Use of Recalcitrant Agriculture Wastes to Produce Biogas and Feasible Biofertilizer.

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Abstract

In the ongoing work, the digestion process of recalcitrant agricultural wastes (olive wastes and citrus pulps) mixed in different proportions with, livestock manures, milk serum and maize silage for biogas production was studied. Additionally, the chemical composition and the phytotoxicity of the digestates (each separated in liquid and solid fraction) were evaluated with the purpose of being used as organic fertilizer in agriculture.The results demonstrated that animal manure and recalcitrant agricultural wastes, if properly mixed, produced high percentage of biogas. The digestate chemical compositions differed and varied in respect to the kind of feedstock, and the ratio of their mixing to feed the digesters. The digestate from the digester named Fattoria, mainly powered with animal manures (poultry, cow and sheep), contained less phenols and more active microbial biomass than the digestate from the digester Uliva, mainly fed with olive waste and citrus pulp and in minor extent with animal manure and maize silage. Our data showed that the digestate composition depended on the mix of biomass input. Additionally, the effects of digestate were plant specie-specific and a positive correlation between the amount of phenols and the phytotoxic effects of digestate on plants was also well evident. These results evidenced that each single digestate has a own chemical feature, suggesting that the sustainable disposal of digestates requires a preliminary screening to select the one which better fits the demands of a particular species for optimizing crop production.

Key words: anaerobic digestion; antioxidant system; biogas; digestate; phytotoxicity; seed germination.

Introduction

Agricultural activities, waste management, and use of energy from fossil fuels, all contribute to global warming and climate change. [1,2], Against these background it is necessary to strengthen waste management activities in the context of climate change, promoting alternative energy derived from natural sources. Biogas technology, also known as anaerobic digestion (AD) technology, can be considered a competitive process for reducing the rate of climate change and global warming managing biodegradable waste streams to produce renewable energy and nearly stable residue (digestate), in a sustainable way [3-5].[3-5]. The energy produced in the form of biogas, is a mixture of methane (45-75%), carbon dioxide (25-55%) and minor amounts of H_2S and H_2 and the actual proportion is dependent on the feedstock (substrate) used, and on the processes employed. While biogas represents an ascertained useful source of renewable energy, the digestate ever-increasing production induces problems related to its sustainable disposal. Consequently, research on agriculture valorization routes to reduce its environmental impact and to improve the economical profitability of AD plants are of great interest [6]. Depending on their chemical features, some digestates can have negative impact on environment or on plant growth [7], while some others may influence soil fertility and plant health positively. However, a digestate cannot be considered positive or negative *in toto*, therefore it is necessary to chemically and biologically characterize each digestate for finding an adequate utilization. Despite all, the use of digestate as fertiliser is legally limited in many countries due to unfamiliarity of the product and insufficient confidence in its quality and safety [8]. Quality assurance is an important prerequisite for increasing market confidence in digestate and for enhancing its use as fertilizer [9]. In many European Union Nations, anaerobic digestion technologies and processes are a widely accepted practice that aims to increase the profitability of dairy farmers and the food processing industry by utilizing organic wastes for better meeting the needs of environmental regulators [10]. Currently, in Italy, issues such as demand for renewable energy, landfill tax on organic wastes, demand for organic fertilizer, pollution of the environment and legislation relating to the treatment and disposal of organic wastes are all important factors influencing investments in AD [11]. Calabria (Southern Italy) is an agricultural land with predominant production of citrus, oil and livestock [12] to produce milk and cheese. Agriculture wastes and livestock manures are highly polluting and difficult to dispose of, with a high cost for farmers [13]. Thus, their anaerobic digestion could be a reliable way to use refuse as resource producing economic benefit [14-17]. This research, in cooperation with two cooperatives **Fattoria della Piana** soc. Agricola, and **Uliva Srl soc. Agricola**, owners respectively of two biogas plant each with 998 kWel of installed power, has the aim to evaluate the digestion process of olive wastes and citrus pulps mixed with other organic biomass and animal manure, for biogas production. The specific objectives were: 1) to compare the output and the composition of biogas, obtained from the two plants fed with recalcitrant agriculture wastes (olive wastes and citrus pulps), mixed in different proportions with livestock manures, milk serum and maize silage; 2) to chemically characterize the two digestates, each separated in liquid and solid fractions; 3) to test *in vitro* the effects of the liquid and solid fractions of the two digestates on seed germination, seed performance and antioxidant system of model plants (cucumber, watercress and lettuce).

Materials and Methods

Biogas plants: process temperature and retention time

Each biogas energy plant has an installed power of 998 kWel. The two biogas plants were differently supplied: the first one named **Fattoria (F)** was powered with animal manures (poultry, cow and sheep), milk serum, maize silage and in minor amount with olive waste and citrus pulp. The second one named **Uliva (U)** was mainly powered with olive waste and citrus pulp and in minor amount with animal manure and maize silage.

The time of residence of the feedstock inside the digester (retention time), at constant process temperature, influences the digestate quality. Retention times are quoted as hydraulic retention time (HRT) and as minimum guaranteed retention time (MGRT). HRT is the nominal time that feedstock remains inside the digester at the process temperature. HRT is usually expressed in days and it depends, to a large extent, on the digestibility of the feedstock mixture.

HRT [h or days] = Digester volume [m^3] / the influent flow rate [m^3/h or days].

Combinations of thermophilic or mesophilic process temperatures and MGRT can provide pathogen reduction in the digestate obtained, equivalent to the EU sanitation standard of 70°C for 1 hour and are thus allowed, depending on the feedstock mixtures. Biogas plant operators have selected process temperatures and retention times which are appropriate for the feedstock that had to be digested.

Fattoria: process temperature: 40 °C, pH 7.8, total volume of the two digesters: 7500 m^3 (2500 DIG.1 + 5000 DIG.2), total volume loaded per day: 120 m^3/ day, hydraulic retention time (HRT): 60 days, minimum guaranteed retention time (MGRT) 16 h at 40°C.

Uliva: process temperature: 40 °C, pH 8.0, total volume of the two digesters: 7420 m^3 (3180 DIG.1 + 4240 DIG.2), total volume loaded per day: 120 m^3/day, hydraulic retention time (HRT) 60 days, minimum guaranteed retention time (MGRT) 16 h at 40°C.

The digestates coming from both plants were separated in liquid and solid fractions (Solid Uliva, **SU**; Liquid Uliva, **LU**; Solid Fattoria, **SF;** Liquid Fattoria, **LF,** and analyzed for chemical and biological characteristics.

Chemical analysis

Chemical parameters were determined in three replicates. Dry matter (dm) content was determined at 105°C until the mass loss of the sample during 24 h was lower than 0.5% of its weight [18]; moisture content, after drying to constant weight at 105 °C; volatile solids, reflect the content of OM which can be decomposed by combustion at 550 °C for 24 h up to constant weight; pH was measured in distilled water using a 1:2.5 (digestate/water) suspension; organic carbon was determined by the Walkley–Black procedure [19], and it was converted to organic matter by multiplying the percentage of carbon by 1.72; total nitrogen was measured by Kjeldahl method [20]; electric conductibility was determined in distilled water by using 1:5 digestate:water suspension, mechanically shacken at 15 rpm for 1 hour to dissolve soluble salts, and then detected by Hanna instrument conductivity meter. Available P was determined by the Bray II method [21].

Exchangeable K was extracted with 1 M NH_4OAc, and determined using a flame-photometer. The NO_3–N was measured using a nitrate-ion selective electrode (USEPA, 2011), while NH_4–N was determined by a colorimetric method based on Berthelot's reaction [22]. All values refer to material dried at 105 °C for 24 h. The 5-day biochemical oxygen demand (BOD) was measured with a respirometric Oxitop® IS 6 (WTW, Germany) based on pressure measurement, which is automatically transformed into mg O_2 L^{-1}. In the Oxitop® system, cumulative oxygen consumption measurements were made each day during a 5-day period. COD was determined by dichromate oxidation of dried ground samples, according to an adaptation of the standard method described for liquid samples [18] and using an automatic titration device (Metrohm Titrando-Dosino device); total water-soluble phenols were measured by using the Folin–Ciocalteau reagent, following the Box method [23]. Tannic acid was used as a standard and the concentration of water-soluble phenols was expressed as tannic acid equivalents (mg TAE/g dm) [24]. Fluorescein diacetate hydrolysis (FDA) reaction was determined according to the methods of Adam and Duncan [25]. Briefly, to 2 g of digestate (fresh weight, sieved <2 mm) 15 ml of 60 mM potassium phosphate pH 7.6 and 0.2 ml 1000 µg FDA ml^{-1} were added. The flask was then placed in an orbital incubator at 30 °C for 20 min. Once removed from the incubator, 15 ml of chloroform/methanol solution (2:1 v/v) was added to terminate the reaction. The content of the flask was centrifuged at 2000 rpm for 3 min. The supernatant was filtered and the filtrates measured at 490 nm on a spectrophotometer (Shimadzu UV–Vis 2100, Japan).

Germination test

The germination test on the Petri dish is a promising test in predicting the phytotoxicity [26]. Additionally, seed germination and germination index of model species [27-28], are recognized as indicators particularly sensitive and may be adopted as test to determine the phytotoxicity of new compounds /products [29-31].

The seeds of watercress, lettuce and cucumber were surface-sterilized for 20 min in 20% (v/v) sodium hypochlorite, rinsed and soaked in distilled water (for a total of 1 h). Five 50-seed replicates for germination test were carried out with different concentrations of solid and liquid digestate fractions from Uliva and Fattoria. In the experiments five different concentrations of liquid fraction of Fattoria and Uliva digestates were used (0, 10, 25, 50 and 100%); Uliva and Fattoria solid digestate were extracted in water (1:5 w/v) for 24 h at room temperature in agitation and then diluted with distilled water to have 5 concentrations (0, 10, 25, 50 and 100%). Fifty seeds of each species were placed on filter paper in 9 cm Petri dishes containing 3 cm^3 of each solution. The Petri dishes were hermetically sealed with Parafilm to prevent evaporation and kept in a growth chamber at a temperature of 25±1°C in the dark with a relative humidity of 70%. Seeds were considered germinated when the radicle had extended at least 2 mm. Three replicates were analyzed for each treatment.

Germination indexes. The number of seeds germinated was recorded daily for up to 7 d. From these germination counts several germination attributes were calculated to characterize the phytotoxicity, including total germination percentage (TG) (%) at 7 d, coefficient of velocity of germination (CVG) [32], germination rate index (GRI) [33], and mean germination time (MGT) [33] as follow:

$$\mathrm{CVG}\ (\%\ \mathrm{day}^{-1}) = \sum Ni/\sum (NiTi) \times 100,$$

$$\mathrm{GRI}\ (\%\ \mathrm{day}^{-1}) = \sum Ni/I,$$

$$\mathrm{MGT}\ (\mathrm{day}) = \sum (NiTi)/\sum Ni$$

Where N is the number of seed germinated on day i, Ti is the number of days from sowing and I is the number of germinated seeds at 7 d. The CVG gives an indication of the rapidity of germination: it increases when the number of germinated seeds increases and the time required for germination decreases. The GRI reflects the percentage of germination on each day of the germination period. Higher GRI values indicate higher and faster germination. The lower the MGT, the faster a population of seeds has germinated.

Determination of enzyme activities

Seeds (0.5 g) that had been soaked for 3 d in the test solutions were ground using a chilled mortar and pestle and homogenized in 0.1 M phosphate buffer solution (pH 7.0) containing 100 mg soluble polyvinylpolypyrrolidone (PVPP) and 0.1 mM ethylenediamine tetra acetic acid (EDTA). The homogenate was filtered through two layers of muslin cloth and centrifuged at 15000 g for 15 min at 4°C. The resulting supernatant was used to evaluate the activity of catalase (CAT, EC 1.11.1.6), peroxidase (POX, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase (GR EC 1.15.1.1). All enzyme activities were measured at 25°C by a UV: visible light spectrophotometer (UV-1800 CE, Shimadzu, Japan).

CAT activity was determined by monitoring the disappearance of H_2O_2 at 240 nm, calculated using its extinction coefficient (ε) = 0.036 mM^{-1} cm^{-1}. The reaction mixture contained 1 mL potassium phosphate buffer (50 mM, pH 7.0), 40 μL enzyme extract and 5 μL H_2O_2 [34].

APX activity was assayed according to Nakano and Asada [35]. The reaction mixture (1.5 mL) contained 50 mM phosphate buffer (pH 6.0), 0.1 μM EDTA, 0.5 mM ascorbate, 1.0 mM H_2O_2 and 50 μL enzyme extract. The reaction was started by the addition of H_2O_2 and ascorbate oxidation measured at 290 nm for 1 min. Enzyme activity was quantified using the molar extinction coefficient for ascorbate (2.8 $mM^{-1}cm^{-1}$)

GR activity was assayed spectrophotometrically at 30 ºC in a mixture containing 3 mL 100 mM potassium phosphate buffer (pH 7.5), 1 mM 5,5'-dithio-bis (2-nitrobenzoic acid), 1 mM oxidized glutathione (GSSG) and 0.1 mM NADPH. The reaction was initiated by the addition of 50 µL of enzyme extract. The rate of reduction of GSSG was followed by monitoring the increase in absorbance at 412 nm over 2 min [36].

POX activity was measured on the basis of determination of guaiacol oxidation at 436 nm for 90 s [37]. The reaction mixture contained 1 mL potassium phosphate buffer (0.1 M, pH 7.0), 20 μL guaiacol, 40 μL enzyme extract and 15 μL H_2O_2. POX activity was quantified by the amount of tetraguaiacol formed using its extinction coefficient (ε) = 25.5 $mM^{-1}cm^{-1}$.

For CAT, APX, GR and POX activities, the results were expressed as enzyme units (U) per mg protein. One unit of enzyme was defined as the amount of enzyme necessary to decompose 1 μmoL of substrate per min at 25ºC.

Total antioxidant capacity determination

Seeds treated with different salt solutions for 3 d were homogenized in a chilled mortar with distilled water at a ratio of 1:4 (seeds/water; w/v) and centrifuged at 14000 g for 30 min. All steps were performed at 4 °C. The supernatants were filtered through two layers of muslin cloth and were used to determine the total antioxidant capacity by the spectrophotometric method of Prieto et al. [38]. Aqueous extracts of the seeds were combined in Eppendorf tubes with 1mL of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate, 4 mM ammonium molybdate mixture). The tubes were incubated for 90 min at 95ºC, then cooled to room temperature and the absorbance read at 695 nm against a blank (mixture without seed extract). The assay was conducted in triplicate and the total antioxidant activity expressed as the absorbance of the sample at 695 nm. The higher the absorbance value, the higher the antioxidant activity [39].

Total phenolic content determination

Total phenolic content was determined with the Folin-Ciocalteu reagent according to a modified procedure described by Singleton and Rossi [40]. Briefly, 0.50 mL of the aqueous extract of the seeds was reacted with 2.5 mL of Folin-Ciocalteu reagent (1:10 diluted with distilled water) for 4 min, and then 2 mL saturated sodium carbonate solution (about 75 g/L) was added into the reaction mixture. The absorbance readings were taken at 760 nm after 2 hours of incubation at room temperature. Tannic acid was used as a reference standard, and the results were expressed as milligram tannic acid equivalent (mg TAET/g fresh weight).

Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA). Separate ANOVAs were performed for each of digestate fractions and concentrations. The response variables for these ANOVAs were: seed germination, seedling growth, enzyme activities. Since the concentration of each digestate fraction had five levels, on all significant ANOVAs we performed Tukey multiple comparison tests to compare all pairs of means. The germination percentage data were previously subjected to arcsine transformation but are reported in tables as untransformed values. All data collected were statistically analyzed using SYSTAT 8.0 software (SPSS Inc.).

Results

Biogas and Digestate composition

No differences in the biogas composition between the two plants were observed (Table 1). In both plants, the biogas production reached 440-450 m^3/h with a methane content of ~60% (Table 1).

Both digestates had higher ammonium (NH_4^+) to total nitrogen (N) ratios, decreased OM, total and organic carbon (C) contents, reduced biological oxygen (O_2) demands (factor), elevated pH values, smaller carbon to nitrogen ratios (C:N ratios), and a greater amount of nutrients than the respective input materials (ingestate) (Table 2)

The two biogas digestates were chemically and qualitatively different one from the other. Fattoria (**F)** had less total phenols, lower COD and BOD, but greater amount of K^+, P, NH_4^+, and NO_3^- than Uliva (**U)** (Tables 3-4), **U** contained more Mg^{++} and Ca^{++}. From a biological point of view, **F** had a greater amount of bacteria, than **U**. Apart from the differences observed between the two digestates, we detected chemical and biological differences, between the solid and liquid fractions of the same digestate (Tables 3-4). In **Fattoria,** the solid fraction had less total phenols, total oil, saponifiable fat and total hydrocarbon than the liquid one. Additionally the **SF** had greater amounts of bacteria, K^+, P, Mg^{++} and Ca^{++} and NO_3^- than **LF (**Table 3**)**. In uliva digestate, the solid fraction had a lower pollution load (BOD and COD) and contained less total phenols, total oil, saponifiable fat and total hydrocarbons than **LU** (Table 4). T. Additionally, **SU** contained more P, NO_3^- and Ca^{++} and less K^+, NH_4^+ and Mg^{++} with respect to **LU**.

Seed germination

Germination differed significantly among the species in respect to the type of fractions, to the dilution levels, and to the combination of these factors. (Tables 5-6). Maximum germination percentage (100%) was observed in water. **LF** at the lowest concentration, decreased (-20%) seed germination percentage of lettuce and watercress. Higher LF concentrations, completely inhibited the germination percentage of lettuce and watercress, while did not affect the total germination of cucumber. Even if the germination percentage was reduced in seeds of lettuce and watercress treated with 10% **LF**, there were no significant differences in germination rapidity (CVG), in medium germination time (MGT) and in GRI, an index reflecting the percentage of germination on each day (Table 5). These parameters were not affected in cucumber seeds by all **LF** concentrations. **SF** at the lowest concentration speeded up the germination of lettuce and watercress. Increasing its concentrations the germination percentage of these species decreased in a concentration dependent manner. **SF** at all concentrations did not affect seed germination percentage, germination rapidity (CVG), GRI and MGT of cucumber (Table 4). Lettuce and watercress appeared the most sensitive species to the **F** treatments. Uliva digestate was more detrimental than Fattoria on germination of all species assayed (Table 6). In presence of **LU**, no germination was detected for lettuce and watercress and only a 50% of germination in presence of the lowest **LU** concentration was observed for cucumber seeds. Increasing the **LU** concentrations, the cucumber seed germination decreased accordingly, and no germination was observed with 100%**LU** (Table 6). **SU** was less detrimental than **LU** for all the three species. With the lowest **SU** concentration, germinated only 40 % of lettuce and watercress and 59% of cucumber seeds. Increasing the concentrations, the germination percentages decreased, much more for lettuce and watercress than for cucumber. Also germination velocity, percentage of germination on each day, and mean germination time were significantly affected by **SU** at all concentrations (Table 6). The analysis of variance (Table 7) showed that the inhibitory effect on total germination of lettuce and watercress was mainly due to the concentrations rather than to the type of fractions used. Germination percentages of cucumber seeds were not affected by **F** digestate. Differently, **U** digestate negatively affected TG of cucumber seeds and the effect was mainly due to the concentrations rather than to the fractions used. The two digestates were responsible for the significant changes on MGT (Table 7). In lettuce and watercress the effect was mainly due to the fractions. In cucumber, the effect of **F** on MGT, was mostly dependent on the fraction and it was much lower than that detected for lettuce and watercress (lower F-ratios). Differently, the effect of Uliva, on cucumber MGT, was mainly due to the combinations of fraction x concentration in comparison with changes induced by the two parameters individually considered.

Enzyme activities, phenols and antioxidants

In lettuce treated with the lowest concentration of **LF** no significant changes in enzyme activities in respect to control were observed (Table 8). Increasing **LF** concentrations all enzyme activities were inhibited. The lowest **SF** concentrations (10 and 25%) did not affect the enzyme activities, but increasing its concentration, all the activities increased (Table 8). No activities were detected in lettuce seeds treated with **LU**. Conversely, **SU** positively affected all the activities in a concentration dependent manner. In watercress no enzyme activities were detected in seeds treated with **LU** (Table 9). The two lowest concentrations of **SU** did not induce significant changes in the enzymatic activities with respect to control, but when **SU** concentration increased all the activities in watercress decreased. Differently, in cucumber **LU** and **SU** significantly increased the antioxidant enzyme activities, linearly with the concentration (Table 10), while both Fattoria fractions did not affect the enzyme activities even at the highest concentrations compared to control. Regarding the non enzymatic antioxidants in lettuce and watercress, in presence of both liquid fractions (Fattoria and Uliva), total antioxidant activity and total phenols were under the detection limit (Table 11); the only exception was for seeds treated with **LF** at the lowest concentration, where ToA and TP values were similar to the control. Conversely, in lettuce and watercress seeds, increasing the concentrations of both solid fractions, the non enzymatic antioxidants increased compared to control (Table 11). Cucumber showed a different trend, **LU** increased the concentration of non enzymatic antioxidants with respect to the control (even if less than in watercress and lettuce), except for the highest concentration that completely inhibited seed germination. No significant differences with respect to the control were instead induced in the amounts of ToA and TP by **SU**, **LF** and **SF** fractions at all concentrations (Table 11). It was found a significant correlation between FDA and phenols of digestates and MGT (Table 12). A linear inverse correlation was noted between the concentration of phenols and MGT in all the species analyzed, while a linear positive correlation was observed between the amount of FDA and MGT (Table 11).

Discussion

From an energetic point of view, our results evidenced that the two digesters differently fed, produced the same amount of biogas with a high percentage of methane. Additionally, comparisons between digested and undigested materials showed that the biomass have been transformed during the digestion process and that the digestates have a higher content of plant-available nitrate, ammonium, Ca, and Mg than indigested materials. Anaerobic digestion could therefore help farmers to maximize the return of nutrients to the soil, reducing agricultural dependence from inorganic fertilizer that are becoming increasingly expensive for their energy-intensive production process, and responsible for the greenhouse-gas (GHG) emissions and water-pollution incidences from agriculture. Even though olive and citrus wastes, which are used in a greater proportion to feed the Uliva digester, contained major amounts of recalcitrant substances, such as lignocelluloses and phenolic compounds [41] with the potential to reduce the activities of microorganisms [42-44], unexpectedly, we didn’t find an inverse relationship between the amount of biogas produced, and the amount of phenols contained in the organic substrate utilized, as previously demonstrated by

Battista et al. [45]. This could be the result of having used a multi-component mixture of wastes in such proportions as to allow the easily degradable compounds they contained to act as buffer, or release important micro- and macro-nutrients able to increase bacteria number and activity, thereby overcoming the bacteriostatic effects of the phenolic compounds present in the starting biomass. Surprisingly, even though the biomass input had no effect on the quantity and quality of the biogas produced, it had a great impact on the quality and composition of the digestates. The different mixture of biomass input, affected the composition of digestates from an agronomic point of view, conditioning the content of phenols, a class of compounds with adverse effects on plant growth and soil microorganisms [46-47]. Digestate Fattoria that was found to be more suitable for agronomic purpose, contained less phenols than uliva, probably for the high amount of active bacteria (derived from the livestock manure, the main waste that fed Fattoria digester) which used phenols as carbon source for their own metabolic needs [48] and[46]. This is confirmed by the data of FDA (higher in F digestate) that is a measure of total microbial activity and a marker of total enzyme activity [25], [49], [50-51]. In general, Fattoria with high nutrients, lower phenols and pollutant load, was less phytotoxic than **U**, evidencing a strong direct relationship between phenol content and phytoxicity. In presence of **LU** the fraction by far richer in phenols, no watercress and lettuce seeds germinated. Data on antioxidant system confirmed that **U** mainly at the high concentrations, represented a stress factor for lettuce and watercress. Conversely, cucumber seeds were able to germinate in presence of **LU** 50% and **SU** 100% but with a low germination percentage and speed. Both fractions of **U**, most at the highest concentrations, were phytotoxic, and the seeds protected themselves from the stressful conditions, activating the antioxidant system to scavenge the ROS for completing their germination [52]. Traditionally, reactive oxygen species (ROSs) in plants are considered as by-product of aerobic metabolism and also as cellular indicators of stress and signals for the activation of stress-response and defense pathways. The major defense systems against ROS injury are based mainly on enzymes and antioxidant compounds that remove ROSs [53] since the production of ROSs under stress conditions may negatively affect seed metabolism and in turn the whole germination process [54-58]. In our study, to correlate, the chemical composition and the biological effects of the single fractions a significant inverse linear correlation was found among the concentration of phenols, MGT and antioxidant system activation, in all the species analyzed, while the amount and activity of bacteria present in the digestates and MGT were positively correlated. Among the three species investigated watercress was the most negatively affected by the digestates showing the minimum germination and the maximum activation of the antioxidative system, while cucumber resulted the species that better answered to the amendment with the digestates. These results confirm the hypothesis of Fuchs et al. [59] in which they suggested that for obtaining positive results, it is important not only to take into account the chemical characteristics of the digestate but also to use it by leveraging its species-specificity.

Conclusion

This study demonstrated that animal and recalcitrant agriculture wastes represent a great resource in producing biogas with high methane percentage. The results evidenced that digestate composition is strictly dependent on the amount and kind of wastes used, and on the ratio in which they are mixed. It is incorrect to generalize on the use of digestate as organic fertilizer, but it is imperative to test preliminarily the digestate phytotoxicity every time new mixtures of biomass are used to feed the digesters. In this study, a specificity between the kind of digestate and plant species was really evident. Additionally an increase in antioxidant compounds (ToA and TP) in crops treated with **LU** and **SU** were also identified. Even though, the digestate richer in phenols reduced crop productivity, at the same time it increased the antioxidant content in plants. Thus, if used as fertilizer, it may represent, an additional resource for agriculture to produce food with nutraceutical values. Numerous ongoing studies continue to evidence that antioxidant rich foods or antioxidant supplements reduce the risk of chronic disease and promote wellness. Thus the cultivation of species in lands amended with digestate may provide enormous environmental and economic benefits increasing the green economy when the species and the optimal cultivation conditions are identified. Therefore, AD should not only considered a source of renewable energy, waste management system, pollution-abatement technology, but also an opportunity for providing value-added byproducts.

Conflict of interest statement

The authors declare they have no competing financial interests

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Table 1. Composition of biogas from Fattoria (F) and Uliva (U) plants.

Constituents	**Units**	**Fattoria**	**Uliva**
Biogas production	cm/h	440	450
Methane	Vol %	59	61
Ethane	Vol %	0	0
Propane	Vol %	0	0
Butane	Vol %	0	0
Pentane	Vol %	0	0
Carbon Dioxide	Vol %	41	39
Nitrogen	Vol %	0-2	0-2
Hydrogen	Vol %	0	0
Hydrogen Sulphide	ppm	~50	~50
Ammonia	ppm	~100	~100
Carbon monoxide	ppm	0	0
Volatile Organic Compounds	Vol %	0	0

Table 2 Chemical and biological characteristics of biomass (ingestate) used to feed **Fattoria and Ulivo plants**. Values are means ± SE (n=4). Different letters in the same row indicate significant differences $P \leq 0.05$

Parameters	**Units**	**Ingestate Fattoria**	**Ingestate Ulivo**
Total solids	%	$40^{b} \pm 3$	$48^{a} \pm 2$
Volatile Organic Compounds	Vol%	$21^{a} \pm 2$	$13.5^{b} \pm 1$
Moisture	%	80 ± 4	$85^{b} \pm 5$
COD	mg/L	$80000^{b} \pm 24$	$180000^{a} \pm 41$
BOD	mg/L	$25000^{b} \pm 34$	$50000^{a} \pm 33$
Fluorescein diacetate hydrolysis	μg fluorescein g^{-1} dm	$1.1^{a} \pm 0.5$	$0.74^{b} \pm 0.05$
Bacteria	CFU	$90 \times 10^{3a} \pm 3$	$15 \times 10^{3b} \pm 1$
Total phenols	mg/L	$514^{b} \pm 6$	$1424^{a} \pm 4$
Total oil	mg/L	$400^{b} \pm 11$	$600^{a} \pm 9$
pH		$6.1^{a} \pm 0.4$	$5.5^{a} \pm 0.5$
Conductivity	μS/cm	$1640^{a} \pm 14$	$1326^{b} \pm 12$
Total Carbon	% dm	$144^{a} \pm 5$	$130^{b} \pm 3$
Organic matter	% dm	$248^{a} \pm 4$	$224^{b} \pm 4$
Total nitrogen	% dm	$6.0^{b} \pm 1$	$6.5^{a} \pm 2$
C/N		$24^{a} \pm 2$	$20^{b} \pm 3$
K^{+}	mg/L	$840^{a} \pm 5$	$340^{b} \pm 8$
K_20	mg/L	$1340^{a} \pm 9$	$952^{b} \pm 7$
P	mg/L	$631^{a} \pm 8$	$560^{b} \pm 12$
P_20_5	mg/L	$1340^{a} \pm 47$	$1378^{a} \pm 38$
NO_3^{-}	mg/L	$112^{b} \pm 4$	$190^{a} \pm 7$
NH_4^{+}	mg/L	$149^{a} \pm 6$	$54^{b} \pm 6$
Ca^{++}	mg/L	$1300^{b} \pm 14$	$1800^{a} \pm 15$
Mg^{++}	mg/L	$149^{b} \pm 11$	$230^{a} \pm 13$

Table 3 Chemical and biological characteristics of solid and liquid digestate fractions from **Fattoria** Plant. Values are means ± SE (n=4). Different letters in the same row indicate significant differences P≤0.05.

Parameters	Units	Liquid fraction	Solid fraction
Total solids	%	$7^{b} \pm 3$	$25^{a} \pm 4$
Volatile Organic Compounds	Vol%	$63^{b} \pm 3$	$79^{a} \pm 5$
Moisture	%	$93^{a} \pm 5$	$75^{b} \pm 6$
COD	mg/L	50000 ±121	-
BOD	mg/L	8500 ± 12	-
Total phenols	mg/L	$395^{a} \pm 12$	$325^{b} \pm 9$
Total oil	mg/L	200 ± 6	-
Fat (saponifiable)	mg/L	180 ± 6	-
Total hydrocarbons	mg/L	33 ± 1	-
pH		$8.3^{a} \pm 0.6$	$8.4^{a} \pm 0.5$
Fluorescein diacetate hydrolysis	µg fluorescein g^{-1} dm	$1.68^{b} \pm 0.5$	$2.45^{a} \pm 0.4$
Bacteria	CFU	$110 \times 10^{3b} \pm 5$	$140 \times 10^{3\,a} \pm 7$
Conductibility	µS/cm	$1879^{ab} \pm 10$	$1707^{a} \pm 11$
Total Carbon	% dm	$39.5^{a} \pm 4$	$43^{a} \pm 5$
Organic matter	% dm	$69^{a} \pm 2$	$74^{a} \pm 5$
Total nitrogen	% dm	$4.9^{a} \pm 2$	$5.3^{a} \pm 3$
C/N		$8.1^{a} \pm 3$	$8.1^{a} \pm 2$
K^{+}	mg/L	$480^{b} \pm 9$	$960^{a} \pm 8$
K_20	mg/L	$576^{b} \pm 11$	$1152^{a} \pm 7$
P	mg/L	$290^{b} \pm 9$	$560^{a} \pm 12$
P_20_5	mg/L	$664^{b} \pm 16$	$1282^{a} \pm 11$
NO_3^{-}	mg/L	$140^{b} \pm 11$	$1500^{a} \pm 17$
NH_4^{+}	mg/L	$340^{a} \pm 12$	$30^{b} \pm 6$
Ca^{++}	mg/L	$600^{b} \pm 11$	$900^{a} \pm 15$
Mg^{++}	mg/L	$9^{b} \pm 2$	$100^{a} \pm 13$

Total solid	%	$8^{b} \pm 3$	$40^{a} \pm 6$
Volatile substances	%	$73^{b} \pm 5$	$85^{a} \pm 4$
Moisture	%	$92^{a} \pm 8$	$60^{b} \pm 6$
COD	mg/L	94000 ± 16	-
BOD	mg/L	16000 ± 16	-
Total phenols	mg/L	$940^{a} \pm 12$	502^{b}
Total oil	mg/L	230 ± 10	-
Fat (saponifiable)	mg/L	200 ± 13	-
Total hydrocarbons	mg/L	36 ± 6	-
pH		$8.3^{a} \pm 1$	$8.4^{a} \pm 1.5$
Fluorescein diacetate hydrolysis	µg fluorescein g^{-1} dm	$1.18^{a} \pm 0 .5$	$2.15^{a} \pm 0.6$
Bacteria colonies	CFU	$30 \times 10^{3\,b} \pm 2$	$55 \times 10^{3\,a} \pm 3$
EC	µS/cm	$1438^{a} \pm 3$	$1298^{b} \pm 5$
Total Carbon	% dm	$37.5^{b} \pm 2$	$42.9^{a} \pm 2.5$
Organic matter	% dm	$65^{b} \pm 4$	$74^{a} \pm 3$
Total nitrogen	% dm	$4.7^{a} \pm 0.5$	$5.5^{a} \pm 0 .6$
C/N		$7.97^{a} \pm 0.9$	$7.8^{a} \pm 0 .8$
K^{+}	mg/L	$660^{a} \pm 15$	$300^{b} \pm 11$
K_20	mg/L	$792^{a} \pm 8$	$360^{b} \pm 5$
P	mg/L	$250^{b} \pm 7$	$450^{a} \pm 5$
P_20_5	mg/L	$573^{b} \pm 12$	$1030^{a} \pm 25$
NO_3^{-}	mg/L	$100^{b} \pm 5$	$400^{a} \pm 15$
NH_4^{+}	mg/L	$260^{a} \pm 15$	$40^{b} \pm 3$
Ca^{++}	mg/L	$700^{b} \pm 8$	$1400^{a} \pm 17$
Mg^{++}	mg/L	$150^{a} \pm 7$	$50^{b} \pm 5$

Table4 Chemical and biological characteristics of solid and liquid digestate fractions from **Uliva** Plant. Values are means ± SE (n=4). Different letters in the same row indicate significant differences $P \leq 0.05$.

Table 5 Germination indexes: Total germination (TG); Coefficient of germination velocity (CVG), Germination Rate Index (GRI) and Mean Germination Time (MGT) determined for lettuce, watercress and cucumber seeds treated with different concentration of Fattoria digestate.

	Lettuce				Watercress				Cucumber			
Treatment	TG %	CVG %	GRI %	MGT days	TG %	CVG %	GRI %	MGT days	TG %	CVG %	GRI %	MGT days
Control	100	27.8	27.7	3.9	100	26.7	27.5	3.8	100	28.9	27.8	3.4
LF 10%	80	27.5	27.4	4.1	80	26.0	27.1	4.0	100	28.2	27.9	3.5
LF 25%	nd	nd	nd	nd	nd	nd	nd	nd	100	28.9	28.4	3.3
LF 50%	nd	nd	nd	nd	nd	nd	nd	nd	100	28.6	27.9	3.5
LF 100%	nd	nd	nd	nd	nd	nd	nd	nd	100	28.5	27.6	3.6
SF 10%	100	29.8*	29.9*	3.4*	100	29.6*	31.1*	3.3*	100	28.9	27.8	3.7
SF 25%	70	28.5*	29.6*	5.1*	70	28.8*	28.6*	3.4*	100	28.8	27.7	3.5
SF 50%	48	14.3**	16.8**	19***	55	14.0**	19.8**	10***	100	28.7	27.7	3.7
SF 100%	20	14.2**	2.28***	15***	18	14.0**	2.12***	16***	100	28.4	27.3	3.7

***p<0.001; ** p<0.01: *p<0.05; *p<0.1

Table 6 Germination indexes: Total germination (TG); Coefficient of germination velocity (CVG), Germination Rate Index (GRI) and Mean Germination Time (MGT) determined for lettuce, watercress and cucumber seeds treated with different concentration of liquid and solid fractions of Uliva digestate.

	Lettuce				**Watercress**				**Cucumber**			
Treatment	TG %	CVG %	GRI %	MGT days	GP %	CVG %	GRI %	MGT days	GP %	CVG %	GRI %	MGT days
Control	100	28.8	27.7	3.7	100	28.7	27.5	3.6	100	28.9	27.8	3.4
LU 10%	nd	nd	nd	nd	nd	nd	nd	nd	50	26.5*	23.0**	4.4*
LU 25%	nd	nd	nd	nd	nd	nd	nd	nd	29	24.6**	20.8**	4.8*
LU 50%	nd	nd	nd	nd	nd	nd	nd	nd	10	15.5*	15.3**	30***
LU 100%	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SU 10%	40	25.8*	21.4**	4.2*	35	25.6*	21.1**	4.5*	59	26.8*	23.4**	4.0*
SU 25%	20	19.6**	6.6***	5.1*	18	19.8**	6.4***	5.4*	35	21.6**	21.8**	4.4*
SU 50%	15	14.3**	0.28***	35***	10	14.0**	0.28***	35***	20	19.7*	18.6***	4.9*
SU 100%	8	14.2**	0.28***	35***	5	14.0**	0.28***	35***	10	15.7*	15.4**	28***

***p<0.001; ** p<0.01: *p<0.05; *p<0.1

Table7 Analysis of variance of different treatments of Fattoria and Uliva digestate fractions (solid and liquid) on total germination (TG) and mean germination time (MGT) of seeds of lettuce, watercress and cucumber

	Lettuce		Watercress		Cucumber	
Fattoria						
	TG	MGT	TG	MGT	TG	MGT
R^2	0.999	0.995	1.00	0.995	0.437	0.784
Source of Variance*: F-ratio*						
Concentrations	5478.28***	183.53***	8740.86***	199,631***	n.s	7.00**
Fractions	4150.21***	1382.25***	6981.82***	1564.50***	n.s	30.73***
Conc x Fractions	647.66***	428.45***	1125.23***	472.95***	n.s	3.46*
Uliva						
	Lettuce		Watercress		Cucumber	
	TG	MGT	TG	MGT	TG	MGT
R^2	0.999	0.998	1.00	0.999	0.999	0.998
Source of Variance*: F-ratio*						
Concentrations	4513.91***	378.47***	19193.37***	638.18***	6442.46***	732.76***
Fractions	955.862***	1914.50***	2376.56***	3053.74***	260.12***	10.11***
Conc x Fractions	157.61***	454,16***	487.19***	745.84***	21.04***	1554.10***

***p<0.001; ** p<0.01: *p<0.05; *p<0.1

Table 8 Activities of ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase, (POX) and catalase (CAT) enzymes in 7 day old lettuce seedlings under Solid (SU) and Liquid (LU) Uliva and Solid (SF) and Liquid (LF) Fattoria treatments at different concentrations. Values are means ± SE (n=4). Different letters in the same column indicate significant differences P≤0.05.

Treatment	**APX** U mg^{-1} prot	**GR** U mg^{-1} prot	**POX** U mg^{-1} prot	**CAT** U mg^{-1} prot
Control	1.41 ± 0.2^{d}	0.05 ± 0.03^{e}	0.26 ± 0.1^{f}	25 ± 2.0^{f}
LU 10%	nd	nd	nd	nd
LU 25%	nd	nd	nd	nd
LU 50%	nd	nd	nd	nd
LU 100%	nd	nd	nd	nd
SU 10%	2.92 ± 0.2^{b}	0.11 ± 0.02^{d}	0.29 ± 0.2^{f}	56 ± 2.1^{c}
SU 25%	3.44 ± 0.3^{b}	0.16 ± 0.02^{c}	0.56 ± 0.3^{d}	99 ± 4.5^{b}
SU 50%	4.40 ± 0.5^{a}	0.20 ± 0.01^{b}	1.10 ± 0.2^{b}	107 ± 3.5^{b}
SU 100%	4.97 ± 0.4^{a}	0.25 ± 0.03^{a}	1.26 ± 0.1^{a}	135 ± 4.0^{a}
LF 10%	1.98 ± 0.1^{c}	0.08 ± 0.03^{e}	0.28 ± 0.1^{f}	29 ± 1.5^{e}
LF 25%	nd	nd	nd	nd
LF 50%	nd	nd	nd	nd
LF 100%	nd	nd	nd	nd
SF 10%	1.55 ± 0.2^{d}	0.06 ± 0.01^{e}	0.27 ± 0.2^{f}	24 ± 2.1^{f}
SF 25%	1.76 ± 0.2^{d}	0.07 ± 0.02^{e}	0.30 ± 0.3^{f}	29 ± 2.0^{f}
SF 50%	2.40 ± 0.3^{b}	0.12 ± 0.01^{d}	0.39 ± 0.2^{e}	37 ± 3.1^{d}
SF 100%	3.22 ± 0.5^{b}	0.16 ± 0.02^{c}	1.06 ± 0.1^{c}	35 ± 2.0^{d}

Table 9 Activities of ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase, (POX) and catalase (CAT) enzymes in 7 day old watercress seedlings under Solid (SU) and Liquid (LU) Uliva and Solid (SF) and Liquid (LF) Fattoria treatments at different concentrations. Values are means ± SE (n=4). Different letters in the same column indicate significant differences P≤0.05.

Treatment	**APX** U mg^{-1}prot	**GR** U mg^{-1} prot	**POX** U mg^{-1} prot	**CAT** U mg^{-1} prot
Control	2.21±0.5^{c}	0.09±0.03^{d}	0.32±0.1^{d}	27±2.0^{f}
LU 10%	nd	nd	nd	nd
LU 25%	nd	nd	nd	nd
LU 50%	nd	nd	nd	nd
LU 100%	nd	nd	nd	nd
SU 10%	2.42±0.2^{c}	0.16±0.02^{b}	0.33±0.2^{f}	67±2.1^{c}
SU 25%	2.74±0.3^{c}	0.16±0.02^{b}	0.36±0.3^{d}	108±4.5^{b}
SU 50%	4.00±0.5^{a}	0.25±0.01^{a}	1.4±0.2^{b}	109±3.5^{b}
SU 100%	4.38±0.4^{a}	0.28±0.03^{a}	1.76±0.1^{a}	144±4.0^{a}
LF 10%	1.77±0.4^{c}	0.06±0.03^{d}	0.33±0.1^{d}	27±2.5^{d}
LF 25%	nd	nd	nd	nd
LF 50%	nd	nd	nd	nd
LF 100%	nd	nd	nd	nd
SF 10%	1.23±0.2^{d}	0.07±0.01^{d}	0.29±0.2^{d}	27±2.1^{d}
SF 25%	1.76±0.2^{c}	0.09±0.02^{d}	0.31±0.3^{d}	31±2.0^{d}
SF 50%	3.00±0.3^{b}	0.15±0.01^{c}	0.47±0.2^{e}	33±3.1^{d}
SF 100%	3.42±0.5a^{b}	0.19±0.02^{b}	0.98±0.1^{c}	33±2.0^{d}

Table 10 Activities of ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase, (POX) and catalase (CAT) enzymes in 7 day old cucumber seedlings under Solid (SU) and Liquid (LU) Uliva and Solid (SF) and Liquid (LF) Fattoria treatments at different concentrations. Values are means ± SE (n=4). Different letters in the same column indicate significant differences P≤0.05.

Treatment	**APX** U mg^{-1} prot	**GR** U mg^{-1}prot	**POX** U mg^{-1} prot	**CAT** U mg^{-1} prot
Control	3.34±0.5^{b}	0.11±0.01^{c}	2.5±0.1^{b}	31±2.0^{e}
LU 10%	4.10±0.5^{a}	0.15±0.01^{b}	5.4±0.7^{a}	47±2.0^{d}
LU 25%	4.33±0.5^{a}	0.17±0.01^{b}	6.1±0.5^{a}	56±2.0^{c}
LU 50%	5.44±0.7^{a}	0.28±0.01^{a}	6.6±1.0^{a}	61±2.0^{b}
LU 100%	nd	nd	nd	nd
SU 10%	3.82±0.2^{ab}	0.16±0.02^{b}	5.1±0.6^{a}	37±2.1^{e}
SU 25%	3.94±0.^{ab}	0.19±0.03^{b}	6.0±0.9^{a}	48±4.5^{b}
SU 50%	4.55±0.5^{a}	0.26±0.04^{a}	6.3±0.5^{a}	69±3.5^{a}
SU 100%	5.38±0.4^{a}	0.29±0.04^{a}	7.4±1.2^{a}	74±4.0^{a}
LF 10%	2.77±0.4^{b}	0.09±0.03^{c}	2.3±0.5^{b}	26±1.5^{e}
LF 25%	2.94±0.5^{b}	0.08±0.02^{c}	2.4±0.3^{b}	31±2.0^{e}
LF 50%	2.78±0.7^{b}	0.11±0.03^{c}	2.5±0.5^{b}	33±3.1^{e}
LF 100%	3.04±0.5^{b}	0.10±0.03^{c}	2.3±0.5^{b}	33±2.0^{e}
SF 10%	2.54±0.7^{b}	0.07±0.01^{c}	2.1±0.2^{b}	30±2.1^{e}
SF 25%	2.49±0.65^{b}	0.08±0.02^{c}	2.3±0.4^{b}	31±2.0^{e}
SF 50%	2.77±0.4^{b}	0.11±0.03^{c}	2.2±0.2^{b}	30±3.1^{e}
SF 100%	3.01±0.5^{b}	0.09±0.02^{c}	2.4±0.3^{b}	31±2.0^{e}

Table 11 Total Antioxidant Activity (ToA, μmol α-tocopherol/ g FW) and Total Phenols (TP, μg TAET/g DW) in 7 day old seedlings of lettuce, watercress and cucumber with Solid (SU) and Liquid (LU) Uliva and Solid (SF) and Liquid (LF) Fattoria digestates at different concentrations. Values are means ± SE (n=4). Different letters in the same column indicate significant differences P≤0.05.

	Lettuce		**Watercress**		**Cucumber**	
Treatment	ToA	TP	ToA	TP	ToA	TP
Control	0.65 ± 0.02^{c}	209 ± 10^{d}	0.73 ± 0.02^{c}	231±11 ^{b}	0.77 ± 0.01^{c}	244±14^{c}
LU 10%	nd	nd	nd	nd	1.91 ± 0.10 ^{b}	555 ± 25^{b}
LU 25%	nd	nd	nd	nd	2.62 ± 0.03 ^{a}	521± 10 ^{b}
LU 50%	nd	nd	nd	nd	2.99 ± 0.02 ^{a}	625± 20^{a}
LU 100%	nd	nd	nd	nd	nd	nd
SU 10%	2.06 ± 0.4^{b}	275±10^{c}	3.13±0.15^{ab}	285±10^{b}	1.80 ± 0.04^{b}	223±22^{c}
SU 25%	2.91 ± 0.9^{b}	299±8^{b}	3.55±0.4^{a}	317±8^{b}	2.44 ± 0.02^{a}	243±18^{c}
SU 50%	3.39 ± 0.2^{a}	345 ±10^{a}	3.97±0.2^{a}	356±12^{b}	2.68 ± 0.03^{a}	229±19^{c}
SU 100%	3.94 ±0.5^{a}	367 ±12^{a}	4.43 ± 0.5^{a}	398±15^{a}	2.84 ± 0.05^{a}	233±20^{c}
LF 10%	0.85 ± 0.01^{c}	223±10^{d}	0.75 ± 0.08^{c}	256±13	0.79 ± 0.01^{c}	234±16^{c}
LF 25%	nd	nd	nd	nd	0.87 ± 0.01^{c}	235±15^{c}
LF 50%	nd	nd	nd	nd	0.84 ± 0.01^{c}	241±14^{c}
LF 100%	nd	nd	nd	nd	0.79 ± 0.01^{c}	245±13^{c}
SF 10%	0.69 ± 0.04^{c}	219±9^{d}	1.01 ± 0.09^{c}	233±12^{b}	0.78 ± 0.01^{c}	232±11^{c}
SF 25%	1.77 ± 0.4^{b}	224±10^{d}	1.33 ± 0.12^{d}	243± 6^{b}	0.67 ± 0.01^{c}	236±14^{c}
SF 50%	2.40 ± 0.9^{b}	305±10^{b}	2.24 ± 0.5^{b}	277±15^{a}	0.88 ± 0.01^{c}	247±11^{c}
SF 100%	3.10 ± 0.2^{a}	317±9^{b}	2.97 ± 0.7^{b}	299±10^{a}	0.97 ± 0.01^{c}	249±10^{c}

Table 12 Correlation coefficient between fluorescein diacetate hydrolysis (FDA) and total phenol (TP) on mean germination time (MGT) of lettuce, watercress and cucumber seeds.

		Fattoria					
		Lettuce		Watercress		Cucumber	
Fractions		SF	LF	SF	LF	SF	LF
FDA	r	0.957***	0.872*	0.959***	0.795	0.657**	0.696**
	R^2	0.915	0.761	0.921	0.631	0.432	0.485
TP	r	0.783**	0.901*	0.782***	0.837*	0.586*	0.693**
	R^2	0.613	0.811	0.611	0.700	0.344	0.480
		Uliva					
		Lettuce		Watercress		Cucumber	
Fractions		SU	LU	SU	LU	SU	LU
FDA	r	0.885***	-	0.882***	-	0.902***	0.900***
	R^2	0.783	-	0.778	-	0.813	0.810
TP	r	0.883***	-	0.880***	-	0.903***	0.899***
	R^2	0.780	-	0.775	-	0.816	0.808

***p<0.001; ** p<0.01: *p<0.05; *p<0.1