The effects of heavy metals, ammonia and electrical conductivity in compost derived from swine solid fraction on seed germination and root elongation of *Lepidium sativum* L.

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Abstract

Phytotoxicity of four different composts derived from swine solid fraction were tested with a bioassay method. The effect of water extracts of compost on seed germination and primary root growth of *Lepidium sativum* L. was measured. The composts were chemically analyzed to determine electrical conductivity, total N, NH_4^+ and heavy metals. The percentage seed germination was not affected by the water extracts of composts. The root lengths of all composts were more than 100% of the control (deionized water) throughout the experiment. Electrical Conductivity showed a significant and negative correlation with relative seed germination at the 50% and 75% concentrations. A strong positive correlation was found for water-extractable Cu with relative root growth and germination index at the 10% concentration. Water-extractable Zn showed a high and significant positive correlation with relative root growth and significant but less high correlation with germination index at the 10% concentration.

Introduction

Compost derived from swine solid fraction can be re-used as a new resource material, such as soil fertilizer and conditioner, to substitute for the more costly commercial fertilizers for crop production. However, non-composted manure or immature compost could generate adverse effects on plant growth and/or seed germination [1]. Phytotoxic effects of organic wastes are the result of the combination of several factors like ammonia [2] and heavy metals [3]. These factors have been shown to be responsible for inhibitory effects [4].

Pig manure often contains high concentrations of Cu compared with other animal manures, because Cu supplements are normally added to pig rations to accelerate weight gain and increase food conversion rates of fattening pigs [5]. In addition, Zn is also added to pig diets to counteract any toxicity which might be caused by the high Cu content. Only a small proportion (5-10%) of dietary Cu and Zn is absorbed by the pig and the rest is voided in the pig faeces [6]. These elements, at high concentrations, can impose detrimental effects on seed germination, development of young seedlings, maturation, root and shoot growth. In order to guarantee that compost derived from swine solid fraction can be re-cycled back to agricultural land without causing any environmental risk, a quick, simple and cost effective method to evaluate the toxicity of compost is required.

Plant seed germination and root elongation tests have been used as a simple and sensitive techniques for detection of the toxicity of various environmental pollutants such as heavy metals [7].

In the present study, seed germination and root elongation of cress (*Lepidium sativum* L.) were used to evaluate the toxicity of four different compost derived from swine solid fraction and to verify if the composts could affect plant growth. The composts were chemically analyzed for total N, NH_4^+, Electrical Conductivity (EC), pH and heavy metals and correlate to phytotoxicity indexes.

Material and Methods

Composting process and chemical parameters

Composting process were performed in four windrows using different materials. A pile consisted in 6000 kg of swine solid fraction from screw press separator (SSFC). Following three heaps were realized as follows: 5000 kg of swine solid fraction from screw press separator mixed with 720 kg of wheat straw (WSC), 8000 kg of swine solid fraction from screw press separator mixed with 2400 kg of woodchips (WCC) and 5000 kg of swine solid fraction obtained from decanting centrifuge mixed with

500 kg of sawdust (SC). Materials were mixed in order to obtain a theoretical C/N ratio equal to 30 and to optimize the composting process development.
The experimental composting process was observed for 150 days.
At the end of the composting process, for each pile, about 200 g sample was collected from 5 random locations and thoroughly mixed to generate composite samples. The samples were stored for 24 h in a cooling cell at 0-7°C.
Total nitrogen (Total N) and ammonium (NH_4^+) were determined according to UNI 10780:1998 (Table 1).

Table 1. Some chemical characteristics of the four study composts (WSC: wheat straw compost, WCC: woodchips compost, SC: sawdust compost, SSFC: swine solid fraction compost).

Compost	Compost Sample[a]			
	NH_4^+ (mg/g)	Total N (mg/g)	Ext. Zn[b] (mg/kg)	Ext. Cu[b] (mg/kg)
WSC	2.9	14.6	16.0	2.8
WCC	4.0	17.3	18.0	1.9
SC	5.2	25.5	22.0	3.2
SSFC	2.9	11.1	24.0	4.0

[a] All characteristics are on dry weight basis
[b] Ext.: water extractable

Concentrations of water-extractable Cu and Zn were determined by inductively coupled plasma-mass spectrometry with the Elan 6000 (Perkin-Elmer Corporation, Norwalk, CT, USA), after shaking dried compost with water (1:20 w/w) for 2 h (Table 1).

Seed germination test
The effect of compost phytotoxicity on seed germination, root length and germination index were determined with cress (*Lepidium sativum* L.) seeds.
After determining the dry matter content of the four compost samples, the moisture content of the samples was standardized at 85% by adding deionized water [8]. Composts water extracts were obtained by shaking 200 g samples with deionized water in a volumetric flask for 2 h using a horizontal shaker. After shaking, the suspension was centrifuged at 6000 rpm for 15 min and filtered. As not much is known about the phytotoxic level of compost derived from swine solid fraction, four different concentrations were made of this suspension: 75, 50, 25 and 10%. The pH and EC of the extracts were determined (Table 2).

Table 2. Some chemical characteristics of the four compost extract (WSC: wheat straw compost, WCC: woodchips compost, SC: sawdust compost, SSFC: swine solid fraction compost).

Compost	Compost Extract							
	Electrical Conductivity ($\mu S \cdot cm^{-1}$)				pH			
	75%	50%	25%	10%	75%	50%	25%	10%
WSC	1904	1308	692	292	6.6	6.7	7.2	6.7
WCC	1688	1156	608	277	5.5	5.4	5.7	6.1
SC	7960	5690	1970	1160	7.4	6.3	6.3	5.9
SSFC	3890	2830	1561	748	6.7	6.5	7.1	6.4

Ten cress seeds were placed on layer of filter paper (Schleicher and Schuell no. 595, 85 mm rundfilter) in 90 mm Petri dishes and 1 ml of each concentration was added [8]. Distilled water was used as a control. The experiment had a complete randomized block design with three blocks and two pseudo-replications (i.e. two Petri dishes with the same dilution). The Petri dishes were incubated in a growth chamber at 27±2°C and 70% relative humidity without photoperiod. A visible root was used as the operational definition of seed germination. After 72 h, the percentage of germination was recorded and the length of the roots was measured.
The percentages of relative seed germination (RSG), relative root growth (RRG) and germination index (GI) after 72 h of exposure to compost extracts were calculated as follows:

RSG (%) = (n° of seeds germinated in compost extract / n° of seeds germinated in control) x 100

RRG (%) = (mean root length in compost extract / mean root length in control) x 100

GI (%) = (RSG x RRG) / 100

Statistical analysis

The effect of compost type and its concentration on RSG, RRG and GI were analyzed using an ANOVA.

The effect of the chemical properties of the compost extracts within the concentrations was evaluated by correlation analyses. All statistical analysis were performed with the statistics package SPSS 17.0.

Results and discussions

Composts and concentrations analyzed in this study did not affect seed germination and the germination percentages were significantly higher than those found in the control (deionized water).

The mean values of germination index obtained were 160.7, 187.9, 200.9 and 264.4% for wheat straw compost, woodchips compost, sawdust compost and swine solid fraction compost, respectively. Zucconi *et al*. [9] reported that the compost with GI values greater than 80% was mature and phytotoxic-free. Therefore, the four composts showed GI values higher than this limit.

According to the results of other investigation on phytotoxicity [10], ammonium appeared not to be significantly affecting seed germination and root growth (Table 3).

Table 3. Linear correlations (shown by asterisks) between RSG, RRG and GI at four compost concentrations with five chemical parameters of the compost extracts.

Concentration		NH_4^+	Total N	Ext. Zn[b]	Ext. Cu[b]	EC
	RSG	0.21 NS	0.22 NS	-0.08 NS	-0.14 NS	0.19 NS
10%	RRG	-0.14 NS	-0.24 NS	0.52**	0.63**	0.34 NS
	GI	-0.06 NS	-0.16 NS	0.49*	0.56**	0.37 NS
	RSG	-0.21 NS	-0.19 NS	-0.05 NS	-0.01 NS	-0.29 NS
25%	RRG	0.03 NS	-0.04 NS	0.34 NS	0.31 NS	0.20 NS
	GI	-0.01 NS	-0.08 NS	0.34 NS	0.32 NS	0.16 NS
	RSG	-0.34 NS	-0.32 NS	-0.02 NS	0.04 NS	-0.46*
50%	RRG	0.01 NS	-0.04 NS	0.20 NS	0.20 NS	0.13 NS
	GI	-0.05 NS	0.09 NS	0.20 NS	0.21 NS	0.05 NS
	RSG	-0.27 NS	-0.25 NS	-0.06 NS	0.02 NS	-0.43*
75%	RRG	-0.01 NS	-0.11 NS	0.37 NS	0.32 NS	0.08 NS
	GI	-0.08 NS	-0.17 NS	0.38 NS	0.34 NS	0.01 NS

* $p < 0.05$

** $p < 0.01$

Electrical Conductivity showed a significant and negative correlation with RSG at the 50% and 75% concentrations (Table 3). Salinity can have a detrimental effect on seed germination and plant growth, especially in the seedling stage, though the response of various plant species to salinity differs considerably [11]. In general, salinity effects are mostly negligible in extracts with EC readings of 2000 $\mu S \cdot cm^{-1}$ or less [10]. This critical level was exceeded in the SC and SSFC extracts in the 50 and 75% concentrations.

Concentration of water-extractable Cu, which was highest in SSFC, appeared to be positively correlated with RRG and GI at the 10% concentration. However it is known that metals can cause a marked delay in germination and that they can inhibit plant growth severely [3]. Concentration of water-extractable Cu in the compost extracts was maximally 0.21 $\mu g ml^{-1}$, whereas 0.04 $\mu g ml^{-1}$ of Cu inhibited root growth of plants [10]. However, it should be mentioned that critical concentrations of heavy metals for toxicity in compost extracts are likely to be higher than critical values mentioned in literature, because of the relatively high amount of organic compounds, which can bind heavy metals [10].

Water-extractable Zn showed a high and significant positive correlation with RRG and significant but less high correlation with GI at the 10% concentration (Table 3). Concentration of water-extractable Zn was below phytotoxic levels as mentioned in the literature. The maximum concentration of water-extractable Zn in the compost extracts was 1.2 $mg \cdot l^{-1}$ compared to critical values ranging from 75 to 600 $mg \cdot l^{-1}$ [10]. This might explain the fact that no significant negative correlations of water-extractable Zn with RSG, RRG and GI were found.

Conclusion and perspectives

The mature compost extract may have stimulatory effects on plant growth due to the presence of mineral nutrients, beneficial microorganisms, humic substances and the physical characteristics of mature compost. From the results it may be concluded that the four composts could be used as a soil amendment with positive effects on seed germination and plant growth.

References

[1] Garcia C, Hernandez T, Costa F, Pascual JA, 1992. Phitotoxicity due to the agricultural use of urban wastes. Journal of the Science of Food and Agriculture 59, 313-319.
[2] Wong MH, Cheung YH, Cheung CL, 1983. The effects of ammonia and ethylene oxide in animal manure and sewage sludge on the seed germination and root elongation of *Brassica parachinensis*. Environmental Pollution Series A 30, 109-123.
[3] Wollan E, Davis RD, Jenner S, 1978. Effects of sewage sludge on seed germination. Environmental Pollution 17, 195-205.
[4] Gouider M, Feki M, Sayadi S, 2010. Bioassay and use in irrigation of untreated and treated wastewaters from phosphate fertilizer industry. Ecotoxicology and Environmental Safety 73, 932-938.
[5] Liu S, Wang X-d, Lu L-l, Diao S-r, Zhang J-f, 2008. Competitive complexation of copper and zinc by sequentially extracted humic substances from manure compost. Agricultural Sciences in China 7(10), 1253-1259.
[6] Tam NFY, Tiquia S, 1994. Assessing toxicity of spent pig litter using a seed germination technique. Resources, Conservation and Recycling 11, 261-274.
[7] Ko HJ, Kim KY, Kim HT, Kim CN, Umeda M, 2008. Evaluation of maturity parameters and heavy metal contents in composts made from animal manure. Waste Management 28, 813-820.
[8] Regione Piemonte. 1998. Metodi di analisi dei compost. Collana ambiente no. 6, pp 84-87.
[9] Zucconi F, Pera A, Forte M, De Bertoldi M, 1981. Evaluating toxicity of immature compost. BioCycle 22(2), 54-57.
[10] Hoekstra NJ, Bosker T, Lantinga EA, 2002. Effects of cattle dung from farms with different feeding strategies on germination and initial root growth of cress (*Lepidium sativum* L.). Agriculture, Ecosystems & Environment 93, 189-196.
[11] Adriano DC, Chang AC, Pratt PF, Sharpless R, 1973. Effect of soil application of dairy manure on germination and emergence of some selected crops. Journal of Environmental Quality 2, 396-399.

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