Suitability and impacts of 'CascadeReUse Systems' on irrigation water and soil

Deliverable D5.2

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List of abbreviations

AGRINUPES	Integrated monitoring and control of water, nutrients and plant protection products towards a sustainable agricultural sector
A-W	Autumn-Winter period

CRUS	CascadeReUse System
EC_{20}	Effective concentration of 20% - The concentration that causes an effect of 20% (e.g. 20% reduction in growth) or that affect 20% of the test organisms (e.g. 20% of mortality)
EC_{50}	Effective concentration of 50%
ELV	Emission Limit Value
FRes	Final reservoir
IRes	Initial reservoir
PPP	Plant Protection Products
Res	Reservoir
S-S	Spring-Summer period
Sub	Substrate
Sub_{1y}	Substrate with 1 year of use
Sub_{3y}	Substrate with 3 years of use
Sub_{5y}	Substrate with 5 year of use
WHC_{max}	Maximum Water Holding Capacity
WP	Work Package

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Summary

This report describes the results and conclusions of the Work Package (WP) 5 from AgriNuPes project, and aims at assessing the suitability and impact of horticultural semi-open systems (CascadeReUse systems; CRUS) – which reuse the drainages from the soilless main crop in the fertigation of a secondary crop grown on soil. The quality and safety of the drainages and the impacts on soil quality were assessed. Thus, to meet these goals, a set of tasks were performed in Portugal, at the Faculty of Sciences of the University of Porto, from April 2017 to March 2019 (M4-M24 of AgriNuPes). The assessment of the safety and quality of drainages was done through the quantification of the three main macronutrients for plants (nitrogen, phosphorus and potassium) and Plant Protection Products (PPP) residues, and through ecotoxicological

assays using aquatic species (*Aliivibrio fischeri*, *Raphidocelis subcapitata* and *Daphnia magna*) and following standard protocols to assess drainages from three selected Portuguese CRUS used for growing different crops (rose, strawberry and tomato) and in two periods (Autumn-Winter and Spring-Summer). The soil quality of one of the CRUS (strawberry) was assessed through ecotoxicological assays using the same aquatic species as for the drainages, terrestrial species (*Eisenia fetida* and *Folsomia candida*), soil enzymes´ activity, potential nitrification and potential mineralization, to assess the impacts of the fertigation with drainages from CRUS on soil retention (by testing soil elutriates) and habitat functions, as well as soil fertility, respectively.

Drainage samples were taken immediately after draining through the substrates (Sub), with different years of use when possible (Sub_{nY}), and from the reservoirs (Res) where the drainages were retained before reuse.

In general, the main macronutrients showed to remain along the systems, which corroborates the potential for drainages reuse. The higher concentrations of N and K were observed in drainages from the tomato CRUS, while the highest values for P were observed in the drainages from the rose CRUS. Nonetheless, the importance of using technological tools to support decision-making is reinforced by a crop-dependent consumption of nutrients in different proportions. The drainages showed to stimulate the bioluminescence of *A. fischeri* and the growth of *R. subcapitata*, which indicates the potential of the drainages to cause eutrophication in water bodies. Moderate toxicity to *R. subcapitata* was found for drainages from the substrates of the rose CRUS (Sub_{3y} EC_{20}=63%; Sub_{5y} EC_{50}=84%). This was the CRUS with higher levels of PPP residues recorded in the drainages. The drainages showed to be highly toxic to *D. magna*, as shown by 48h-EC_{50} as low as 9.2% (Sub_{3y}) or 10.8% (Res) from the strawberry CRUS. The high levels of conductivity may have been responsible for the acute response of this test organism.

Through the exposure of organisms to the whole soil samples fertigated with drainage from CRUS (terrestrial species) and to elutriates of those soils (aquatic species), it was shown that run-offs and leaching from those soils can cause toxicity to aquatic organism (*A. fischeri* EC_{50}=10.3 g L^{-1}; *R. subcapitata* EC_{50}=223 g L^{-1}) and that the CRUS can cause soil degradation by increased salinization, accumulation of PPP residues (under evaluation) and loss of fertility through reduction of soil microbial activity. The habitat function of these soils, as assessed in terms of survival and reproduction of both *E. fetida* and *F. candida*, is also affected.

In addition to what was defined in WP5, it was made a survey on the Emission Limit Values (ELV) for N and P for wastewater discharge and on environmental quality standards for surface water, according to the Portuguese legislation in force. These standards were used as referential for

the concentrations of macronutrients and PPP residues of the drainages. As such, according to those standards, it was also concluded that the drainages from soilless cultivation can be unsuitable for being discharged to the public sewage system, since the concentration of N and P were much higher than the ELV specific for each element.

While the CRUS are undoubtedly a better option than open systems (free drainage), procedures should be taken in order to mitigate their environmental impact and degradation of the agricultural area itself. The promotion of closed systems should be addressed to contribute to the European Union goals on climate changes adaptations, on soils biodiversity protection and to guarantee the sustainability and competitiveness of the horticulture sector under future forecasted environmental scenarios.

1. Introduction

In horticultural crops grown in substrate (semi-hydroponics), fertigation[1] is supplied ad libitum for an adequate growth of crops. Typically, the growers control fertigation through drainage volumes, which are commonly maintained between 30 to 40% of the total volume of the nutrient solution provided (Kooten et al., 2004). In respect to the reuse of the drainages, three types of systems can be considered: closed system, open system and semi-open system (CascadeReUse System, CRUS).

In the closed system, the drainage is collected in reservoirs and then recirculated to the same crop, after being mixed with fresh nutrient solution and corrected for electrical conductivity. In the open system the drainages are not reused and often simply released to the environment, and in the CRUS the drainages are reused, often only partially, for the fertigation of secondary crops (i.e., the crop which receives the drainage) grown on soil. As such, the non-recirculation of the drainages poses problems either in terms of efficiency of the soilless production or in terms of environmental impact in soilless production, since large amounts of water and nutrients are not recycled.

The WP5 of AgriNuPes project includes a set of five tasks focused on the CRUS in Portugal. After the conclusion of the first task, reported in D5.1 (Santos et al., 2017), where a general characterization of the CRUS was made through a questionnaire applied to horticultural growers, the following two tasks aimed at assessing the impact of CRUS on the quality and safety of irrigation/drainage water (T5.2) and on the quality of soil from the secondary crops fertigated with those drainages (T5.3).

[1] Fertigation is the plants' irrigation enriched with nutrients that were injected in the irrigation system.

For T5.2, three CRUS were taken as case studies from the cultivation of rose, strawberry and tomato. These crops were selected for their representativeness in terms of soilless cultivation in Portugal, and because of the expected different strategies in terms of nutritional management of each crop. Moreover, the samples were collected in the Autumn-Winter (A-W) and in the Spring-Summer (S-S) periods given the seasonality of the production management, which influences the fertigation management and the control of pests and diseases (Pardossi et al., 2011).

For T5.3, soil from the greenhouse under which the secondary crop from the strawberry CRUS was installed (in this case tomato), was assessed in terms of retention function for nutrients and PPP, habitat function and fertility, and compared with reference soils. For those purposes, the ecotoxicological assays were performed with aquatic species exposed to soil elutriates, terrestrial species directly exposed to the soil and it was assessed the soil´s enzymatic activity.

These tasks were performed from April 2017 to March 2019 (M4-M24 of AgriNuPes) in Portugal (FCUP), and the results are presented in this report.

2. Materials and methods

2.1. Chemical quality and safety of drainages from CRUS

Drainages were collected from three selected Portuguese horticultural growers that use CRUS for soilless cultivation of rose, strawberry and tomato. The rose and the strawberry CRUS were located in the Entre-Douro e Minho region and the tomato CRUS was located in the Beira Litoral region, in Portugal. After being collected and properly conditioned, the chemical quality of the drainages, its safety and subsequently its suitability for being recycled were assessed through (i) quantification of nitrogen and phosphorus levels, (ii) quantification of PPP residues levels (iii) and ecotoxicological assays.

2.1.1. Drainages sampling

The drainage samples were collected in the A-W and the S-S except for the tomato CRUS, of which only the S-S collection was performed until M24 of AgriNuPes. The sample collection for the A-W at this CRUS was planned for February 2019, but no drainages were available at that time due to a delay on the start of the cropping cycle. Those results will be made available later.

Drainages samples were collected immediately after draining through the substrate and from each reservoir used by the grower for drainage collection and storage. Whenever as possible, drainages from substrates with different years of use were collected. As such, at the rose CRUS, samples from substrates with three and five years of use in both sampling periods were

collected, and at the strawberry CRUS samples from substrates with one and three years of use were collected in A-W period. During the S-S sampling, only a substrate with the same age was being used in the strawberry CRUS. In what regards the reservoirs, two tanks were available at the rose and the tomato CRUS, while only one reservoir was being used by the strawberry CRUS. The scheme from Figure 1 and Table 1 describe the sampling design followed for the three CRUS, as well as sampling points and dates.

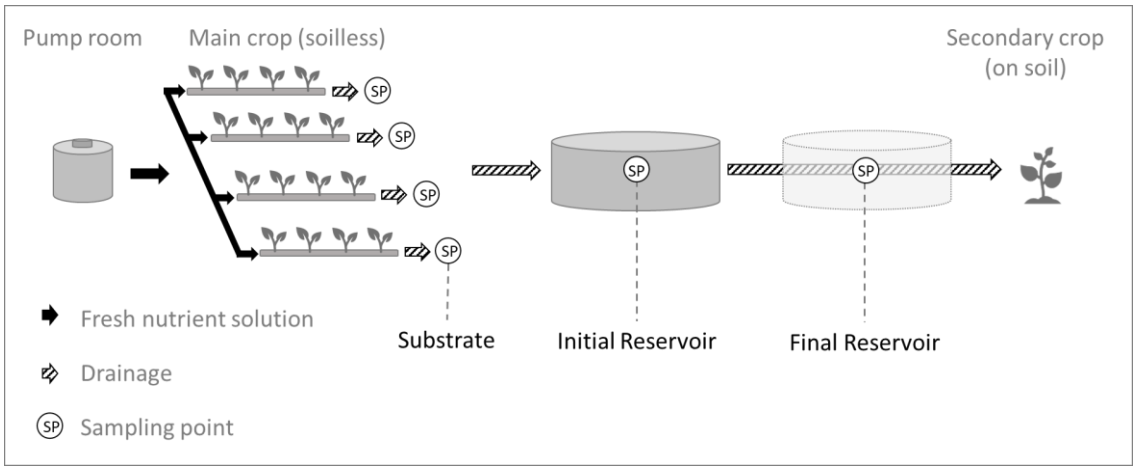

Figure 1. Scheme of the (a) irrigation flow, from the fresh nutrient solution until the drainage reuse on soil and scheme of the (b) CascadeReUse Systems (CRUS) from the three CRUS where drainage samples were collected. The main components of each production system and sampling points (SP) are shown in the scheme.

Table 1. Summary of sampling point identification and sampling collection from the CascadeReUse Systems from the three CascadeReUse systems where drainages were collected.

Sampling point (SP) identification	Sampling period	
Rose	**A-W (date)**	**S-S (date)**
Sub_{3y}	√ (15-11-2017)	√ (10-07-2018)
Sub_{5y}	√ (15-11-2017)	√ (10-07-2018)
IRes	√ (15-11-2017)	√ (10-07-2018)
FRes	√ (15-11-2017)	√ (10-07-2018)
Strawberry		
Sub_{1y}	√ (09-03-2018)	√ (18-09-2018)
Sub_{3y}	√ (09-03-2018)	nc
Res	√ (09-03-2018)	√ (18-09-2018)
Tomato		
Sub	nc	√ (14-09-2018)
IRes	nc	√ (14-09-2018)
FRes	nc	√ (14-09-2018)

Sub – drainage collected immediately after draining through the substrate, with indication of years of use (Sub_{ny}) when applicable| **IRes** – drainage collected from the initial reservoir | **FRes** – drainage collected from the final reservoir | **Res** – drainage collected from the only reservoir.
Note: nc indicates not-collected samples (see 2.1.1).

The drainage samples were collected in glass bottles, previously washed with a basic solution of NAOH (4%) , (i) immediately after draining from the substrate (Figure 2-a) and (ii) at the drainage reservoirs of each CRUS. For (i), composite samples were obtained by collecting a similar volume at the end of the gutter of lines of plants randomly selected. For (ii), samples were collected by dipping the bottles into the reservoir (Figure 2-b).

Each crop was installed in a different substrate. Rose was cultivated in 100% coco peat, strawberry was cultivated in a mixed substrate (humus, coco peat, blond peat and coconut fiber) and tomato was cultivated in a mixture of coco peat and coconut fiber. The exact composition of the substrates as well as its origin is not provided by the manufacturers.

The electrical conductivity (EC), the pH and the dissolved oxygen were measured in the samples with a multiparametric probe (HI 9828 Hanna Instruments, UK).

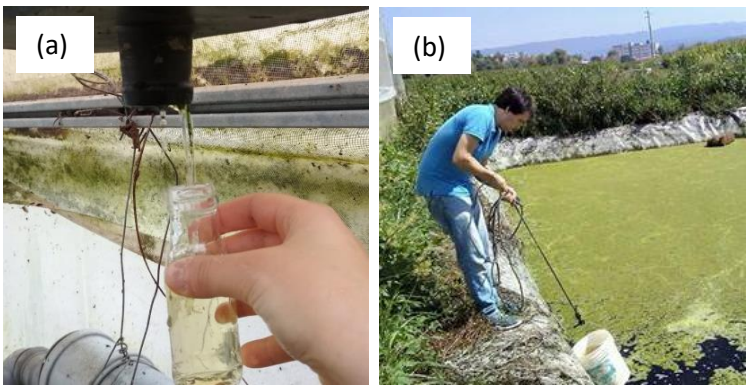

Figure 2. Drainage sampling at different sampling points in each CascadeReUse System: (a) immediately after draining through the substrate and at (b) the reservoirs were the drainages remain for later reuse on soil.

2.1.2. Quantification of nitrogen, phosphorus and potassium

The drainage samples for the determination of nitrogen (N) and phosphorus (P) were kept frozen at -18 °C from the day of collection. Before the quantification of total N and total P, the samples were slowly defrosted at 5 °C. The determination of N content was done colorimetrically through the chromotropic acid method (HI-93767A-50, Hanna Instruments, UK). The determination of P content was done colorimetrically following an adaptation of the Standard Methods for the Examination of Water and Wastewater, 20th edition, 4500-P C, vanadomolybdophosphoric acid method (HI-93758-0, Hanna Instruments, UK). Readings for N and P were performed using a multiparameter photometer (Hanna Instruments, C214).

The samples for the quantification of potassium (K) were acidified with nitric acid (1%) and kept at 4 °C. The determination of K content was performed by atomic absorption spectroscopy (GBC Avanta Sigma AAS).

For all macronutrients, the concentration recorded for each sample was the mean of three replicates. After a statistically significant difference was found between groups (sampling points of each CRUS) by one-way ANOVA ($p<0.05$), a Tukey post-hoc test was used to compare the means (IBM SPSS V25.0).

2.1.3. PPP residues in the drainages

The samples for PPP residues were kept frozen at -18 °C from the day of collection until being sent for analysis. The quantification of PPP was done by an external laboratory (Eurofins Hydrologie Est, UK). For the selection of the active ingredients to be analysed, it was taken into account the information previously collected from growers through a questionnaire (Deliverable 5.1) (Santos et al., 2017) on the most used PPP (strobilurins, organophosphates, carbamates, pyrethroids, triazoles and others).

2.1.4. Ecotoxicological assays using aquatic species

For the assessment of safety of the drainages, three aquatic species were used from different trophic levels in the food chain, including the marine bacteria *Aliivibrio fischeri*, the green microalgae *Raphidocelis subcapitata* and the microcrustacean *Daphnia magna* to perform standard ecotoxicological assays.

The acute toxicity of the drainages to *A. fischeri* was assessed using the Basic Test Protocol (81.9%) and the M500 analyzer from Microtox® (Modernwater, USA), by determining the inhibition of the bioluminescence of *A. fischeri* after 30 min of exposure to the different dilutions of the drainages.

Growth inhibition assays with the unicellular green algae *R. subcapitata* were performed according to the OECD 201 protocol (OECD, 2011). The algae were exposed for 72h in 24-well plates (Figure 3) to different dilutions of the samples, starting at 100% and sequentially diluting with Woods Hole – MBL (Marine Biological Laboratory) medium, using a dilution factor of 1.25, to compare their growth with control conditions (100% MBL).

The Effective Concentrations[2] (EC_{50} and EC_{20}) and corresponding 95% confidence intervals were calculated through non-linear estimation using the least squares method with the STATISTICA Version 8 software (StatSoft, inc).

[2] Effective Concentration is the concentration of the test substance expected to cause a given effect (EC_{50}, 50%; EC_{20}, 20%) in the test organism.

Figure 3. Daily resuspension of the unicellular green algae *Raphidocelis subcapitata* in the 24-well plate used for the growth inhibition assay.

In order to assess the impact of drainages on *D. magna*, it was used the immobilization test according to the OECD 202 protocol (OECD, 1984). The cladocerans were exposed for 48 h to different concentrations of drainages in glass tubes (Figure 4).

The EC_{50}, EC_{20} and corresponding confidence intervals were calculated through PROBIT non-linear regression (IBM SPSS V25.0).

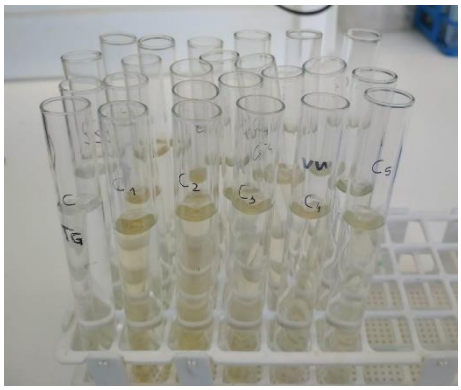

Figure 4. Immobilization test with the microcrustacean *D. magna* exposed to different dilutions of drainages from CascadeReUse systems in glass tubes.

2.2. Soil quality

Several ecotoxicological assays were performed to assess the soil habitat and retention functions, and soil's fertility. Furthermore, several soil physical and chemical parameters such as pH, electrical conductivity, maximum water holding capacity (WHC_{max}) and organic matter content were analysed.

2.2.1. Sampling and conditioning of soil

The strawberry CRUS mentioned above (in 2.1) was selected for assessing the impact of the drainages from CRUS on soil quality. In this CRUS, the drainages from the soilless main crop

(strawberry) are reused to irrigate a secondary crop (tomato) growing on soil and planted in ridges, under a plastic greenhouse with a covered area of 390 m^2.

The sampling was done in July 19th 2018. For sample identification, the ridges were numbered from R1 to R5, from the north side to the south side of the greenhouse. Composite soil samples were collected superficially (0-20 cm) along both sides of each ridge, which are drip irrigated with the drainages, and also two reference soils were sampled as controls, including soil from inside the greenhouse but not irrigated with the drainages (identified as INT) and soil from the outside of the greenhouse and representative of the area (identified as EXT).

A schematization of the soil sampling is presented in Figure 5.

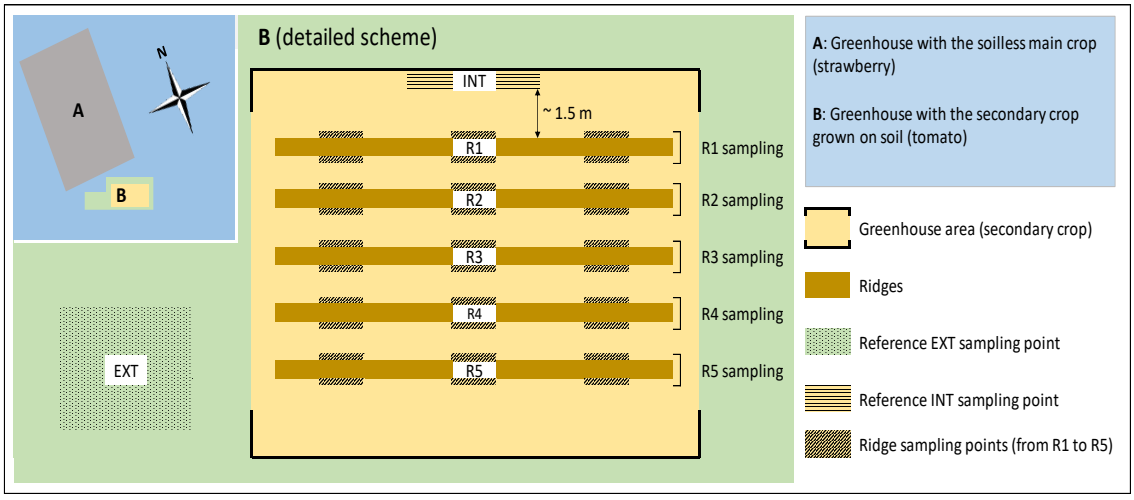

Figure 5. Schematic representation of the soil sampling design at the CascadeReUse System from the strawberry CRUS that reuses the drainages from the soilless main crop (strawberry) for the fertigation of a secondary crop (tomato) grown on soil (in ridges) under a plastic greenhouse.

Samples to be used for the ecotoxicological assays were maintained refrigerated at 5 °C, whereas samples for measuring soil enzymes activity, potential nitrification and potential mineralization and PPP residues were stored at -18 °C. For the PPP residues, before storage the samples were wrapped in aluminium foil.

For the soil porosity and bulk density determinations, plastic cylinders with an internal diameter of 4.3 cm and 5 cm height (volume core of 72.6 cm^3) were collected in every sampling point.

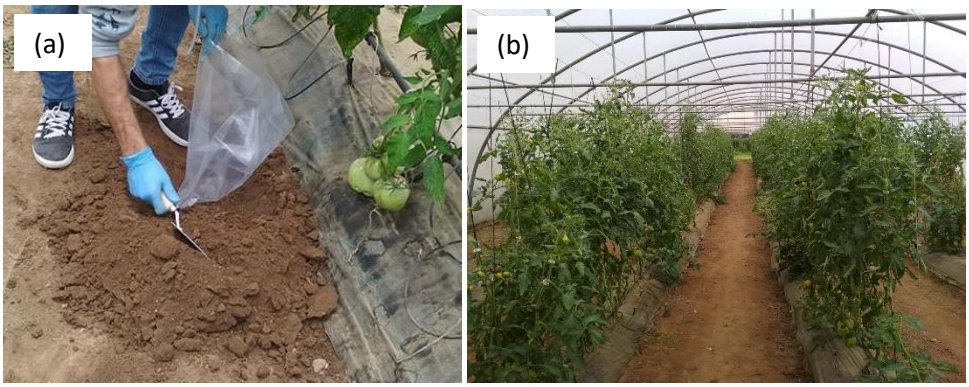

Figure 6. (a) sampling of soil along the ridges at the secondary crop. (b) overall view of the greenhouse covering the secondary crop.

2.2.2. Soil physical and chemical parameters

For the soil porosity and bulk density, the determinations were performed according to the USDA method (USDA, 2014).

The soil pH was determined according to the method described by the standard protocol ISO 10390 (ISO, 2005). After the pH has been measured, the soil:water suspension (1:5 m/v) was left overnight and the electrical conductivity was measured as described in Annex C of the ISO 11268-2 (ISO, 2012), by introducing the probe into the suspension without touching the settled soil. The WHC and the organic matter content were determined as described in ISO 17512-1 (ISO, 2008).

After a statistically significant difference was found between the means for each soil by one-way ANOVA ($p<0.05$), a Tukey's post-hoc test was used for multiple comparisons of the means (IBM SPSS V25.0).

2.2.3. Soil retention function: ecotoxicological assays with aquatic species

The aquatic species *R. subcapitata* and *A. fischeri* were used in the ecotoxicological assays with soil elutriates.

For the assays with *R. subcapitata* it was used the same protocol as for assessing the toxicity of the drainages (OECD 201 protocol). The Effective Concentrations (EC_{50} and EC_{20}) and corresponding 95% confidence intervals were calculated through non-linear estimation using the least squares method with the STATISTICA Version 8 software (StatSoft, inc).

For *A. fischeri*, it was used the solid-phase Basic Test protocol (Modernwater, USA). The acute toxicity of the elutriates to *A. fischeri* was assessed after 30 min of exposure to the different

dilutions. The data were processed with the Microtox® Omni Software, and whenever as possible EC values were estimated after adjusting a Probit model to data.

2.2.4. Soil habitat function

For assessing the habitat function of soil, two reproduction tests were performed using soil invertebrates, including the earthworm *Eisenia fetida* (Annelida: Oligochaeta) and the springtail *Folsomia candida* (Insecta: Collembola) which were exposed to 4 mm fraction of the soils sampled.

Survival adults and fecundity are presented as the average of each treatment. After a statistically significant difference was found between the means for each soil by one-way ANOVA ($p<0.05$), a Tukey's post-hoc test was used for multiple comparisons of the means (IBM SPSS V25.0).

Earthworms

In addition to sampled reference soils not irrigated by the drainages (internal and external to the greenhouse), it was also used OECD artificial soil as control. This soil was prepared by mixing 70% dry sand, 20% kaolin clay and 10% defaunated sphagnum peat. Adult and sexually mature earthworms from the species *E. fetida* were exposed to soil samples plus the control soil (Figure 7) for assessing the effects on reproduction according to the OECD 222 protocol. Survival was registered after 28 days of exposure and fertility was assessed after 56 days from the beginning of the assay.

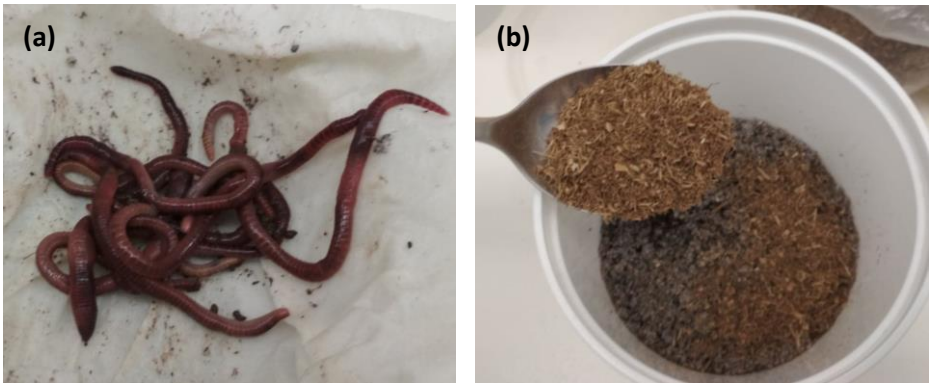

Figure 7. Preparation of the earthworms´ reproduction test. After (a) selection of adult and sexually mature individuals from the species *Eisenia fetida*, (b) exposure to soil samples were performed in plastic containers with 500 g of soil and feeding consisted of horse manure.

Springtails

The reproduction test with *F. candida* was performed following the ISO 11267 protocol (ISO, 2014). Survival of the springtails was registered after 21 days of exposure.

Figure 8. Eggs from the springtail (*Folsomia candida*) being transferred to new culturing medium, a procedure for the maintenance of the cultures and for obtaining juveniles with the same age for the reproduction assays.

2.2.5. Enzymes' activity

For assessing the fertility of soils, it was assessed the activity of dehydrogenase soil enzymes as well as enzymes involved in the cycles of carbon (CM-cellulase), nitrogen (urease), phosphorus (phosphatase) and sulfur (arylsulfatase). Potential nitrification and N-mineralization process were also assessed and compared with reference soils.

The soil samples were sieved with a 2 mm sieve and slowly defrosted at 5 °C, 48 h before the assays. The enzymatic activity was evaluated in three sub-replicates per soil sample, each one consisting in 1 g of soil in a 15 mL falcon tube. After incubation at optimal temperatures and oxidation of specific substrates, the enzymes´ activity was determined colorimetrically in 96-well microplates using a Multiskan™ FC Microplate Photometer (Thermo Fisher Scientific, USA) at the wavelength specified in the methodology used in each determination (Table 2).

Table 2. Summary of the methods used and incubation conditions for the determination of the enzymes activity of enzymatic process.

Enzyme or enzymatic process	Method	Incubation
CM-cellulase	Schinner and von Mersi (1990)	24 h; 50 °C
Nitrification process	modified method from Berg and Rosswall (1985)	5 h; 25 °C
N-mineralization process	Kandeler and Gerber (1988)	7 days; 40 °C
Urease	Kandeler and Gerber (1988)	2 h; 37 °C
Dehydrogenase	modified method from Thalmann (1968)	24 h; 40 °C
Phosphatase	adapted method from Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977)	1 h; 37 °C
Arylsulfatase	Strobl et al. (1996)	1 h; 37 °C

The values are presented as the mean of the three sub-replicates per sample. After a statistically significant difference was found between the means for each soil by one-way ANOVA ($p<0.05$), a Tukey post-hoc test was used for multiple comparisons (IBM SPSS V25.0).

3. Results and discussion

3.1. Chemical quality and safety of drainages from CRUS

3.1.1. Physical-chemical parameters

In the A-W period, the drainages had a pH ranging from 5.95 to 6.72. The drainages from the S-S period showed pH ranging from weakly acidic to neutral from 6.40 to 7.30. In terms of electrical conductivity, the drainages from the tomato CRUS showed the highest values, ranging from 3.1 dS m^{-1} (FRes) to 6.0 dS m^{-1} (Sub). The drainages from the rose CRUS showed slightly higher electrical conductivity at the S-S period (1.6 dS m^{-1}, IRes; 2.3 dS m^{-1}, Sub_{5y}) than in the A-W period (1.3 dS m^{-1}, Sub_{5y}; 1.6 dS m^{-1} Sub_{3y}) and the strawberry CRUS showed slightly higher electrical conductivity at the A-W period (1.3 dS m^{-1}, Sub_{3y} and Res; 1.5 dS m^{-1}, Sub_{1y}) than in the S-S period (0.9 dS m^{-1} Res; 1.0 dS m^{-1} Sub_{1y}).

The dissolved oxygen for the rose CRUS ranged from 66.4% (Sub_{5y}) to 82.0% (IRes) in A-W and from 59.6% (Sub_{5y}) to 72.1% (FRes) in S-S. For the strawberry CRUS the dissolved oxygen ranged from 46.2% (Sub_{1y}) to 57.3% in A-W and was 48.7% (Res) and 56.7% (Sub_{1y}) in S-S, and for the tomato CRUS the dissolved oxygen ranged from 57.0% (FRes) to 66.7% (Sub) in S-S.

3.1.2. Quantification of nitrogen, phosphorus and potassium

The overall results of the quantification of total nitrogen, total phosphorus and potassium in the drainages are presented in Table 3.

In terms of [N] in the drainages, the highest values were observed for the tomato CRUS in the S-S period, with [N] ranging from 175.3 mg/L (FRes) to 459.7 mg/L (IRes). The drainage from IRes of this CRUS showed N content 1.2-fold higher compared with drainage from Sub ($p<0.01$) and 2.6-fold higher content compared with FRes ($p<0.001$).

The drainages from the rose CRUS showed higher [N] in the A-W period than in the S-S period, but with no significant differences among sampling points, for each period. In the A-W period the values ranged from 121.3 mg/L (IRes) to 140.7 mg/L (FRes), and in the S-S period the values ranged from 49.3 mg/L (FRes) to 68.7 mg/L ($Sub5_{y}$). The lowest [N] were found in the drainages from the strawberry CRUS, ranging from 51.3 mg/L (Res) to 86.0 mg/L (Sub_{1y}) in the A-W period, and from 52.0 mg/L (Res) to 91.3 mg/L (Sub_{1y}) in the S-S period.

Relatively to the [P], the highest values were observed in drainages from the rose CRUS. The highest [P] was observed in the A-W period in drainage from Sub_{3y}, with 42.2 mg/L, which was

1.5-fold higher ($p<0.001$) compared with the drainage from the FRes (28.7 mg/L). In the S-S period, the drainages from the rose CRUS showed [P] ranging from 20.9 mg/L (IRes) to 29.7 mg/L (Sub_{3y}), but without significant differences among sampling points.

The drainages from the tomato CRUS showed significantly higher [P] in the drainages from the reservoirs compared with the drainage from Sub. The IRes showed [P] of 24.1 mg/L and the FRes showed [P] of 20.6 mg/L, being 2.1-fold ($p<0.001$) and 1.8-fold higher ($p<0.01$) compared with the drainage from Sub, respectively.

The [K] was higher in drainages from the tomato CRUS, ranging from 396.7 mg/L (FRes) to 691.7 mg/L (IRes).

The drainages from the rose CRUS showed higher [K] in the A-W period than in the S-S period, and a tendency to have higher [K] in the drainages from the substrates compared with the drainages from the reservoir. For example, in the A-W period, the drainage from Sub_{3y} showed [K]=395.5 mg/L, which was 2.3-fold higher ($p<0.001$) than the [K] in the drainage from the IRes (171.0 mg/L). In the S-S period, the drainage from the Sub_{5y} showed [K]=200.8 mg/L, which was 1.3-fold higher ($p<0.001$) than the drainage from the IRes (152.8 mg/L).

The drainages from the strawberry CRUS showed the lowest [K] of all CRUS, with [K]=15.0 mg/L in the drainage from Res, and [K]=13.1 mg/L in the drainage from Sub_{1y}.

In overall terms, it is worth to mention the tendency for N and P to remain, to a large extent, along the production systems, as generally observed for the rose and the strawberry CRUS. The much lower value for N from the FRes of the tomato CRUS might have been due to high consumption resulting from the algal bloom (Figure 2-b) and to microbial activity occurring at the reservoir. The nutrient concentrations observed for the generality of the drainages were well above levels that cause eutrophication (Baxter et al., 2016), but the higher nutrient content in the drainages from the tomato CRUS might have favored this process. Moreover, several other factors such as temperature (season), light exposure (open or closed reservoir) or pH might explain the differences on the maintenance of nitrogen in the reservoirs, not only by means of algal proliferation, but also by favoring the denitrification process carried out by microorganisms (Ni et al., 2016).

Taking into consideration the possibility of discharge of the drainages into the public sewage system – as could be the case in open systems or even in CRUS with partial reuse of the drainages – it is highly relevant to infer about the suitability of this action, concerning the nutrient content of the drainages. Regarding the discharge of wastewater, the Portuguese legislation stablishes the emission limit values (ELV) of 15 mg/L for N, and 10 mg/L for P, whereas no thresholds are indicated for K (Ministério do Ambiente, 1998). As such, in overall terms the drainages from the selected CRUS showed [N] and [P] well above the ELVs. Considering only the drainages from the

reservoirs of each CRUS, the [N] were 31-fold higher (S-S period) in the drainage from the tomato CRUS, 9-fold (A-W period) and 4-fold higher (S-S period) in the drainage from the rose CRUS, and 3-fold higher (A-W and S-S periods) in the drainage from the strawberry CRUS, compared with the ELV.

The [P] were 3-fold higher than the ELV in the drainage from the rose CRUS (A-W and S-S periods), 2-fold higher in the drainage from the tomato CRUS (S-S period) and 1.5-fold higher in the drainage from the strawberry CRUS (A-W period). Only the drainage from the strawberry CRUS, in the S-S period, was below the ELV for P.

Table 3. Total nitrogen, total phosphorus and potassium concentration of the drainages from the CRUS visited in the Autumn-Winter (A-W) and Spring-Summer (S-S) periods for sample collection at the different sampling points. Results are expressed as the mean ± SE of three replicates. Different letters indicate significant differences through Tukey´s test ($p<0.05$), after one-way ANOVA.

Drainage	N content (mg L^{-1})		P content (mg L^{-1})		K content (mg L^{-1})	
Rose	**A-W**	**S-S**	**A-W**	**S-S**	**A-W**	**S-S**
Sub_{3y}	124.7 ± 9.8 ^{a}	61.0 ± 33.0 ^{a}	42.2 ± 0.2 ^{a}	29.7 ± 5.2 ^{a}	395.5 ± 0.7 ^{a}	197.0 ± 0.4 ^{b}
Sub_{5y}	137.0 ± 5.0 ^{a}	68.7 ± 22.9 ^{a}	32.7 ± 0.8 ^{b}	26.7 ± 1.6 ^{a}	241.4 ± 1.5 ^{b}	200.8 ± 0.3 ^{a}
Ires	121.3 ± 6.4 ^{a}	53.7 ± 11.1 ^{a}	34.7 ± 0.9 ^{b}	20.9 ± 1.6 ^{a}	171.0 ± 5.3 ^{c}	152.8 ± 0.3 ^{d}
Fres	140.7 ± 4.1 ^{a}	49.3 ± 3.3 ^{a}	28.7 ± 0.2 ^{c}	25.2 ± 0.6 ^{a}	247.9 ± 0.9 ^{b}	156.6 ± 1.0 ^{c}
Strawberry						
Sub_{1y}	86.0 ± 12.1 ^{a}	91.3 ± 35.9 ^{a}	20.6 ± 0.6 ^{a}	0.9 ± 0.5 ^{a}	145.7 ± 0.4 ^{a}	13.1 ± 0.0 ^{b}
Sub_{3y}	43.7 ± 13.7 ^{a}	nc	11.5 ± 0.1 ^{c}	nc	73.9 ± 0.0 ^{b}	nc
Res	51.3 ± 3.7 ^{a}	52.0 ± 3.1 ^{a}	14.8 ± 0.2 ^{b}	0.7 ± 0.1 ^{a}	71.5 ± 0.1 ^{c}	15.0 ± 0.1 ^{a}
Tomato						
Sub	nc	394.0 ± 7.2 ^{b}	nc	11.3 ± 1.6 ^{b}	nc	448.3 ± 1.5 ^{b}
Ires	nc	459.7 ± 9.1 ^{a}	nc	24.1 ± 0.5 ^{a}	nc	691.7 ± 2.0 ^{a}
Fres	nc	175.3 ± 7.1 ^{c}	nc	20.6 ± 0.1 ^{a}	nc	396.7 ± 2.2 ^{c}

Sub – drainage collected immediately after draining through the substrate, with indication of years of use (Sub_{ny}) when applicable| **IRes** – drainage collected from the initial reservoir | **FRes** – drainage collected from the final reservoir | **Res** – drainage collected from the only reservoir.
Note: nc indicates non-collected samples (see 2.1.1).

3.1.3. PPP residues in the drainages

The main results of the PPP residues are briefly presented and discussed here. However, the results are not thoroughly presented, because they are reserved for publication in a scientific journal.

Relatively to the drainages from the rose CRUS, the obtained PPP residue levels were, in general, higher in the A-W period. The PPP with readings above the limit of detection for the drainages from rose CRUS were methiocarb, pyraclostrobin, chlorpyrifos-ethyl, dimethoate, lufenuron, difenoconazole, myclobutanil, abamectin, azoxystrobin, boscalid, clofentezine and trifloxystrobin. The highest values were observed for dimethoate (12.1-300.0 µg/L), myclobutanil (0.660-3.304 µg/L), boscalid (0.93-2.89 µg/L) and methiocarb (0.445-2.140 µg/L), all of them from the A-W sampling period. In general, the PPP residues were lower in the FRes, indicating the PPP might be degraded during the retention time at the reservoir. The most important exception, however, was for boscalid, which was 2.8-fold higher in FRes than in Sub_{5y}.

The drainages from the strawberry CRUS showed values of PPP residues above the limit of detection for eight PPP from the 15 analyzed. The highest values for the A-W period were observed for dimethoate (1.2-36.2 µg/L) and boscalid (3.97-14.80 µg/L), whereas the highest values for the S-S period were for boscalid (1.96-4.23 µg/L) and azoxystrobin (0.085-0.430 µg/L). As for rose, the drainages from strawberry production showed, in general, lower values of PPP in drainages from the reservoir than from the substrate, except for the much higher value for dimethoate at the A-W period (36.2 µg/L) compared with Sub_{1y} and Sub_{3y} (1.2 µg/L, for both).

Relatively to the drainages from the tomato CRUS, six PPP showed values above the limit of detection, but generally in only part of the sampling points and often with values only slightly above the limit of detection. It should be highlighted, however, the values for boscalid, ranging from 15.10 µg/L (Sub) to 29.80 µg/L (IRes).

In summary, the drainages from the rose CRUS presented more PPP residues in overall terms, especially in the A-W period. The results suggest a tendency of decreasing PPP residues after retention at the reservoirs, and that apparently there was not a clear relationship between the age of the substrates and the concentration of PPP residues in drainages. Nevertheless, in this respect it is worth mentioning the much higher value for dimethoate concentration from Sub_{5y} than from Sub_{3y} (7.4-fold) and for chlorpyrifos-ethyl from Sub_{3y} which was 2.8-fold higher than Sub_{1y}, both cases being from the A-W sampling period.

From the PPP analyzed, only the organophosphate chlorpyrifos-ethyl is considered a priority substance by the European Union, as referred in the Directive 2008/105/EC (European Comission, 2008), which defines environmental quality standards for water bodies. For this particular PPP, the maximum allowable concentration in surface waters is 0.1 µg/L. The drainages from the rose CRUS always showed residues of chlorpyrifos-ethyl, ranging from 0.010 µg/L to 0.033 µg/L in the A-W period, and 0.007 µg/L to 0.026 µg/L in the S-S period, whereas

for tomato CRUS, only the drainages from the substrate in the S-S period showed residues of chlorpyrifos-ethyl (0.007 µg/L) and in the drainages from the strawberry CRUS, this PPP was not detected.

The Portuguese legislation establishes an environmental quality standards for surface waters, a maximum value of 2.5 µg/L for total PPP and 0.5 µg/L for a single PPP (Ministério do Ambiente, 1998). Taking those thresholds as guidelines, it becomes apparent the unsuitability of the drainages from CRUS to be released into water bodies, since values for total PPP as high as 300.6 µg/L (rose CRUS, A-W period), 40.8 µg/L (strawberry CRUS, A-W period) and 29.9 µg/L (tomato CRUS, S-S period) were found for drainages from the reservoirs of the different CRUS. Thus, the cumulative release of these drainages on surface waters, without a previous treatment, could contribute for attaining these thresholds, affecting the chemical quality of the water of receptor freshwater reservoirs.

3.1.4. Ecotoxicological assays using aquatic species

For the A-W period, only the drainages from the substrate of the rose CRUS showed to be toxic to *R. subcapitata*, and higher toxicity was observed in drainages from Sub_{5y} (EC_{20}=81%; EC_{50}=84%) when compared with Sub_{3y} (EC_{20}=63%). The other samples from A-W were non-toxic. For the S-S period all the drainages from the rose CRUS, except for drainages from IRes, showed to stimulate the growth of the algae, from 23 to 51%. The same was observed in the drainage from the reservoir of the strawberry CRUS, which increased the growth of the algae in 9%. Despite not being possible to determine EC values for the drainages from the strawberry CRUS and the tomato CRUS, a decrease equal or lesser than 20% was observed in drainage from $Sub_{1y,}$ and from the reservoirs from the tomato CRUS.

The increased growth of the algae in the S-S period compared with A-W might be due to higher nutrient contents in the drainages, as supported by the electrical conductivity.

The effect of the drainages on the growth of *R. subcapitata* was quite variable, including moderate toxicity, lack of toxicity and even stimulus on the growth. Stimulation in high nutrient concentrations, especially nitrogen and phosphorus, were already demonstrated, not only for *R. subcapitata* (Expósito et al., 2017) but also for other green microalgae grown in hydroponic wastewater (Bertoldi et al., 2009).

The differences in toxicity of drainages from the substrates compared with the drainages from the reservoirs of the rose CRUS, did not seemed to be the result of either the electrical conductivity level or pH, since the variation was low and no trend could be unraveled through those parameters.

The results from the Microtox® assays revealed a clear trend for the drainages to stimulate the growth of *A. fischeri*, as estimated by increased bioluminescence. In this respect, the drainages from the tomato CRUS showed the highest effects, stimulating *A. fischeri* from 58 to 92%.
The drainages from Sub_{3y} (rose) and Sub_{1y} (strawberry) from the S-S period showed to be non-toxic with an effect below 10% and the highest decrease (19%) in bioluminescence was observed in drainage from Sub_{3y} (rose) but for A-W period. As such, no EC values could be determined for *A. fischeri*.
Despite that both *R. subcapitata* and *A. fischeri* are sensitive to numerous pesticides and metals, the lack of toxicity that was generally observed indicates that the PPP and metal residues from the phytosanitary management of the crops were below harmful values for these species. Even the highest value for dimethoate for the rose CRUS (300.0 µg/L) was substantially below the EC_{50} previously indicated for *A. fischeri* (800 µg/L) in other study (Lopez -Roldan et al., 2012).
The results for *D. magna* showed something clearly different. The drainages showed to be highly toxic to the crustaceans *D. magna*, as indicated by low EC_{50} values that were determined. The drainages from the IRes of the rose CRUS showed EC_{50} values of 9.1% and 20.5% in the S-S and A-W periods, respectively. The drainages from Sub_{3y} showed EC_{50}=27.6% in S-S and EC_{50}=8.2% in A-W, and the drainage from Sub_{5y} in A-W was less toxic, with EC_{50}=66.4%. The drainages from FRes showed to be highly toxic as well, with EC_{50}=17.8% and EC_{50}=29.0% in the A-W and S-S, respectively.

For the strawberry CRUS, high toxicity to *D. magna* were found for the drainages from Sub_{3y} (EC_{50}=9.2%) and Res (EC_{50}=10.8%), while lesser toxicity was found in the drainage from Sub_{1y} (EC_{50}=44.7%).

The crustaceans *D. magna* are considered sensitive to PPP, but often less sensitive than *A. fischeri* (Mansour, 2015). Since the drainages were non-toxic to the bacteria, most probably the high toxicity observed for *D. magna* might have been due to some other factors. Salinity, for example, can be suggested as a probable cause for these results. Working with NaCl salt, Santos et al. (2007) estimated 50% lethality of *D. magna* with [NaCl]=0.46 dS m^{-1}. The drainages from the rose CRUS and the strawberry CRUS showed electrical conductivity always well above this threshold, ranging from 0.86 to 3.6 dS m^{-1}. Obviously, these values corresponded to the 100% drainage concentration in the tests, but even after the subsequent dilutions with the growing medium, the electrical conductivity levels might have been detrimental to the organisms. Nevertheless, since the electrical conductivity is merely indicative of the overall nutrients concentrations, rather than the result of the concentration of harmful salts, reserves have to be maintained when doing comparisons. Besides salinity, it should also not be discarded the

possibility of presence of metal residues, such as copper, in the drainages, since copper compounds are commonly use as fungicide and bactericide in agriculture.

3.2. Soil quality

3.2.1. Physicochemical parameters

All the sampled soils showed to be acidic. The reference soil EXT showed pH of 5.40 and INT showed pH of 5.70. The soil from the ridges also showed to be acidic, with pH ranging from 4.90 (R1) to 5.41 (R3 and R5).

The values for the electrical conductivity were lower in the reference soils, with 0.10 dS m^{-1} for EXT and 0.26 dS m^{-1} for INT. In the soils from the ridges, the values ranged from 0.57 dS m^{-1} (R3) to 1.65 dS m^{-1} (R1).

The reference EXT was the less compacted soil with bulk soil density of 1.00 g cm^{-3}, whereas INT showed bulk density of 1.24 g cm^{-3} and the soil between ridges showed bulk density of 1.13 g cm^{-3}. The soil EXT showed significantly higher WHC compared with INT ($p<0.001$) and with the rest of the soils. In terms of organic matter content, the highest values were observed in the soil from the ridges, ranging from 5.76% (R5) to 6.05% (R2). The reference soil EXT showed lower organic matter content with 5.68% and the reference INT showed the lowest organic matter content, with 5.37%.

3.2.2. Soil Retention function: ecotoxicological assays using aquatic species

The soil irrigated with drainages from the strawberry CRUS showed to be moderately toxic to *R. subcapitata*, as assessed through the growth test. Soil from R1 showed to be the more toxic (EC_{20}=202 g L^{-1}; EC_{50}=223 g L^{-1}), followed by R2 (EC_{20}=162 g L^{-1}). For the soil from the other ridges, it was not determined neither the EC_{50} nor the EC_{20}, but highest effects from 20.5 % to 31.5% were observed. The reference EXT showed highest effect of 29.0% and INT showed to be non-toxic to *R. subcapitata*.

It should be highlighted the fact that despite the soil elutriates from the secondary crop of the strawberry CRUS have been toxic to *R. subcapitata*, the same was not verified in the drainages from this CRUS. This might suggest an accumulation effect of nutrients, and salts in particular, which caused the much higher EC in soils irrigated with drainages, compared with the reference soils, and consequent detrimental effect on the growth of the freshwater algae, already reported as being salt-sensitive (Santos et al., 2007).

Relatively to the solid-phase Microtox® test, the soil affected by the drainages showed to be more toxic to *A. fischeri* than the reference soils. Higher toxicity was observed when the

crustaceans were exposed to soil elutriates from R1 (EC_{50}=10.3 g L^{-1}) and R2 (EC_{50}=20.2 g L^{-1}). The reference soils also showed toxicity to *A. fischeri* but to lesser degree, since higher EC_{50} values were determined for INT (58.0%) and EXT (51.3%).

3.2.3. Habitat function: ecotoxicological assays using terrestrial species

The impact of the CRUS on earthworms from the species *E. fetida* was assessed in terms of both survival and fecundity. No significant differences were found in the survival rate amongst the three reference soils used in the reproduction assay. Survival rate was maximum in OECD soil (100.0 ± 0%) and was followed by EXT (97.5 ± 2.5 %) and INT (95.0 ± 2.9 %). Significantly lower survival was observed in all soils from the ridges compared with any of the references. Soils from R4 (25.0 ± 6.5%) and R1 (0.0%) showed the lowest survival rates.

Earthworms are susceptible to salinity and *E. fetida* has been referred as a particularly sensitive species (Owojori et al., 2009b). In our study, a highly significant negative correlation was found between survival and EC level ($r=-0.94$; $p<0.01$), indicating that salinity might have been the most detrimental factor on the high mortality of the earthworms.

In terms of fecundity, the OECD reference showed the highest number of juveniles after 56 days of incubation (72.8 ± 4.6 juveniles). No differences were found for survival among controls, but INT and EXT showed much lower fecundity compared with OECD, with less 60% ($p<0.001$) and 72% ($p<0.001$), respectively.

Despite the high variability observed in terms of survival in soils from the ridges, no juveniles were born in any of the treatments from R1 to R5, indicating that reproduction was more affected than survival. This is in accordance to other studies, which considered cocoon production as a more sensitive parameter than survival or growth, in respect to salt stress (Guzyte et al., 2011; Owojori et al., 2009a).

The impact of the CRUS on springtails from the species *F. candida* was also assessed in terms of both survival and fecundity, after 21 days of exposure. Amongst the three reference soils, the highest value for survival was observed in INT (88.0 ± 3.7%) and was followed by EXT (80.0 ± 0.0%) and OECD (64.0 ± 5.1%) but the differences were not statistically significant. Compared with the reference INT, significantly lower survival rate was found in R5 (46.0 ± 13.3%, $p<0.001$) and R1, which did not showed survivors among the ten individuals of any of the replicates. The results for fecundity followed the same pattern relatively to the references, with no significant differences among them and, except for R5, the soils from the ridges significantly affected fecundity compared with INT, the decreases ranging from 50% in R3 ($p<0.05$) to 82% in R1 ($p<0.001$).

Highly significant negative correlations were found between survival and EC (r=-0.73; p<0.01) and between fecundity and EC (r=-0.78; p<0.01), in *F. candida*. Despite being less susceptible to salinity than *E. fetida*, even the mild salinity of the soils from the ridges might have been the most detrimental factor, especially in terms of fecundity, as previously shown before by other authors (Owojori et al., 2009b).

Whereas our results might corroborate the higher susceptibility of *E. fetida* compared with *F. candida*, it should not be disregarded, the possibility of accumulation of PPP residues in the soil from the CRUS, causing harmful effects on soil organisms.

Yet, since no clear relationship was found between PPP residues in the drainages and toxicity to some aquatic groups here tested, we can therefore suggest salinity as the most important factor to justify the impact on soil habitat function.

A weak positive correlation, but highly significant, was found between survival and pH (r=0.53; p<0.01), while a strong and positive correlation was found between fecundity and EC (r=0.80; p<0.01), in *F. candida*.

3.2.4. Enzymes' activity

The overall results of the enzymes´ activity are shown in Table 4. The origin of the soil did not show significant impact on CM-cellulase activity (p>0,961). The soil from CRUS significantly affected the nitrification process (11.73, p<0.001). Whereas no significant difference was found between the references EXT and INT (p=0.495), the activity of nitrification was significantly reduced in the rest of the groups, except for R5, that was just marginally above the threshold (p=0.053). Soil from R1 and R2 showed to be the most affected, with reductions of 69% and 73%, respectively, compared with EXT.

Significant effect was also found for N-mineralization process (16.10, p<0.001). The lowest value was found for the reference INT, which showed N-mineralization activity 79% lower than that observed in the reference EXT (p<0.001). Soil from every ridge showed much lower activity compared with EXT, the decreases ranging from 38% to 56%. In contrast, except for R5, the soil from the rest of the ridges showed significantly higher activity compared with the reference INT. For example, R3 and R4 showed 3-fold higher N-mineralization compared with INT.

The soil from the CRUS significantly decreased the urease activity (63.56, p<0.001). Compared with EXT, the reference INT showed 68% lower urease activity (p<0.001). Considering only the soil from inside the greenhouse, two significant differences compared with the reference INT

can be pointed out, the activity from R3 being significantly higher (63%, p=0.024) and the activity from R1 being significantly lower (68%, p<0.05).

Relatively to dehydrogenase activity, despite the ANOVA indication of difference in at least one group (4.33, p=0.011), no significant differences were found through Tukey's post hoc test, probably due to its conservativeness, or the low overall values of the means, since low activity of dehydrogenase is often observed in cultivated soils (Błońska et al., 2017). Nevertheless, it is worth mentioning the considerably high variability shown by the soils from the ridges.

The CRUS significantly affected the phosphatase activity (p<0.001), but in an inverse way, since soils from all the ridges showed much higher activity compared with the reference INT. This reference showed very low activity, 99% lower compared with the reference EXT (p<0.001). Compared with EXT, R2 showed phosphatase activity 38% higher (p<0.05), whereas R1 was the most affected, showing activity 64% lower than EXT (p<0.001).

The reference INT showed a reduction of 80% in arylsulfatase activity compared to EXT (p<0.001). While the effect of the origin of the soil is very clear for this trait (39.50, p<0.001), no differences were found in the comparison of the reference INT and soil from any of the ridges.

In summary, clear differences between EXT reference and the soil from the ridges were found in four from the total of seven enzymatic processes analyzed, namely nitrification, N-mineralization, urease and arylsulfatase. In general, the reference INT showed enzyme activity similar or lower than those observed in the soil from the ridges.

The correlation analysis (Table 5, Annex) indicates that the EC negatively affected the activity of nitrification (r=-0.78; p<0.01), urease (r=-0.76; p<0.01), dehydrogenase (r=-0.65; p<0.01) and arylsulfatase (r=-0.59; p<0.01). It is widely known that salinity affects the microbial communities and, thus, the soil enzymatic activity (Pathak and Rao, 1998; Rietz and Haynes, 2003). As such, salinity can be pointed out as one of the main factors which caused the poor microbial activity in the soils irrigated with the drainages. The results from the reference INT did not point to this direction but, despite being presumably not affected by excessive applications of nutrients and by PPP residues, the INT sampling point was, simultaneously, deprived from rainfall and away from the irrigation area. Thus, the low moisture might have affected the soil microbial community (Błońska et al., 2017) in this part of the greenhouse, while being compacted to the highest degree among all soils. Actually, neither the reference INT, nor the soils from the ridges (R1 to R5) are under natural climate conditions, since they are under a plastic greenhouse. Despite the negative effects that might result from excessive nutrients or PPP applications along

the ridges, even so the frequent irrigations might, in part, alleviate the detrimental impact of the drainages in the soil from the ridges compared with the reference INT.

The reference EXT might have been benefited from coverage with spontaneous vegetation and lack of soil mobilization (Błońska et al., 2017), compared with the soil from the greenhouse.

In addition to salinity, the PPP residues that were found in the drainages, most likely considerably contributed to the drastic reduction in the enzyme activity that were found in soils affected by the CRUS, while pH, organic matter content and soil compacting might have been factors of lower influence on those processes (Błońska et al., 2017).

Table 4. Enzymes activity of the soils sampled from the selected CascadeReUse System, including reference soil from outside the greenhouse (EXT), reference soil from inside the greenhouse (INT) and soil collected along five ridges (R1-R5) irrigated with drainages from soilless cultivation of strawberry and reused for the fertigation of tomato grown on soil under a greenhouse. The activity is expressed in mass of a given product (see table bottom) per mass of dry matter and per hour of incubation (mean ± SE). Different letters indicate significant differences through Tukey´s test ($p<0.05$).

	Soil enzymes activity						
Soil	Cel (µg GE $dm^{-1} h^{-1}$)	Nit (ng N $dm^{-1} h^{-1}$)	N-min (µg N $dm^{-1} h^{-1}$)	Ure (µg N $dm^{-1} h^{-1}$)	Deh (µg TPF $dm^{-1} h^{-1}$)	Pho (µg pNP $dm^{-1} h^{-1}$)	Ary (µg pNP $dm^{-1} h^{-1}$)
EXT	2.7 ± 1.8^{a}	114 ± 12^{a}	0.30 ± 0.04^{a}	0.97 ± 0.06^{a}	0.55 ± 0.23^{a}	470 ± 44^{b}	74.3 ± 8.3^{a}
INT	1.6 ± 1.2^{a}	91 ± 4^{ab}	0.06 ± 0.01^{c}	0.31 ± 0.01^{cd}	0.57 ± 0.11^{a}	3 ± 3^{d}	14.8 ± 2.7^{b}
R1	2.6 ± 1.2^{a}	36 ± 4^{cd}	0.17 ± 0.02^{b}	0.10 ± 0.02^{e}	0.04 ± 0.04^{a}	168 ± 28^{c}	5.3 ± 3.9^{b}
R2	2.6 ± 1.6^{a}	31 ± 4^{d}	0.15 ± 0.01^{b}	0.17 ± 0.02^{de}	0.14 ± 0.04^{a}	646 ± 35^{a}	10.5 ± 1.4^{b}
R3	1.1 ± 1.1^{a}	72 ± 8^{bcd}	0.19 ± 0.00^{b}	0.50 ± 0.05^{b}	0.49 ± 0.18^{a}	516 ± 30^{ab}	5.1 ± 2.9^{b}
R4	1.6 ± 1.5^{a}	72 ± 6^{bcd}	0.19 ± 0.01^{b}	0.34 ± 0.05^{bcd}	0.07 ± 0.07^{a}	471 ± 22^{b}	2.7 ± 1.6^{b}
R5	1.6 ± 0.7^{a}	72 ± 15^{abc}	0.13 ± 0.01^{bc}	0.37 ± 0.02^{bc}	0.00 ± 0.00^{a}	453 ± 21^{b}	6.7 ± 3.4^{b}

Soil:
EXT – reference soil from outside the greenhouse | **INT** – reference soil from inside the greenhouse | **R1-R5** – soil collected along five ridges irrigated with drainages from soilless cultivation of strawberry and reused for the fertigation of tomato grown on soil under a greenhouse.
Enzymes:
Cel – CM-cellulase (Glucose Equivalents, GE) | **Nit** – nitrification (nitrite) | **N-min** – N-mineralization (nitrogen) | **Ure** – urease (nitrogen) | **Deh** – dehydrogenase (triphenyl-formazan) | **Pho –** phosphatase (p-nitrophenol | **Ary** – arylsulphatase (p-nitrophenol).

4. Conclusions

This study allowed to draw a set of conclusions on the suitability of CRUS based on the quality of the drainages from these systems and their impact on aquatic and terrestrial organisms and on soil quality. The conclusions are as follows:

- The good reuse potential of the drainages from CRUS were here corroborated by means of the maintenance of large part of the main macronutrients;
- The main macronutrients are consumed differently, depending on factors such as crop or season, which reinforces the importance of improving the methods of correcting new fertigation cycles, in the case of recirculation (closed system);
- The sustainability of CRUS is here called into question, owed to the possibility of the drainages to affect aquatic and terrestrial organisms, and the quality of soils;
- The drainages from soilless cultivation can have a negative impact on the environment, mainly due to the large amounts of nutrients that they carry, but also due to PPP residues that remain in them;
- The results indicate that the drainages from soilless cultivation can be unsuitable for being discharged to the public sewage system, owed to their high concentration of N and P;
- If the utilization of CRUS is to be maintained, we strongly recommend the elaboration of guidelines for good agricultural practices in the management of such production systems and surveillance of the compliance with the regulations.

At the time of publication of this report, tasks T5.2, T5.3 and T5.4, from WP5, were already concluded. However, part of the data collected in T5.2 and T5.3, and all the data from T5.4 are being analyzed and soon will be made available in a public report, to be annexed to this document.

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6. Annex

Table 5. Correlation analysis, indicated by Pearson´s coefficient (r) between the soil enzyme´s activity and physicochemical parameters.

	Cel	Nit	N-mi	Ure	Deh	Pho	Ary	OM	pH	EC
Cel	-									
Nit	-0.18	-								
N-mi	0.11	0.32	-							
Ure	-0.05	**0.74****	**0.64****	-						
Deh	0.11	**0.44***	-0.04	**0.53***	-					
Pho	-0.06	-0.20	0.40	0.26	-0.16	-				
Ary	0.05	**0.65****	**0.69****	**0.85****	**0.45***	0.11	-			
OM	0.12	**-0.73****	0.25	-0.37	**-0.53***	**0.57****	-0.28	-		
pH	-0.16	**0.69****	-0.28	**0.47***	**0.57****	-0.36	0.26	**-0.93****	-	
EC	0.07	**-0.78****	-0.10	**-0.76****	**-0.65****	0.00	**-0.59****	**0.78****	**-0.85****	-

Cel – Cellulase | **Nit** – Nitrification | **N-mi** – N-mineralization | **Ure** – Urease | **Deh** – Dehydrogenase | **Pho** – Phosphatase | **Ary** – Arylsulphatase | **OM** – organic matter | **EC** – Electrical conductivity.

* Correlation is significant at 0.05 level

** Correlation is significant at 0.01 level