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A Review on the Use of Phytotoxicity as a Compost Quality Indicator

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ABSTRACT

Given the common use of compost in agriculture, forestry, landscaping and environmental restoration, it is essential that it is perfectly compatible with plant growth and, if possible, that it increases production and quality of plant biomass. It is therefore necessary to evaluate the absence of phytotoxic substances in the compost, which not only informs about its quality, but also on the proper handling of the composting process. Procedures for assessing the phytotoxicity as an indicator of compost maturity can be grouped into two types: a) seed germination and seedling elongation tests with extracts of compost; b) direct seeding tests on substrates made wholly or partly by compost. For the compost to be considered mature, the results of germination and elongation, or plant biomass, should be at least 80-90% of those obtained in a control. These tests have proven to be sensitive to various toxins, such as salts, organic acids, ammonia, or metals, and can be used to evaluate the process conditions and the degree of stabilization of organic matter, and the suitability of the raw materials. However, the great variability in testing methods make difficult the comparison and interpretation of results, so it is necessary to advance in the standardization of procedures and determine phytotoxicity thresholds for potential phytotoxic compounds in compost.

Keywords: composting, germination index, ecotoxicity, cress, barley, growing media
Abbreviations: **$FMr_{25\%}$**, plant production in fresh weight of 25% compost mixture; **$FMr_{50\%}$**, plant production in fresh weight of 50% compost mixture; **GI**, germination index; **MSW**, municipal solid waste

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INTRODUCTION

Phytotoxicity can be defined as a delay in germination, inhibition of plant growth or any other adverse effect caused by specific substances (phytotoxins) or by inadequate growth conditions (Baumgarten and Spiegel 2004). It can also be described as detrimental deviations from the normal growth and appearance pattern of plants in response to a given substance (OECD 2006). If this concept is applied to compost, phytotoxicity can be understood as the condition or quality of the material which negatively influences plant growth.

Since all the potential applications of compost consider its contact with plants, phytotoxicity is one of the most important criteria to assess its quality and suitability for agricultural purposes, landscaping and environmental restoration, and it is particularly relevant with respect to the compost to be used in high value horticultural applications.

Moreover, phytotoxicity assessment is a valuable way to assess the stage of the composting process (decomposition, stabilization or maturity) that has been achieved (Zucconi *et al*. 1985). It also informs about the composting conditions, since some of the components that can cause toxicity originate during the composting process, and can be observed mainly during the intermediate stages of the transformation of the organic matter or when the process is not properly managed.

Phytotoxic effects of compost are usually the result of several factors. Thus, it has been shown that several compounds can cause phytotoxicity: ammonia (Wong *et al*. 1983; Wong 1985; Barberis and Nappi 1996), which is especially important when composting materials with low C/N ratio (Zucconi *et al*. 1985); ethylene oxide, which is synthesized during the decomposition of the compost after being applied to the soil (Wong 1985); organic acids, which are produced during the decomposition of fresh organic residues, including acetic acid, propionic acid, and butyric and isobutyric acids (Chandrasekaran and Yoshida 1973; Kuwatsuka and Shindo 1973; DeVleeschauwer *et al*. 1981; Chanyasak *et al*. 1983; Zucconi *et al*. 1985; Shiralipour *et al*. 1997; Himanen *et al*. 2006); phenols, which are present in some agricultural wastes (Pascual *et al*. 1997; Alburquerque *et al*. 2006); salts (Adriano *et al*. 1973; Barral *et al*. 2007), which are present mainly in food wastes; and heavy

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metals (Wollan *et al.* 1978; Pahlsson 1989; Prasad and Hagemeyer 1999), which are of concern especially in urban waste composts, since they contain a number of potential sources of heavy metals (batteries, paints, electronic, ceramics, plastics, etc.).

Some toxic components can be identified by means of analytical methods but these can be expensive and time consuming. Moreover, unexpected contaminants can be present in the compost, which are not detected in routine analyses. Further, at present there are no analytical procedures that can measure the effects of synergy and antagonism of toxic compounds (Emino and Warman 2004). In these circumstances, biological tests are the most realistic and thorough way to assess the compatibility of composted materials with plants, because they allow the assessment of the combined effects of several phytotoxic factors present in the compost (Zucconi *et al*. 1981a, 1981b; Emino and Warman 2004). The main difficulty lies in distinguishing the toxic responses due to substances produced during the composting process – and therefore susceptible to reveal the degree of the evolution and stabilization of the compost – from those due to salts, heavy metals or other pollutants, introduced along with the raw materials or incorporated into the final product. In other words, is open to debate whether a phytotoxicity test is equivalent to a compost maturity test or not, although several authors and associations consider it as such (Warman 1999). The meaning of phytotoxicity as a compost quality indicator and the available methodologies for its assessment are reviewed in this work, and the need to agree on standardized methods for its determination is highlighted.

PHYTOTOXICITY: PROOF OF IMMATURITY/ INSTABILITY?

There is a general consensus with respect to the meaning of the term 'stability', which refers to the degradability/degradation of organic matter (Wu *et al*. 2000; Benito *et al*. 2003; Brewer and Sullivan 2003; Rynk 2003), considering a stable compost as one that has a low mineralization rate and a low concentration of easily biodegradable substances. Nevertheless, there are various interpretations of the term 'maturity' as applied to compost. A simple and frequently used meaning refers to the suitability of the compost for plant growth, including therefore the absence of toxic substances that may delay or reduce the germination of seeds, damage or kill plants (Butler *et al*. 2001; Benito *et al*. 2003; Cooperband *et al*. 2003; Said-Pullicino *et al*. 2007). Another more complete meaning of the term considers maturity as the combination of the stability and humification of the organic matter, besides the absence of phytotoxic substance (Iglesias Jiménez *et al*. 2008). The document 'Assessment of options and requirements for stability and maturity testing of composts, FRAP Standards Research Report' (ADAS 2005) offers a simple comparison of both terms:

- Stability is defined as a "biological activity rate" and is generally measured as an aerobic respiration ratio.
- Maturity is defined as "suitability for use" and is assessed by means of stability and phytotoxicity, as well as other relevant parameters for the required use.

Phytotoxicity may be due to the production of substances of transient nature during the early stages of composting (short chain organic fatty acids, phenols) (Madejón *et al*. 2001), but can also be attributed to the characteristics of the materials undergoing composting (high salinity, high content of heavy metals, presence of alcohols or phenols). Consequently, biological tests should be considered maturity indicators –meaning the non-restriction of plant growth– rather than stability indicators. Notwithstanding, given that microorganisms produce phytotoxins during the decomposition of organic matter, an appropriate response during biological tests should be an indication of sufficiently stable organic matter. Since composting is able to eliminate most of the phytotoxic substances found in fresh wastes, and the toxic compounds that are produced during the initial stages of composting are also degraded in time, the best results of phytotoxicity tests are obtained with 'mature' (stable) composts (Pascual *et al*. 1997), whereas 'immature' (unstable) compost may still cause a toxic response (Kapanen and Itävaara 2001).

Table 1 Compost maturity parameters according to the California Compost Quality Council.

C:N ratio	
Group A (stability)	**Group B (maturity)**
Respirometric Test	Ammonia/Ammonium
• Oxygen Consumption Ratio	Ratio ammonia:nitrate
• Carbon Dioxide Production Ratio	Biological Trials
• Dewar Self-Heating Test	• Seedling Vigour and Emergence
• Solvita CO_2	• Germination and Root Elongation *in vitro*
Carbon available for organisms	• Bio-trial with earthworms
	Solvita NH_3
	Volatile Organic Acids

As a result, the stability of organic matter is normally a prerequisite for the absence of phytotoxicity, but when this negative effect occurs, it may be due to other reasons rather than the lack of stability. For example, some potentially toxic components do not disappear during composting, as in the case of salts, heavy metals and some persistent herbicides. In fact, salinity probably represents the main limiting factor for the use of certain composted residues (Smith and Hall 1992; Vogtmann and Fricke 1992), especially those obtained from municipal solid waste (MSW) (Moldes *et al*. 2006).

Thus, there is not a clear and uninfluenced relationship between phytotoxicity and stability, mainly because of the potential presence of phytotoxic substances in compost (salts, heavy metals) not related to compost stability. It is therefore appropriate to combine several measures for the evaluation of compost maturity, including a determination of phytotoxicity. Based on the premise that mature compost has completed the composting process and presents a minimum risk of affecting plant growth, the California Compost Quality Council (CCQC 2001) has proposed a Maturity Index, which, after identifying the C/N ratio, continues with the description of at least one parameter of the lists A and B (**Table 1**). List A includes respiration and self-heating measures, whilst List B considers the risk of phytotoxicity through the analysis of the ammonia: nitrate ratio, the concentration of ammonium, the concentration of volatile organic acids, or a plant test. Similarly, Paradelo *et al*. (2010a) recommend examining at least three groups of properties: a measurement of the degree of humification, a measurement of the microbial activity or of the water-soluble organic matter, and a plant germination or growth test.

THE RELATIONSHIP BETWEEN PHYTOTOXICITY AND COMPOSTING PROCESS STAGES

As mentioned above, a peculiarity of the metabolic phytotoxicity is that it is associated to some composting stages (Zucconi *et al*. 1985). The production of phytotoxic compounds represents a transient situation during composting, since it is high in the initial stage, when there is a rapid degradation of the organic matter, and decreases during the stabilization, when 'humification' and mineralization predominate. This decrease in toxicity towards the end of composting may be due to several factors, primarily the metabolic degradation of some phytotoxic organic compounds, or to the incorporation of certain phytotoxic molecules to the 'humic acids' fraction (Zucconi *et al*. 1985). The functional groups existing in the humified organic matter can help reducing the bioavailability and thus the phytotoxicity of heavy metals and organic substances such as pesticides, by means of mechanisms such as sorption or complexation

(Smith 2009).

Stabilization occurs faster when oxygen concentration increases and therefore phytotoxicity quickly disappears in ventilated systems. The disappearance of phytotoxic compounds has been observed in a few weeks in systems with forced aeration, before concluding the thermophilic stage. On the contrary, anaerobic processes are characterized by high levels of toxicity that remain in the stabilized products for months or more than one year, and which can be mostly attributed to the ammonia produced during the anaerobic decomposition of organic wastes (Wong 1985; Barberis and Nappi 1996; Tiquia and Tam 1998).

METHODS TO DETERMINE PHYTOTOXICITY

General considerations

Phytotoxicity tests have been applied to environmental matrices (soils, sediments) and materials of anthropic origin (compost, sewage sludge) (Czerniawska-Kusza *et al*. 2006). A review of compost bio-maturity tests was carried out by Mathur *et al*. (1993). Likewise, a compilation of ecotoxicity tests can be found in Kapanen and Itävaara (2001), which although initially designed for soils, sediments or wastes, can be applied to compost.

Phytotoxicity tests should be simple, reproducible and quick. Long field experiments are not recommendable, because the sensitiveness to the toxic compounds can be overcome by means of the adaptation of plants (Zucconi *et al*. 1985). Determination of "*in vitro*" seed germination and plant growth are the most common techniques to assess the phytotoxicity of compost (Kapanen and Itavaara 2001). Some protocols use compost extracts, while others are based on direct seeding on compost. Several parameters are used for phytotoxicity assessment (seed germination percentage, plant length, root length and biomass), and also a great variety of plant species are tested. The main difficulty in the application and interpretation of these techniques is the variety of existing methods which are not always consistent and sensitive enough (Warman 1999; Rynk 2003; Emino and Warman 2004), and are not sufficiently standardized.

Therefore, although biological tests are the most realistic way to analyse the compatibility with plants, specific targeted studies are needed to establish the cause-effect relationship between the concentration of potentially toxic constituents such as ammonium, organic acids, salts or heavy metals, and the germination response and plant growth. It is also necessary to select index plant species to differentiate whether the toxicity is due to a lack of stability in the organic matter, process defects such as inadequate aeration, or to unsuitable characteristics of the raw materials. The standardization of methods, in order to easily compare results from different laboratories, is also necessary.

Plant species used to determine phytotoxicity

Garden cress (*Lepidium sativum* L.) is the most commonly used species, because it is easy to manage and shows fast germination and growth. Many other species have also been used, among which it is worth mentioning horticultural species such as tomato (*Lycopersicum esculentum* Mill.), carrot (*Daucus carota* L.), cucumber (*Cucumis sativus* L.), cabbages (*Brassica oleracea* L. var. italica, *Brassica rapa* L. var. pekinensis, *Brassica parachinensis* B.), radish (*Raphanus sativus* L.), lettuce (*Lactuca sativa* L.) and beans (*Phaseolus vulgaris* L.); cereals such as barley (*Hordeum vulgare* L.), Italian rye grass (*Lolium multiflorum* Lam.), rice (*Oryza sativa* L.), wheat (*Triticum vulgare* L.), rye (*Secale cereale* L.), soya (*Glycine max*. L.) or corn (*Zea mays* L.), besides sunflowers (*Helianthum annuus* L.), petunia (*Petunia* x hybrida) or amaranth (*Amaranthus tricolor* L.), among others. References to various studies using these species can be found in Warman (1999). Also, an extensive experiment in terms of number of species tested has been carried out by Emino and Warman (2004). It should be noted that some protocols (ISO11269-2:2005) recommend the use of at least one monocotyledonous and one dicotyledonous species in these tests.

Some species are more sensitive than others to the different toxic components that may exist in the compost, although the results obtained by different authors do not always coincide. Thus, cress is very sensitive to salinity (FCQAO 1994), but it does not seem to distinguish between mature (stable and humified) and immature composts (Emino and Warman 2004). Barley seems to be less sensitive to salinity than cress, but it is more affected by organic acids produced during the decomposition of organic matter (FCQAO 1994). Chinese cabbage (Tiquia *et al*. 1996), lettuce and amaranth (Emino and Warman 2004) appear to be more sensitive than cress to immature composts, while lettuce and tomato are affected by phenolic compounds (Ortega *et al*. 1996). In conclusion, although cress is the most used plant species (Emino and Warman 2004), the choice of species susceptible to the toxic components that are to be identified is an essential element of the biotests.

Types of tests used to determine the phytotoxicity of compost

Phytotoxicity tests can be grouped into two wide-ranging categories: germination and/or elongation tests with compost extracts, and direct growth tests.

1. Germination and root elongation in contact with compost extracts

Procedures

In 1981, Zucconi *et al*. (1981b) described a germination test with cress seeds in contact with compost extracts, obtained by pressure from moistened compost, which determined the relative seed germination and root elongation, as compared with that obtained in distilled water. Many researchers have used germination tests based on this procedure, but making changes in the plants used and the mode of preparation of the extracts, since in the modified methods the extracts are obtained by centrifugation and/or filtering of suspensions with different compost-water ratios, and used directly or after dilution. An extensive review of the literature on germination tests can be found in Warman (1999). In **Table 2** the conditions used in this type of tests are compared. A common description of the procedure is as follows: seeds are placed on Petri dishes on filter paper moistened with the compost extracts, and placed in incubation chambers for time periods ranging from one to six days, with temperatures ranging between 20°C and 28°C. The results are generally expressed as an index GI [GI = (% G × % L)/100] combining relative germination (% G) and relative root elongation (% L), compared to a distilled water control.

Interpretation of results

Germination index is considered the most sensitive parameter for identifying the phytotoxicity of compost and assess its suitability for use as soil amendment or growing media. It is an integrated parameter, which combines the relative germination and relative root elongation, although several authors (Emino and Warman 2004; Fuentes *et al*. 2004; Di Salvatore *et al*. 2008; Paradelo *et al*. 2008) have reported that root elongation is more sensitive to the presence of toxins than seed germination. According to Zucconi *et al*. (1981a, 1981b, 1985) and Emino and Warman (2004), GI values below 50% indicate high phytotoxicity; values between 50% and 80% indicate moderate phytotoxicity; and values above 80% indicate the absence of phytotoxicity. When the index exceeds 100%, the compost can be considered a phytonutrient or phytostimulant. So, for example, GI values > 125% were observed in rye grass seeds germinating in extracts of composted grape marc, which was

Table 2 Some conditions used to determine phytotoxicity using germination tests with compost extracts.

Volume (mL)	Extract	Species	Seeds	Replicates	T (ºC)	Time (h)	Reference
0.5	Pressure[1] at 60% moisture; subsequent dilutions	Watercress	6-8	-	27	24	[a]
2	1:10	Barley	10	4	28	120	[b]
10	1:10	6 plants	10-30	3	22	120	[c]
5	1:2.5	Watercress, radish and cabbage	5-10	4	20	24-48	[d]
1	Pressure at 60% H; later dilution 1:10	Rye grass and watercress	5	8	22	72	[e]
5	1:2 (v:v), later dilutions 0, 25, 50, 75 and 100%	Lettuce	10	10	25	72	[f]
5	1-30%	Watercress	10	2	24	72	[g]
3	1:10	14 species	5-10	4	20	Most seeds germinated in control and 3-5 cm roots	[h]
1	1:15 (dry weight/v)	Watercress	8	10	27	48	[i]
5	1:10	Watercress, barley and oats	5-10	5-10	25	48 watercress, 144 barley and oats	[j]
5	1:10	Barley and watercress	8	5	20	72 watercress, 144 barley	[k]
9	Metal solutions	Radish and tomato	20	6	21	72-96	[l]
3	1:10	Watercress, barley and rye grass	10-15	3	28	120	[m]
5	1:10	Chinese cabbage	50	3	25	72	[n]

[1] 5 min 2.5 atm/cm^2

[a] Zucconi *et al*. (1985); [b] Pascual *et al*. (1997); [c] Tiquia *et al*. (1997); [d] Warman (1999); [e] Madejón *et al*. (2001); [f] Gariglio *et al*. (2002); [g] Hoekstra *et al*. (2002); [h] Emino and Warman (2004); [i] Alburquerque *et al*. (2006); [j] Walter *et al*. (2006); [k] Alvarenga et al. (2007); [l] DiSalvatore *et al*. (2008); [m] Paradelo *et al*. (2008, 2010b); [n] He *et al*. (2009)

highly phytotoxic before being composted in controlled microaerobic conditions in the laboratory (Moldes *et al*. 2007).

Germination tests seem to be sensitive to compost stability, for Pascual *et al*. (1997) observed that the rate of germination and length of roots of barley were higher in mature composts, and correlated negatively with water soluble carbon (WSC) and WSC/N ratio. Aslam *et al*. (2008) also noted a direct relationship between phytotoxicity and parameters indicative of instability of compost, such as C mineralization rate or potentially mineralizable C concentration, while Fang and Wong (1999) found a relationship between the GI and the $C/N_{organic}$ ratio. Furthermore, the information provided by GI coincided with that provided by the ratios of soluble organic components in water $C_{soluble}/N_{soluble}$ and by the total $C_{soluble}/N$ ratio (Sánchez-Monedero *et al*. 2001), which increased during the composting process as the phytotoxic substances decomposed (Bernal *et al*., 1998). GI is also sensitive to the conditions of composting. The persistence of phytotoxic compounds under conditions of insufficient aeration has been shown by Zucconi *et al*. (1985). On the contrary, when the supply of O_2 is appropriate, several experiments have demonstrated that the GI value increases during composting (Wong 1985; Tiquia *et al*. 1996; Gariglio *et al*. 2002), as the phytotoxins produced by the organic matter decomposition gradually disappear under conditions of adequate temperature, oxygen, humidity and nutrient concentration. Therefore, several authors have observed an increase in the GI in parallel with the decrease of the concentration of toxic compounds (Lasaridi and Stentiford 1998) such as ammonia (Tiquia and Tam 1998), phenols and lipids (Alburquerque *et al*. 2006), and ethylene oxide (Wong 1985), during the composting process.

A high salinity may also cause phytotoxicity. In fact, it has been observed that GI is more affected by this property than by the degree of stability, in organic amendments with a wide range of electrical conductivities (Lasaridi and Stentiford 1998). Campitelli and Ceppi (2008) also observed a high negative correlation between GI and electrical conductivity. Hauke *et al*. (1996) demonstrated different degrees of tolerance to salts among vegetable species and put forward that the chloride content should not exceed 0.6 g/L if the growing media contains 40% (v/v) of compost, and 1.0 g/L if only 20% compost is incorporated. EC values over 2.45 dS/m are considered phytotoxic for cress (Sesay *et al*. 1997). Moldes *et al*. (2006) attributed the phytotoxicity of MSW compost to excessive salinity, when they noted that the GI increased by diluting the extracts or using previously washed compost. On the other hand, Gariglio *et al*. (2002) deduced that the toxicity of compost made of *Salix* sp. sawdust was due to very active compounds which inhibited germination even at low concentrations, as GI did not increase with dilution of the compost extracts.

Although many laws establish certain maximum levels of heavy metals in compost, their phytotoxic effect is not clear enough. So, for example, Wong (1985) found that the phytotoxic effect of MSW compost on the germination of Chinese cabbage (*B. parachinensis* Bailey) decreased during the composting time, despite heavy metals concentrations remained practically unchanged. This suggested that the metals were not the main cause of the toxic response, which was attributed to degradable organic compounds. Similarly, Madejón *et al*. (2001) found that heavy metals did not seem to play an important role in the phytotoxicity of the tested composts, since in their experiments the highest values of GI were obtained in a material with high metal concentrations. Besides, the total concentrations of potentially toxic elements can be difficult to link to phytotoxicity, as the mobility and bioavailability of heavy metals depend on a number of factors, such as their speciation, and will be variable through different amendments, even if the total concentrations remain the same (Tisdell and Breslin 1995; Greenway and Song 2002; Paradelo *et al*. 2011).

Moreover, the assessment of heavy metals phytotoxicity using germination or elongation tests in compost extracts presents some experimental difficulties. According to Wang (1994), there is a direct relationship between the toxicity of metals and the degree of exposure to the pollutant, so that the highest inhibitory effect is observed when the roots are completely exposed to heavy metals. Wierzbicka and Obidzinska (1998) observed that when a germination test is carried out in a Petri dish containing a limited quantity of Pb dissolution, the penetration of the metal does not follow a regular pattern; consequently, they consider that the absolute quantity of the metal in relation to the seed surface is more important than the metal concentration. To overcome this limitation, they proposed a modification in which seeds are pre-soaked in a large volume of the solution being evaluated. The modified test is more reproducible and more sensitive (Wierzbicka and Obidzinska 1998; Paradelo *et al*. 2010b).

Another limitation may arise when the filter paper used in germination tests interacts with the metallic ions, reducing their availability (Ratsch and Johndro 1986). To avoid this problem, DiSalvatore *et al*. (2008) replaced the

filter paper by agar when testing the effect of various heavy metals on the germination and growth of broccoli, lettuce and radish roots. At the concentrations studied, the heavy metals had no effect on germination, but did affect the growth of roots. More consistent and sensitive results were obtained using agar, which was attributed to a greater bioavailability of the metals and to a more complete exposure of the roots to the pollutant.

For a better interpretation of the results of the germination test, it is necessary to establish dose/response relationships and toxicity thresholds, at least approximate. Paradelo *et al.* (2010b) assessed the toxicity of solutions of heavy metals using a germination/elongation test, obtaining a toxicity threshold for Cu and Pb at 5 mg L^{-1} and a toxicity threshold for Zn at 25 mg L^{-1}. Comparing these results with water-soluble metal concentrations in composts from several sources, they considered unlikely to reach levels high enough to produce a phytotoxic response in the germination-elongation test. In the same study of Paradelo *et al.* (2010b) the phytotoxic concentrations of acetic acid, propionic acid and butyric acid were set between 50 and 100 mg L^{-1}, while the toxic concentration was 2 g L^{-1} for ethanol.

2. Direct growth tests

Procedures

The use of aqueous extracts provides relevant information, but does not offer a complete description of the toxicity, which should take into account not only the fraction of contaminant dissolved in water at the time, but also the fraction of the contaminant associated with the matrix (Oleszczuk 2008). García *et al.* (1992) observed a greater inhibition in direct growth tests than in tests with aqueous extracts, suggesting that either the phytotoxic substances were not soluble in water or intrinsic adverse effects, such as increased temperature, occurred due to the direct use of compost. Direct growth tests allow overcoming this problem. They can be short tests focused on the assessment of root germination and elongation, or longer growth trials focused on the evaluation of the effect of compost at later stages of plant development.

Although the seed germination test has been widely used, it should be noted that this stage of the plant is relatively insensitive to many toxic substances, because the embryo is isolated from the environment and many chemicals are not absorbed by the seed, which supplies the necessary nutrients to the embryo (Kapustka 1997). In fact, some authors consider that plant growth is more sensitive to toxic substances than seed germination (Kapustka 1997; Araújo and Monteiro 2005; Cendón *et al*. 2005). Pot trials are quite comprehensive, because they provide productivity data, but they are slow and require complex installations, either a growth chamber or a greenhouse, with controlled temperature, humidity and illumination conditions.

Direct growth tests used to evaluate the quality of compost use substrates made of compost alone or mixed with other constituents. To assess if compost exhibits any toxicity which is not due to salinity, it is interesting to dilute the compost with other suitable material (ADAS 2005). The results obtained with the substrates tested are compared with a control substrate, which may be a good quality soil (ISO 1993; ISO 2005), a reference soil (FCQAO 1994), a commercial substrate (Emino and Warman 2004), a mixture of peat and perlite (Iannotti *et al*. 1994; Thompson *et al*. 2002), or a mixture of a standard substrate and vermiculite (Australian Standards 1999).

If the objective is to determine only germination and root length, the experiment is conducted in an incubator, in the darkness. At the end of the experiment a germination index can be calculated, as explained above. If the objective is to determine plant production, the experiment is carried out under controlled lighting and temperature, for a period ranging between 7 and 77 days, at the end of which the fresh and dry weight of the aerial part of the plants is determined, and production is calculated in relation to control (FCQAO 1994; CCME 1996; Gariglio *et al*. 2002; Araújo and Monteiro 2005). A commercial biotest (Phytoxkit test) has been developed recently; using digital photographs, it determines seed germination and early root growth after selected superior plant seeds are exposed to the contaminated matrix for three days, compared to controls in a reference soil (Oleszczuk 2008).

Some standardized procedures can be used with appropriate modifications, to assess the phytotoxicity of compost, such as regulation 208-OCDE (2006) for the assessment of chemical substances, or regulations ISO 11269-1 (1993) and ISO 11269-2 (2005) for the evaluation of soil quality. **Tables 3** and **4** present a comparison of the conditions used in direct seeding and/or growth experiments.

Interpretation of results

Using a direct germination test, Aslam *et al*. (2008) found a negative correlation between relative germination, on the one hand, and electrical conductivity, C mineralisation rate and mineralizable C concentration in the compost, on the other hand. Also using direct seeding, Emino and Warman (2004) observed a greater germination and growth of several species in mature compost than in immature compost; however, they did not observe differences when the composts were mixed at 50% with commercial substrate.

Using the protocol put forward by FCQAO (1994), Cendón *et al*. (2005) compared the growth of cress in mix-

Table 3 Substrate characteristics used in direct growth tests.

Substrate	Control	Proportion	Reference
Problem soil	Good quality soil or sand	Various dilutions	ISO 11269-1
Polluted/Contaminated soil	Soil with no pollutant/contaminant	Different concentrations of pollutant/contaminant	ISO 11269-2
Compost	Reference soil	-	FCQAO (1994)
Compost + reference soil, with fertilisation	Reference soil	25 and 50 % compost	FCQAO (1994)
Washed compost	1:1 mixture of peat and perlite, limed and fertilised	-	AS 4454
Compost + Peat + Perlite	70% peat+30% perlite, limed and fertilised	0 to 50 % compost	Iannoti *et al*. (1994)
Sand and organic matter	Sand	1: 1: w/w	Madejón *et al*. (2001)
Problem soil + artificial soil	Artificial soil	25-50-75-100%	Alvarenga *et al*. (2007)[1]
Polluted/Contaminated soil and compost	-	-	Gong *et al*. (2001)[2]
Compost diluted with vermiculite to adjust to EC at 400μS/cm	Limed and fertilised peat	According to EC	ADAS (2005)
Artificial soil with compost	Artificial soil[3]	6 or 12 t/ha compost	Moreira *et al*. (2008)[2]
Soil and compost	Water	0-5-50%	Aslam and VanderGheynst (2008)

[1] According to ISO/DIS 15799, 2003
[2] According to ISO 11269-1 and/or 2
[3] OCDE 208, 2006

Table 4 Experiment conditions used to determine phytotoxicity in direct growth plant tests.

Plants	Seeds	Replicates	T (°C)	Light (h/d)	Time[1] (days)	Parameter	Reference
Barley	6 pre-germinated seeds	3	20	12-16 25000 lm/m^2	5	Length of the longest root	ISO 11269-1
At least one monocotyledon and one dicotyledon	10	4	Adequate for selected seed	16 7000 lx	14-21	Emergence and aerial part weight	ISO 11269-2
Watercress	1 gram		18-20	12 >3000 lx	7 (after sowing)	Relative fresh weight	FCQAO (1994)
Barley	50	3	18-20	12 >3000 lx	10	Relative fresh weight	FCQAO (1994)
Radish	10	2	25	12	5	Relative radicle length	AS 4454
Cucumber	8 initial 4 final	2	23-26	14 225[4]	21	Germination and aerial part biomass	Iannoti *et al*. (1994)
Rye grass; watercress	5	8	22	-	72	Germination and radicle length	Madejón *et al*. (2001)
Watercress and barley	16	4	20	16	14	Relative dry weight	Alvarenga *et al*. (2007)[2]
Watercress, oats, turnip, broad beans	10 initial 5 final	4	20 d/16 n	16 17000 lx	14	Emergence and fresh and dry weight	Gong *et al*. (2001)[3]
Radish, lentils	8	3	15-25	10000 lx	7 radish 14 lentils	Emergence and relative weight	ADAS (2005)
Oats and turnip	10	4	22	12	19-21	Dry weight	Moreira *et al*. (2008)[3]
Watercress	20	2	22	10 71[4]	5	Relative germination	Aslam and VanderGheynst (2008)

[1] Frequently after reaching 50% emergence of the control group
[2] According to ISO/DIS 15799, 2003
[3] According to ISO 11269-1 and/or 2
[4] µeinsteins/m^2

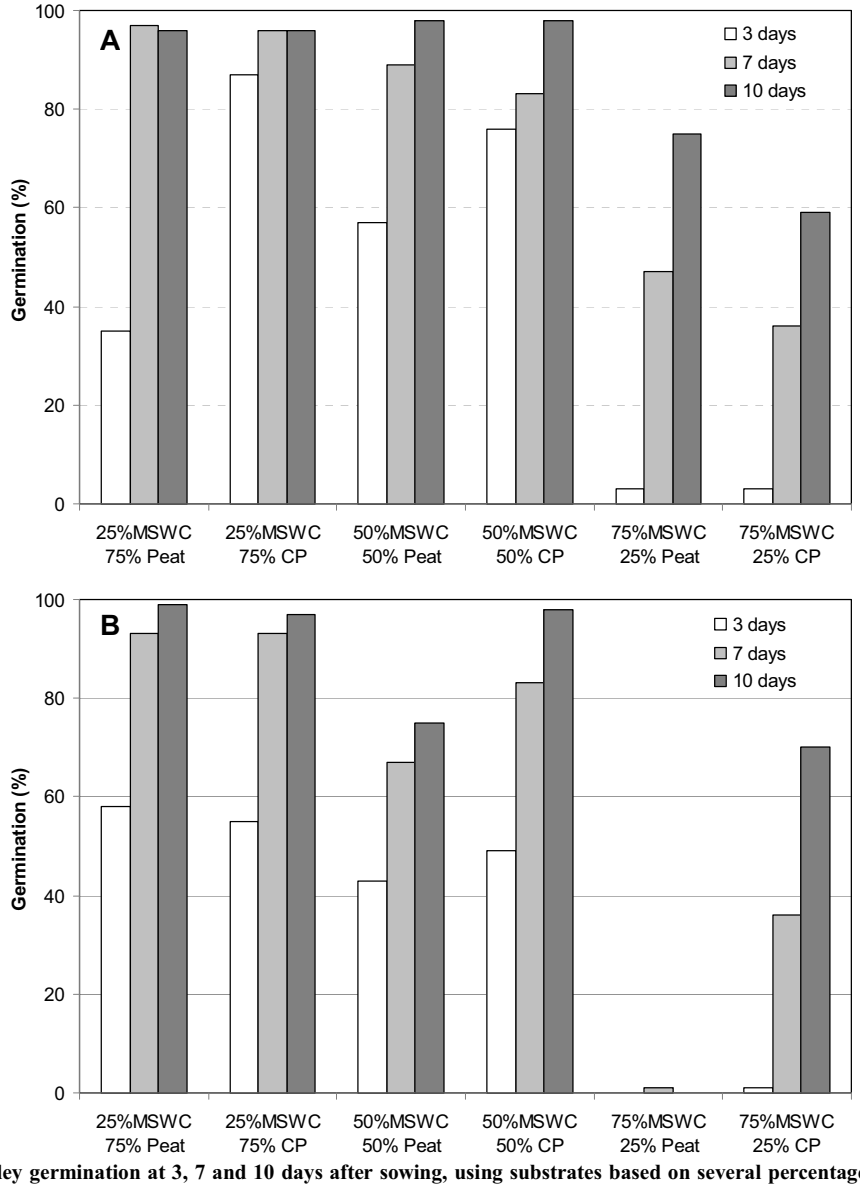

Fig. 1 Percentage of barley germination at 3, 7 and 10 days after sowing, using substrates based on several percentages of MSW compost from aerobic (A) and anaerobic (B) treatments, mixed with peat or composted pine bark (CP).

tures of compost and fertilised peat, with the growth obtained in a peat substrate. Relative yield, in fresh weight, ranged between 90-110% in mixtures with MSW compost from aerobic treatment, while was only slightly over 50% in mixtures with MSW compost from a previous biomethanization stage, attributable to its higher ammonium concentrations, which highlights the importance of process conditions.

Another FCQAO (1994) protocol assesses the growth of barley in mixtures with 25% and 50% of the compost compared with a reference substrate. It is considered that plants tolerate the compost when there is no visible chlorosis or necrosis in the leaves, and production in fresh weight of 25% compost mixture ($FMr_{25\%}$) is at least 90% compared to the control substrate; if this condition is met, the compost can be used as an amendment and fertilizer. When the mixture with 50% compost produces yields ≥ 90% compared with the reference substrate, compost can also be used as a component of substrates, an application which requires higher quality compost. Cendón *et al*. (2008) used a similar method to assess the use of MSW compost as a partial substitute of peat in the production of growing media, concluding that peat could be substituted up to 75% (v/v) by MSW compost, with satisfactory growth results. Barral *et al*. (2006), using the same test, observed that barley was less tolerant to MSW compost obtained after a previous biomethanization phase, than that obtained under permanent aerobic conditions (**Fig. 1**). Therefore the compost obtained after the biomethanization should not exceed 50% (v/v) of growing media, and is preferable to use it as an organic soil amendment.

STANDARD PHYTOTOXICITY TEST REQUIREMENTS

For a plant test to be comprehensive, it should be: fast (although a longer test may be an option), easy to perform, reproducible in different laboratories, easy to interpret, and adaptable to the desired use of the compost. ADAS (2005) recommends a direct growth test with the compost assessed, diluted with another substrate, depending on its electrical conductivity. Sand, peat, vermiculite or perlite may be some of the materials used as diluents. The container size should be sufficient to test coarse compost.

In the context of the PROJECT HORIZONTAL (CEN/TC 223), Baumgarten (2003) describes what could be a draft European standard protocol. This, as previous tests, would be based on monitoring of germination and growth of indicator plants – 20 seeds of each species, of at least one monocotyledonous and one dicotyledonous – in the sample tested, with or without dilution, compared with a standard substrate, under controlled growth conditions (temperature between 15 and 25°C, according to the species; illumination 16 h d^{-1}, intensity ≥7000 lux). The results would be expressed as a percentage of germinated fresh mass in the sample tested compared with the standard substrate. Additionally, germination percentage and germination delay compared with the standard substrate could be reported. As a result of this and other works, two CEN/TC 223 regulations – Soil improvers and growing media – are still being drafted, and their publication is expected in 2012: a) WI number 00223091- Soil improvers and growing media- Determination of plant response - Part 1: Pot growth test with Chinese cabbage; b) WI number 00223091- Soil improvers and growing media- Determination of plant response - Part 2: Petri dish test with cress.

SYNOPSIS

Phytotoxicity tests can be used to assess the maturity of compost, which is an indicator of compost stability and the presence of substances potentially inhibiting plant development. Seed germination in extracts of compost and direct seeding in compost alone or mixed with other substrates, are the most commonly used procedures. Seed germination and root elongation, or plant growth (fresh or dry weight), are the parameters determined. It is necessary to agree on standardized methods for the determination of phytotoxicity of compost, in order to make comparisons between different laboratories and improve consumer information. Also, when there is a toxic response it is important to establish what is due, in order to act on raw materials or on process conditions to correct this problem.

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