











LIFE17 ENV/IT/000347

### SUBSED TIMETABLE



	Action	20	)18			201	9		20	20		2	021	L
Number	Name of the action		111	IV	I	11	II IN	/ 1	П		IV I		1	IV
A	Preparatory actions													
A1	Review of the EU and national regulations on the use of sediments for plant nursery and analytical protocol													
В	Implemenation actions (obligatory)													
B1	Phytoremediated sediment trated via landfarming process													
В2	Demostrationof the use of remediated sediment as a substrate for nursery production													
В3	Demostrationof the use of remediated sediment as a substrate for non food crop cultivation													
В4	Demostrationof the use of remediated sediment as a substrate for food crop production													
В5	Training courses, workshop and guidelines for project replicatibility and transferability													
B6	SUBSED business plan													
с	Monitoring of the impact of the project actions (obligatory)													
C1	Monitoring and validation of the treated sediment													
C2	Monitoring and validation of the used of remediated sediment as a substrate for plant nursery and cultivation: non food crops													
С3	Monitoring and validation of the used of remediated sediment as a substrate for plant nursery and cultivation: food crops													
C4	Monitoring of socio-economic impact of the project and LCA													
C5	Performances indicators monitoring													
D	Public awareness and dissemination of results (obligatory)													
D1	Project dissemination plan (materials, web-site, articles, video, Layman's report)													
D2	Project dissemination plan (evenst and networking)													
E	Porject management (obligatory)													
E1	Porject management (FLORA)													
E2	SUBSED audit													
E3	SUBSED after-life													





## Action B2: Demonstration of the use of remediated sediments as a substrate for nursery production

	Action	2018 2019		9		20	)20			202:	1				
Number	Name of the action	Т	II	Ш	IV	I	11 1	II IV	1	п	ш	IV	I	11 17	ii iv
В	Implemenation actions (obligatory)														
В2	Demostrationof the use of remediated sediment as a substrate for nursery production														

Foreseen start date: 01/04/2019 Foreseen end date: 31/03/2021 Actual start date: 08/06/2020 Actual end date: -----

- Action performed by CREA and FLORA
- Nursery production of semi-hardwood cuttings of Prunus laurocerasus (laurel)

Evergreen ornamental: Prunus laurocerasus 'Novíta'







### Action B2: Demonstration of the use of remediated sediments as a substrate for nursery production

**B2.1: Nursery production of ornamentals** Procedures for *Prunus laurocerasus* (rooted cuttings)

Starting material: Cherry Laurel (common name), Cultivar Novìta

### Substrates:

- LMix 1: 100% peat-based substrate, taken as control treatment (60% peat, 40% pumice)
- LMix 2: 75% peat-based substrate and 25% treated sediment (v/v)
- LMix 3: 50% peat-based substrate and 50% treated sediment (v/v)
- LMix 4: 75% coconut fiber-based substrate and 25% treated sediment (v/v)
- LMix 5: 50% coconut fiber-based substrate and 50% treated sediment (v/v)
- LMix 6: 75% wood fiber-based substrate and 25% treated sediment (v/v)
- LMix 7: 50% wood fiber-based substrate and 50% treated sediment (v/v)

Water regimes: WR1: normal water regime WR2: low water regime

### **Experiment period:**

Laurel plants were planted at the Azienda Agricola Franceschini (Pescia, Italy) on 08/06/2020 in a greenhouse. The experimentation will last one growing cycle (one year).







### Action B2: Demonstration of the use of remediated sediments as a substrate for nursery production

**B2.1: Nursery production of ornamentals** Procedures for *Prunus laurocerasus* (rooted cuttings)

#### **Cultural practices:**

Pest and disease control as usual; Fertilization is being performed weekly with 4 g per pot with a water-soluble ternary fertilizer (15% N, 7% P2O, 30% K2O)

#### **Experimental design:**

For each substrate and water regime, 4 pots (each once consisting in 2 rooted cuttings) were prepared. Each substrate x water regime were replicated three times for a total of 336 laurel rooted cuttings.





Dop 1	WR1	LMix 7	LMix 4	LMix 5	LMix 2	LMix 1	LMix 6	LMix 3
керт	WR2	LMix 7	LMix 4	LMix 5	LMix 2	LMix 1	LMix 6	LMix 3
Dan 1	WR1	LMix 6	LMix 1	LMix 3	LMix 7	LMix 5	LMix 4	LMix 2
кер 2	WR2	LMix 6	LMix 1	LMix 3	LMix 7	LMix 5	LMix 4	LMix 2
Dan 2	WR1	LMix 5	LMix 3	LMix 2	LMix 6	LMix 4	LMix 7	LMix 1
керэ	WR2	LMix 5	LMix 3	LMix 2	LMix 6	LMix 4	LMix 7	LMix 1





	Action	2018 2019		2018 2019		2018 2019				2	2020			21	
Number	Name of the action	I	П	ш	IV	I	11 1	II IN	1	I	ı III	IV	Ι	11	III IV
В	Implemenation actions (obligatory)														
В3	Demostrationof the use of remediated sediment as a substrate for non food crop cultivation														

Foreseen start date: 01/04/2019Actual start date: 01/06/2020Foreseen end date: 31/03/2021Actual end date: -----

- Action performed by CREA and FLORA
- Non-food crops cultivation (from plantlets to final production: flowers/ornamentals)

Cut flower: Calla lily (*Zantedeschia aethioica*)



Potted plant: King Protea (Protea cynaroides)



Evergreen ornamental: Prunus laurocerasus







**B3.1: Flower and ornamentals** Pocedures for *Zantedeschia aethiopica* (cut flower production)



Starting material: Calla lily (Common name)

### Substrates:

**CMix 1:** 100% peat-based substrate (60% peat and 40% pumice v/v), considered as control treatment **CMix 2:** 75% peat-based substrate (45% peat and 30% pumice v/v) and 25% treated sediment (v/v) **CMix 3:** 50% peat-based substrate (30% peat and 20% pumice v/v) and 50% treated sediment (v/v)

#### Water regimes:

WR1: normal water regimeWR2: low water regime (30% reduced water flow)WR3: very low water regime (50% reduced water flow)

#### **Experimental period:**

Calla rhizomes were planted at Azienda Agricola Salvestrini (Pescia, Italy) in late summer (12/09/2020) under a greenhouse, as usually done for this species. Calla lily plants will be cultivated over two growing cycles (2 years).





### **B3.1: Flower and ornamentals**

Pocedures for Zantedeschia aethiopica (cut flower production)

### **Cultural practices:**

Pest, disease control and fertilization are being performed as typically done in the area of production (Tuscany) throughout the cultivation cycle

#### **Experimental design:**

Calla rhizomes were planted in 30-L containers, obtained from a specific arrangement of benches (21 m<sup>2</sup> surface, 20 cm depth), respecting a distance of 50 cm from each other.





Bench 1	WR1	Cmix 3	CMix2	Cmix 1	Cmix 3	Cmix 1	CMix2	Cmix 3	CMix2	Cmix 1
		Rep1	Rep1	Rep1	Rep 2	Rep 2	Rep 2	Rep 3	Rep 3	Rep 3
Bench 2	WR3	Cmix 3	CMix2	Cmix 1	Cmix 3	CMix2	Cmix 1	Cmix 3	Cmix 1	CMix2
		Rep1	Rep1	Rep1	Rep 2	Rep 2	Rep 2	Rep 3	Rep 3	Rep 3
Bench 3	WR2	CMix2	Cmix 1	Cmix 3	Cmix 1	CMix2	Cmix 3	CMix2	Cmix 1	Cmix 3
		Rep1	Rep1	Rep1	Rep 2	Rep 2	Rep 2	Rep 3	Rep 3	Rep 3





**B3.1: Flower and ornamentals** 

Pocedures for Protea cynaroides (flower pot plant)

Starting material: King Protea (Common name), cultivar Little Prince

### Substrates:

PMix 1: 100% peat-based substrate (79% peat, 8.5% agroperlite, 12.4% pomice v/v)

PMix 2: 25% treated sediment and 75% peat-based substrate

PMix 3: 50% treated sediment and 50% peat-based substrate

PMix 4: 25% treated sediment and 75% coconut fiber-based substrate (60% fiber, 40% midollo)

Pmix 5: 50% treated sediment and 50% coconut fiber-based substrate (60% fiber, 40% midollo)

Pmix 6: 25% treated sediment and 75% coconut fiber-based substrate (70% midollo, 30% fiber)

**Pmix 7**: 50% treated sediment and 50% coconut fiber-based substrate (70% midollo, 30% fiber)

### Water regimes:

WR1: normal water regimeWR2: low water regime (30% reduced water flow)WR3: very low water regime (50% reduced water flow)

### **Experimental period:**

Protea plants were planted indoor at the Azienda Agricola Simoncini (Pescia, Italy) on 02/06/2020. Potted plants will be cultured over two growing cycles (2 years) under a greenhouse.







### **B3.1: Flower and ornamentals**

Pocedures for Protea cynaroides (flower pot plant)

### **Cultural practices:**

Before the cultivation, each pot was fertilized with 4g per pots with Osmocote fertilizer. Other cultural practices, such as weed and pest control, were performed as typically done in the area of production throughout the cultivation cycle.

### **Experimental set up:**

For each "combination substrate and water regime", 10 rooted cuttings were planted in 2-L pot, replicated three times, for a total of 630 plants.







	RW3	RW2	RW1
	PMix 2	PMix 5	PMix 6
	PMix 6	PMix 6	PMix 3
	PMix 1	PMix 4	PMix 2
Rep 1	PMix 4	PMix 1	PMix 4
	PMix 3	PMix 7	PMix 7
	PMix 7	PMix 3	PMix 1
	PMix 5	PMix 2	PMix 5
	PMix 2	PMix 7	PMix 3
	PMix 3	PMix 2	PMix 2
	PMix 5	PMix 1	PMix 5
Rep 2	PMix 7	PMix 6	PMix 4
	PMix 4	PMix 3	PMix 6
	PMix 6	PMix 5	PMix 1
	PMix 1	PMix 4	PMix 7
	PMix 7	PMix 5	PMix 3
	PMix 3	PMix 6	PMix 6
	PMix 2	PMix 3	PMix 5
Rep 3	PMix 4	PMix 2	PMix 2
	PMix 1	PMix 4	PMix 4
	PMix 6	PMix 1	PMix 7
	PMix 5	PMix 7	PMix 1





### **B3.1: Flower and ornamentals**

Pocedures Prunus laurocerasus (rooted cuttings)

Starting material: Cherry Laurel (common name), Cultivar Novita

#### Substrates:

- LMix 1: 100% peat-based substrate, taken as control treatment (60% peat, 40% pumice)
- LMix 2: 75% peat-based substrate and 25% treated sediment (v/v)
- LMix 3: 50% peat-based substrate and 50% treated sediment (v/v)
- LMix 4: 75% coconut fiber-based substrate and 25% treated sediment (v/v)
- LMix 5: 50% coconut fiber-based substrate and 50% treated sediment (v/v)
- LMix 6: 75% wood fiber-based substrate and 25% treated sediment (v/v)
- LMix 7: 50% wood fiber-based substrate and 50% treated sediment (v/v)

Water regimes: WR1: normal water regime WR2: low water regime

### **Experimental design**

The laurel cultivation tests will be carried out on the same plants used in the propagation phase for a further vegetative cycle. Same growing substrates, water regimes, replicates and experimental design will be applied as described in action B2.







	Action		20	18			203	19			202	20		2	021	
Number	Name of the action	I	Π	ш	١V	Т	Ш	III	١V	Т	П	ш	IV	1 1	П	I IV
В	Implemenation actions (obligatory)															
В4	Demostrationof the use of remediated sediment as a substrate for food crop production															

Foreseen start date: 01/04/2019 Foreseen end date: 31/03/2021 Actual end date: ------

Actual start date: 01/09/2020

- Action performed by CREA, FLORA and CARBONSINK •
- **Basil cultivation**

Ocimum basilicum (seeds)







### Action B4: Demonstration of the use of remediated sediments as a substrate for food crops production BMix 1

**B4.1: Basil cultivation** 

Procedures for Ocimum basilicum (seeds)

Starting material: Cultivar Genovese and Valentino

### Substrates:

- BMix 1: 100% peat-based substrate, taken as control treatment
- BMix 2: 50% peat-based substrate and 50% treated sediment (v/v)
- BMix 3: 100% treated sediment (v/v)

Water regimes:

WR1: normal water regimeWR2: low water regime (30% reduced water flow)WR3: very low water regime (50% reduced water flow)

### **Experiment period:**

Basil seeds were sown in plastic pots (12 cm of diameter and 0.75 L) placed indoor at the Azienda Agricola Simoncini (Pescia, Italy) on 08/06/2020. The experimentation will last at the growth stage of 2-4 couple of true leaves. Basil experimentation will also be replicated next spring 2021.











**B4.1: Basil cultivation** 

Procedures for Ocimum basilicum (seeds)

### **Cultural practices:**

Pest, disease control and fertilization are being performed as typically done in the area of production (Tuscany) throughout the cultivation cycle.

### **Experimental design:**

For each cultivar, the combination "substrate-water regime" consisted of 12 pots (each containing 23 seeds) and was replicated three times, for a total of 324 pots per cultivar.

	Rep 1	Rep 2	Rep 3
	Mix 2	Mix 3	Mix 1
WR3	Mix 1	Mix 2	Mix 3
	Mix 3	Mix 1	Mix 2
	Mix 1	Mix 3	Mix 2
WR2	Mix 3	Mix 2	Mix 1
	Mix 2	Mix 1	Mix 3
	Mix 1	Mix 3	Mix 2
WR1	Mix 2	Mix 1	Mix 3
	Mix 3	Mix 2	Mix 1

For each pot, 23 seeds were sown



Seeds were covered with a thin layer of vermiculite







### Action C2: Monitoring and validation of the use of remediated sediments as a substrate for plant nursing and cultivation: non-food crops

	Action		20	)18		2	2019	)		20	20		20	021	
Number	Name of the action	1	11	Ш	IV	1	11	IIV	1	П	III   I	/ 1	11	Ш	IV
С	Monitoring of the impact of the project actions (obligatory)														
C2	Monitoring and validation of the used of remediated sediment as a substrate for plant nursery and cultivation: non-food crops														

Foreseen start date:	01/07/2019	Actual start date:	
Foreseen end date:	01/06/2021	Actual end date:	

- Action performed by CREA
- This action was started two month in advance for better preparing field and laboratory protocols
- Sub-action C2.1 regarding the monitoring of nursery ornamental plants (i.e. laurel)
- Sub-action C2.2 regarding the monitoring of non-food crops cultivation (i.e. calla, protea and laurel)





### Action C2: Monitoring and validation of the use of remediated sediments as a substrate for plant nursing and cultivation: non-food crops

### C2.1: Procedures for laurel nursery production (rooted cuttings)

#### Plants and leaves

Data collection (non-destructive analysis). Recurrence: every 60 days from planting time (included) or more

- Plant mortality (number; percentage)
- Base stem diameter
- Maximum plant height
- Number of vegetative sprouts
- Length of primary vegetative shoot
- Number of fully expanded leaves on primary vegetative shoot
- Leaf blade colour (L, a, b coordinates) and chroma index (a<sup>2</sup> + b<sup>2</sup>)<sup>1/2</sup>
- Photosynthetic activity by CIRAS-2

Data collection (destructive analysis) - Recurrence: only at the end of the trial

- Leaf area
- Chlorophyll content
- Malondialdehyde (MDA) analysis for oxidative stress
- Heavy metal analysis







## Action C2: Monitoring and validation of the use of remediated sediments as a substrate for plant nursing and cultivation: non food crops

### C2.2: Procedures for Zantedeschia aethiopica (cut flower production)

#### Plants and leaves

Data collection (non-destructive analysis). Recurrence: according to the phenological phases (vegetative initial growth/re-growth, full bloom, vegetative dormancy)

- Plant mortality (number; percentage)
- Number and maximum height of stems
- Number of flowers
- Length of spathe
- Spathe colour and leaf blade colour (L, a, b coordinates) and chroma index  $(a^2 + b^2)^{1/2}$
- Photosynthetic activity by CIRAS-2

Data collection (destructive analysis). Recurrence: only at the end of the trial

- Leaf area
- Chlorophyll content
- · Fresh and dry weight of the aerial part, stem and whole plant
- Length, fresh and dry of root system
- Malondialdehyde (MDA) analysis for oxidative stress
- Heavy metal analysis

#### Phenology

- Re-growth time (second year)
- Flowering time (first flower first two completely open flowers in one plant; peak of flowering 50% of completely open flowers; last flower)







# Action C2: Monitoring and validation of the use of remediated sediments as a substrate for plant nursing and cultivation: non food C2.2: Procedures for Protea cynaroides (flower pot plant)

#### Plants and leaves

Data collection (non-destructive analysis). Recurrence: according to the phenological phases (vegetative initial growth/re-growth, full bloom, vegetative dormancy)

- Plant mortality (number; percentage)
- Number of stems with flowers
- Number, base diameter and maximum height of stems
- Number of flowers
- Shape and size of inflorescence
- Colour of flower cluster, surrounding bracts and paddle-shaped leaves
- Photosynthetic activity by CIRAS-2

#### Data collection (destructive analysis) - Recurrence: only at the end of the trial

- Leaf area
- Chlorophyll content
- Fresh and dry weight of the stem and whole plant
- Fresh and dry weight and length of root system
- Malondialdehyde (MDA) analysis for oxidative stress
- Heavy metal analysis

#### Phenology

- Re-growth time (second year)
- Flowering time (first flower first two completely open flowers in one plant; peak of flowering 50 % of completely open flowers; last flower)







# Action C2: Monitoring and validation of the use of remediated sediments as a substrate for plant nursing and cultivation: non-food crops

C2.2: Laurel nursery production (evergreen ornamental)

### 1. Plants and leaves

Data collection (non-destructive analysis). Recurrence: every 60 days or more

- Plant mortality (number; percentage)
- Base stem diameter
- Maximum plant height
- Number of vegetative sprouts
- Length of primary vegetative shoot
- Number of fully expanded leaves on primary vegetative shoot
- Leaf blade colour (L, a, b coordinates) and chroma index (a<sup>2</sup> + b<sup>2</sup>)<sup>1/2</sup>
- Photosynthetic activity by CIRAS-2

#### Data collection (destructive analysis) - Recurrence: only at the end of the trial

- Leaf area
- Chlorophyll content
- Fresh and dry weight of the whole plant
- Fresh and dry weight of stem (aerial part)
- Length, fresh and dry weight of root system
- Malondialdehyde (MDA) analysis for oxidative stress
- Heavy metal analysis

#### 2. Phenology

- Re-growth time
- Sprouting time





### Action C3: Monitoring and validation of the use of remediated sediments as a substrate for plant nursing and cultivation: food crops

	Action		20	18		2	2019	9		202	20		20	)21
Number	Name of the action	I	П	ш	IV		1	II IV	Т	П	III IV	v i	п	III IV
С	Monitoring of the impact of the project actions (obligatory)													
С3	Monitoring and validation of the used of remediated sediment as a substrate for plant nursery and cultivation: food crops													

Foreseen start date:	01/07/2019	Actual start date:	
Foreseen end date:	01/06/2021	Actual end date:	

- Action performed by CREA, FLORA and Carbosink
- This action was started two month in advance for better preparing field and laboratory protocols
- Sub-action C3.2 basil cultivation





### Action C3: Monitoring and validation of the use of remediated sediments as a substrate for plant nursing and cultivation: food crops

C3.2: Procedures for Ocimum basilicum (seeds)

#### **Plants and leaves**

Recurrence: every 7 days from planting time (included) or more

- Seed germination (number; percentage)
- Plant mortality (number; percentage)
- Number of leaves

Data collection at the growth stage of 2-4 couple of true leaves (end of demonstration)

- Number of leaves
- Maximum plant height
- Leaf blade colour (L, a, b coordinates) and chroma index  $(a^2 + b^2)^{1/2}$ ٠
- Leaf area ٠
- Chlorophyll content
- Fresh weight of the whole seedling
- Dry weight of the whole seedling

#### **Chemical parameters**

- Total polyphenols content
- Antioxidant activity: FRAP, ABTS and DPPH
- Organic acids (malic, citric, ascorbic)
- Sugars (glucose, fructose, sucrose)
- Essential oil content
- Analysis of organic and inorganic contaminants



BMix 1

BMix 2

BMix 3

