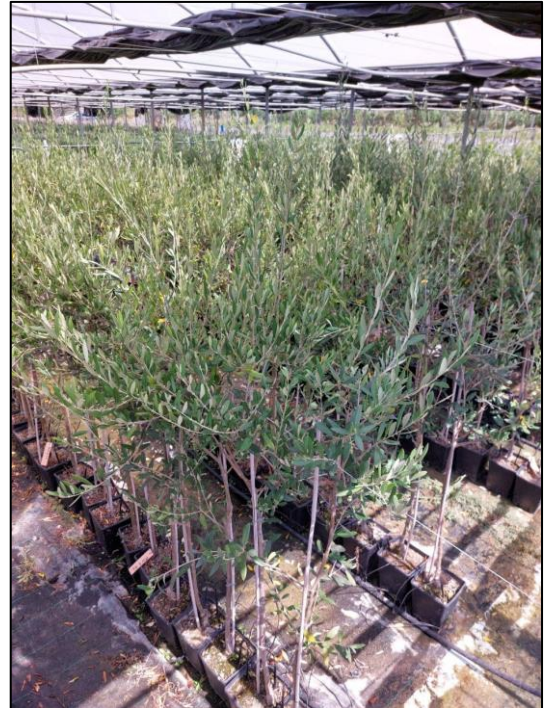


Figure 20. Production of grafted olive plants in pots

a standard commercial peat-based substrate (Pe = 60% peat and 40% inert pumice): i) TS0 = 100% Pe (control); ii) TS25 = 75% Pe and 25% TS; iii) TS50 = 50% Pe and 50% TS; iv) TS75= 25% Pe and 75% TS; v) TS100 = 100% TS. Moreover, two different average daily water regimes (WR) were applied to each substrate mixture: WR1 = 240 cc water per pot; WR2 = 325 cc water per pot.



Figure 21. First fruiting on TS50

Pots were arranged in a randomized block design with 3 blocks, each consisting of 5 pots per GM* WR, for a total of 150 pots (5 pots x 5 substrate mixtures x 2 water regimes x 3 blocks).

The demonstration trial was performed at the Franceschini facility (Montecarlo, LU, Italy) under greenhouse conditions during 15/04/2020 - 30/05/2022.

Hardening and development of grafted olive trees were monitored during two-growing seasons and biomass production was determined at the end of the propagation cycle.

No significant differences between substrates were observed as regards morphological parameters. Concerning plant development similar values were recorded for olive trees that were cultivated either on peat alone or sediment-based growing media. In fact, even plants grown in TS100 displayed values of plant height and stem diameter comparable with those obtained in the control. Furthermore, results

showed that a mild water deficit does not affect development and hardening of young, grafted olive trees regardless of the substrate used for cultivation.



Figure 22. Leaf area analysis

On the other hand, results of leaf area exhibited a different pattern: a larger leaf area was recorded from plants grown in substrate mixes containing smaller amounts of TS (TS0 and TS25). The greater leaf development of olive grown on peat-based media is most likely correlated to peat organic matter content and other available nutrients.

The water availability significantly affected fruit production of olive trees, being yields reduced under water deficit, and particularly when plants were cultivated in TS75 and TS100. However, these data must be interpreted carefully as two-year-old trees have not yet entered full production. Previous studies on vegetation development showed that olive tree growth is mainly controlled by temperature, water availability and competition for assimilates. Plants cultivated in the sediment-based growing media that received little water showed a reduced leaf expansion and assimilate production.

Recommendations

The similar growth performances (absence of differences recorded on plant height and stem diameter) observed in plants cultivated in peat alone and peat/treated sediment suggests that young, grafted olive trees can be grown in both sediment free and sediment-based substrates, corroborating the thesis that phytoremediation sediment can be used as a component of a substrate for soilless cultivation.

Water supply given by irrigation do not affect plant development in terms of shoot expansion; nevertheless, had a significant effect on leaf area and crop production. Limited water availability affects leaf development, especially if associated with TS100 and TS75, which in turn affects, light capture, and thus production of assimilates and yield.

However, in this phase of production of two-year-old grafted olive trees to be transplanted in open field, plant performance in terms of fruit yield is of little relevance. Thus, reduced water scheduling can be considered in more arid climate environments.

Producing *Citrus limon* L. seedlings using remediated substrate

Lemon is one of the most *Citrus* species cultivated in the southeast of Spain and also in Murcia area.

The demonstration trial was carried out at the Caliplant nursery facility in order to evaluate the possibility to produce *Citrus* commercial seedlings using the treated sediments (TS) mixed with a standard cocopeat substrate (CP). Five different growing media combining different proportions of the treated sediment (TS) with cocopeat substrate were tested: i)

100% CP, control (CMix1 = TS0); ii) 75% CP and 25% TS (CMix 2 = CP:TS25); iii) 50% CP and 50% TS (CMix 3 = CP:TS50); iv) 25% CP and 75% TS (CMix 4 = CP:TS75); v) 100% TS (CMix 5 = TS100).



Figure 23. Production of lemon rootstocks seedlings in pots



Figure 24. One-year-old *Forner alcaide* n°5 rootstocks grown on TS100, TS75, TS50, TS25, TS0 (from left to right)

Seedlings of three species most commonly employed species for rootstocks creation were used as plant material: *Citrus macrophylla*, *Citrus aurantium* and *Forner alcaide* n°5. Trunk diameter, plant height evolution and drainage composition over time were recorded. Totally, 375 lemon trees (25 plants x 5 substrates x 3 rootstocks x 3 blocks) were grown in 2.5-L polypropylene pots.

Seedlings were cultivated until they reached and fulfilled the standard commercial requirements, that is 1-year-old citrus rootstock: height 60-70 cm.

In general, all seedlings showed an adequate vegetative and development growth, although seedlings of the three species showed statistically lower values when grown on CMix4 and

CMix5, both containing high percentages of the treated sediment (TS 75% and TS 100%, respectively). Seedlings cultivated in CMix3 (CP:TS50) showed a similar agronomical behaviour in terms of trunk size, height development, and drainage composition over time compared to the control treatment.

Once the seedlings reached the optimal commercial rootstock requirements (height and trunk diameter), they were grafted with the cv. 'Fino', which is the most common cultivar in Spain. The

main flowering period for this lemon tree falls between April and May, and the fruit is harvested between October and May. At the end of summer, the ‘Fino’ lemon tree has a second flowering period that produces lemons, which are harvested the following summer. This cultivar is prized for its abundant juice, citric acid and essential oils. This important commercial cultivar was chosen in order to verify and consolidate the previous observations on plant performances according to different sediment-based growing media.

Interestingly, the results confirmed the same growth and development trend, with scions (fruiting cultivar) grafted on different *Citrus* rootstocks that were able to grow normally and to reach a standard size, regardless of the growing medium. In particular, the presence of 50% treated sediment in the growing media did not produce any growth variation in ‘Fino’ lemon compared to the control, with values that remained statistically comparable onto all rootstock species.

Recommendations

The results indicate that it is possible to produce a commercial *Citrus* seedling using the treated marine sediment mixed with cocopeat.

‘Fino’ lemon grafted on different *Citrus* rootstocks and cultivated on CP:TS50 performed similarly to the control, therefore this substrate mixture is recommend for the production of citrus rootstock seedlings and grafted ‘Fino’ lemon two-year-old trees.

Growing *Citrus limon* L. (lemon) cv. ‘Verna’ in pots

The lemon tree (*Citrus limon* (L.) Burm f.) is considered the third most important citrus species in the world, only behind orange and tangerine, and is socially known for its valuable nutritional,



Figure 25. Cultivation of lemon plants in pots

medical, pharmacological, industrial and cosmetics properties and uses. Although the lemon is present in the world market, Spain is the main lemon producer in the Mediterranean region with 42% of the total European production followed by Italy (38%), Greece (5%) and Portugal (1.6%) among other minority countries. Due to the climatic specifications of lemon trees, in Spain, its production is concentrated on the southeast region, with emphasis on the south of the Valencian Community, where the experimental SUBSED plot was carried out (Orihuela, Alicante).

Among all the cultivated varieties of lemons, 'Verna' was chosen because is the second most relevant in the Valencian Community. The main characteristic of this lemon variety is that, in general, present an abundant harvest with medium/big sized fruits, a good acidity of its juice and few seeds.

The lemon trees were planted at the Polytechnic School of Orihuela (EPSO) of Miguel Hernandez University (Orihuela, Spain) in late spring (May 20th, 2020). Nine different treatments were evaluated based on two controlled variables: the rootstock (n=3) and the growing media (n=3). Three different growing media (GM), combining different proportions of the treated sediment (TS) with a commercial peat (Pe), were tested: i) 75% Pe and 25% TS (TS25); ii) 50% Pe and 50% TS (TS50); iii) 25% Pe and 75% TS (TS75).



Figure 26. Production of grafted lemon plants in pots

Among the most common rootstocks used in commercial lemon cultivation three *Citrus* species were selected: i) *Citrus macrophylla*, which is the most important rootstock used for lemon trees, mainly because of its vigorous, which translates into great productivity; however, when grafted with 'Verna' it can produce oversized lemons; ii) *Citrus aurantium*, which is the traditional lemon rootstock that confers the tree great resistance to hydro edaphic environments; however, its affinity with 'Verna' variety is limited; iii) a combination between *Citrus aurantium* and *Citrus sinensis*, usually called sweet orange intermediate wood, which is commonly used to avoid possible incompatibilities between rootstock and variety.



Figure 27. Citrus plant in pot (2 years)

Trees were arranged in a randomized block design with 5 blocks, each consisting of 2 trees per each GM*Rootstock combination. Totally 90 two-year old lemon trees were grown in 40-L polypropylene pots (2 trees x 3 growing media x 3 rootstocks x 5 blocks). Both the environment conditions and the crop management were kept homogeneous throughout the trial to minimize external influences and to study the treated sediment impact as growing media. Growth parameters, fruit production, fruit quality (morphological, chemical and nutraceutical), soil microbiome (bacterial and fungi communities), and sensitive indicators of the cellular metabolic state (leaf gas exchange measurements and metabolomic analysis) were measured (Table 5 and 6).

In general, all the lemon trees showed an adequate vegetative development, growth and production. However, lemon trees grown on TS75 showed a reduced development in terms of both trunk diameter and leaf size. Moreover, lemon trees grown on TS50 and TS75 required less water than those cultivated on TS25, that is, the amount of irrigation water applied was lower as the content of treated sediment was higher. This is due to the physical-chemical characteristics of the treated sediment that retains a more significant amount of water in the growing media.

Table 5. Effect of the growing media and rootstock on ‘Verna’ lemon production

Factor	Fruits/tree (n°)	Yield (kg tree ⁻¹)	Fruits (total n°)	Total yield (kg)
Growing medium				
TS25	9.63 c	1.68 a	289 c	50.51 b
TS50	19.66 a	3.12 b	590 b	90.53 a
TS75	4.13 b	0.58 c	124 a	17.28 c
Rootstock				
<i>C. macrophylla</i>	14.83 a	2.35 a	445 b	70.78 b
<i>C. aurantium</i>	3.03 b	0.65 b	91 a	18.86 a
<i>C. aurantium/C. sinensis</i>	15.56 a	2.28 a	467 c	68.67 c

Mean values within each factor followed by different letters are significantly different at $P \leq 0.01$.

Table 6. Effect of the growing media and rootstock on ‘Verna’ lemon morphological characteristics

	Fruit weight (g)	Equatorial diameter (mm)	Fruit length (mm)	Mamelon length (mm)	Peel thickness (mm)	Carpelly (n°)	Seeds (n°)	Juice content (w:w)
Growing medium								
TS25	168.15 a	63.10 a	93.66 a	15.24 a	7.08 a	9.31 a	1.59 a	0.27 a
TS50	168.91 b	63.32 b	96.39 ab	16.18 ab	6.73 a	8.92 a	1.66 a	0.27 a
TS75	142.42 b	59.78 b	94.10 b	16.69 b	6.75 a	9.06 a	1.31 a	0.24 a
Rootstock								
<i>C. macrophylla</i>	163.25 a	62.60 ab	94.26 a	15.01 a	6.74 a	9.15 a	1.36 a	0.26 a
<i>C. aurantium</i>	176.13 b	64.07 b	98.05 b	16.90 b	6.83 a	9.02 a	2.31 b	0.27 a
<i>C. aurantium/C. sinensis</i>	159.25 a	61.96 a	94.37 a	16.51 b	6.97 a	9.08 a	1.35 a	0.25 a

Mean values within each factor followed by different letters are significantly different at $P \leq 0.01$.

Recommendations

The results indicated the potential of the treated marine sediment mixed with other substrates as an agricultural substrate applied to the cultivation of lemon. Indeed, the substrate composed by 50% mixture of treated sediment and peat presented the highest fruit production (both in number of fruits and in weight). The impact of treated sediment on the reduction of the fruit size/weight could be used as a tool to control excessive fruit development in some lemon varieties as ‘Verna’.

The lemons obtained in the nine substrate/rootstock combinations studied, presented appropriate characteristics for their categorization as commercial. The nutritional value of the ‘Verna’ lemon juices was very homogeneous among the treatments studied, hence, the limited influence of both the rootstock and the culture substrate on the chemical characteristics of the fruits is confirmed.



Figure 28. Lemon fruit characterization

Soilless cultivation of *Fragaria vesca* L. (wild strawberry) cv. ‘Regina delle valli’



Figure 29. Wild strawberry plants in rectangular (80x50 cm) pot

Strawberry covers an important place in the horticultural industry, particularly in the Mediterranean countries, which produce around 1.6 million tons annually, comprising almost 18% of the world production.

Fragaria vesca is a perennial plant that produce small, very fragrant fruits in a copiously way. These plants can adapt to different kinds of situations thanks to its rhizomes.

Certified plants of the strawberry cultivar ‘Regina delle valli’ were used as plant material. Different proportion (0, 50 and 100% v/v) of the treated sediment (TS) were mixed with a standard commercial peat-based substrate, composed by 60% peat and 40% of inert pumice: i) TS0 = 100% TS; ii) TS50 = 50% peat-based substrate and 50% TS; iii) TS100 = 100% treated sediment. Two different water regimes were applied in order to evaluate their effect on plant growth and productivity in relation to the substrate mixtures tested: normal = 960 cc average annual water supply per pot and per day (WR1); reduced by 45% = 540 cc average annual water supply per pot and per day (WR2). A total of 90 certified micropropagated plants were grown for each ‘substrate x water regime’ combination (3 substrates x 2 water regimes, replicated in 3 blocks, each consisting of 5 plants). Certified plants were planted in 80x50 cm plastic plant-boxes filled with different substrates and water regimes at the Franceschini facility (Montecarlo, LU, Italy) during the period June 2020 – March 2021. Fertigation was carried out once a week, providing Universol® water soluble fertilizer NPK 15:7:30 + 3.0 MgO + TE in the range of 200 g per plant in the periods May-June and September-April, and 400 g per plant from July to August. These doses were recommended for enhancing crop development and improving gustatory qualities and shelf life of final product. Plant growing and physiological parameters (crown diameter, maximum shoot length, number of leaves per plant, leaf area, leaf colour and leaf chlorophyll content) were monitored and collected at

monthly intervals. Fruit yield was determined as total production over the whole plant growth and fruits were harvested when considered “marketable” (characterized by a red colour all over the fruit). Only fruits without any anomalies were considered. For each treatment, 30 fresh strawberries were randomly selected and used for pomological characterization: fruit diameter, fruit height, fruit weight, fruit colour

expressed by chroma index, fruit pulp titratable acidity and soluble solids content were determined. At the end of the second growing season the aerial and root plant fresh and dry weight were measured on randomly selected plants of each treatment.

The results of the morphological traits analysis showed that wild strawberries grown on TS50 and TS100 substrate mixtures exhibited significant higher average values of crown diameter and shoot development. Conversely, leaf area was considerably reduced in plants grown on sediment-based media. The greater leaf development of strawberries grown on peat-based media might be attributed to peat organic matter content and other available nutrients. No noticeable differences were detected in the number of leaves, leaf chlorophyll content (SPAD index) and chroma index among the three tested substrate mixtures.

The water regime affected only leaf chlorophyll content index. SPAD values were higher when plants were watered with WR1 than WR2, denoting a decreased photosynthesis in treatments with lower water availability.

An increase in the number of fruits in strawberries with larger crown diameter has been previously reported. It was supposed that larger

crown diameters, besides having a higher energy reserve, have also a high number of buds that can

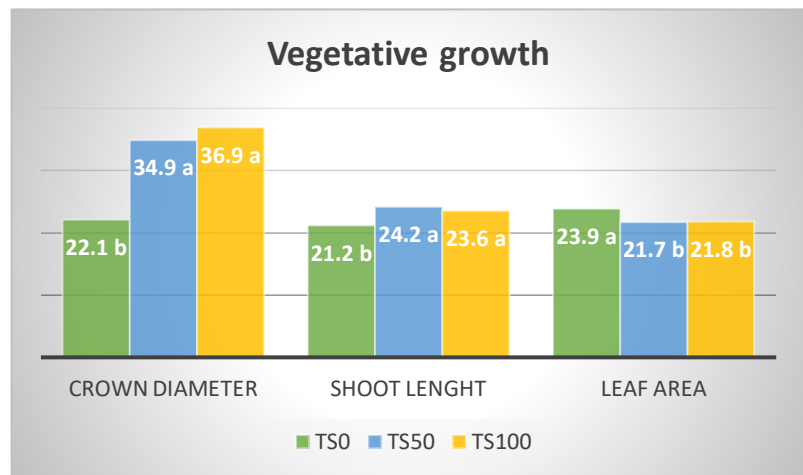


Figure 30. Influence of growing medium (TS) on *F. vesca* growth. Crown diameter (mm); shoot length (cm); leaf area (mm²). Mean values within each column followed by different letters are significantly different at $P \leq 0.01$

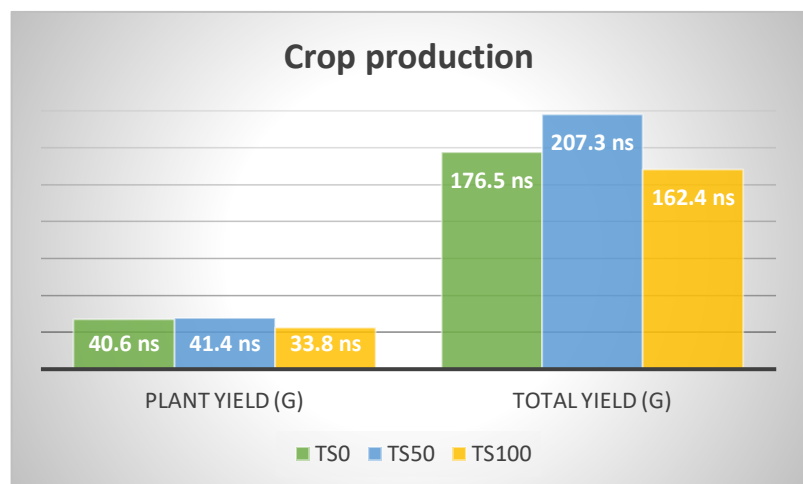


Figure 31. Effect of growing medium (TS) on *F. vesca* fruit production. Total yield = total fruits per growing medium over one growing season. ns = non-significant

differentiate into flowers and fruits. However, in our study, the growing medium composition did not significantly affect productive plant parameters.

On the other hand, a significant effect on crop yield was observed according to the different applied water regime. The highest average total production was obtained in control plants with greater water availability, but when the water supply was reduced by 30% fruit production obtained on TS0 dropped down dramatically. In TS50 and TS100, the reduction of water volumes resulted of much less importance on fruit production probably due to the greater water retention capacity of the sediment matrix compared to peat.

Diameters of fruits were statistically influenced by growing media; TS50 and TS100 produced larger fruits than control (TS0). Water regime had a significant effect on fruit weight, chroma index and soluble solids content, that exhibited higher values with WR1. Fruit height and titratable acidity showed similar trends for all types of growing media and water regime.

Among the tested growing media, no significant differences were found for all of the considered biomass parameters, while the water regime statistically affected both the above ground biomass and



Figure 32. Comparison between strawberry plants grown on TS0 (left) and TS50 (right) 40 days after planting



Figure 33. Wild strawberry plant grown on TS50: inflorescence and fruit bearing shoot

total plant dry weight.

Analytical methods for determining organic and inorganic contaminants presence are very complex and therefore were carried out on fresh product and certified by accredited laboratories.

Analyses were made to assess the health risk for humans, evaluating the health risk index for Cr, Ni, Cu, Pb, Cd, Mn and Zn by consumption of contaminated fruits. Other contaminants were compared with the European and Italian legislation safe limits. Analysis of organic and inorganic contaminants showed no risk for human health for every growing media, indicating the marketability of strawberry fruits.

Nutraceutical properties are also an important aspect of fruits quality. Consumers prefer tasty fruits, but an important added value is the contribution that their consumption can offer to

human health. Nutraceutical qualities of strawberry fruits were assessed for each cultivar x growing media treatment. The following nutraceutical parameters were analyzed: total soluble substances, total polyphenol, oxidizing capacity, percentage of glucose, percentage of fructose and the percentage of total sugar. The nutraceutical quality of fruits was similar in TS0 and TS50, while decrease in TS100 if compared to TS0, regardless of the water supply.

Recommendations

The use of treated sediment in proportion less than 50% can allow for growth and development of plants similar or better to that observed on commercial soil.

Wild strawberry grows well on all types of substrates; however, it is recommended to use a percentage of marine sediment not exceeding 50% in order to avoid a decrease in fruit yield and quality, especially from a sensorial and nutraceutical point of view.

The use of sediment-based growing media can reduce water consumption, due to the greater water retention capacity of the sediment matrix compared to peat.

Soilless cultivation of *Vaccinium corymbosum* L. (blueberry) cvs. ‘Duke’ and ‘Bluecrop’

Blueberry is a fruit belonging to the Ericaceae family and native to several regions of Europe and the United States, where this species has a significant commercial importance. Blueberries are very appreciated both for the taste and quality, being an important source of phenolic compounds that confer these berries very high antioxidant and antiradical properties, declared as a “source of longevity” for human life.

Plants are deciduous shrubs with a spreading habitus that grow best in well-drained but moisture-retentive acid soils (pH 4.0 to 5.5) with high organic matter (superior to 5%). In soils where the pH is superior to 5.5 it is necessary to add sulfur, otherwise the plant will not correctly develop.

Certified blueberry plants cvs. ‘Duke’ and ‘Bluecrop’ were planted at Franceschini facility (Montecarlo, LU, Italy) in 35-L plastic pots and grown under greenhouse conditions. Three different growing media (GM), combining different proportions of the treated sediment (TS) with a standard peat-based substrate (peat 60%, 40% pumice v/v), were tested: i) TS0 = 100% standard peat-based substrate; ii) TS50 = 50% peat-



Figure 34. Fruit set of ‘Bluecrop’ grown in TS0

based substrate and 50% TS; TS100 = 100% phytoremediated sediment. Two different water regimes were applied in order to evaluate their effect on plant growth and productivity in relation to the substrate mixtures tested: normal = 480 cc average annual water supply per pot and per day (WR1); reduced by 25% = 360 cc average annual water supply per pot and per day (WR2). Pots were arranged in three blocks, each consisting of 3 pots per GM* WR*CV, for a total of 108 pots (3 pots x 3 substrate mixtures x 2 water regimes x 2 cultivars x 3 blocks).

Plant growth and productivity were monitored in terms of stem diameter, plants height, number of sprouts, number of flowers, and leaf area during each growing season. After two years of cultivation, disruptive analyses were performed one plant for each GM*WR*CV*Block combination. Fresh and dry weight of aerial and root parts was assessed, and the ratio between fresh and dry weight was calculated.



Figure 35. Inflorescence of 'Bluecrop' plant grown in TS50



Figure 36. 'Bluecrop' berries obtained on TS50 at veraison stage

Results showed that all growing parameters very significant affected by the growing media regardless of the tested cultivar (Table 5). Even though all the plants suffered during the summer period from the unfavorable environmental conditions imposed by the crop protection, blueberry plants grown on TS0 exhibited increased stem diameter, plant height, number of sprouts and fruit production compared to sediment-based substrate mixes. These data indicate that the remediated marine sediment is not suitable for the cultivation of this species, due to its high pH and bulk density as well as low TOC percentage. Fruits of cv 'Bluecrop' displayed better morphological and physiological patterns than 'Duke'. Water supply did not affect plant development, flower differentiation and fruit set.

Table 7. Influence of growing medium on blueberry growth

Cultivar	Growing medium	Plant height (cm)	Diameter (mm)	Sprouts (n)
Duke	TS0	119.28 a	25.27 ab	10.75 a
	TS50	65.52 c	18.64 c	7.17 b
	TS100	63.61 c	15.16 d	6.33 b
Bluecrop	TS0	126.03 a	27.35 a	12.56 a
	TS50	86.54 b	24.61 b	6.83 b
	TS100	78.59 b	24.33 b	6.46 b

Mean values within each column followed by different letters are significantly different at $P \leq 0.01$.

At the end of the cultivation trial, analyses of organic and inorganic contaminants were conducted on different fresh portions of the plants (root, stem and leaf) in order to assess the ascendant translocation and allocation of pollutants in the different plant organs. Analytical methods certified by accredited laboratories were used. Data on heavy metals (Cr, Ni, Cu, Pb, Cd, Mn and Zn) and polycycles aromatic hydrocarbons harmful to human health showed average concentrations under the risk limits for human health.

Recommendations

Root system quality involves root number and root length, and these factors are desirable because they favour water and nutrient adsorption and are related to substrate physical and chemical characteristics. According to the results obtained in our trial, the phytoremediated sediment is not favourable for root development and shoot sprouting of blueberry plants. In fact, this matrix exerts an evident negative effect on root counts and length, due to its not very porous structure and high bulk density. Ultimately, this entails a lowered developmental rate in plants grown on sediment-based growing media, especially TS100.

The incorporation of treated sediment in a dose $\geq 50\%$ is not recommended for blueberry cultivation, although cultivar ‘Bluecrop’ performs quite better than ‘Duke’ on TS50. Reducing the sediment percentage in the substrate ($> 50\%$) could allow a greater plant development and fruit production.

Blueberries are plants that grow on acid, well-drained, moist soils with high organic matter. On the other hand, the remediated sediment is characterized by a high bulk density and an alkaline pH, contains relatively large amounts of silt and clay fractions and is poor in organic carbon and, compared to peat, has lower porosity, water capacity and air capacity. Therefore, an improvement of the physico-chemical parameters of sediment-based growing media is necessary for achieving adequate blueberry yields and this could be obtained by varying the ratio and type of substrate components and adjusting the pH of the media before planting.

Growing *Ocimum basilicum* L. (basil) cvs ‘Genovese’ and ‘Valentino’ in pots

Basil, cvs ‘Genovese’ and ‘Valentino’, was chosen as model plants as aromatic leaf species seedling production. This aromatic plant was chosen for its importance in Italy regarding food and medicine. Basil is cultivated by seed and is the most loved and popular herb in Italy, with a very short spring-summer cycle. Annual herb, suitable for the Mediterranean climate. It grows well in all types of soils, but does its best in well-drained, moist - nutrient rich soil with a neutral pH.



Figure 37. Basil cultivation

Basil seeds were sown at Simoncini facility (Pescia PT, Italy). The study consisted of two separate trials.

The first one was performed in Autumn 2020 by testing the peat substrate (TS0 = control) in comparison with Pe:TS50 and pure treated sediment (TS100). Totally 7,452 seeds (3,726 for each cv) were sown manually in 0.75-L pots. The second trial was started in Spring 2021 reducing by half the percentages of TS used in the first test (Pe:TS25 and Pe:TS12.5 vs T0). Totally 7,452 seeds (3,726 for each cv) were sown manually in 1-L pots. Pots received different amounts of water: WR1: normal; WR 2: low (reduced by 30%); WR3: very low (reduced by 57%). Twelve pots (containing 23 seeds) for each combination “substrate-water regime” were replicated 3 times, for a total of 162 pots per cultivar. Germination rate, physiological parameters and the transfer of nutrients and toxic metals to the leaves were analysed.

Basil production was significantly influenced by the growing media, being total fresh weight and leaf area significantly reduced in plants obtained on growing media containing high amounts ($\geq 50\%$) of treated sediment. However, the phytoremediated sediment was successfully and safely used in the proportion from 12.5 to 25% (v/v) with peat to produce fresh basil herb pots with spring sowing. Under these growing conditions, plants did not show any visible phytotoxic symptoms or damages.

Recommendations

Basil is an annual herb that grows well in all types of soils, but does its best in well-drained, moist - nutrient rich soil with a neutral pH. The sediment is predominantly a mixture of silts and clays, therefore its portion in the substrate mixture must be well balanced so as not to make the substrate too heavy. High amounts of treated sediment affect basil production and quality; however, decreasing

the sediment percentage in the substrate (< 50%) could allow crop growth and productivity very similar to those obtained using a common peat based commercial substrate.

It is recommended to use 12.5 - 25% (v/v) mixed treated sediment with peat to get good quality of basil pots. The sediment-based substrate allows to save water for irrigation.