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## Refuse derived soluble bio-organics enhancing chlorophyll yield, growth and productivity for tomato plants.

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### ABSTRACT

A municipal bio-refuse (CVD), containing kitchen wastes, home gardening residues and public park trimmings, was treated with alkali to yield a soluble bio-organic fraction (SBO) and an insoluble residue. These materials were characterized by elemental analysis, potentiometric titration, and <sup>13</sup>C NMR and IR spectroscopy, and then applied as organic fertilizers to soil for tomato greenhouse cultivation. Their performance was compared with a commercial product obtained from animal residues. Plant growth, fruit yield and quality, and soil and leaves chemical composition, were the selected performance indicators. The SBO exhibited the best performance by enhancing leaves chlorophyll content, and improving plant growth, and fruit ripening rate and yield. No product performance-chemical composition relationship could be assessed. The SBO solubility and photosensitizing properties appeared the main parameters connected to its superior performance as tomato growth promoter.

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## 1. Introduction

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Soluble substances of biological origin (SBO) isolated from urban biowastes (UBW) of different composition and aging conditions have been reported (Montoneri et al., 2011) to have promising performances as chemical auxiliaries for a number of technological applications in the chemical industry and in environmental remediation. These results, coupled with the easy, virtually no cost, availability of UBW offer scope to develop sustainable products and processes from bio-refuse at industrial and commercial level.

The above SBO have been shown to have chemical similarities with natural soil-organic matter. They are described as mixtures of macromolecules with weight average molecular weight ( $M_w$ ) values ranging from 67 to 463  $\text{kg mol}^{-1}$  and number average molecular weight ( $M_n$ ) yielding polydispersity index values ( $M_w/M_n$ ) in the 6 to 53 range (Montoneri et al, 2010). These macromolecules contain several functional groups and C types of different polarity: i.e. long aliphatic C chains substituted by aromatic rings and several functional groups as COOH, CON, C=O, PhOH, O-alkyl, OAr, OCO, OMe, and NRR', with R and R' being alkyl C or H. These chemical features determine their behavior as surfactants and/or polyelectrolytes. The most recent data published for SBO indicate that their chemical composition (Montoneri et al., 2011) and physical-chemical properties (Montoneri et al., 2010) vary over a wide range as a function of the composition and aging conditions of the sourcing bio-refuse. Thus UBW, by virtue of their variability due to biological factors and treatment types, may provide a wide range of bio-based products differing for chemical composition and properties and, therefore, may potentially be used in place of or in conjunction with commercial synthetic chemicals for a large variety of uses. Exploration of other fields of application for SBO seems at this point a worthwhile effort to undertake in order to evaluate the full potential of UBW as sustainable source of chemicals to replace or reduce the use of synthetic chemicals.

One application of the above SBO which has not been reported is in agriculture. Eco-friendly agriculture has been a new trend to ensure sustainable productivity and conserve environmental quality of

soil and water, reduce pollution, recycle organic resources, and produce safe foods (Dorais, 2007). Indeed, synthetic chemicals have been dumped continuously over years and have made the land infertile, leading to yield losses. Bio fertilizers have become an ideal substitute for chemical fertilizers for conditioning the soil fertility and to maintain the agro-ecosystem. Investigation of the performance of SBO for these purposes seemed highly worthwhile to the authors of the present work in view of the relevant share of the agriculture market for chemicals and of the expected world demand increase for fertilizers (Freedonia Group, 2011), particularly for organic agriculture (Dorais, 2007), of the positive effects in agriculture reported for similar substances isolated from peat (Böhme, 1999; Hoanf,g et al., 2001) or other sources (Atiyeh et al., 2002; Valdrighi et al., 1996), and of the market price of peat based commercial products (Montoneri et al., 2011) rated up to 10-15 \$ kg<sup>-1</sup>. These facts pointed out a rather high stake for investigating SBO as growth promoters or soil activators in agriculture (Dorais, 2007), either from the economic and the environmental point of views. In essence, extending the range of applications of these substances to the agriculture market offered the perspective, in case of technological transfer of the research results to industrial and commercial level, to attain a net revenue from SBO production and sale up by 10 xs more than that expected from their use in the chemical technology and environmental remediation.

Investigation of SBO in this work required a different approach from the above referenced work reporting their technological applications in the chemical industry and in environmental remediation. In the latter cases the need to undertake a chemical process for the isolation of SBO from its sourcing matter was justified by the need to have a water soluble product. For agriculture use, the product solubility, although desirable, did not appear strictly necessary or critical to attain the desirable effect on plant growth. Previous work on similar substances isolated from peat or other sources has not specifically addressed this point. Raw UBW are known to contain humic-like substances, which although insoluble or not readily soluble, may still contribute amelioration of soil properties for agricultural purposes. Composted UBW added to soil have been reported to bring about significant changes of the physicochemical parameters of soil (Adani et al., 2007), such as cation exchange capacity and N and

organic C content, which may improve soil fertility (Ferrer and Gupta, 1983; Haber, 2008). Based on these considerations, an experimental plan was designed for the present work to test the performance of SBO as aids in greenhouse cultivation of tomato, one of the most important vegetables with a yearly world production of 130 million tons (Bingqing, 2010; Growtomatoes.com, 2005).

The experimental cultivation plan in the present work was performed in a private farm located in south-east Sicily, Italy, where greenhouse installations have contributed since 1950 to develop horticulture production and export throughout Europe and, thus, to raise greatly the social and economic level of the local population. For the experimental plan, a readily available composted UBW in metropolitan areas was digested as previously reported (Montoneri et al., 2011) with alkaline water to yield an SBO solution which was allowed to settle in order to separate from the insoluble organic residue (IOR). All materials, UBW and its SBO and IOR fractions, were characterized for their chemical nature and tested for effects on tomato plant growth and fruit quality and yield by comparison with a commercial product (RCP). This latter product, derived from animal wastes, was normally used in the hosting farm with the intent to supply soil in organic field-vegetable production where high nutrient demands must be covered within relatively short periods (Dorais, 2007). As one concern in using the above bio-refuse derived material in agriculture might be their content of trace metals for potential ecotoxicologic effects (Dorais, 2007; Ferrer and Gupta, 1983; Haber, 2008; Gallardo-Lara et al. 1999), both soil and leaves were analyzed for these elements. The results of the agriculture tests were analyzed against the chemical nature and properties of the materials applied to the soil.

## **2. Materials and Methods**

### *2.1. Starting materials and chemical characterization.*

The compost, hereinafter referred to by the CVD acronym, was supplied by Acea Pinerolese Industriale SpA, Pinerolo (TO), Italy in October 2009. This company has a urban waste treatment plant performing anaerobic digestion for 14 days of the organic humid fraction of urban refuse (FORSU) obtained by separate source collection practice to yield biogas and digestate (FORSUD) containing residual

lignocellulosic material. To obtain CVD, FORSUD was mixed with gardening and park trimming residues (GR) at 33/67 w/w FORSUD/GR ratio and composted for 110 days. Composting took place in 1.5 m high piles laid over a 3x70 m<sup>2</sup> area. During the first 21 days the pile was turned once a week and reached temperature up to 70 °C. Afterwards, the solid residue was aged for 90 days and turned only one more time at the 75th day. The product was identified by the supplier code 96 P09 M05 at the August 2009 production date. The SBO in 10 % w/w yield and the IOR materials were obtained using a pilot production facility comprising a 500 liter capacity reactor which operated according to a previously reported procedure (Montoneri et al., 2011). Thus, CVD was treated under stirring with alkaline water at 4 ml g<sup>-1</sup> liquid/solid and 0.02 w/w NaOH/CVD ratios at 60 °C for 4 h. The reaction mix was allowed settling to separate the supernatant liquid containing the SBO fraction from the solid IOR residue. The IOR residue was washed once with fresh water at 4 ml g<sup>-1</sup> added water/IOR ratio. The total collected liquid phase was centrifuged to separate fine solid particles. The recovered SBO solution and IOR solid products were allowed to concentrate and/or dry in ventilated oven at 60 °C to yield the analytical values reported in Table 1. The reference commercial product (RCP) was supplied by the farm (see 2.2) where the cultivation trials were performed. This product, normally used in the farm soil treatment, was declared by the supplier to contain meat, bone and animal blood meal (Dorais, 2007). It was found very poorly soluble in water. All products were characterized for their chemical compositions by the analytical data reported in Table 1 obtained by methods and instruments previously described (Montoneri et al., 2011).

## 2.2 Set up of cultivation trials.

The cultivation trials were carried out in the Gambuzza farm located along the sea shore in Punta Secca in the province of Ragusa, Italy, starting from November 2009 through August 2010. The farm soil was identified sandy according to its texture analysis: coarse sand 79.9 ± 2.5 % w/w, fine sand 5.3 ± 0.7 % w/w, silt 10.6 ± 1.8 % w/w, clay 4.2 ± 0.4 % w/w. The experiment was set up as a completely randomized design with 3 replications. The soil covered by greenhouse was divided into 15 parcels, each covering 25 m<sup>2</sup> soil surface. The parcels were arranged randomly to be treated as follows: 12 parcels with

the four products listed in Table 1, each tested in triplicates, and 3 control parcels not treated. The test plant seedlings were transplanted to the field on November 6, 2009 in all parcels in sets of double rows to have 3 plants per square meter (Ho, 1984) at 33 cm distance from each other along the single row, whereas the distance between rows in each single set was 50 cm and the distance between sets was 150 cm. The hosting green house fabricated with 0.15 mm thick polyethylene sheets supported by cement and wood was equipped with automatic watering and mineral fertilizer dispenser. The soil was treated according to the protocol adopted by the Gambuzza farm in their normal cultivation activity for commercial tomato production, except for the addition of CVD, IOR and SBO in place of RCP. Thus, all parcels had the following basal mineral fertilization (Dorais, 2007; Chapagain and Wiesman, 2004):  $P_2O_5$  60 kg ha<sup>-1</sup> supplied as mineral ammonium phosphate,  $K_2O$  100 kg ha<sup>-1</sup> supplied as potassium sulfate. This was followed by the addition of the product listed in Table 1 intended to contribute organic matter to soil, and then by tomato seedlings transplanting. Application of organic materials, following mineral fertilizers and before transplanting, is an important practice for restoring the fertility of soil by improving its structure, maintaining the level of organic C and N, and controlling the microflora. Under normal practice conditions at the Gambuzza farm and in the other farms located in the same area, the amount of RCP supplied to the soil was intended to contribute 1.1-1.2 ton ha<sup>-1</sup> of organic matter and about 130 kg ha<sup>-1</sup> of N as reported in literature for tomato plants cultivation (Dorais, 2007). Thus, based on the chemical composition data in Table 1, different amounts of CVD, IOR and SBO were supplied to the soil in order to guarantee the same o.m. application rate contributed with RCP. The experimental plan covered the whole production time from tomato seedling transplanting in November 2009 through harvesting in June 2010. Over this time, soil irrigation was performed with the drip irrigation system (Ho, 1984) to supplement the natural soil water and mineral depletion and fulfill the plant needs over the plant growth and production cycle. To this end, the above phosphate and sulfate products were used in conjunction with a mineral nitrogen fertilizer containing 32.4 % total N as nitrate, ammonia and urea in 1:1:2.5 ratio. The frequency of irrigation depended on weather conditions and was the same for all test parcels. Irrigation was performed approximately every 10-15, covering the whole crop production cycle,



to provide different N/K ratios depending on the plant growth/production stage; i.e. 1:3 N/K from transplanting to flowering N to encourage plant flowering, 1:2 N/K at beginning fruit-setting of the fourth cluster, then 1:1 N/K after the start of fruit ripening to promote leaf development. Throughout the entire crop cycle the total amounts of supplied nutrients were at about 0.80 N ton ha<sup>-1</sup> and 1 K ton ha<sup>-1</sup> level. The plants were pruned in early March in order to stop vegetative growth and enhance fruit production. Thus, monitoring of the plant biometric data ended at the same date. Soil, plant, leaves and fruits were analyzed at time intervals for chemical and physical features. Results are reported only for surveys performed at dates where significant differences were found relatively to the previous survey.

### *2.3 Plant and harvest analyses and measurements.*

Four plants in the center row of each parcel were sampled. Considering the three replicates per treatment, values for each treatment reported hereinafter are averages of measurements performed over 12 plants (3 replicate plots and 4 plants per replicate), unless otherwise indicated. Out of each plant, 4 flower or fruit clusters were sampled. Thus values reported in Tables 4 and 5 are averages of measurements of 48 flower-fruit clusters. Plant height, branches diameter and inter-nodes length were measured at different dates from seedlings transplanting. For each plant, internode length values were calculated as averages of the measurements performed on the first four consecutive internodes. Plant vigor was visually evaluated. Vigor was rated by an arbitrary index scale ranging from 1 (not vigorous plant) to 9 (very vigorous plant) established by the evaluators panel constituted by three local agriculture experts. According to this scale a plant very vigorous is in a more-than-optimal nutrition; on the contrary, a not vigorous plant is a barely fed plant. The leaves chlorophyll content was measured by means of a portable SPAD-502 Minolta chlorophyll meter. Fruits were analyzed immediately after harvesting. Fruits total soluble solids, titratable acidity, pH, electrical conductivity and firmness were measured according to established procedures (Chapagain and Wiesman, 2004; Thybo et al., 2006; Jones and Scott, 1983; Carli et al, 2011; Mata et al, 2000; Ünlü et al, 2011). Total soluble solids (TSS) were measured on tomato juice samples extracted from fully ripe fruits using a hand-held digital refractometer Model HB 32 ATC, ERREPI, Solarolo (RA), Italy. The fruit titratable acidity and pH were determined on the whole fruit

homogenate water suspension. The fruit titratable acidity (as % citric acid) was determined by titration using standard 0.1 N NaOH solution in the presence of phenolphthalein as an indicator. The pH was measured using Hanna pH 211 pH meter. Conductivity was measured by conductivity meter Jenway 4200. Firmness was determined by means of model FT327 Bertuzzi, Italy, fruit pressure tester. Measurements were taken from the opposite cheeks of each fruit (skin removed). The maximum force required to reach the bioyield point was recorded.

#### *2.4 Soil and leaves analyses.*

Soil samples were taken at 0-30 cm depth at three dates covering the crop production cycle from start to end. Four samples per parcel were taken and homogenized. The homogenized sample was analysed in triplicates according to the official methods for soil analysis issued by the Italian Ministry for Agriculture (Ministero per le Politiche Agricole, 1997 and 1998). The pH and electrical conductivity were determined in water at 1:2.5 solid/water ratio. Microanalyses for C and N content were performed on 0.5 mm sieved samples. Analyses were performed for exchangeable cations, held on negatively charged soil sites, and assimilable nutrients, i.e. those which may be absorbed by the roots. The assimilable P concentration was determined colorimetrically (phosphomolybdic complex), after NaHCO<sub>3</sub> extraction. The assimilable Na, Mg, Fe, Zn, Cu, and Mn concentrations were measured by atomic absorption spectrophotometry after ammonium acetate/ethylenediaminetetraacetic acid extraction. The exchangeable K, Ca, and Mg concentrations were determined by atomic absorption spectrophotometry after BaCl<sub>2</sub> extraction. The total silicon concentration has been measured by hot mineralization with concentrated HNO<sub>3</sub>-HF (1:3, v/v). Leaves were also sampled at the start of the crop production and at the full production time. For each plot 10 plants were selected to sample complete leaves (petiole and flap). The sampling was performed by taking the 4th-6th leaf down starting from the plant apex. The leaves were taken from both sides of the plant to avoid possible effects due to leaves orientation. The samples were analysed according to literature (Gallardo-Lara et al, 1999; Uni, 1998). C and N contents were measured by elemental analysis. The total P concentration was determined colorimetrically (phosphomolybdic complex), after hot mineralization (HClO<sub>4</sub>-HNO<sub>3</sub>). The total Na, K, Mg, Fe, Zn, Cu, and Mn concentrations were determined

by atomic absorption spectrophotometry after hot mineralization with  $\text{HNO}_3$ . The total B concentration was determined colorimetrically (azomethine-H method) on the calcined sample. Soil and leaves samples were analyzed in triplicates for each treatment. Treatments were compared for their average values by Anova analysis of variance and Student test.

### 3. Results and Discussion

#### 3.1 Chemistry of materials applied to soil.

The UBW sourced materials investigated in this work are products of biological origin with quite complex chemical compositions. Assessment of their chemical nature is difficult, owing to the broad distribution of molecular weight and to the content of many organic moieties from the main constituents of vegetable matter which are not completely mineralized by biodegradation. They are normally analyzed for their content of organic matter (o.m.) obtained from the weight loss after calcination at  $650\text{ }^\circ\text{C}$ , their C and N elemental analytical data, and their organic moieties and functional groups content obtained by  $^{13}\text{C}$  NMR spectroscopy (Montoneri et al, 2011). At best, organic matter in these materials can be virtually represented by molecular fragments such as those shown in Fig. 1, where aliphatic and aromatic C moieties and functional groups can be varied to fit analytical data.

Tables 1 report chemical data for the compost (CVD) of a 1: 2 w/w mix of kitchen waste left over organics after anaerobic digestion and fresh vegetable residues, and for the SBO and IOR fractions recovered after CVD alkali digestion (see 2.1). Data are also reported for the commercial product used in this study for comparison, i.e. RCP of animal origin (see see 2.1). All these materials are confirmed to contain both inorganic and organic matter (Table 1). Compared to their sourcing CVD material, SBO and IOR have lower (29 %) and higher (62 %) ash contents respectively. This shows that the alkaline extraction process allows some selectivity in separating the starting inorganic matter from the organic matter, to yield the soluble fraction with relatively higher organics, and coherently the IOR fractions with poorer organics content than CVD. Table 1 also shows that o.m./C and C/N ratios are strongly affected by

the alkaline treatment. The o.m./C ratio values varying between 1.5 and 2.1 indicate a significant chemical composition difference among the investigated materials. The higher N/C ratio for SBO compared to CVD and IOR indicates that the alkaline treatment of CVD allows to concentrate the CVD starting organic N in soluble form as SBO. By comparison, RCP has the highest N/C ratio. This points out a strong difference in chemical constituents between RCP and the other three CVD, SBO and IOR materials, due to their different.

Table 1 reports also some useful data related to the organic matter and its fate determined from the reaction of CVD with alkali. It may be readily observed that, compared to CVD and IOR, SBO contains more aliphatic and aromatic C, much higher carboxyl C and much lower O-R C. These data are consistent with soluble substances formed following hydrolysis of CVD organic matter. The O-R C shown in Table 1 is likely to be contributed by ROR and RCO(OR') moieties. Following hydrolysis of ester functional groups in CVD, RCO(OH) and R'OH functional groups are obtained. The results are then likely to indicate that the RCO(OH) molecular fragments are soluble and become components of the soluble SBO products, while R'OH are insoluble molecular fragments constituting the IOR fraction. This latter product contains therefore the memories of ether linkages in the CVD organic matter which resist hydrolysis under the alkaline treatment. Further support to the above interpretation was obtained by IR spectroscopy. The IR spectra in Fig. 1a exhibit several broad bands which are assigned according to literature (Montoneri, 2005; Smidt, 2007) to bonds vibration involving the elements or functional groups listed in Table 1, i.e. the band at  $3422\text{ cm}^{-1}$  to total O-H and N-H bonds in  $\text{NH}_2$ , RCONH, ArCONH, COOH, ArOH, ROH functional groups, the band at  $2933\text{ cm}^{-1}$  to aliphatic C-H bonds, the band at  $1540\text{--}1750\text{ cm}^{-1}$  to C=O bonds in carboxylic acid or salts, amide and ester functional groups, the band at  $1380\text{ cm}^{-1}$  to aromatic skeletal C=C bonds, the band at  $1000\text{--}1200\text{ cm}^{-1}$  to C-O bonds in ether, phenol and ester functional groups and to M-O bonds with  $M = \text{Si}$  and other metal elements. The most significant detectable differences in the spectrum of SBO compared to CVD and IOR are (i) the change of the C=C to C=O bands ratio (CVD 0.40, IOR 0.86, SBO 0.56), (ii) the lower wavelength of the C=O absorption maximum ( $1595\text{ cm}^{-1}$  for SBO and at  $1648\text{ cm}^{-1}$  for CVD and IOR) and (iii) the lower relative

absorbance of the band falling at  $1030\text{ cm}^{-1}$ . These features are respectively consistent with (i and ii) the higher content of carboxylate ( $\text{RCOO}^-$ ) groups in SBO and (iii) the lower content of mineral elements. To validate this interpretation SBO was washed with HCl and HF, as reported in the experimental section, to further separate the organic matter from mineral matter. The expected ash free product ( $\text{SBO}_{\text{af}}$ ), recovered as insoluble material in the acid reagents, contained only 0.8 % of mineral matter and accounted for nearly 55 % of the organic matter content present in the pristine SBO sample. The remaining 45 % organics and 99 % of starting mineral matter were soluble in the acid reagents. The data in Table 1 show that the C types and functional groups relative composition in  $\text{SBO}_{\text{af}}$  is quite different from that in the sourcing SBO sample. The former shows more aromatic C, particularly phenol C, and less O-R C than the latter. Consistently with the higher organics content, relatively higher concentrations of COOH and PhOH functional groups in  $\text{SBO}_{\text{af}}$  compared to SBO were confirmed from data obtained by potentiometric titration: i.e. COOH  $3.42\text{ meq g}^{-1}$  and PhOH  $1.87\text{ meq g}^{-1}$  in  $\text{SBO}_{\text{af}}$  compared to COOH  $3.17\text{ meq g}^{-1}$  and PhOH  $1.03\text{ meq g}^{-1}$  in SBO. Also, in agreement with Table 1 data the PhOH/COOH ratio in  $\text{SBO}_{\text{af}}$  (0.55) was higher than in SBO (0.32). The difference between SBO and  $\text{SBO}_{\text{af}}$  should not surprise. Presumably, the HCl/HF washing allows a further separation of the molecular mix constituting SBO into acid insoluble molecules ( $\text{SBO}_{\text{af}}$ ) richer in phenol functional groups and acid soluble molecules relatively richer in aliphatic and carboxyl C.

The IR spectrum of  $\text{SBO}_{\text{af}}$  in Fig. 1 showing no clearly detectable absorption band at  $1030\text{ cm}^{-1}$  in  $\text{SBO}_{\text{af}}$  validates the above assignment of this band to M-O bonds in the CVD, SBO and IOR spectra. Other features that are strictly connected to the nature of the organic matter in  $\text{SBO}_{\text{af}}$  are the well distinct absorption at  $1710\text{ cm}^{-1}$  and the rather strong absorption at  $1220\text{ cm}^{-1}$ . The band at  $1710\text{ cm}^{-1}$  is assigned to the C=O vibration in free COOH functional groups (Silverstein et al., 1991). The broad band peaking at  $1220\text{ cm}^{-1}$  is assigned to phenols. Solid phenols are known in fact to absorb at  $1390\text{-}1330\text{ cm}^{-1}$  and at  $1260\text{-}1180\text{ cm}^{-1}$ , with the long wavelength band being stronger, as a result of the interaction between O-H bending and C-O stretching (Silverstein et al., 1991). Consequently, since the COOH C=O band is well observed to fall at  $1710\text{ cm}^{-1}$ , the band at  $1614\text{ cm}^{-1}$  may well be assigned to the vibration of C=O bonds

in amide or amino acids functional groups. These spectral features clearly distinguish the SBO<sub>af</sub> spectrum from that of its sourcing SBO matter. The high intensities of the bands due to free carboxylic acids, amide and/or aminoacids, and phenol functional groups observed in the IR spectrum of SBO<sub>af</sub> are well consistent with the high content of the same functional groups reported in Table 1. It may indeed be observed that COX and Ph-O C concentration values in Table 1 are nearly the same. The data collected on SBO<sub>af</sub> further confirm that SBO solubility arises from the hydrolysis reaction performed on its sourcing matter.

For the scope of the present work, characterization of the products inorganic matter was also necessary. This was found mainly constituted by Si, Ca, Mg, Fe, Al and alkali metal. However, for CVD, SBO and IOR being new materials for use as soil amending agents for agriculture purpose, matter of specific concern was their trace elements content and the fate of these elements in soil. For this reason, Table 1 reports also the content of trace elements in the above products. It may be observed that the starting Cu, Zn, Ni and Pb elements in CVD part preferably into SBO rather than into the IOR fraction. The data suggest that SBO has strong affinity for these elements, particularly for Cu and Zn. Indeed Table 1 also shows that, at nearly equal product organic matter application rate to soil, the amounts of Cu and Zn contributed by SBO are higher than CVD and IOR. This fact might be connected to the higher content of COO<sup>-</sup> functional groups in SBO. Although these results offered intriguing perspectives for further studies, investigation of the products binding capacity for trace elements was beyond the scope of this work.

### *3.2 Products Effects on Plant Growth, and Yield and Quality of Fruits.*

The experimental plan to assess effects of SBO, CVD and IOR municipal bio-refuse products added to soil as organic amending agent for the cultivation of tomato was carried out in 8 months, from November 2009 through June 2010. This time covered the entire plant growth/fruit production cycle (Ho, 1984) from tomato seedling transplanting to plants growth and flowering, and fruit setting, turning color, ripening and harvesting. The test plant, *Solanum Lycopersicum*, was a hybrid tomato variety for red

cluster ripening harvest (Fig. 2), much appreciated for its disease resistance and high productivity. It is transplanted in autumn to express most of its productive potential.

The cultivation trials were designed to maintain the soil treatment protocol of a typical large farm producing horticulture products in south east Sicily (see 2.2), except for the application of SBO, CVD or IOR to soil in place of the animal source derived commercial product (RCP) which was used routinely. Table 1 reports products amounts applied to soil. As SBO was proposed in this work for its high concentration of soluble organic matter, products applied quantities were normalized with respect to their organic matter (o.m.) content in order to compare the four materials under investigation at nearly equal o.m. application rate comprised in the 1.1-1.2 ton ha<sup>-1</sup> range. Supply of this o.m. amount to soil was normally practiced by the farms in the area where the cultivation trials were carried out.

Several parameters connected to soil, plant and fruit were monitored as indicators for comparing products performance. Fig. 3 reports plant biometric data calculated as averages of measurements performed over 12 plants per treatment (4 plants per replicate) at different dates from seedlings transplanting. These data provide an assessment of the plants nutritional status. With reference to stature, in three surveys performed in November and December, the application of SBO was shown to lead to good plant growth. Significant differences in plant stature appeared already on November 17, eleven days after transplanting. At this date, 17.8 cm average plant height was reached by the plants growing in the SBO treated soil compared to 20 cm measured for the plants growing in the untreated soil and to 16 cm measured for all other treatments. In the two subsequent December surveys, the plants grown in the SBO treated soil exhibited the highest stature, not significantly different from the plants grown in the untreated soil, but significantly higher than in all other treatments. Plant growth until March was further followed by monitoring the diameter of the main stem and the inter-node length. According to the results shown in Fig. 3 these indicators seem to definitely prove the superior effect of the SBO treated soil on plant growth compared to the untreated and all other treated soil parcels. Consistently with the above biometric data, the plants grown in the SBO treated soil were also shown to have greater vigour (Fig. 4) than those grown in the control soil and by all other soil treatment.

Leaves were analyzed for the content of chlorophyll and elements such as C, N, P, Na, K, Ca, Mg, Fe, Zn, Cu, Mn and B. These parameters bear complex interactions, one with the other, depend on the status of soil and affect the plant nutritional status and the productivity and quality of fruits. The presence of chlorophyll is an index of photosynthetic activity which is essential for plant life. Fig. 5 reports the results of the analysis of the chlorophyll content performed monthly from February through May and calculated as average of five replicates in SPAD units. It may be readily observed that the SBO soil treatment yields plant leaves with the highest chlorophyll content compared to the control and all other treatments throughout the entire monitoring time span. At the last May measurements, the leaves of the plant grown in the untreated soil had the lowest chlorophyll content. Contrary to chlorophyll, no significant differences ascribed to the soil treatment were detected for leaves elements composition in most cases. Leaves chemical composition data are discussed in details further in the text below together with soil data.

Mostly important are the effects of the different treatments on crop production rate and quality, due to the direct impact of these parameters on farm revenue. Precocious fruit ripening allows the farmer early product sales and, thus, to benefit from the higher sale price at the start of the seasonal market. Table 2 reports the plant flowering-fruit production stages measured in days from seedlings transplanting. It may be observed that SBO allows precocious start of all stages (see also Fig. 2a and b), resulting at whole ripening in a significant advance of 3-10 days for the first harvest, 4-8 days for the second harvest and 5-8 days for the third harvest, compared to the control and/or the other treatments. Generally, the control and all other treatments did not exhibit significant differences one from the other at all stages, except in a few cases. Table 3 reports production data. The SBO treatment is shown to yield the highest per plant fruit production amounting to  $5.97 \text{ kg plant}^{-1}$ . The reference commercial product (RCP) and the control treatment yield apparently lower values, which however are not proven to be statistically different from the former one. The IOR and CVD treatments yield lower production rates compared to the SBO treatment. Indeed, commercial production was strongly correlated with the number of fruits produced by



each plant after subtracting the poor quality discarded fruits; the IOR and CVD treatment gave the highest percentage of not-commercial fruit and/or the lowest fruit unit weight and per cluster number of fruits.

Fresh tomato quality and consumer's acceptability is a complex matter involving physicochemical and sensory parameters connected to physical appearance, firmness and flavor (Dorais, 2007; Thybo et al., 2006; Jones and Scott, 1983). Although visual appearance is a critical factor driving initial consumer choice, in subsequent purchases eating quality becomes mostly influential (Carli et al., 2011). For the fruits harvested in the experimental plan of this work, no differences in physical appearance parameters such as size and redness due to the different soil treatments were evident (Fig. 2c). However, other parameters (Table 3) connected to firmness and flavor revealed significant differences between fruits. Firmness, as measured by the resistance of the fruit to withstand crushing pressure, allows fruits to withstand mechanical impact and to have longer shelf life. This parameter is particularly important for the fruit product handling in the early stages of collecting, transporting and pre-processing. Tomato organoleptic features depend on its sourness to sweetness ratio (Bingqing, 2010). This dependence is however quite complex. Tomato flavor, for instance, is defined by a wide range of interactions among several physicochemical and sensory parameters and is influenced by plant nutritional regime, stage of ripening at harvest, genotypic differences and environmental conditions (Carli et al, 2011). The presence of salts, as indirectly evidenced by electrical conductivity (EC) measurements, of osmotically effective substances, measurable as total soluble solids (TSS), and of carboxylic acids (mainly citric acid), as evidenced by titratable acidity (TA) measurements, is well known to influence pH and the fruit taste (Krauss et al., 2006). The main components of TSS are sugars (fructose and glucose), while citric acid is mainly responsible for TA values. Thus TSS is a good indicator of the sugar content (Bingqing, 2010), and the TA/TSS ratio is frequently used as flavor index. A high TA/TSS ratio should be associated to a sharp acid flavor, presumably preferred (Mata et al., 2000) by European consumers, while a lower TA/TSS ratio should indicate a sweeter flat taste.

The data in Table 3 show no significant differences in dry matter content of berries and electrical conductivity of their juice due to the different soil treatments. Conversely, all the other quality parameters

appear affected by the soil treatment. Compared to the control, all other treatments yield fruits with lower total soluble solids (TSS) content, although the minimum value attained by this parameter still remains in the range reported for several tomato varieties (Krauss et al., 2006; Carli et al., 2011; Mata et al., 2000). Particularly CVD and SBO gave the lowest TSS values. Also, the SBO, RCP and IOR treatments seem to cause a significant increase in the fruits pH compared to control and CVD. The SBO and CVD treatment however appear to cause the highest 0.11 acidity/TSS ratio, 22 % higher than for the RCP and IOR treatments and 38 % higher than for the control soil. Firmness, the last parameter listed in Table 3, appears lower in fruits grown in the SBO, IOR and RCP treated plots compared to the CVD and control treatments. However no significant difference is shown for the SBO treatment compared to the RCP treatment. The higher firmness of the fruits grown in the control soil could reflect the lower maturity of these fruits compared to those grown in the other treated plots and harvested at the same time (Ünlü et al., 2011). The data in Table 2 show indeed that tomatoes reach full maturity at different times depending on the soil treatment. Except for the IOR treatment, for all other treatments the order of firmness in Table 3 seems to follow the order of the fruits ripening rate in Table 2.

Tables 4 and 5 report soil and leaves chemical and physicochemical data. The importance of these parameters and their relationships in soil and leaves are reported in more details in the specialized literature (Dorais, 2007; Pagliarulo, 2000). Generally however the chemical analysis of leaves is an indicator of the plant nutritional status and soil analyses are intended to provide guidance on the ability of soil to supply plants with the elements properly essential during the growing season. It should however be known that every element has a range of optimal concentration beyond which the plant physiology and nutritional status is adversely affected. As the potential ecotoxicologic effects of the bio-refuse derived products used in this work was specific matter of concern, in addition to plant and fruit biometric and production data, the following foliar and soil data have been collected.

Table 4 reports soil analyses performed before and after treatment at different dates covering the whole cultivation trial time. Anova analysis of average values calculated over triplicate soil samples per each treatment indicated no significant differences among treatments in most cases. Thus, Table 4 data

are reported as average values over all treatments at the same sampling time, while specific differences between soil treatments are reported in Table 5. The farm soil was a sandy type one (see 2.2). As shown in Table 4, the sampling time elapsed after treatment and seedlings transplanting affected significantly many soil parameters. From February to June significant decrease of pH, electrical conductivity, salinity and depletion of C, N, Ca, Mg, Fe and Cu occurred. The change may be caused by washing out these elements due to the continuous soil irrigation and/or by the nutrients transfer from soil to plants for their needs. Few significant differences were however detected in soil chemical composition due to the different treatments. Table 5 shows relatively higher Na content in all treated soil parcels compared to control soil appearing significant in the February survey. In the detection of early May, the plots treated with SBO and IOR exhibited higher N content than the other plots. At the same date, the Mg and Zn contents in the treated soil parcels were shown respectively higher and lower than in the control soil. In June, at the end of the crop cycle, all above differences caused by the different treatment relatively to the control soil appeared leveled out.

Leaves chemical composition are reported for the months where the most significant differences were detected, i.e. in February and May. As for most parameters no significant differences ascribed to the soil treatment were evident, the results are reported in Table 4 as average values over the treated and control soils vs. time. This appeared to be the parameter mostly affecting leaves chemical composition. Detailed data for each treatment are reported in Table 5 only for the cases showing statistically significant differences between treatments. Table 4 shows that depletion of C and N upon increasing the cultivation time appeared also in tomato plant leaves, as in soil. However, contrary to the changes observed in soil, over the same time leaves showed decrease of P and K, and increase of Mg, Na, Fe and B concentration. Changes in foliar mineral elements are known to occur during fruit development in tomato plants. The P and K decreases in leaves have been reported to be accompanied by increases of these elements in the fruit (Chapagain and Wiesman, 2004). Specific differences on leaves chemical composition due to the different soil treatments are reported in Table 5 and can be summarized as follows: (i) relatively lower P content observed in February for leaves grown in the control soil compared to the other treatments, (ii)

relatively higher P content observed in May for leaves grown in the CVD treated soil compared to all other treatments, (iii) relatively higher Fe content observed in May for leaves grown in the control soil and (iv) relatively higher Zn content in leaves grown in the control soil and in the CVD treated soils compared to all other treatments.

The collected soil and leaves data do not allow speculation on their relationship with plant growth and fruit production. However, in relation to the potential ecotoxicologic issue inherent to the bio-refuse products used in this work, it appears that CVD, SBO and IOR refuse derived products do not seem to cause important changes in the farm soil compared to the commercial product normally used in the farm cultivation practice. Thus, the higher amounts of trace elements contributed to soil by SBO (Table 1) does not seem to have any significant effect for the chemical composition of soil and leaves under the experimental conditions of this work, and therefore no adverse environmental impact from these substances may be assessed.

### *3.3 Relationship between product performance and chemical composition.*

The experimental data demonstrate that, although no significant effect on soil chemical composition is apparently observed by addition of the investigated products, SBO improves significantly plant growth and fruit production in the cultivation of tomato. From the farm economic point of view, the most important effect is the earlier fruit ripening (Table 2) and higher plant productivity (Table 3) induced by SBO compared to the other treatments. These results appear associated with the higher biometric data (Fig. 3) and the higher leaves chlorophyll content (Fig. 5) of the plants grown in the SBO treated soil and, therefore, presumably due to increased photosynthetic activity in these plants (Rodriguez et al., 2007). The capacity of SBO at very low concentration to promote photochemical reactions under solar light for the abatement of organic pollutants from aqueous streams has already been demonstrated (Bianco Prevot et al., 2011). Thus, even if the soil total organic matter content does not appear to be significantly affected by addition of SBO, presumably due to its low supply rate, some of this material, due to its water solubility, must be readily absorbed by the plant roots from soil and transferred to leaves to exert its photosensitizing property. Leaves, indeed, are considered to be the primary suppliers of photosynthetic

assimilates to fruit (Hetherington et al., 1998). The enhancement of photosynthetic activity caused by SBO may be particularly desirable in cultivations performed in high latitudes countries or under conditions where the light environment is characteristically low (Dorais, 2007).

The SBO effects on the fruit ripening rate and yield do not seem to be accompanied by negative changes on product quality. On the contrary, with specific reference to flavour, the SBO treatment seems to yield fruits which, by virtue of the higher TA/TSS ratio, should presumably meet European consumers' likes (Mata et al., 2000). As the products used in the different treatment have been compared at the same level of organic matter supply rate to soil, the superior performance of SBO is likely to be ascribed to the chemical nature of its organic matter.

Table 1 shows that the main chemical composition difference between SBO and the CVD and IOR products is its higher content of carboxyl groups. RCP however exhibits the highest content of carboxyl groups, together with the highest content of aliphatic C. Due to the animal origin of this product stated by its supplier (see 2.1), proteins and fats (Wikipedia, 2011) are supposed to be main contributors to its total organic C content. On the contrary, CVD, SBO and IOR, having mainly vegetable origin, are mostly constituted by lignin-like matter characterized by higher content of aromatic C. The difference in the chemical nature of the protein RCP and the ligno-cellulosic CVD, SBO and IOR materials is well consistent with the data in Table 1 and the IR spectra in Fig. 2. The aliphatic to total aromatic C ratio (al/ar) data reported in Table 1 readily evidence the relatively higher content of aliphatic C in RCP compared to the municipal bio-refuse products. The higher relative concentration of aliphatic and carboxyl C in RCP shown by Table 1 data appears well associated with the higher relative intensities of the aliphatic C-H band at 2923-2845  $\text{cm}^{-1}$  and of the COOH C=O band at 1745  $\text{cm}^{-1}$  exhibited by the IR spectra in Fig. 1. The absorption at 1745  $\text{cm}^{-1}$  is typical of esters as in fats. Other main absorptions in the IR spectrum of RCP cover the 1400-1700  $\text{cm}^{-1}$  range due to the  $\text{NH}_3^+$  bending and  $\text{COO}^-$  stretching vibrations of protein matter. By comparison, The IR spectra of CVD, SBO and IOR do not exhibit any prominent and/or distinct absorption in the 1735-1750  $\text{cm}^{-1}$  range where the esters band is expected to fall.

The available chemical data do not allow at this time to assess a product performance-chemical composition relationship. It has also been inferred in the above 3.1 subsection that SBO is the soluble product obtained by the hydrolytic reaction performed on CVD. Solubility indeed seems the most evident difference between SBO and the other CVD, IOR and RCP products. Whereas SBO was found soluble in water at 10-11 % level, the other three products were practically insoluble. It may then be assessed that, aside from or in addition to chemical composition, solubility is likely to have a main role in the products effects on plant growth and fruit production. Solubility in the present investigation appears even more important than the content of organic N. Indeed, more organic N was supplied to soil with RCP (130 N kg ha<sup>-1</sup>) than with the same amount of SBO organic matter (83 N kg ha<sup>-1</sup>), although the latter is still within the range of the required N amount for tomato cultivation (Dorais, 2007). Nevertheless, depending on the used performance indicators, SBO has shown equal or better performance than RCP.

Particularly interesting appears the effect of SBO to enhance chlorophyll formation in tomato plant leaves (Fig. 5). This effect allows to connect the herewith observed SBO performance in agriculture with the previously reported performance of the same bio-organic substances in environmental remediation chemical technology. Previous work has in fact reported the photosensitizing properties of several soluble bio-organic substances isolated from yard trimmings and/or food urban residues aged under aerobic digestion for different time. These substances, at very low concentration, have been demonstrated capable to enhance photochemical transformation of several organic molecules, such as azo-dyes and phenols (Bianco Prevot et al., 2011) under solar light. The observed effect of the soil SBO treatment to enhance tomato leaves chlorophyll content might be connected with the same photosensitizing property of SBO. To the authors knowledge, this is a new perspective to guide the search for new soil fertilizers. Many products from animal or vegetable source have been investigated as bio-fertilizers, and their primary mode of action has been claimed to arise from the presence of plant growth substances, acting alone or in combination with other nutrients, proteins or enzymes components (Edmeades, 2002), but the photosensitizing activity has never been directly addressed.

While posing a number of questions on the relationship between effects and product chemical composition, this work certainly provides a positive answer to the issue set forth in the introduction section, i.e. whether it was worthwhile to perform the hydrolytic reaction of compost matter to use the soluble product in agriculture rather than using the sourcing material itself. Nevertheless, for a proper utilization of bio-wastes as source of soluble organics for use in agriculture, further studies are required to assess source-product and product chemical composition-properties-performance relationships. This seems feasible in view of previous work reporting the availability of soluble bio-organic substances with chemical composition and properties varying over a wide range depending on the sourcing refuse (Montoneri et al., 2011). Similar products are likely obtainable from agriculture residues such as plant and fruit left over after harvesting. This offers worthwhile scope to carry on further cultivation trials with the above bio-organic substances isolated from urban and agriculture residues to assess chemical composition and dose effects and to develop both chemical and agricultural sustainability.

#### **4. Conclusions**

Refuse derived bio-organics (SBO) seem to have two important properties for use in agriculture: i.e. solubility and photosensitizing activity. These properties are shown related to plant growth and fruit ripening rate enhancement. These findings broaden the range of applications (Montoneri et al., 2011) for SBO and confirm that development and/or optimization of technology for cost-effective isolation of SBO is worthwhile and should be pursued to make bio-residues viable sources of products to use in place of synthetic chemicals. Due to their origin, no adverse environmental impact is expected from recycling SBO, and none is indeed proven by the present study.

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## Abbreviations

CVD, composted municipal bio-refuse containing kitchen wastes, home gardening residues and public park trimmings; SBO, soluble bioorganic substances isolated from CVD; IOR, insoluble organic residue; RCP, reference commercial product; UBW, urban biowastes; FORSU, organic humid fraction of urban refuse obtained by separate source collection; FORSUD, digestate from FORSU anaerobic digestion; GR, gardening and park trimming residues; EC, electrical conductivity; TSS, total soluble solids; TA, titratable acidity; al/ar, aliphatic to total aromatic C ratio.

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(1)

Product	Composition, % w/w referred to total sample weight (t.w.s.)							Applied quantities to soil (kg per 25 m <sup>2</sup> parcel)			
	H <sub>2</sub> O	d.m. <sup>a</sup>	o.m. <sup>b</sup>	C	N	o.m./C	N/C	t.w.s.	o.m. <sup>b</sup>	C	N
RCP	4.66	95.34	53.22	28.4	5.92	1.87	0.208	5.0	2.66	1.42	0.296
CVD	19.5	80.5	32.65	22.0	1.70	1.48	0.077	9.6	3.13	2.11	0.163
IOR	73.8	26.2	9.90	4.64	0.346	2.13	0.075	30	2.97	1.39	0.104
SBO	86.1	13.9	9.87	5.68	0.716	1.74	0.126	27	2.66	1.53	0.193
	% organic moieties and/or functional groups C over total C										
		aliphatic	OMe+NR <sup>c</sup>	OR <sup>c</sup>	anomeric	Ph <sup>d</sup>	PhOY <sup>e</sup>	COX <sup>f</sup>	keto	al/ar <sup>g</sup>	
RCP		43.9	11.3	12.2	2.0	7.0	3.4	20.0	0.2	4.2	
CVD		39.1	9.5	27.3	4.2	7.8	5.0	6.5	0.6	3.0	
SBO		40.9	7.3	14.2	3.8	12.3	6.0	12.9	2.6	2.4	
SBO <sub>af</sub>		34.0	4.3	3.9	4.6	24.1	13.5	13.4	2.2	0.9	
IOR		31.7	9.4	29.3	6.4	9.8	6.2	5.9	1.3	1.9	
		43.9	11.3	12.2	2.0	7.0	3.4	20.0	0.2	4.2	
	Product trace elements content (ppm in dry matter)										
		Cu	Ni	Zn	Cr	Cd	Pb	Hg			
CVD		92±1	71±4	199±4	44±1	<0.5	54±1	0.33±0.01			
SBO		249±2	97±2	427±2	27±2	<0.5	99±1	0.26±0.01			
IOR		73±1	61±2	199±1	44±1	<0.5	54±1	0.22±0.02			

**Table 1.**

Chemical data for products used in the tomato greenhouse cultivation experimental plan.

		Product trace elements weight contribution (mg per 25 m <sup>2</sup> parcel) to soil								
		Cu	Ni	Zn	Cr	Cd	Pb	Hg		
CVD		711	548	1538	340	<3.9	417	2.5		
SBO		935	364	1602	101	<1.9	371	1.0		
IOR		574	479	1564	345	<3.9	424	1.7		

<sup>a</sup>Dry matter; <sup>b</sup>organic matter; <sup>c</sup>R = alkyl C; <sup>d</sup>Ph = aromatic C, except PhO C; <sup>e</sup>PhO = aromatic C bonded to O as in phenols, diaryl ethers and alkyl aryl ethers according to Y = H, R, Ph; <sup>f</sup>COX = carboxyl C as in carboxylic acids, esters and amides according to X = OH, OR, N; <sup>g</sup>aliphatic /total aromatic C ratio, total aromatic = aromatic<sup>d</sup> + PhO<sup>e</sup>C.

**Table 2.**

Days from tomato seedlings transplating as averages of values measured in three subsequent floor-fruit cluster sets (cs)<sup>a</sup> at different plants flowering-fruit production stages (Fig. 2) and significance level (p) of data differences according to Student test.<sup>b</sup>

Treatment	Stage	1st cs <sup>a</sup>	2nd cs <sup>a</sup>	3rd cs <sup>a</sup>
	beginning flowering			
CVD		29 a	40 ab	53 a
RCP		26 a	37 b	45 b
IOR		31 a	44 a	55 a
SBO		21 b	30 c	39 c
Control		27 a	37 b	45 b
p		≤ 0.01	≤ 0.001	≤ 0.001
	beginning fruit-setting			
CVD		47 a	54 a	69 a
RCP		42 ab	47 b	60 b
IOR		46 a	58 a	71 a
SBO		35 c	42 c	52 c
Control		40 b	48 b	59 b
p		≤ 0.001	≤ 0.001	≤ 0.001
	beginning turning colour			
CVD		120 a	136 a	149
RCP		117 a	134 ab	148
IOR		120 a	136 a	153
SBO		113 b	129 b	142
Control		118 a	135 ab	148
p		≤ 0.01	≤ 0.05	ns
	beginning ripening			
CVD		131 a	146 a	158
RCP		127 a	143 a	157
IOR		127 a	146 a	162
SBO		122 b	136 b	152
Control		129 a	143 a	157
SL		≤ 0.01	≤ 0.01	ns
	whole ripening			
CVD		149 a	162 a	178 a
RCP		142 bc	158 a	175 a
IOR		145 ab	162 a	176 a
SBO		139 c	154 b	170 b
Control		144 ab	160 a	177 a
SL		≤ 0.01	≤ 0.01	≤ 0.05

<sup>a</sup>Each set containing 36 clusters; <sup>b</sup> within each column, values with no letter in common differ significantly: a > b > c; ns = not significant.

**Table 3.**

Plant productivity<sup>a</sup> and berries quality data for the different soil treatments, and significance level (p) of data differences according to Student test.<sup>b</sup>

Plant production average data						
Treatment	Per plant fruit net production (kg) <sup>c</sup>	Discarded fruit %	Per fruit weight (g)	Per cluster number of fruits		
CVD	5.26 d	0.98 b	67.24 b	7.20 b		
RCP	5.72 ab	0.95 b	68.49 a	8.35 ab		
IOR	5.42 cd	1.68 a	68.05 ab	7.96 b		
SBO	5.97 a	0.91 b	68.53 a	8.71 a		
Control	5.66 bc	0.99 b	68.70 a	8.24 ab		
p	≤ 0.01	≤ 0.05	≤ 0.05	≤ 0.05		
Berries physicochemical data <sup>d</sup>						
	d.m. <sup>e</sup> (% w/w)	TSS <sup>f</sup> (°Brix)	pH	TA <sup>g</sup> (% CA)	EC <sup>h</sup> (mS)	Firmness (kg)
CVD	7.24	5.02 d	3.08 b	0.54 a	3.27	3.73 a
RCP	7.20	5.29 bc	3.11 a	0.48 c	2.95	3.14 bc
IOR	7.63	5.47 b	3.13 a	0.51 b	3.12	2.95 c
SBO	7.12	5.13 cd	3.13 a	0.54 a	3.15	3.34 b
Control	7.49	5.80 a	3.08 b	0.47 c	3.28	3.59 a
p	ns	≤ 0.001	≤ 0.05	≤ 0.001	ns	≤ 0.001

<sup>a</sup>Calculated over the three replicate parcels per treatment, four plants per parcel in the mid row being sampled; <sup>b</sup>within each column, values with no letter in common differ significantly: a > b > c > d, ns = not significant; <sup>c</sup>per plant fruit net production after subtracting the poor quality discarded fruits = per fruit weight x per cluster number of fruits x 10, with 10 being the number of clusters per plant being sampled; <sup>d</sup>average data over ten harvests per treatment (300 fruits over the three replicates); <sup>e</sup>dry matter; <sup>f</sup>total soluble solids; <sup>g</sup>titratable acidity as % citric acid; <sup>h</sup>electrical conductivity.

**Table 4.** Averages for soil<sup>a</sup> and leaves<sup>b</sup> analytical data<sup>c</sup> over the four treated soils and the control soil vs. sampling time from seedlings transplanting: soil K, Ca, Mg reported as exchangeable ions and other elements as assimilable ions, unless otherwise indicated; leaves data reported as elements total concentration.

Sampling date	Soil data				Leaves data	
	11.1.2009 <sup>d</sup>	2.1.2010	5.5.2010	6.17.2010	2.1.2010	5.6.2010
pH	7.63 ± 0.15	7.99 ± 0.05	7.25 ± 0.08	7.33 ± 0.09		
EC, <sup>e</sup> μS cm <sup>-1</sup>	2261 ± 51	791 ± 70	648 ± 124	414 ± 53		
Salinity, μeq g <sup>-1</sup>	287 ± 5	106 ± 10	85.5 ± 15.5	51.8 ± 6.6		
Total C, % w/w	4.75 ± 0.16	4.61 ± 0.18	4.44 ± 0.16	4.52 ± 0.26	43.1 ± 0.6	37.7 ± 0.3
Total N, % w/w	0.147 ± 0.006	0.166 ± 0.018	0.146 ± 0.016	0.132 ± 0.013	4.84 ± 0.08	3.08 ± 0.18
C/N	32.5 ± 2.3	28.1 ± 1.8	30.9 ± 2.7	34.6 ± 2.3	8.92 ± 0.20	12.3 ± 0.7
K, μeq g <sup>-1</sup>	4.67 ± 1.50	3.07 ± 1.40	6.40 ± 0.95	4.93 ± 0.36	3.21 ± 0.16 % w/w	1.59 ± 0.20 % w/w
Ca, μeq g <sup>-1</sup>	229 ± 4	231 ± 6	103 ± 4	92 ± 2	2.42 ± 0.16 % w/w	2.23 ± 0.17 % w/w
Mg, μeq g <sup>-1</sup>	4.0 ± 1.0	4.20 ± 0.56	4.53 ± 0.38	3.93 ± 0.28	0.38 ± 0.02 % w/w	0.59 ± 0.03 % w/w
Na, % w/w	0.550 ± 0.040	0.549 ± 0.024	0.569 ± 0.052	0.548 ± 0.007	0.16 ± 0.02% w/w	0.49 ± 0.11% w/w
Mg, <sup>f</sup> mg kg <sup>-1</sup>	575 ± 33	509 ± 36	369 ± 90	360 ± 20		
Fe, mg kg <sup>-1</sup>	303 ± 16	314 ± 35	223 ± 12	188 ± 11	90.9 ± 8.9	151 ± 32
Zn, mg kg <sup>-1</sup>	33.3 ± 2.1	30.8 ± 0.5	32.7 ± 1.2	32.1 ± 0.4	29.6 ± 1.1	33.0 ± 8.0
Cu, mg kg <sup>-1</sup>	11.3 ± 0.6	10.9 ± 0.2	8.27 ± 0.28	8.73 ± 0.15	8.87 ± 0.69	7.93 ± 0.55
Mn, mg kg <sup>-1</sup>	189 ± 11	151 ± 10	173 ± 4	160 ± 5	70.6 ± 3.1	69.3 ± 1.8
P, mg kg <sup>-1</sup>	125 ± 4	179 ± 6	103 ± 3	170 ± 6	0.34 ± 0.04 % w/w	0.15 ± 0.04 % w/w
B, mg kg <sup>-1</sup>					57.7 ± 10.1	86.9 ± 5.3

<sup>a</sup> Three soil samples per treatment were analyzed to calculate average values; <sup>b</sup> twelve plants per treatment were analyzed to calculate average values; <sup>c</sup> dimensional units indicated in 1<sup>st</sup> left column, unless otherwise indicated; <sup>d</sup> before treatment; <sup>e</sup> electrical conductivity; <sup>f</sup> assimilable ion.

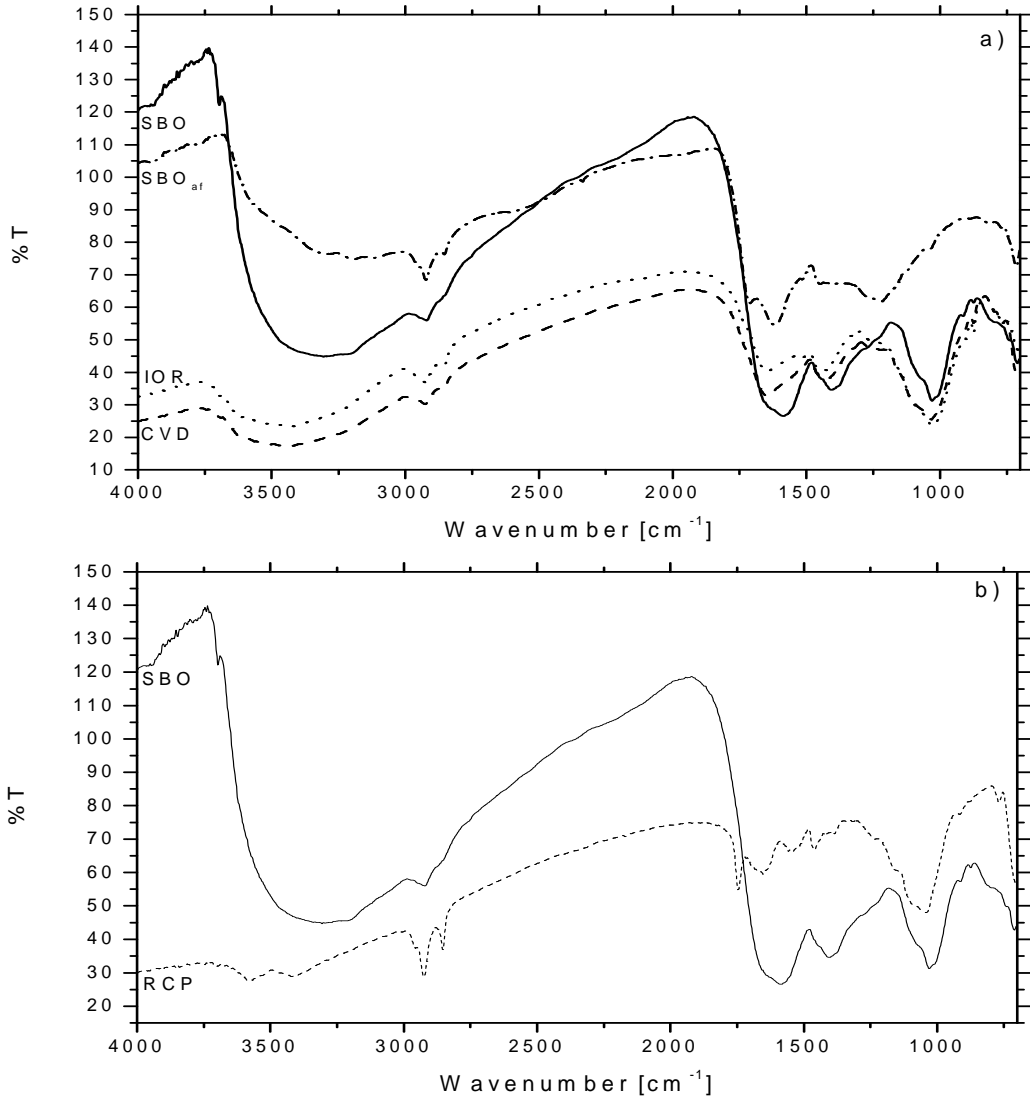


**Table 5.**

Differences<sup>a</sup> in soil and tomato plant leaves chemical data due to soil treatment with municipal bio-refuse (CVD, IOR, SBO) and animal (RCP) sourced products.

Sample/Date	Element	Control	RCP	CVD	SBO	IOR
soil 1.2.2010	Na, % w/w	0.51 b	0.56 a	0.58 a	0.56 a	0.54 ab
soil 5.5.2010	Na, % w/w	0.55ab	0.60 a	0.62 a	0.58 ab	0.49 b
soil 5.5.2010	Mg, ppm	276 d	431 b	493 a	338 c	307cd
soil 5.5.2010	Zn, ppm	34.7 a	32.0 b	31.7 b	32.0 b	33.0 b
soil 5.5.2010	N, % w/w	0.13 b	0.14ab	0.13 b	0.17 a	0.16 a
leaves 1.2.2010	P, % w/w	0.28 b	0.35ab	0.35ab	0.34ab	0.39 a
leaves 6.5.2010	P, % w/w	0.15ab	0.18ab	0.20 a	0.11 b	0.13ab
leaves 6.5.2010	Fe, ppm	195 a	123 ab	116 b	157 ab	161 ab
leaves 6.5.2010	Zn, ppm	42.7 a	25.4 b	38.7 a	33.0ab	25.3 b

<sup>a</sup>Within each row, values with no letter in common differ significantly with the following order and probability level (p) according to Student test: a > b > c > d;  $p \leq 0.001$  for Mg data and  $p \leq 0.05$  for all others.

**Fig. 1.**



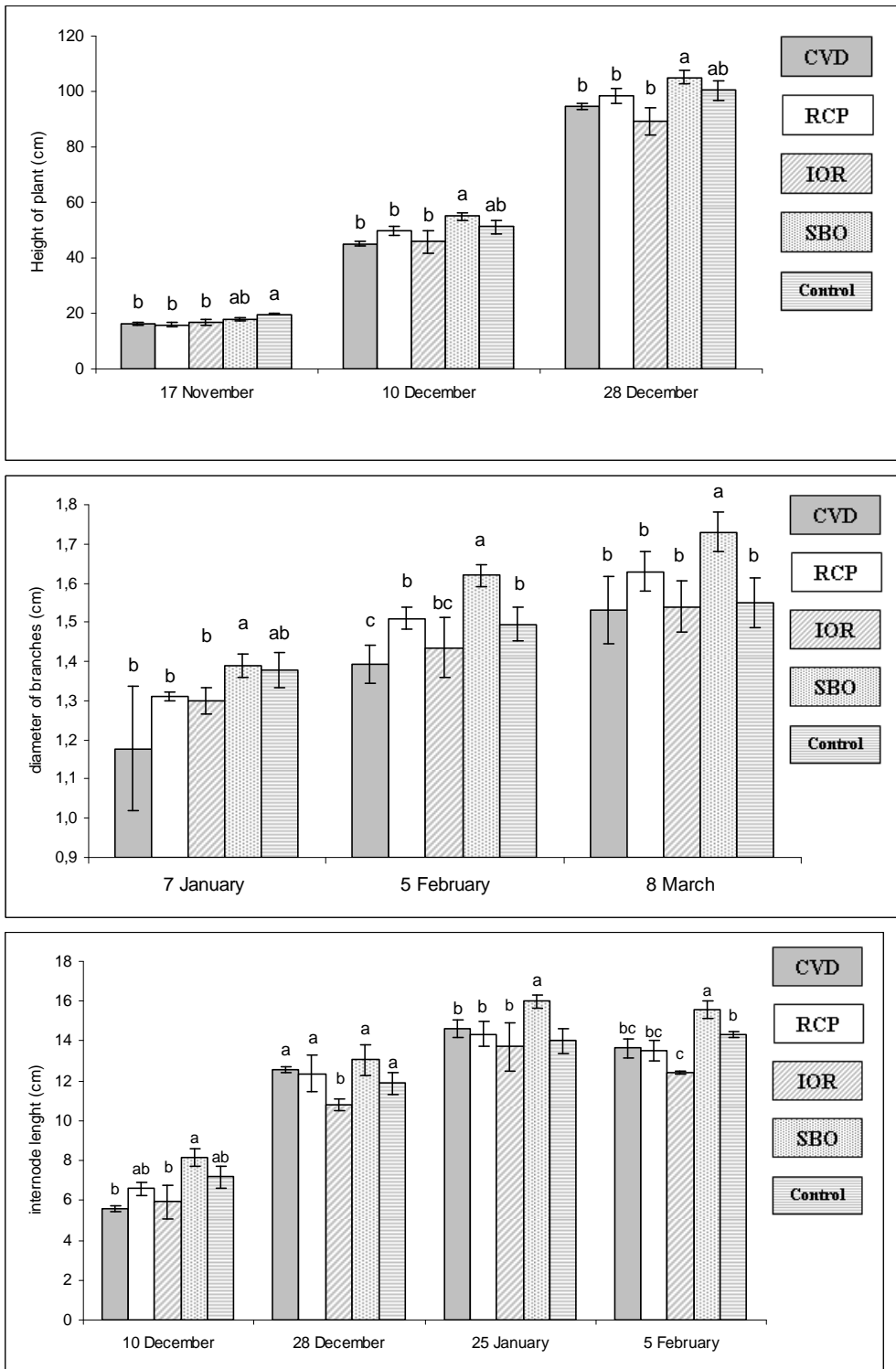
(a) SBO treatment

(b) RCP treatment

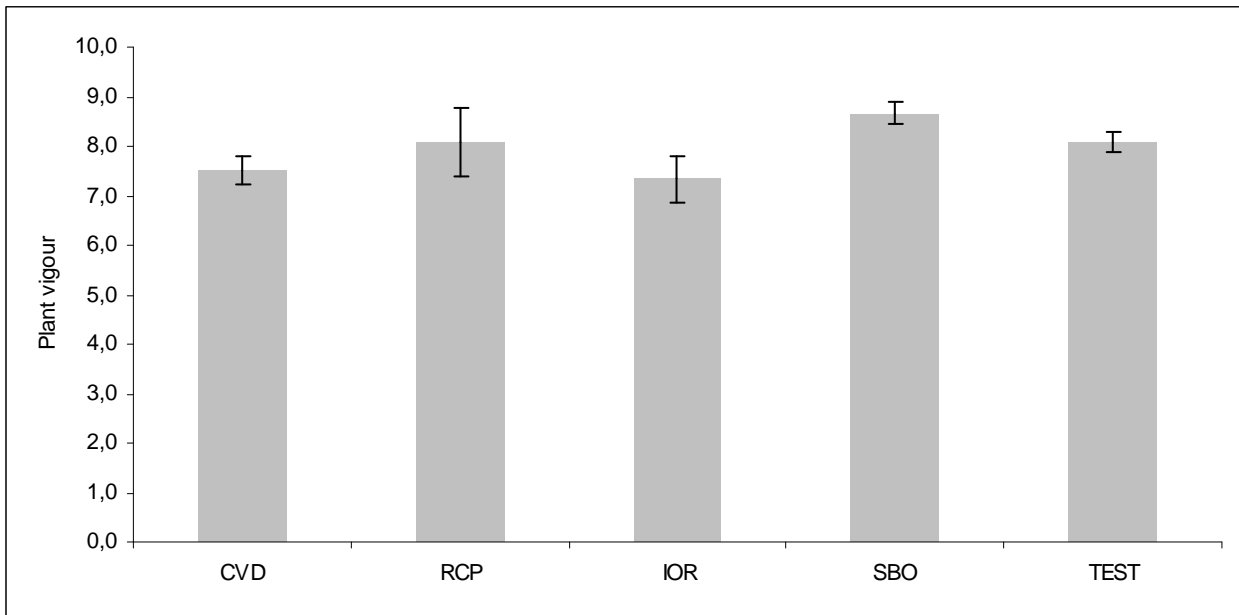


(c) all treatments: SOS = SBO; compost acea = CVD; ROI = IOR;  
 testimone = control; compost commercial = RCP

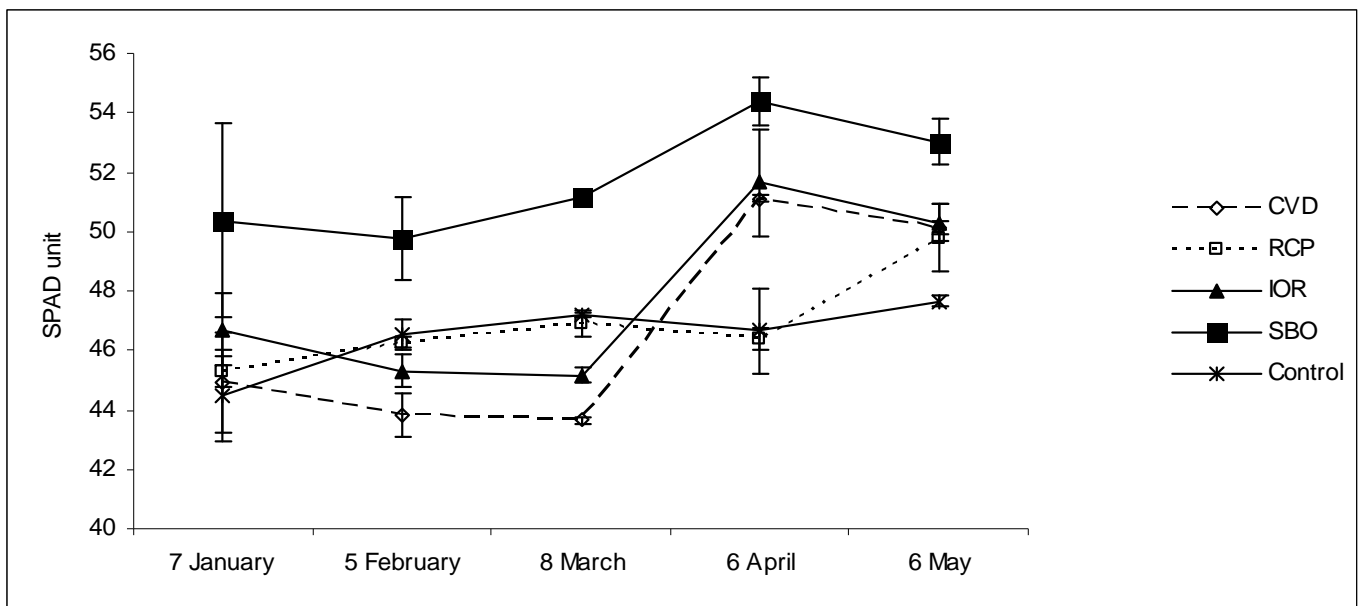
**Fig. 2.**



**Fig. 3.**



**Fig. 4.**



**Fig. 5.**

#### Figure captions

Fig. 1. IR spectra of products used in the tomato cultivation trials.

Fig. 2. Tomato test plant at beginning turning color stage (a and b) of first fruit cluster set (see Table 2) grown in soil treated with SBO and in soil treated with commercial product (RCP) and harvested fruits (c).

Fig. 3. Tomato plant height, branches diameter and internode length at different dates from seedlings transplanting vs. soil treatment. Columns at the same abscissa date having no letter in common differ significantly according to Student test:  $a > b > c > d$ .

Fig. 4. Tomato plant vigor on January 10, 2010 vs. soil treatment.

Fig. 5. Chlorophyll content (SPAD unit) in tomato plant leaves at five different dates from seedlings transplanting vs. soil treatment.