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Evaluation of hormone-like activity of the dissolved organic matter fraction (DOM) of compost and digestate

Barbara Scaglia *, Michele Pognani, Fabrizio Adani

GRUPPO RICICLA, Dipartimento di Science Agrarie e Ambientali: Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy

HIGHLIGHTS

- Biomasses can have biostimulating effect for the presence of hormone-like molecules.
- No data have yet been reported on the bio-stimulating effects of digestate.
- Auxin-like activity was associated with digestate dissolved organic matter (DOM).
- DOM bio-stimulating activity was due to neutral hydrophobic DOM fraction.
- Hormone-activity was due to the auxin coming from aromatic amino acid biodegradation.

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ABSTRACT

Biomasses are usually applied to soil for their agronomic properties (fertilization and amendment properties). Biomass can also have bio-stimulating effects on plants because of the presence of hormones and hormone-like molecules. Although compost has been the subject for studies of this aspect, no data have yet been reported on the extraction of this kind of molecule from digestate biomass. The aim of this work is to study the auxin- and gibberellin-like activity of pig slurry digestate in comparison with those of pruning and garden wastes compost's dissolved organic fraction (DOM). DOM (i.e., fraction < 0.45 μm) is the most reactive among the organic matter fractions readily available to microbial and plant metabolism. No gibberellin-like activities were found for either compost or digestate, whereas digestate showed auxin-like properties which were found to be located in its neutral hydrophobic (NHo) DOM fractions. Hormone activity was due principally to the presence of auxin coming from the anaerobic digestion of aromatic amino acids.

1. Introduction

Organic matter (OM) plays a central role in maintaining soil fertility, providing nutrients and maintaining favorable physical and biological properties of soil (Smith et al., 2007). Soil OM-balance in agricultural soil depends on natural inputs and outputs and on the application of biomasses such as manure and waste-derived products, i.e. sewage sludge, compost and digestate (Tambone et al., 2007; Ahmed et al. 2010; Saruhan et al., 2010). Sewage sludges are commonly applied to soil as fertilizer because of their high N and P content and low C/N ratio (Adani and Tambone, 2005; Ziembia and Peccia, 2011). On the other hand, composts are normally applied to soil as organic amendments because of their high OM content and quality (Tambone et al., 2007). Digestate, (the by-product of anaerobic digestion for biogas production) has also been recently proposed for use as both an organic amendment (solid fraction) (Tambone et al., 2010, 2013) and as an inorganic fertilizer (liquid fraction) (Ledda et al., 2013).

Biomasses also have bio-stimulating effects on plant growth because they contain hormones, hormone-like molecules and other growth-promoting substances (Xu et al., 2012). Compost aqueous extracts, for example, have been reported to stimulate plant growth because of the presence of hormones, vitamins and other biostimulant components (Pedurand and Reynaud, 1987; Du Jardin, 2012). Phytohormones such as auxin, gibberellin, cytokinins, abscisic acid and ethylene, have been searched for in biomass or biomass-derived fractions in the light of their importance in positively influencing many plant physiological processes, such as root system expansion, biomass accumulation, nutrient absorption and mobilization, tolerance to stress, resistance to diseases and retardation of senescence (Crouch and van Staden, 1993). Phytohormones can be present in organic raw material (Arthur et al., 2007) or they can be produced during biomass transformation; for example

* Corresponding author.
E-mail address: barbara.scaglia@unimi.it (B. Scaglia).

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abscisic acid, cytokinin, isopentenyladenosine (iPA), gibberellin and auxin were found into aqueous extracts of green waste compost (Arthur et al., 2007; Pant et al., 2010), whereas gibberellins (GA4, GA34, GA24) were extracted from manure-based compost, vermicompost and green compost (Arthur et al., 2007; Pant et al., 2010). Composting process parameters (i.e., temperature and the duration of treatment) seem to affect the presence of phytohormones in compost (Arthur et al., 2007).

Several articles have reported the ability of biomass to stimulate plant growth independently of the presence of identified hormones, ascribing this effect to the presence of specific organic molecules, named hormone-like compounds (e.g., Nardi et al., 2002; Trevisan et al., 2010; Quilty and Cattle, 2011; Jindo et al., 2012). Auxin-like activities and gibberellin-like activities were found for humic acids extracted from soil and biomasses and for water-dissolved organic matter (DOM, i.e. organic carbon fraction below 0.45 μm) of biomasses (Pizzeghello et al., 2006).

Among the biological processes employed to treat organic wastes, anaerobic digestion (AD) has attracted increased interest in recent years in the EU countries (Tani et al., 2006). AD is an anaerobic biological process by which, in the absence of oxygen, OM is transformed into biogas, which principally consists of methane (50–80 v/v) and carbon dioxide (Tani et al., 2006), and leaves a digestate as the residue of the process (Møller et al., 2009; Tambone et al., 2010). AD treatment plants are typically located at farms, and the biomass used is mainly composed of animal slurries, agro-industrial by-products and dedicated biomass. Digestate is a biologically-stable and partially hygienic organic product with good fertilizing properties because of the high nutrient (N, P and K) content in available forms which makes it a suitable candidate to replace inorganic fertilizers (Ledda et al., 2013).

In contrast to the well-studied biomasses such as compost and sewage sludge, the bio-stimulant properties of digestate are not well known and its potential is unexplored. Literature data are very few; recently Ertani et al. (2013), studying the bio-stimulant effect of digestates' humic-like substances, found a significant auxin-like effect on maize plants, and concluded that these digestate-fractions are bioactive plant growth promoters. Nevertheless humic substances represent an operational fraction, i.e. the alkali soluble fraction, which do not fully represent the soil organic matter (Kelleher and Simpson, 2006). However, the DOM fraction is likely to be of great ecological importance as it represents a readily available carbon and nutrient source for microorganisms and plants (Metting, 1993; Schnabel et al., 2002; Marschner and Kalbitz, 2003; Scaglia and Adani, 2009).

DOM consists of a miscellany of organic molecules such as organic acids, carbohydrates, and fats (Dilling and Kaiser, 2002; Said-Pullicino et al., 2007), the composition of which depends upon the raw materials and the biological treatment which the biomass has undergone (i.e. aerobic or anaerobic biological degradation) (D'Imporzano and Adani, 2007; Salati et al., 2013).

Auxin and cytokinin-like activities have been tested for in compost extract and in stable compost leachate without any responsible molecules being identified (Arthur et al., 2007; Garcia et al., 2002). Later, Pizzeghello et al. (2006) linked DOM auxin and gibberellin-like activities to the presence of oxalic, tartaric, and phenolic acids (Nardi et al., 2002; Pizzeghello et al., 2006).

Since DOM is a complex mixture of molecules, a fractionating procedure can be used to separate DOM into the hydrophilic (Hi), the hydrophobic (Ho) and the neutral hydrophobic (NHo) fractions (Qualls and Haines, 1991). In the past, DOM fractionation has been applied to study biomass evolution during aerobic (composting) (Said-Pullicino et al., 2007; D'Imporzano and Adani, 2007) and anaerobic (landfill) (Salati et al., 2013) biodegradation processes, and to characterize natural DOM (Leenheer, 1981), but the isolation of the DOM-fraction responsible of the bio-stimulant effect of DOM has not yet been reported.

The aim of this work was to study the hormone-like activity (auxin-like and gibberellin-like effects) of DOM extracted from a digestate in comparison with that of compost, by applying, for the first time, the DOM fractionation procedure to isolate the fraction responsible for the stimulant effect and subsequently, to investigate the organic molecules responsible for this effect.

2. Materials and methods

2.1. Biomass

Compost and digestate samples were obtained from composted pruning waste mixed with garden waste, and from anaerobically digested pig slurry, respectively. Compost was produced in a full scale composting plant in northern Italy. The active composting phase took place in an in-vessel system for 3 weeks; at the end of the active phase the material was transferred to an open area, where the curing phase (turned piles) took place for 8 weeks. After the curing phase the material was (sieved) using an industrial mesh (hole diameters of 30 mm) to produce the final compost.

The pig slurry-digestate was collected at the end of the anaerobic process at a full scale agricultural plant in northern Italy. The treatment plant consisted of a continuously-stirred anaerobic digester working at 37 °C with a retention time of 40 days. The pig slurry was produced every day at farm level, stored in a pre-digester container and fed into the plant. The digestate obtained at the end of the anaerobic treatment is routinely used at farm level as fertilizer.

2.2. Biomass characterization

Total solids (TSs), volatile solids (VSs), total organic carbon (TOC), total Kjeldahl nitrogen (TKN) and pH assessments were performed using standard analytical procedures (The U.S. Composting Council, 1997). Electrical conductivity (EC) was measured using a conductivity meter (XS COND 510, Tecnovetro, Monza, Italy). All tests were run in triplicate and results are presented as average values followed by standard deviations.

2.3. Dissolved organic matter extraction, fraction separation and characterization

Dissolved organic matter (DOM) was extracted from compost (DOM_c) and digestate (DOM_d) by using deionized water, using 5 g of equivalent dry material (1:20 solid:liquid ratio, w/w) in a Dubnoff bath at 60 rpm for 30 min at 40 °C. The suspension obtained was centrifuged for 15 min at 6500 rpm and then vacuum filtered twice: firstly, by using a fast glass fiber filter (Whatman GF 6), and then by using a 0.45 μm cellulose acetate membrane filter (Whatman OE 67) (Salati et al., 2013). DOM was successively fractionated into hydrophilic (Hic and Hid for compost and digestate, respectively), hydrophobic (Hoc and Hod for compost and digestate, respectively) and neutral hydrophobic (NHoc and NHod for compost and digestate, respectively) fractions, according to the procedure published by Salati et al. (2013) and briefly described below.

One hundred millimeters of DOM, previously acidified (pH < 2) with 0.5 mol l^{-1} of H_2SO_4, was loaded (at the velocity of 2 ml min^{-1}) onto a column (40 cm high glass column, 2.5 cm in diameter) filled with activated Amberlite XAD-7 (Sigma-Aldrich Steinheim, Germany). The resin was first washed with distilled water (2 bed volume) to obtain the Hi fraction. Then, the resin was washed with NaOH 0.05 mol l^{-1} followed by distilled water (2 bed volume) to get the Ho fraction. Sodium cations were then removed from Ho by using a strong cation exchange resin (Amberlite IR 120, Merck, Darmstadt, Germany). Finally the NHo fractions were obtained from the XAD-7 resin by a Soxhlet extraction procedure with MeOH. DOM, Hi and Ho fractions were then characterized for dissolved organic carbon, total organic nitrogen (DON), pH and electrical conductivity (EC), as previously reported (see Section 2.2). In addition cation contents (Ca^{++}, Na^{+}, K^{+}, Mg^{++} and Mn^{++}) were determined by inductively coupled plasma mass