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Molecular characteristics of water-extractable organic matter from different composted biomasses and their effects on seed germination and early growth of maize

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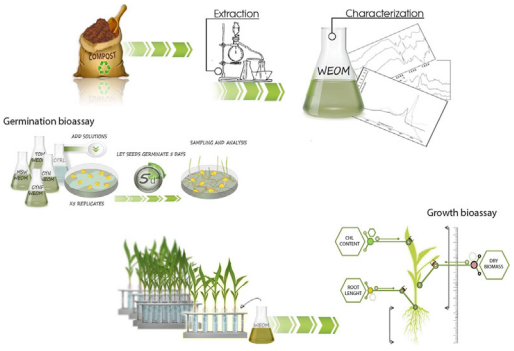
HIGHLIGHTS

- WEOMs from composted green residues may be useful as biostimulants for plant growth.
- Little information on the relationship between WEOM chemical composition and its bioactivity
- WEOMs tested have different significant effects on maize growth.
- The effects on plant are related to the intrinsic molecular composition of materials.

GRAPHICAL ABSTRACT



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ABSTRACT

Four water extractable organic matter (WEOM) were obtained from composts made out of residues of: 1. artichoke (C-CYN), 2. artichoke/fennel (C-CYNF), 3. tomato/woodchips (C-TOM), 4. Municipal solid waste (C-MSW), and their bioactivity was tested for maize seed germination and maize seedling growth. The molecular properties of both original composts and their WEOM were characterized by spectroscopic (^{13}C-CPMAS- and ^{1}H NMR, FTIR-ATR), thermochemolysis-GC/MS, and thermal methods (TGA, DSC). While all WEOM had significant effects on plant growth, CYN-WEOM was the only material that concomitantly increased germination rate and primary and lateral root length of maize seedlings. The lignin-rich WEOM from green composts were generally more effective than those obtained from equally hydrophobic, but mainly alkyl-rich municipal organic wastes. A flexible conformational structure, due to the balanced content of aromatic compounds and carbohydrates, appeared to facilitate the release of bioactive molecules from WEOM suprastructures and stimulate plant growth.

1. Introduction

The composting process is a low-cost and sustainable technology to recycle organic biomasses, while its use in agriculture has become popular as alternative to chemical fertilizers and toxic treatments to control

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soil pathogens (Huang et al., 2013; Pane et al., 2015). The biochemical transformation of organic biomasses leads to a process of humification that resembles that occurring in soil, by which apolar compounds progressively accumulate in hydrophobic domains excluded from water and microbial activity (Piccolo, 2002, 2016; Spaccini and Piccolo, 2007). Nevertheless, a fraction of organic matter can be still extracted from compost in aqueous solutions (WEOM), that, similarly to dissolved organic matter (DOM), may be operationally defined as the organic matter passing a filter of 0.4–0.6 μm pore size (Herbert et al., 1995; Puglisi et al., 2010).

DOM and WEOM are composed by heterogeneous mixtures of medium-polar molecules which arrange in relatively complex supramolecular associations (Piccolo, 2002; Nebbioso and Piccolo, 2013). The humic molecules in WEOM reflect the biological and chemical transformation of organic matter (Lv et al., 2013), influence soil biological activity (Flessa et al., 2000), and interact with metals and organic pollutants (Römkens and Dolfing, 1998; Nebbioso and Piccolo, 2009). While a number of studies has followed WEOM changes during composting (Said-Pullicino et al., 2007; Maia et al., 2008; He et al., 2011), only recently a rising attention has been devoted to the role of humic molecules from compost as a source of bioactive compounds (Canellas and Olivares, 2014; Ramos et al., 2015).

It is well-established that natural organic matter exerts a significant and direct influence on plant growth, affecting morphological, physiological and biochemical processes on seed germination, cell differentiation, ion uptake and overall plant growth (Nardi et al., 2007; Canellas and Olivares, 2014; Vaccaro et al., 2015). As in the case of humic substances, compost WEOM at high concentration is recognized to promote plant growth by acting on membrane bound H^+-ATPase in the root system increasing nitrate uptake (Pinton et al., 1999; Canellas and Olivares, 2014). A compost obtained with citrus biomass residues and its WEOM were found to exert an hormone-like activity on melon seedlings, even though the effect of the water extract was significantly lower (Bernal-Vicente et al., 2008). Furthermore, WEOM was believed to contain unidentified chemical properties, such low molecular weight compounds, that appear to play a role as biocontrol agent and induce systemic resistance in plants (Weymann et al., 1995; Zhang et al., 1998).

There is still a relative little information on the relationship between WEOM chemical composition and its biological activity on plant growth. In fact, a detailed molecular characterization is an essential requirement to elucidate the interaction between the intrinsic structural complexity of compost extracts and its plant biochemical activity. Several properties, such as pH and ionic strength of the aqueous extract, determine the WEOM solubility (Chantigny, 2003), while the molecular concentration can affect the strength of the supramolecular arrangement of the humified compounds (Piccolo, 2002; Maia et al., 2008; Šmejkalová and Piccolo, 2008a). The environmental reactivity of WEOM has been recently related to the hydrophobic/hydrophilic ratio of molecular components (Dobbss et al., 2010; Canellas et al., 2012; Canellas and Olivares, 2014). However, the impact of this ratio and other molecular properties on WEOM activity in promoting plant growth has not yet been extensively studied, and existing information are fragmented.

The aim of this work was thus to obtain detailed information on the molecular composition of WEOM from different compost types by using different analytical approaches and relate them to the effects on maize seed germination and seedling growth.

2. Materials and methods

2.1. Compost and water-extractable organic matter (WEOM)

Composts used in this study were obtained by a 45-days on-farm composting process (active or thermophilic phase) of static piles of chipped plant residues under forced aeration, followed by a two months-curing period. The different on-farm composts were selected on the basis of their composition and identified as: 1. C-CYN = 78.0% artichoke, 20% woodchips and 2% mature compost as starter; 2. C-CYNF = 43.5% artichoke, 23.5% fennel, 11.0% escarole residues, 20% woodchips and 2% mature compost as starter; 3. C-TOM = 50.0% tomato residues, 48% woodchips and 2% mature compost as starter; 4. C-MSW = a 1-year old commercial compost made of urban-waste (Gesenu, S.r.l.). For each type, we received a 10 kg bag containing a composite compost sample, collected at different points of each compost pile. The composite sample was well mixed and reduced to 1 kg via the quartile method. This procedure was carried out in triplicate. After, each sample was air-dried, ground using a blender and pulverized with a ball mill to obtain a homogeneous compost sample. An aliquot of each compost was freeze-dried before chemical analyses.

WEOM was obtained from each 1 kg compost samples as it follows: 100 g of each air-dried compost was suspended in 1000 ml of distilled water and mechanically shaken for 24 h. The suspension was then centrifuged at 1000*g* for 15 min and finally filtered through a 0.45 μm Whatman filter. For each compost, the WEOM extraction was carried out in triplicate. An aliquot of each WEOM extract was freeze-dried prior to further analytical analyses.

Some physical and chemical properties of the four composts and WEOM samples are shown in Table 1. The electrical conductivity (EC) and the pH were measured by conventional methods in a water suspension of compost (1:10 w/v). Content of elemental C and N of composts and WEOM (5 mg) were measured by an element analyzer (CHNS, Fison Instruments EA).

2.2. NMR spectroscopy of composts and WEOM

A 300 MHz Bruker Avance spectrometer, equipped with a 4 mm wide-bore MAS probe, was used to run solid-state spectra of compost samples. Each fine-powdered sample (5 mg) was packed into a 4 mm zirconium rotor, stoppered by a Kel-F cap and spun at a rate of 13,000 ± 1 Hz. In particular, ^{13}C NMR spectra were acquired through the Cross-Polarization Magic-Angle-Spinning (CPMAS) technique, by using 2 s of recycle delay, 1 ms of contact time, 30 ms of acquisition time and 4000 scans.

A 400-MHz Bruker Avance spectrometer, equipped with a 5-mm Bruker BBI (Broad Band inverse) probe, was employed to obtain liquid-state NMR spectra of water-soluble extracts from compost. Each sample (5.0 mg ml^{-1}) was dissolved in deuterated water (D_2O) and placed into a 5.0-mm quartz tube. ^1H NMR spectra were acquired with 2 s of thermal equilibrium delay, 90° pulse length ranging within 8.5 and 9.5 μs, 32,768 time domain points, and 64 transients. The residual water signal at around 4.7 ppm was suppressed by adopting the on-resonance pre-saturation technique (~57 dB power attenuation). All spectra were processed by using both Bruker Topspin Software (v.2.1, Bruker Biospin, Rheinstetten, Germany) and MestReC NMR Processing Software (v.4.8.6.0, Cambridgesoft, Cambridge, Massachusetts, USA).

2.3. Offline pyrolysis TMAH-GC–MS of composts

About 500 mg of dried compost were placed in a quartz boat and moistened with 1 ml of TMAH (25% in methanol) solution. After drying the mixture under a gentle stream of nitrogen, the quartz boat was introduced into a Pyrex tubular reactor (50 cm × 3.5 cm i.d.) and heated at 400 °C for 30 min in a round furnace (Barnstead Thermolyne 21,100). The products released by thermochemolysis were continuously transferred by a helium flow (20 ml min^{-1}) into a series of two chloroform (50 ml) traps kept in ice/salt baths. The chloroform solutions were combined and concentrated by roto-evaporation. The residue was redissolved in 1 ml of chloroform and transferred in a glass vial for GC–MS analysis. The GC–MS analyses were conducted with a Perkin-Elmer Autosystem XL by using a RTX-5MS WCOT capillary column (Restek, 30 m × 0.25 mm; film thickness, 0.25 μm), that was coupled, through a heated transfer line (250 °C), to a PE Turbomass-Gold quadrupole mass spectrometer. The chromatographic separation was achieved