



Sustainable maize production by urban biowaste products

Alessandro Rovero¹, Marco Vitali², Daniele Rosso¹, Enzo Montoneri^{3*}, Walter Chitarra², Silvia Tabasso¹, Marco Ginepro¹, Claudio Lovisolo²

¹*Dipartimento di Chimica, Via Giuria 7, 10125 Torino, Italy*

²*Dipartimento di Scienze Agrarie, Forestali e Alimentari, via L. da Vinci 44, 10095 Torino, Italy*

³*Biowaste Processing, Via XXIV Maggio 25, 37126 Verona, Italy*

Article published on April 14, 2015

Key words: Biowaste, Maize, Waste management, Sustainability.

Abstract

For sustainable agriculture productivity and environmental quality, composts are considered as substitutes of chemical fertilizers. They are applied to soil at tens-hundreds dry matter ton ha⁻¹ yearly dose to contribute organic C and N and minerals. Hereinafter, municipal biowaste compost and its hydrolysate products are reported to enhance maize plant growth and productivity at much lower dose. The compost was obtained from a mix of food, gardening residues and sewage sludge. Afterwards, it was hydrolyzed to yield soluble substances (SBO) which were separated from the insoluble residue (IOR). The compost, SBO and IOR contained organic matter (om) and mineral elements (me) in 2.7-0.29 om/me w/w ratio, with SBO and IOR characterized by the highest and lowest weight ratio respectively. The three materials (compost, SBO and IOR) were applied to soil in open field and in pots in hydroponic conditions at 7-9078 kg ha⁻¹ dry matter dose. Urea as conventional fertilizer was used in separate trials at 200 kg ha⁻¹ for comparison. Plant performance indicators were leaf photosynthesis, plant growth, and kernel production and quality. The results demonstrate the higher performance of SBO at 50-140 kg ha⁻¹ both in open field and pot trials. Over double kernel production was obtained in open field by the plant grown on soil treated with 50 kg ha⁻¹ SBO compared to the control soil plant. The pot trials support the importance of the SBO organic matter coupled to mineral elements. The results prospect urban biowastes derived products as viable auxiliaries for ecofriendly sustainable agriculture.

* **Corresponding Author:** Enzo Montoneri ✉ enzo.montoneri@gmail.com

Introduction

The present work concerns two information categories, i.e. agriculture and biowastes. It addresses two issues, (i) the use of municipal biowaste derived products in agriculture and (ii) the development of a viable process to obtain these products. The two issues are very much interrelated. Reciprocal benefits may derive to agriculture and to the management of biowastes by testing biowaste products in agriculture and obtaining feedback from agriculture for further product and process development. The authors have published several papers on this strategy involving municipal (Negre *et al.*, 2012; Sortino *et al.*, 2012, 2013 and 2014) and agriculture (Baglieri *et al.*, 2014) biowastes with the intent to contribute to the development of economically and environmentally sustainable agriculture and biowaste processes (Montoneri *et al.*, 2011). The herewith reported study on maize cultivation contributes new data for the attainment of such goal moving from the following state of art.

The use of biowastes to amend soil and promote plant growth is a current strategy and research topic. A diffuse practice is to apply doses at several tens-hundreds dry matter ton per hectare level over several years (Baldantoni *et al.*, 2010; Pérez-Lomas *et al.*, 2010; Haber, 2008; Dorais, 2007; Maynard, 1995; Ozores *et al.*, 1994). Composted urban biowastes are particularly interesting for several reasons. They are available in large quantities in metropolitan areas and thus constitute a low entropy cost effective source of chemical energy. Their exploitation in agriculture contributes to the development of eco-friendly agriculture and at the same time alleviates the economic burden and environmental impact of the increasing waste production. Very recently soluble substances (SBO) isolated from a composted mix of food and vegetable residues and applied to loamy-sandy soil for tomato (Sortino *et al.*, 2012 and 2014) and red pepper (Sortino *et al.*, 2013) greenhouse cultivation have been reported to enhance leaf chlorophyll content, and also plant growth and fruit ripening rate and yield over the crop production cycle, significantly more than the source compost. A

number of reasons have been proposed in order to explain the observed performance of SBO as plant growth promoter. These substances have been reported to contain 29 % minerals together with organic matter. They could therefore add soluble plant nutrients to soil. They could also act as bio-effectors. They might stimulate the uptake from roots of soil nutrients with a hormone-like effect and/or plant growth by promoting rhizobacteria. In separate studies the SBO have been found to have photosensitizing properties (Avetta *et al.*, 2012). Thus, also a possible link of solubility and photosensitizing properties with the enhancement of leaf chlorophyll content and of plant and crop production in the above tomato and red pepper cultivation studies (Sortino *et al.*, 2013 and 2014) has been suggested. Whereas from the basic science point of view demonstrating the reason for the observed performance of SBO in agriculture is rather intriguing, from the practical point of view the most surprising results was that the highest SBO effect on the above performance indicators was observed at about 140 kg ha⁻¹ dose. At higher dose levels, no improvement or decrease of performance was observed. For red pepper cultivation, the most remarkable results were the maximum productivity increases observed for the 140 kg ha⁻¹ treatment dose compared to the control soil. The increases amounted to 90 % for the precocious crop yield, to 66 % for the total crop production and to 17 % for the per fruit weight. The discovery that these remarkable high effects occurred at such low treatment dose prospected using the SBO to enhance plant growth and productivity, while minimizing the potential environmental impact of conventional fertilizers.

A potentially viable process has been used for the fabrication of the above SBO (Montoneri *et al.*, 2011). This process performs the hydrolysis of biowastes at pH 13 yielding a soluble hydrolysate from which the SBO are recovered. An insoluble organic residue (IOR) is also obtained. In the process, about 30-50 % of the organic matter in the pristine biowaste is recovered with the SBO product. The IOR has lower content of organic matter than the sourcing compost

and the SBO, but relatively higher content of mineral elements. Previous work has demonstrated the remarkable performance of SBO in horticulture (Sortino *et al.*, 2014). No specific use has been proposed for IOR. Yet, due to its high mineral content, this product is a potential source of mineral nutrients for plants. As of now, in the absence of perspectives for allocating the IOR as marketable product, its disposal constitutes a critical factor for the economy of the SBO production process. These circumstances indicated that further studies were necessary with plants other than horticulture plants. Indeed, proving the SBO properties general for other plant species would add a further valuable argument for use of biowaste-derived soluble matter to enhance plant productivity. At the same time, demonstrating the value of IOR in the cultivation of some plant species, would allow to allocate this product in the agriculture market as growth promoter of specific plants. In this fashion, the IOR would become a source of additional revenue and therefore make more cost effective the biowaste process. These motivations generated the present study with the following aims: (i) to extend to other plant species, specifically maize, the previous study performed on the SBO dose effect for horticultural tomato and red pepper plants; (ii) to compare the SBO performance with that of its sourcing compost and of the IOR product; (iii) to find out a possible use of the IOR product in the cultivation of plant species, other than tomato and red pepper plants, and thus to alleviate and/or solve the problem connected to its disposal cost. Pursuing these objectives is worthwhile both to provide practical guides to farmers on the use of biowastes in agriculture and also to contribute to the realization of new added value waste management processes.

Materials and methods

Starting materials

The compost was supplied by Acea Pinerolese Industriale SpA, Pinerolo (TO), Italy in October 2009. The company has an urban waste treatment plant performing anaerobic and aerobic digestion of separate source collection urban biowastes. The

anaerobic digestion generates biogas and a solid digestate (D) containing residual organic matter not converted to biogas. The digestate is mixed with home gardening and park trimmings residues (V) and sewage sludge (F) in 35/55/10 w/w/w D/V/F ratios and composted for 110 days. The SBO and IOR were obtained by alkaline hydrolysis of the compost as previously reported (Montoneri *et al.*, 2013). The starting compost was reacted 4 h with KOH solution at pH 13, 60 °C and 4 V/w water/solid ratio. The liquid/solid hydrolysate mix was allowed to settle to separate the supernatant liquid phase containing the soluble substances from the insoluble substances. The recovered liquid phase was circulated at 40 L h⁻¹ flow rate through the ultra filtration membrane operating with tangential flow at 7 bar inlet and 4.5 bar outlet pressure to yield a retentate with 5–10 % dry soluble substances content. The insoluble substances residue was washed once with fresh water at 4 V/w added water/solid ratio. The recovered ultra filtration retentate and the insoluble substances residue were allowed to concentrate and/or dry in ventilated oven at 60 °C. The products were obtained in 1:4 w/w SBO/IOR ratio. These materials were characterized by the data reported in Table 1 and 2 which were obtained as previously reported (Sortino *et al.*, 2014). First generation Zea Mays maize seeds were acquired from Pioneer Hi-Bred Italia Sementi Srl.

Cultivation and treatments in open field

The study was carried out in a non-irrigated flat field during 2012 summer season, in Corio Farm located in north-western Italy (Marentino, Torino province), altitude 323m. A meteorological station close to the field recorded air temperature, relative humidity and rainfall during the season. The soil was a silty-loamy type according to USDA texture classification: sand 19%, silt 59%, clay 22%. It was divided into 39 parcels each covering 30 m² soil surface. Basic fertilization was performed before seeding according to the host farm routine practice, by adding N-P-K (15-15-15) fertilizer at 260 kg ha⁻¹ dose to each parcel. Seeding was performed on May 27, 2012. All treatments were performed 10 days after plants emergency; i.e. at emission of fourth leaf, corresponding to the Growth

Stage (GS) 12 according to Lancashire *et al.* (1991). Three completely randomized replications for each treatment and for the untreated control soil were performed. The SBO treatments were carried out at 7, 50, 140, 500 and 3000 kg ha⁻¹ doses. The IOR and the pristine compost were tested only in three dosages containing the same organic C (Table 1) of the 7, 140, 3000 kg ha⁻¹ SBO dosage. These were respectively 21, 420, 9078 kg ha⁻¹ for IOR and 10, 204 and 4380 kg ha⁻¹ for the compost. The experimental plan included also a treatment at 220 kg ha⁻¹ dose of urea, which was the conventional fertilizer normally applied by the host farm. Three parcels received no treatment at all, except the above basic N-P-K fertilization, and were used as control. Weed and pest control were conducted with the conventional products and scheduling applied by the host farm.

Cultivation and Treatments in Pots

Maize cultivation in pots was carried under hydroponic conditions, using the same treatments as in the open field. Round plastic pots 20 cm diameter and 25 cm height were used. These were filled up with sand and commercial expanded clay (approximately 66% expanded clay – 33% sand w/w). The expanded clay was previously washed with water to eliminate ions which could be absorbed by the plants, thus interfering with the materials used for the treatments. Drip fertigation of a modified Hoagland solution was applied to the substrate medium. This solution contained the following elements in mg L⁻¹ concentration: N 210, K 235, Ca 200, P 31, S 64, Mg 48, B 0.5, Fe 2, Mn 0.5, Zn 0.05, Cu 0.02, and Mo 0.01. The pots were placed outdoors in a courtyard, and the doses of biowaste products were distributed according to the surface of the pot, maintaining the same concentration of a given dose per hectare as for soil (e.g. 141.27 kg ha⁻¹ of SBO, distributed into a pot of 0.031 m², corresponds to 0.44 g of SBO). The biowaste products were simply mixed with sand, due to the small amount of some doses, and spread on the surface of the pot. Like the cultivation in open field, seeding was performed on May 27, 2012. The administration of Hoagland solution was started after 7 days from plants emergency. All treatments were

performed 10 days after plants emergency; i.e. at emission of fourth leaf, corresponding to the Growth Stage (GS) 12 according to Lancashire *et al.* (1991).

Plant growth and crop yield

Plant growth was assessed as follows. The height was measured with a tape, considering the proximal edge of the emerging leaf as the highest point; stem diameter was measured with a Vernier caliper, by considering the mayor axis of the ellipsoidal maize stem placing the caliper just below the intersection with the first leaf. The number of leaves was determined excluding any withered leaf and emerging leaves lower than 30% of their final dimension. Manual harvest was carried out in October 2012 to estimate the kernel yield in ton per hectare. The ears harvested from each parcel were divided in cobs and kernels using manual machinery and latters were weighted. A part (1 kg) of kernels was dried with a laboratory oven at 110°C for 12 hours and weighted again to assess percentage of dry matter. Yields were expressed in ton ha⁻¹ of dry matter.

Chlorophyll and gas exchange measurements

Leaf chlorophyll was determined by Minolta Spad 502 instrument as previously reported (Sortino *et al.*, 2013). Photosynthetic performance on field (net photosynthetic rate and stomatal conductance) was assessed five times during the season at different increasing growth stages, i.e. GS 16, 32, 36, 65, 71 according to Lancashire *et al.* (1991), on three mature leaves per replicate, with a LCpro+ ADC system (Analytical Development Company, Hoddensdon, UK) equipped with a narrow-leaf chamber (8 cm² leaf area). Measurements were taken during a sunny day, in the central hours of the day, at ambient CO₂ and relative humidity levels under saturating light, according to Vitali *et al.* (2013).

Soil, leaf and caryopsis analyses

Soil samples for analytical purposes (e.g. in the control, and in the urea and 3000 kg ha⁻¹ SBO treated soil plots) were taken at 0-30 cm depth in treatments Control, Urea and SBO 3000. 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 of Agriculture (Ministero per le Politiche Agricole, 1997, 1999). 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 available nutrients, i.e. those which may be absorbed by the roots, P and S. The concentration of macro- and micro-elements was determined using a Perkin Elmer "Optima 2000" ICP-OES; whereas mercury was assessed using a hydride generator Perkin Elmer MHS 20 coupled with an atomic absorption spectrometer Perkin Elmer Mod. 1100 B. Concentration values are referred to the total amount of element which is soluble in aqua regia. Leaf samples were taken from three plants per parcel and caryopsis samples were taken after drying in number of one per parcel, considered as a medium composite. Both leaves and caryopsis were digested in HNO₃ and analyzed in triplicate using ICP-OES technique.

Statistical analysis

Treatments were compared for average values by one-way ANOVA analysis of variance and multiple comparison post-hoc test using SPSS software, version 20 (SPSS IBM, Chicago, IL, USA). The Pearson correlation was used to assess the strength of relationship between variables of interest.

Results

Chemical composition of compost, SBO and IOR

The chemical composition of compost, SBO and IOR was analyzed to attempt understanding differences in the nature and performance of these materials. According to the data reported in Table 1 the highest concentration in C, N, P, K, Na, Cu and Zn were recorded in SBO; on the contrary Si, Fe, Mg were higher in IOR. The sourcing compost shows intermediate values. The SBO is also characterized by the lowest C/N ratio and ash content. This shows that the alkaline treatment of compost allows concentrating relatively more organics and N in the

SBO fraction. Consequently the IOR fraction exhibits the highest C/N ratio and ash content. The higher K content and consequent salinity of the SBO product arises from the added KOH during the sourcing compost hydrolysis. Reduction of the K content can be achieved by diluting the product with water, running the solution through the polysulphone membrane described in Section 2.1 to obtain a retentate with 80 % reduced volume relative to the membrane feed volume, and repeating the dilution/volume reduction cycle to obtain a product with the desired K concentration. Cultivation trials with these products were carried out in open field and in pots, the latter ones to assess the effects of the treatments in absence of the soil contribution.

Treatments in open field

For the carried out on field maize cultivation trials the SBO K content and salinity were not expected to have any impact on soil composition and plant growth due to the low applied doses. Indeed, none of the three investigated materials affected significantly the chemical composition of the starting soil. A few differences were found between the soil treated with the highest 3000 SBO kg ha⁻¹ dose and the control or urea treated soil (Table 2). The former was found to contain 10-22 % more P, Mn and Cu, and 6 % less Ni. Yet, significant effects on maize growth and kernel production by the soil treatments were found.

Fig. 1 reports maize kernel production versus dose for the different treatments compared to the production obtained on the control soil. It may be observed that kernel yields for maize grown in soil treated with SBO, IOR and compost are significantly higher than in the control soil and do not differ from that obtained in soil treated with urea, except for IOR 21 yielding lower kernel production than all other treatments. Usually, kernel yields for non-irrigated maize of North-Western Italy range from 7 to 9 ton ha⁻¹ (Friuli, Romagna, 2013). In this work, only for the control soil and for the soil treated with SBO or IOR at 7 and 21 kg ha⁻¹ dose respectively kernel yields were found significantly lower than 7 ton ha⁻¹. Kernel yields above 7 ton ha⁻¹ were recorded for all other

treatments. The treatment with SBO reached its highest effect already at 50 kg ha⁻¹ dose, with kernel production up to 8.5 ton ha⁻¹, over 2x the kernel production of the control plant. Further SBO dose increases did not result in higher production yield. A similar trend was observed also for the IOR and compost treatments. The trend of plant production versus treatment dose observed in this work for maize

cultivation is similar to that observed by Sortino *et al.* (2013 and 2014) for tomato and red pepper cultivation. The difference between the two cases is that for tomato and red pepper the highest effect is reached at 140 kg ha⁻¹ SBO dose, while for maize cultivation the highest effect is reached at lower dose, most likely comprised in the 7-50 kg ha⁻¹ range.

Table 1. Analytical for the three different refuse derived products used in this study: concentration values (w/w % or ppm, or meq/w) referred to dry matter; averages and standard deviations calculated over triplicates.

	SBO	IOR	Compost
pH	8.2	8.3	7.7
Ash (w/w %)	27.3	77.6	59.4
Salinity (meq per100g)	154.1	24.2	23.1
C (w/w %)	35.47 ± 0.09	11.72 ± 0.22	24.36 ± 0.16
N (w/w %)	4.34 ± 0.17	1.02 ± 0.05	2.25 ± 0.11
C/N	8.17	11.49	10.83
P ₂ O ₅	1.44 ± 0.03	0.53 ± 0.05	1.30 ± 0.22
K (w/w %)	5.49 ± 0.04	1.32 ± 0.02	1.32 ± 0.03
Ca (w/w %)	2.59 ± 0.03	3.20 ± 0.03	3.23 ± 0.05
Mg(w/w %)	0.49 ± 0.01	1.15 ± 0.02	0.83 ± 0.01
Fe (w/w %)	0.53 ± 0.02	1.23 ± 0.03	1.02 ± 0.01
Na (w/w %)	0.15 ± 0.01	0.04 ± 0.01	0.07 ± 0.01
Si (w/w %)	0.92 ± 0.03	7.68 ± 0.06	6.27 ± 0.04
Al (w/w %)	0.44 ± 0.02	1.05 ± 0.01	1.06 ± 0.02
Cu (ppm)	216 ± 1	49 ± 2	89 ± 1
Ni(ppm)	71 ± 0	70 ± 1	53 ± 1
Zn (ppm)	353 ± 3	160 ± 2	211 ± 3
Cr (ppm)	30 ± 1	58 ± 1	41 ± 1
Cd (ppm)	<0.5	<0.5	<0.5
Pb (ppm)	75 ± 1	37 ± 2	6 ± 1
Hg (ppm)	0.45 ± 0.02	0.27 ± 0.02	0.47 ± 0.01

Table 2. Chemical composition (w/w % or ppm) of control or urea treated soil and of soil treated with 3000 SBO kg ha⁻¹ at the end of the maize production cycle. Data are referred to dry soil and reported as mean and standard error calculated over triplicates; values in the same row marked with different letters indicate significant differences (P<0.05; t test): a > b.

	Control or urea treated soil ^a	SBO treated soil	Difference, % ^b
C (%)	2.27 ± 0.02	2.46 ± 0.03	
N (%)	0.18 ± 0.00	0.18 ± 0.00	
C/N	12.63	13.68	
P (ppm)	658 ± 4 b	725 ± 4 a	10
K (ppm)	4246 ± 99	5055 ± 19.2	
Ca(ppm)	38253 ± 359	42473 ± 1253	
Mg (ppm)	22599 ± 278	21957 ± 357	
Fe (ppm)	33661 ± 373	33985 ± 485	
Na (ppm)	324 ± 4	346 ± 3	
Al(ppm)	30163 ± 792	29156 ± 316	
S (ppm)	466 ± 32	431 ± 3	
Mn (ppm)	648 ± 3 b	780 ± 1 a	20
B (ppm)	50.51 ± 0.86	49.67 ± 0.77	
Cu (ppm)	53.6 ± 1.5 b	66.1 ± 0.6 a	22
Ni (ppm)	168.5 ± 0.9 b	157.9 ± 0.4 a	- 6
Zn (ppm)	69.7 ± 0.7	66.6 ± 0.5	

^a Data for control and for urea treated soil are not significantly different one from the other.

^b % increase or decrease by SBO treatment relative to control or urea treated soil.

Data on plant height, diameter and number of leaves (data not shown) were collected from June 22 through July 22. Trends for these indicators were similar to those found for kernel production. On the average, all treatments resulted in higher plant height than no treatment. Plant height throughout the

measurement time span grew from 50-60 to 200-250 cm. The 200-250 cm height was achieved by the plants grown in the treated soil plots, as compared to 160 cm for the plants grown in the untreated soil. No significant or important plant height differences were found among treatments.

Table 3. Macro- and micro-elements concentration in leaves of maize plants growing in Control or Urea treated soil and soil treated with 3000 SBO kg per ha. Leaves were collected at the end of growing season. Data are referred to fresh leaves and reported as mean and standard error calculated over triplicates; values in the same row marked with different letters indicate significant differences ($P < 0.05$; t test): a > b.

Concentration (w/w ppm)	Control or Urea treated soil ^a	SBO treated soil	Difference, % ^b
P	268 ± 51 b	454 ± 29 a	103
K	3163 ± 207 b	4281 ± 339 a	35
Ca	6056 ± 209 a	5110 ± 349 b	- 16
Mg	1152 ± 87	1175 ± 173	
Fe	43 ± 4	39 ± 3	
Na	118 ± 24 a	80.8 ± 1.3 b	- 31
Al	12.6 ± 1.5	12.5 ± 1.7	
S	377 ± 45	433 ± 18	
Mn	7.53 ± 0.67 a	5.99 ± 0.66 b	- 20
B	39.7 ± 10.7	34.0 ± 4.9	
Cu	7.95 ± 2.5	5.79 ± 1.00	
Ni	0.12 ± 0.01 a	0.09 ± 0.01 b	- 25
Zn	18.8 ± 0.66	17.4 ± 2.19	
Cr	0.13 ± 0.03 a	0.086 ± 0.01 b	- 34
Cd	0.031 ± 0.009	0.027 ± 0.005	
Pb	0.15 ± 0.06	0.08 ± 0.02	
Hg	(1.4 ± 0.2) 10 ⁻³	(0.8 ± 0.3) 10 ⁻³	

^aData for control and for urea treated soil are not significantly different one from the other.

^b% increase or decrease by SBO treatment relative to control or urea treated soil.

Leaf chlorophyll by Minolta Spad instrument and net photosynthetic rates were measured. Leaf chlorophyll content measured on July 19 was higher for all treatments compared to no treatment, with no significant difference shown among treatments (data not shown). Fig. 2 reports photosynthetic rate average seasonal values calculated from measurements performed in this work on June 28, July 5 and 19, August 2 and 14. As expected (Sortino *et al.*, 2014), the gas exchange rate measurements were consistent with the Minolta Spad leaf chlorophyll measurements. In essence, in the specific case of this work, both the gas exchange measurement and the

leaf chlorophyll measurements showed no significant difference among treatments.

Meteorological data recorded in the Chieri station (TO, Piedmont) were recorded in order to understand the impact of weather on plant performance (Fig. 3). The evolution of mean temperature showed warm days at the end of June and August, whereas rainfall was concentrated in June. July and August were dry with rainfall regimes lower than 25 mm in both months. Since, physiological performance are strictly linked to weather condition, Fig. 4 reported the seasonal course of net photosynthesis and stomatal

conductance obtained from the daily means of all treatments (June 28, July 5 and 19, August 2 and 14). As attended, the behaviour of these two parameters

was coupled, with high performance at the beginning of July decreasing during the season.

Table 4. □Kernel composition of maize plants growing in control or urea treated soil and in soil treated with 3000 SBO kg ha⁻¹. Kernels were collected at full ripening stage. Data are referred to dry matter and reported as mean and standard error calculated over triplicates; values in the same row marked with different letters indicate significant differences (P<0.05; t test): a > b.

Concentration (w/w ppm)	Control or urea treated soil ^a	SBO treated soil	Difference, % ^b
P	2026 ± 42 b	2546 ± 60 a	21
K	4038 ± 65 b	4691 ± 43 a	16
Ca	137 ± 11	140 ± 9	
Mg	957 ± 18 b	1237 ± 67 a	29
Fe	21.61 ± 0.69	20.82 ± 1.02	
Na	16 ± 5 b	28 ± 3 a	75
Al	0	0	
S	645 ± 21	694 ± 34	
Mn	3.67 ± 0.10 b	3.96 ± 0.08 a	8
Cu	3.17 ± 0.11	3.04 ± 0.07	
Ni	0.79 ± 0.04 b	1.57 ± 0.04 a	98
Zn	18.39 ± 0.16 b	22.4 ± 0.81a	22
Cr	0.05 ± 0.018	0.05 ± 0.01	
Cd	0.02 ± 0.01 a	0 b	
Pb	0	0	

^aData for control and for urea treated soil are not significantly different one from the other.

^b% increase or decrease by SBO treatment relative to control or urea treated soil.

Fig. 5 displays the relationship found among physiological parameter and kernel yield. Height of plant (Fig. 5a) and number of leaves (Fig. 5b) were positively correlated to the grain yield. On the

contrary, no relation was found for the other parameter (photosynthetic rates, stem diameter and chlorophyll SPAD index).

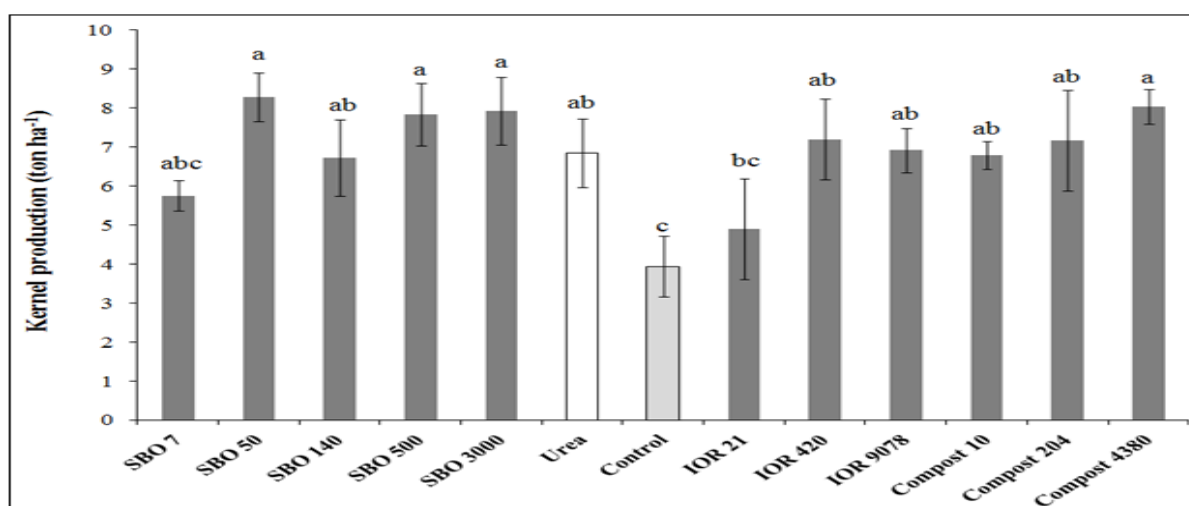


Fig. 1. □Dry weight of maize kernels obtained in summer 2012. Numbers in abscissa indicate dose in kg ha⁻¹; for example SBO 7 stands for SBO at 7 kg ha⁻¹. Production values are means ± standard error calculated over triplicates. Columns with no letter in common indicate significantly different production values (P<0.05): a > b > c.

Leaves and crops were analyzed for macro- and micro-elements composition. Some significant differences were found as arising from the soil treatments. Typical data are reported in Table 3 and 4 for control soil and urea treated soil and for soil treated with the highest 3000 kg ha⁻¹ SBO dose. It may be observed that leaves of plants grown in the

SBO treated soil have higher P and K content, and lower Ca, Na, Mn, Ni and Cr content than those grown in the control soil. Kernel from maize grown in SBO soil has higher P, K, Mg, Na, Mn, Ni, Zn, and lower Cd than kernel from maize grown in the control soil.

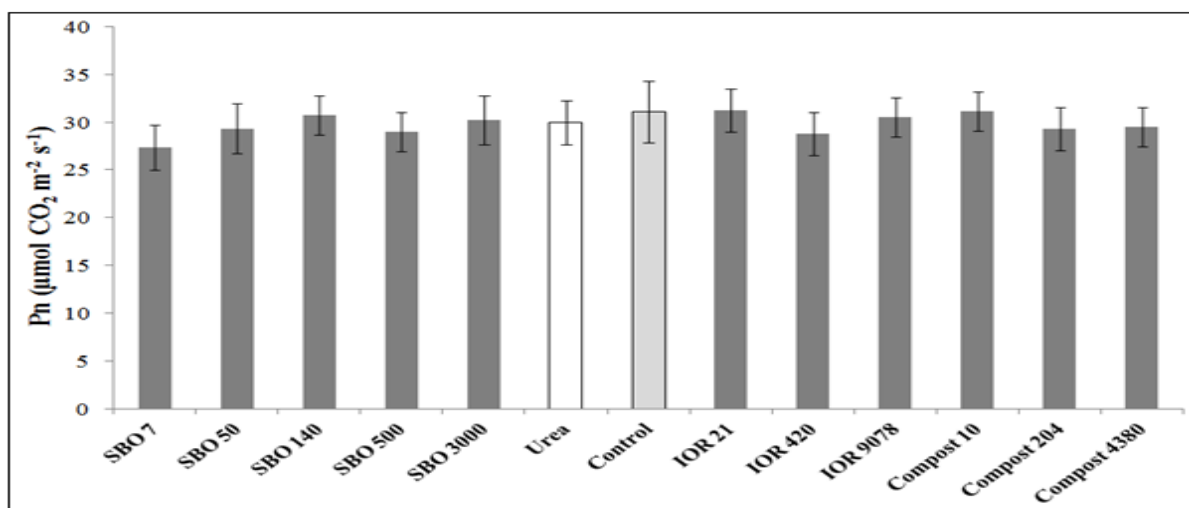


Fig. 2. □□Average seasonal net photosynthesis (Pn) values calculated from measurements performed on June 28, July 5 and 19, August 2 and 14. Data are means calculated from measurements on three leaves per three replicates per five measurements during the summer) ± standard error ($n=45$). Numbers in abscissa indicate dose in kg per ha; for example SBO 7 stands for SBO at 7 kg ha⁻¹.

Treatments in pots

As for field tests, biometric data, number of leaves, and leaf gas exchange were measured for pot cultivated plants on June 28, July 5 and July 19. No significant differences between treated and untreated plant were evident in plant diameter and number of leaves. Effects were however picked out on plant height. On June 22 the height of the plants grown in the pots treated with 7 kg ha⁻¹ SBO and with 420 kg ha⁻¹ IOR was the same (about 40 cm) and significantly higher than the height (about 35 cm) of the plants grown on all other pots (data not shown). Throughout the measurements at the later dates the plant height increased up to 140-160 cm. This height levels were achieved in the last July 22 measurement day. Fig. 6 reports the data recorded at this date. It may be observed that the SBO treatments at 50-3000 kg ha⁻¹ dose, the IOR treatments at 420 and 9078 kg ha⁻¹ and the compost treatments at 204 and 4380 kg ha⁻¹ doses gave higher values than the control and the urea

treatments. These latter treatments gave the lowest 140 cm plant height. All other treatments yielded higher values, but only the SBO 140, 3000 and IOR 420 kg ha⁻¹ treatments were higher from the mean of all treatments.

Leaf photosynthetic rate on June 28 varied from 22 to 32 □mol CO₂ m⁻² s⁻¹ over all treated and control pots. Standard errors were too high to appreciate differences by statistical analysis. On July 5 the gas exchange rate reached its peak values, increasing up to 48 □mol CO₂ m⁻² s⁻¹. This value was recorded for the SBO treatment at 7 kg ha⁻¹ (Fig. 7). All other treatments gave significantly lower values. On July 22 the leaf gas exchange rate decreased to 19-27□□mol CO₂ m⁻² s⁻¹ and no significant differences between treatments and control were proven.

Discussion

Rating treatments based on plant response

indicators

The on field and pot trials show different results in relation to the relative rating of the treatments, particularly for SBO compared to urea. In the on field trials all treatments yield higher plant growth (data not shown) and productivity (Fig. 1) than the control, but exhibit no significant difference among them. However, no differences between treatments and the control are shown for leaf chlorophyll content (data not shown) and plant photosynthetic performance (Fig. 2). This rating is different from that reported by Sortino *et al.* (2013 and 2014) using the same biowaste derived materials for red pepper and tomato cultivation. Sortino *et al.* (2013 and 2014) report that SBO yield higher plant growth and productivity than IOR and the source compost, and that the increase of

these indicators correlates with enhancement of leaf chlorophyll content and gas exchange by SBO. Consistently with other findings (Richards, 2000), these results indicate that different crop yields could be explained by differences in photosynthetic performance. Contrary to this case, the data in Fig. 2 show that for maize cultivation photosynthetic rates are not affected by the dose and or type of product applied to the soil. Also, analyzing the CO₂ exchange rate data in Fig. 2 against the crop production data in Fig. 1 evidences no significant correlation between photosynthesis and kernel yield. In essence, compared to the plants grown in the treated soil plots, the plants grown in the control soil, although characterized by kernel yield, exhibit the same photosynthetic activity as the treated soil plots.

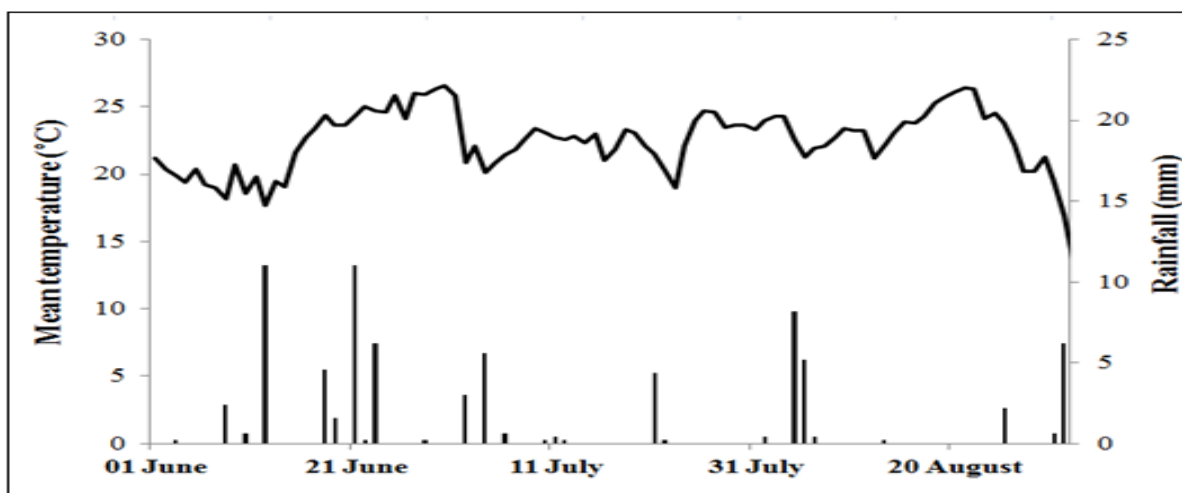


Fig. 3. Seasonal time course of mean temperature (°C) and rainfall (mm) recorded in Chieri (To) 3 km to the experimental field.

The different results obtained in the present work on maize cultivation and in the previous work on red pepper and tomato may indicate that different plants respond differently to the same applied materials. However, the lack of differences of average seasonal photosynthetic rate recorded among plants grown on soil treated with different products and dosages could be hypothetically due to other two factors: (i) a seasonal stomatal regulation of the photosynthesis in consequence to atmospheric events (e.g. water stress) and/or (ii) a putative compensation mechanism developed during the season between photosynthetic sources and productive sinks. Favouring the first

factor, it should be observed that high air temperature and poor rainfall (Fig. 3) was recorded in July and August during the cultivation trials. In response to such unfavourable meteorological conditions and absence of irrigation, photosynthetic rate was in average one half than the optimum, by following a tight decrease in stomatal opening (Fig. 4). The second hypothesis to explain the lack of the expected relationship between kernel yield and plant photosynthetic activity is linked to a supposed occurrence of plant mechanisms which operates to compensate low leaf development with high photosynthetic rates, when photosynthesis is

backward regulated, as lead by the sink strength (Barnett and Pearce, 1983). To corroborate this hypothesis we observe that treatments yielding apparently highest kernel production (i.e. SBO at 50, 500, 3000 and compost at 4380 kg ha⁻¹ doses in Fig.

1) are associated to slightly lower photosynthetic rates (Fig. 2). Vice versa, higher photosynthetic performances are observed for the least crop productive plants grown in control and IOR 21 kg ha⁻¹ treated soils.

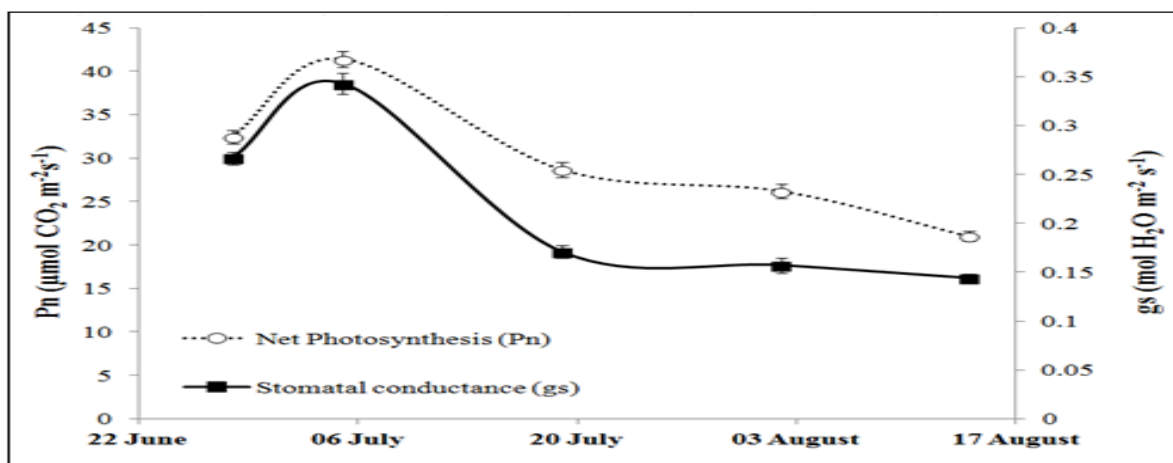


Fig. 4. □ Seasonal time course of net photosynthesis (Pn) and stomatal conductance (gs). Data are means \pm standard error calculated over all treatments (n =120). Measurements were performed on June 28, July 5 and 19, August 2 and 14 corresponding to growth stages 16, 32, 36, 65, and 71 according to Lancashire *et al.* (1991).

Contrary to the above data for photosynthetic rates, a direct relationship among kernel yield, height of plants and number of leaves was found. The data reported in Fig. 5 support the hypothesis that under the experimental conditions of the present work grain yield was mainly linked to the whole-plant leaf area and not (or less) with the photosynthesis occurring in a single leaf. A similar relationship has been proposed by other workers (Tollenaar and Daynard, 1982; Richards, 2000) under different experimental conditions.

Trials in pots were included in this work to provide measurements in the absence of soil organic matter and under well watered conditions. All pots were guaranteed the same mineral nutrient supply by Hoagland solution drip fertigation. In this fashion, the pot trials were expected to allow comparing the performance of the different types of organic matter applied with the compost, SBO and IOR materials without possible interference from organic matter contributed by other sources and unfavourable meteorological conditions. Due to the lower plant growth and little number of plants, compared to the

on field trials, reliable plant crop production data were not expected in the pot trials. Although for the above reason kernel production was not measured, the pot trials provided some interesting hints based on plant biometric and photosynthesis indicators. Contrary to on field trials, the pot trials showed a positive effect of SBO 140, 3000 and IOR 420 treatments on plant height. These treatments, especially the SBO 140 and 3000 ones, resulted in significantly higher plant height than the urea and control treatments (Fig. 6). Based on these results, it could be hypothesized that these products (particularly the SBO) provided an additional nutritional effect (more than the Hoagland solution), probably contributed by the presence of readily available soluble organic matter and/or the bonded mineral elements composing SBO. Under these circumstances, based on the results in Fig. 5 for the on field trials, a higher production of grain could be extrapolated under the experimental conditions of the pot trials. The higher performance of SBO was demonstrated also by the gas exchange measurements recorded when photosynthesis reached its peak level, i.e. on July 5 (Fig. 7). The

reason for the observed differences between pot and on field trials lies most likely in the absence of the climatic stress occurring during the on field trials. For the effects shown on photosynthesis the pot trials

results are consistent with those reported by Sortino *et al.* (2014) on the capacity of SBO to enhance plant photosynthesis.

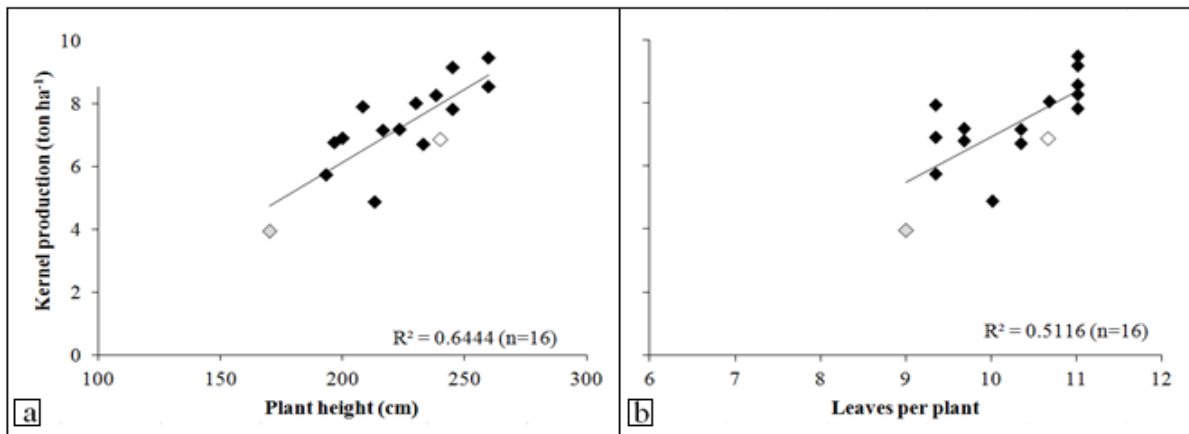


Fig. 5. □ Relationship among crop yield with both height of the plants (a) and number of green leaves (b). R^2 represents the coefficient of the Pearson correlation among variables. Each dot represents the mean of three parcels for either production, height of plants or number of leaves. Gray filled and empty dots correspond respectively to Control and Urea treatments.

Overall, maize is considered one of the most demanding crops in terms of nitrogen fertilization. In this work similar kernel production was obtained by administering the compost, SBO and IOR products with very low N content compared to the urea treatment (Tab. 1), and this occurred in spite of seasonal drought. This result is consistent with previous findings by Sortino *et al.* (2012 and 2014).

These authors have compared SBO and other commercial products containing different C and N amounts for their effects on tomato cultivation and have concluded that C and N contents, although important, are not the only factors that determine the performance ranking order of the investigated products.

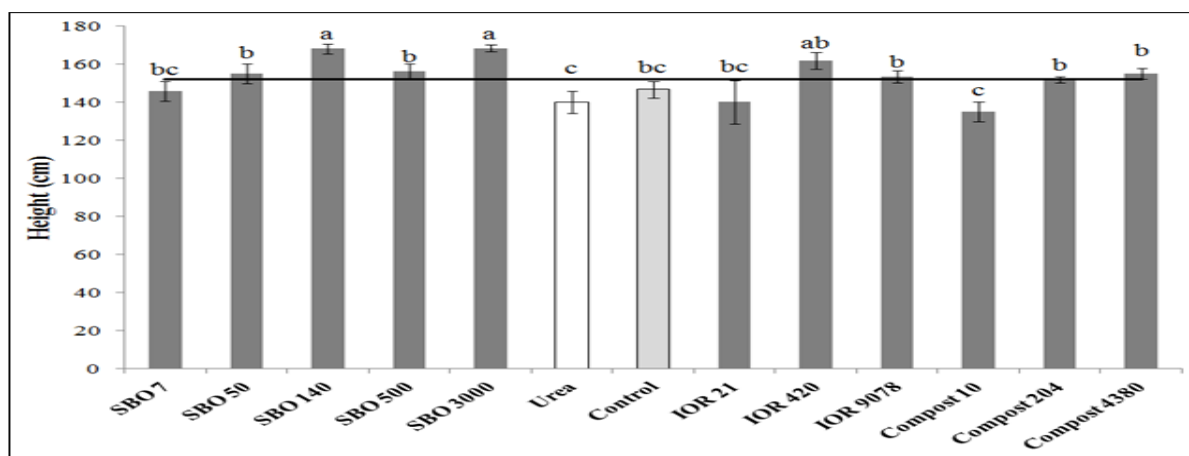


Fig. 6. Plant height in maize pot cultivation recorded on July 22 last measurement day. Production values are means \pm standard error calculated over triplicates. Black line indicates the mean of all treatments (152cm). Columns with no letter in common indicate significantly different production values ($P < 0.05$): $a > b > c$. Horizontal bar indicates average top production level.

The pot trials certainly evidence the importance of the products organic matter. This matter has been reported to be constituted by aliphatic and aromatic C moieties substituted by acid and basic groups capable to bind mineral elements (Montoneri *et al.*, 2011 and 2013). Thus, one role of the above organic matter, particularly that of the water soluble SBO, can be binding mineral elements present in and/or supplied to the growth medium, and so enhancing the rate

and efficiency of the transport of these elements to the plant. However, a number of other roles are possible as anticipated in the Introduction section of this work and discussed by Sortino *et al.* (2014). The available data do not allow further speculation to explain the reasons of the remarkable performance of SBO. Yet, the results of this work are highly relevant for the practical environmental and economic implications discussed hereinafter.

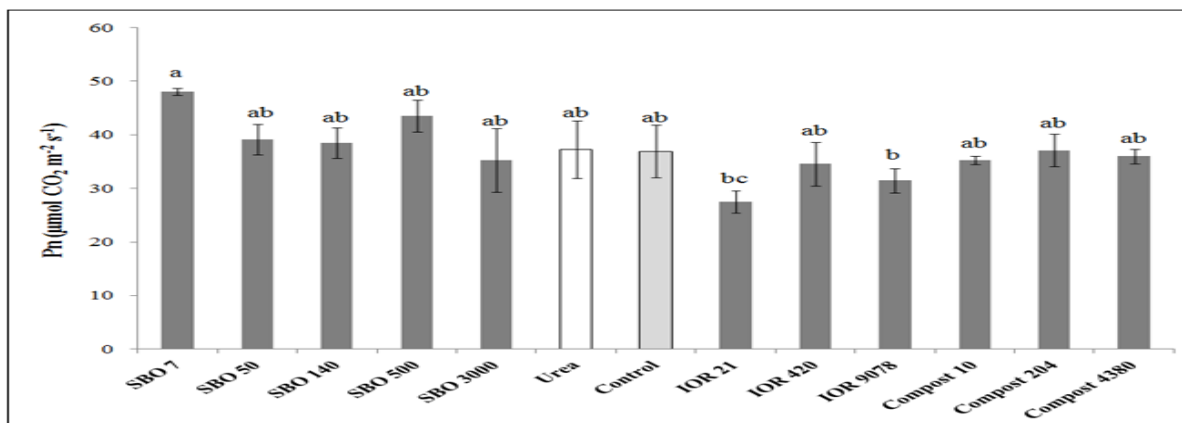


Fig. 7. Leaf gas exchange rate in maize pot cultivation recorded on July 5. Values are means \pm standard error calculated over triplicates. Columns with no letter in common indicate significantly different values ($P < 0.05$): a > b > c.

Environmental and economic implications

With reference to the three objectives stated in the Introduction section of this work, the data obtained in the open field trials indicate that the compost obtained from urban biowastes, and the SBO and IOR products obtained by its alkaline treatment, even under unfavourable meteorological conditions, are effective to promote maize plant growth and productivity better than the control and as well as the conventional urea fertilizer. However, compared to the performance of similar biorefuse derived products in tomato and red pepper cultivation (Sortino *et al.*, 2012, 2013 and 2014), the data collected in these work present two relevant differences: (i) the SBO is not shown significantly more effective than its source compost; (ii) no correlation between plant production and photosynthetic activity is supported. This indicates that different plant species may respond differently to the same biorefuse products. Under the specific experimental conditions of the present work, the photosynthesis or SPAD measurements

performed on single maize leaves are not likely indicator of effective plant potentiality. This may justify the lack of correlation of these indicators with plant productivity.

In the present on field maize cultivation trials, no added benefits are shown from the use of the isolated SBO and IOR products compared to the source compost. Under these circumstances, the collected data may also point out that for maize cultivation the additional cost of further processing the biorefuse compost to yield SBO and IOR is not justified. Nevertheless, as compost processing has been shown worthwhile for the production of SBO to use in tomato and red pepper cultivation, the present work confirms that the co-produced IOR can be used in the cultivation of maize as effectively as compost and urea and therefore can by all means considered potentially marketable. Contrary to the on field trials, the maize pots trials have demonstrated that SBO already at the very low dose of 140 kg ha⁻¹ yields higher plant growth

than urea and all other treatments. This in pot result (Fig. 6) and the on field results (Fig. 1) showing that SBO, even at the lower 50 kg ha⁻¹ dose, exhibits already its highest effect on kernel production, are rather remarkable. Aside from the reasons for the observed effects, for all practical purposes a number of environmental and economic benefits can be envisaged.

The results of this work prospect replacement of conventional commercial fertilizers application with lower doses of materials sourced from urban biowaste and alleviation of the problem connected to the IOR disposal cost in processing compost for the production of SBO. The comparison of data on distribution's specific cost of these products to traditional ones may give the feeling of the feasibility of this perspective. The N, P and K mineral fertilizers world demand is 220 million tons per year, against 240 million tons per year supply (FAO, 2008; OECD, 2014; von Lampe *et al.*, 2014). According to available data (World Bank, Development Prospects Group, 2012; Index Mundi, 2013) market prices for these commodities in 2013 have ranged from 318 to 500 € per ton. Some reports suggest the US fertilizer market to be around \$ 40 billion of which organic fertilizers occupy only about \$ 60 million (15 % of total sales). The rest of it is the share of the various artificial fertilizers (Chemical Fertilizer vs. Organic Fertilizer, 2013). Organic fertilizers whole sale prices range from 140 \$ per ton for solid products containing 10 % soluble organics to 1500 \$ per ton for products with > 90 % soluble organics and to 3000 \$ per ton for products in solution containing 35 % organics and other mineral elements (Organic Fertilizers Market, 2013). Based on their organics' and minerals' content (Table 1), the SBO and IOR products would fall into the higher prices products. The production cost of these materials from urban refuse compost is estimated 100-500 € per ton (Montoneri *et al.*, 2011), depending on the type of sourcing biorefuse. This would allow an interesting profit for the producer and, at the same time, to introduce this new product into the market at competitive price. In the long run, the high performance at relatively low applied doses

should assess the SBO and IOR products as highly desirable by farmers, both from the economic and from the environmental point of views. With specific reference to the environment, the potential impact is connected to the presence of heavy metals and of N. For the potential adverse impact on the environment and human health which might be caused by the heavy metal content in the above products, their relatively low applied doses, composition (Table 1) and lack of effects on the chemical composition of soil, kernel and leaves (Tables 2-4) following their application should raise no more concern than that arising from the use of conventional mineral and organic N fertilizers (Sortino *et al.*, 2012). With reference to N, the possibility to reach the same plant productivity by supplying products with lower N content compared to supplying conventional N fertilizers such as urea is rather important in relation to environmental problems that can be caused by excess N supply to the soil, such as ammonia emission and/or nitrate leaching in groundwater (Al Seadi *et al.*, 2008).

Conclusion

Compost obtained from urban biowastes, which are available in large quantities in metropolitan areas worldwide, and the SBO and IOR products obtained by its alkaline treatment, can be used in place of conventional nitrogen products such as urea to ensure maize yield, or to ameliorate nutrient composition of leaves and kernel. Obtaining same or slight higher kernel yield using these products compared to mineral fertilizer (urea) is an important result. It promotes the use of urban biowastes derived products for ecofriendly sustainable maize cultivation as emancipation from mineral fertilization. The results certainly offer scope for more focused research. Under the experimental conditions of this work water deficit during 2012 summer was certainly the main negative parameter affecting kernel production. Further tests in irrigated conditions to confirm positive effects of bio fertilizers and to comprehend if these products can substitute mineral fertilization through several years are required. In addition, further investigation on the impact of these

substances on soil microbial activity and soil mineral toxicity could be proposed. Such research effort seems worthwhile in view of the potential beneficial environmental and economic impacts on agriculture practises and new waste management processes.

Acknowledgements

This work was carried out partly with funds from the Ministero delle Politiche Agricole as part of the Agrienergia project. The authors are grateful to the following private and/or public Italian institutions: (i) the Corio farm located in Marentino (TO) for making the soil and related cultivation facilities available; (ii) Acea Pinerolese Spa in Pinerolo (TO) for supplying the SBO sourcing biomaterials from its urban waste management plant; (iii) Studio Chiono ed Associati in Rivarolo Canavese (TO) for making pilot equipment and services for SBO production available.

References

- Avetta P, Bianco Prevot A, Fabbri D, Montoneri E, Tomasso L.** 2012. Photodegradation of naphthalenesulfonic compounds in the presence of a bio-waste derived sensitizer. *Chemical Engineering Journal* **197**, 193-198, and references therein.
- Baglieri A, Cadili V, Mozzetti Monterumici C, Gennari M, Tabasso S, Montoneri E, Nardi S, Negre M.** 2014. Fertilization of bean plants with tomato plants hydrolysates. Effect on biomass production, chlorophyll content and N assimilation. *Scientia Horticulturae* **176**, 194-199, <http://dx.doi.org/10.1016/j.scienta.2014.07002>.
- Baldantoni D, Leone A, Lovieno P, Morra L, Zaccardelli M, Alfani A.** 2010. Total and available soil trace element concentrations in two Mediterranean agricultural systems treated with municipal waste compost or conventional mineral fertilizers. *Chemosphere* **80(9)**, 1006-1013.
- Barnett KH, Pearce RB.** 1983. Source-sink ratio alteration and its effect on physiological parameters in maize. *Crop Science* **23(2)**, 294-299.
- Chemical Fertilizers vs Organic Fertilizers.** 2013. http://www.diffen.com/difference/Chemical_Fertilizer_vs_Organic_Fertilizer.
- Dorais M.** 2007. Organic production of vegetables: state of the art and challenges. *Canadian Journal of Plant Science* **87**, 1055-1066, and references therein.
- FAO.** 2008. Current World Fertilizer Trends and Outlook to 2012. <http://www.fao.org/worldfood/Reports/cwfsdo>.
- Friuli VG, Romagna E.** 2013. Fumonisin in italian maize: analysis and considerations *Marketing* 30. http://www.micotossine.it/public/pag_192.pdf.
- Haber N.** 2010. Sustainable Compost Application in Agriculture. ECN INFO PAPER 02,1-36. European Compost Network ECN e.V LTZ, Center for Agricultural Technology, Augustenberg Karlsruhe, Germany. http://www.compostnetwork.info/wordpress/wpcontent/uploads/2011/05/ECN-INFO-02_2010_Sustainable_Use_of_Compost_in_Agriculture_LTZ-Project.pdf.
- Hall AJ, Lemcoff JH, Trapani N.** 1971. Water stress before and during flowering in maize and its effect on yield, its component, and their determinants. *Plant Physiology and Biochemistry* **26**, 19-38.
- Lancashire PD, Bleiholder H, Longelüddcke P, Stauss R, Van Den Boom T, Weber E, Witzemberger A.** 1991. A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology* **119**, 561-601.
- Index Mundi.** 2013. Commodity prices indices. <http://www.indexmundi.com/commodities/?commodity=urea>.
- Maynard AA.** 1995. Increasing tomato yield with MSW compost. *Biocycle* **36**, 104-106.

- Ministero per le Politiche Agricole.** 1997. Metodi ufficiali di analisi fisica del suolo, D.M. del 1° agosto 1997. Gazzetta Ufficiale n. 204 del 2.09.97. <http://www.consiglionazionalegeologi.it/cngwww/AODocumento.asp?iddoc=2842andidcat=7>.
- Ministero per le Politiche Agricole.** 1999. Metodi ufficiali di analisi chimica del suolo, D.M. del 13/09/99. Gazzetta Ufficiale n. 248 del 21.10.99. <http://www.crcnet.it/DOCS/DM-13-09-1999.pdf>.
- Montoneri E, Mainero D, Boffa V, Perrone DG, Montoneri C.** 2011. Biochemenergy: a project to turn a urban wastes treatment plant into biorefinery for the production of energy, chemicals and consumer's products with friendly environmental impact. International Journal of Global Environmental Issues **11(2)**, 170-196, <http://dx.doi.org/10.1504/IJGENVI.2011.043528>. http://www.inderscience.com/search/index.php?action=record&rec_id=43528&prevQuery=&ps=10&m=0 r.
- Negre M, Mozzetti Monterumici C, Vindrola D, Piccone G, Perrone DG, Tomasso L, Montoneri E.** 2012. Horticultural and floricultural applications of urban wastes originated fertilizers. Compost Science and Utilization **20**, 150-155
- Montoneri C, Montoneri E, Tomasso L, Piva A.** 2013. Compost derived substances decrease feed protein N mineralization in swine cecal fermentation. Journal of Agricultural Science **13**, 31-44, <http://dx.doi.org/10.5539/jas.v5n3p>.
- OECD.** 2014. Measuring the incidence of policies along the food chain. [http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=TAD/CA/APM/WP\(2013\)30/FINAL&docLanguage=En](http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=TAD/CA/APM/WP(2013)30/FINAL&docLanguage=En)
- Organic Fertilizers Market.** 2013. <http://www.alibaba.com/showroom/organic-fertilizer-market.html>.
- Ozores-Hampton M, Schaffer B, Bryan HH, Hanlon EA.** 1994. Nutrient concentrations, growth, and yield of tomato and squash in municipal solid-waste-amended soil. HortScience **29(7)**, 785-788.
- Pérez-Lomas AL, Delgado G, Párraga J, Delgado R, Almendros G, Aranda V.** 2010. Evolution of organic matter fractions after application of co-compost of sewage sludge with pruning waste to four Mediterranean agricultural soils. A soil microcosm experiment. Waste Management **30**, 1957-1965.
- Richards RA.** 2000. Selectable traits to increase crop photosynthesis and yield of grain crop. Journal of Experimental Botany **51**, 447-458.
- Sortino O, Dipasquale M, Montoneri E, Tomasso L, Perrone DG, Vindrola D, Negre M, Piccone G.** 2012. Refuse derived soluble bio-organics enhancing tomato plant growth and productivity. Waste Management **32**, 1788-1797.
- Sortino O, Dipasquale M, Montoneri E, Tomasso L, Avetta P, Bianco Prevot A.** 2013. 90% yield increase of red pepper with unexpectedly low doses of compost soluble substances. Agronomy for Sustainable Development **33(2)**, 433-441.
- Sortino O, Montoneri E, Patanè C, Rosato R, Tabasso S, Ginepro M.** 2014. Benefits for agriculture and the environment from urban waste. Science of the Total Environment **487C**, 443-451.
- Al Seadi T, Rutz D, Prassl H, Köttner M, Finsterwalder T, Volk S, Janssen R.** 2008. Biogas Handbook. Ed. Al Seadi, T. <http://lemvigbiogas.com>
- Tollenaar M, Daynard TB.** 1982. Effect of source-sink ratio on dry matter accumulation and leaf senescence of maize. Canadian Journal of Plant Science **62**, 855-860.
- Vitali M, Chitarra W, Galetto L, Bosco D,**

Marzachi’C, Gullino ML, Spanna F, Lovisolo C. 2013. Flavescence dorée phytoplasma deregulates stomatal control of photosynthesis in *Vitis vinifera*. *Annals of Applied Biology* **162**, 335–346.

von Lampe M, Kavallari A, Bartelings HV, Meijl H, Banse M, Ilicic-Komorowska J, Junker F, Van Tongeren F. 2014. Fertiliser and Biofuel Policies in the Global Agricultural Supply

Chain: Implications for Agricultural Markets and Farm Incomes. *OECD Food, Agriculture and Fisheries Papers*, No. **69**, OECD Publishing. <http://dx.doi.org/10.1787/5jxsr7tt3qf4-en>

World Bank, Development Prospects Group. 2013. Commodity price forecast update released January **15**, 2013.