

EFFECTS OF COMPOSTING ON PHYTOTOXICITY OF SPENT PIG-MANURE SAWDUST LITTER

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Abstract

The phytotoxicity of spent pig-manure sawdust litter (spent litter) was evaluated during further composting. Aqueous extracts of the spent litter were prepared by shaking the sample with water (1:10 w/v), and the toxicity of these extracts was determined on relative seed germination, relative root elongation and germination index (GI, a factor of relative seed germination and relative root elongation). The sensitivity of six plant species, namely *Brassica parachinensis* (Chinese cabbage), *Brassica albogalera* (Chinese kale), *Allium sativum* (onion), *Cucumis sativus* (cucumber), *Amaranthus spinosus* (Chinese spinach), and *Lycopersicon esculentum* (tomato) were compared. The effect of different moisture levels during composting on the phytotoxicity of the spent litter was also examined. Phytotoxicity of the spent litter was only evident during the earlier stage of composting (first 14 days) and, that seed germination and root elongation reached 100% (same as the control) towards the end of the composting. The concentrations of the major inhibitors, water-extractable Cu and Zn, and NH_4^+ -N of the spent litter, declined during composting, indicating that these inhibitors were gradually eliminated as composting proceeded. Multiple regression analysis showed that the NH_4^+ -N content of the spent litter was the most important chemical factor affecting phytotoxicity of the plant species selected for this study. Relative root elongation and GI were more sensitive indicators of phytotoxicity than seed germination. In the present study, the GI's of all plant species were >80% at day 60, indicating that the spent litter had reached its maturation by day 60. The responses of different plant species to the water-extracts of the spent litter were different. Among the six species, Chinese cabbage and Chinese spinach were the most sensitive species, and tomato and cucumber were the least sensitive species to indicate phytotoxicity of the spent litter. Moisture adjustment during the composting process did not affect the results of the phytotoxicity test. Copyright © 1996 Elsevier Science Ltd

Keywords: composting, heavy metals, ammonia, seed germination index.

INTRODUCTION

Assessing the phytotoxicity of composts is one of the most important criteria being used to avoid environmental risks before these composts can be recycled back to agricultural land. Previous research work has demonstrated that application of immature compost onto the soil causes negative effects on seed germination, plant growth and development (Morel *et al.*, 1985). These effects occur because an immature compost induces high microbial activity (which reduces oxygen concentration in the soil) and blocks the existing soil-available nitrogen (which gives rise to serious N-deficiencies in crops) (Zucconi *et al.*, 1981a). Immature composts also introduce phytotoxic compounds such as heavy metals (Tam & Tiquia, 1994), phenolic compounds (Wong, 1985) ethylene and ammonia (Wong *et al.*, 1983; Tam & Tiquia, 1994), excess accumulation of salts (Tam & Tiquia, 1994), and organic acids (Manios *et al.*, 1989) which could retard seed germination and growth.

The spent litter disposed from the pig-on-litter (POL) system contains high concentrations of organic matter, nitrogen, phosphorus, potassium and trace elements and also a significant amount of active microbial biomass, which is similar to an immature compost (Tam & Vrijmoed, 1993). Tam & Tiquia (1994) reported that the spent litter disposed from the POL system after being used for 6 months inhibited seed germination and root elongation of some local vegetables. Therefore, in order to avoid the risks listed above, the spent litter must be further composted to achieve full maturation before recycling back onto the soil for crop production. In order to ensure that the spent litter will be safe for land use, a simple method to evaluate its toxicity is necessary. Plant seed germination and root elongation tests have been used as simple, rapid, reliable and reproducible techniques to evaluate the damage caused by toxic compounds present in various composts (Wang & Keturi, 1990). Many species, including cabbage, lettuce, carrot, cucumber, tomato and oats have been recommended for the phytotoxicity test (USEPA, 1982; FDA, 1987).

The process of composting is ecologically complex as it is influenced by a wide range of environmental variables.

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Moisture availability appears to be a significant factor in composting systems since water affects gaseous exchange. As water content increases, the rate of gas transfer decreases, and as the rate of oxygen transfer becomes insufficient to meet the metabolic demands of the microorganisms, the composting system will become restricted in activity and eventually anaerobic (Miller, 1991). Frassinetti *et al.* (1990) studied the changes in patterns of phytotoxicity of sewage sludge compost as affected by the pattern of oxygenation (aerobic and anaerobic conditions). The experimental results showed that sludge processed under anaerobic conditions had increased toxicity. In aerobic conditions, the microflora develops and is capable of transforming organic matter and eliminating biological toxicity.

In this study, the seed germination and root elongation of six plant species were used to evaluate the toxicity of the spent litter. The changes in certain chemical parameters related to plant toxicity and the effects of different moisture contents during composting on the reduction of the compost's phytotoxicity were also determined.

MATERIALS AND METHODS

The spent litter was collected from a pig pen employing the POL system for 12 weeks with 40 piglets raised inside the pig pen (Tam & Vrijmoed, 1990). Three piles of the spent pig-sawdust litter with moisture contents adjusted to 50%, 60%, or 70% were set up and left for further composting according to Tiquia *et al.* (in press). The composting process lasted for 91 days. Composite samples were collected from each pile before the adjustment of moisture (pre-expt.), immediately after the adjustment of moisture (Day 0), and then weekly until the end of the composting period. The spent litter was analysed for total and water-extractable Cu, Zn, and K by atomic absorption spectrophotometry. The concentrations of NH_4^+ -N and $(\text{NO}_3^- + \text{NO}_2^-)$ -N were determined by extracting the sample with 2N KCl and the extracts were distilled using a steam distillation apparatus. MgO was added to the extract to remove the NH_4^+ -N from the sample. After the removal of NH_4^+ -N, Devarda's alloy was added to determine the amount of $(\text{NO}_3^- + \text{NO}_2^-)$ -N (Page *et al.*, 1982). The quantity of nitrite was found to be very low ($< 1 \text{ mg litre}^{-1}$), therefore the third distillation was not performed to distinguish NO_3^- -N from NO_2^- -N. Subsamples of the spent litter were oven dried at 105°C for 48 h to obtain its moisture content, all data are presented on a 105°C dry wt basis.

Aqueous extracts of the spent litter were prepared by shaking the fresh sample with distilled water at 1:10 w/v for 10 min using a stomacher (a shaker), then filtered. The phytotoxicity of these extracts was evaluated by seed germination and the test procedure is summarized in Table 1. Six plant species, namely *Brassica parachinensis* (Chinese cabbage), *Brassica albogalera* (Chinese kale), *Allium sativum* (onion), *Cucumis sativus* (cucumber), *Amaranthus spinosus* (Chinese spinach), and

Lycopersicon esculentum (tomato) were chosen. After 5 days of incubation in the dark, the seed germination, root elongation, and germination index (GI, a factor of relative seed germination and relative root elongation) were determined. A 5-mm primary root was used as the operational definition of germination (USEPA, 1982). The percentages of relative seed germination, relative root growth, and GI (Table 1) were calculated (Tam & Tiquia, 1994).

The mean and standard deviation of triplicate samples from each pile were calculated. Simple correlation coefficients were computed to show the relationship between phytotoxicity assays and chemical properties. A stepwise multiple regression analysis was performed to determine the most important chemical factor retarding seed germination and root elongation of the selected plant species.

RESULTS

Phytotoxicity assay: relative germination percentage, relative root elongation and germination index

The initial relative germination percentage of Chinese cabbage, Chinese kale, onion and tomato was as high as 90%, reaching 100% at the end of the composting period (Fig. 1). The relative seed germination of Chinese

Table 1. Seed germination test conditions

1. Test type	Static (batch)
2. Pre-treatment	Soak in distilled water overnight
3. Temperature	$22 \pm 3^\circ\text{C}$
4. Light	None
5. Test vessel	$10 \times 100 \text{ mm}$ Petri dish plus Whatman Number 1 filter paper
6. Test volume	10 ml per dish
7. Number of seeds	10–30 per dish (depending on the size of the seeds*)
8. Replicates	3
9. Control	Distilled water
10. Test duration	5 days
11. End point	Germination, primary root $\geq 5 \text{ mm}$

$$\text{Relative seed germination (\%)} = \frac{\text{number of seeds germinated in litter extract}}{\text{number of seeds germinated in control}} \times 100 \quad (1)$$

$$\text{Relative root growth (\%)} = \frac{\text{Mean root length in litter extract}}{\text{Mean } \sqrt{\text{root length in control}}} \times 100 \quad (2)$$

$$\text{GI} = \frac{(\% \text{ Seed germination}) \times (\% \text{ Root growth})}{100\%} \quad (3)$$

*Chinese cabbage, 30 seeds; Chinese kale, 30 seeds; Onion, 15 seeds; Cucumber, 10 seeds; Chinese spinach, 30 seeds; Tomato, 20 seeds. The reason for using different numbers of seeds was to balance the size of each type of seeds with the space available for them to germinate. Therefore, a small number of seeds would be used if the seeds are bigger in size.

spinach and cucumber, on the other hand, was significantly retarded by the immature spent litter water-extracts and had an initial relative germination percentage around 60–70% (Fig. 1). The relative seed germination of cucumber increased very rapidly and reached 100% by day 11 (Fig. 1(D)), whereas that of Chinese spinach increased gradually and achieved about 100%, but only after 56 days of composting (Fig. 1(E)).

The responses of the six plant species to the toxicity of the spent litter water-extracts in terms of relative root elongation were different (Fig. 2). The relative root elongation of Chinese cabbage and Chinese spinach were significantly inhibited by the spent litter water-extracts (Fig. 2(A) and (E)). The relative root elongation of these two plant species was only about 40–50% (Chinese cabbage) and 60–70% (Chinese spinach) at day 0 but then continuously increased to about 70–80% and 95%, respectively by day 91. Although the relative root elongation of Chinese cabbage was to a large extent inhibited, its relative seed germination was not affected by the phytotoxic compounds in the spent litter (compare Fig. 1(A) and Fig. 2(A)). These results suggested that relative root elongation was a more sensitive test than relative seed germination. The relative root elongation of Chinese kale (Fig. 2(B)), onion (Fig. 2(C)) and tomato (Fig. 2(F)) also increased with time, but the readings fluctuated during composting. On the contrary,

the initial root length of cucumber in spent litter water-extracts was similar to that of seeds germinated in water (i.e. 100%) but as composting proceeded, the root lengths became less than 80% of those in water. Results of this study indicated that the inhibitory effect of the spent litter appeared to decrease as the spent litter became mature, but the actual response depended on the plant species.

The germination index (GI) values of Chinese cabbage, Chinese kale, onion, Chinese spinach and tomato, increased with time of composting (Fig. 3). Cucumber, on the other hand, behaved differently from the other five test species. Cucumber had a GI of about 70–80% at the beginning of the experimental period, and this index increased rapidly to about 95–115% (~105%) by day 11 but decreased gradually to 93% by day 91 (Fig. 3(D)). In the present study, the GIs of all the plant species tested increased to over 80% by day 60. This increase suggested that the spent litter did not pose any toxicity on the plant growth as phytotoxic inhibitors had been eliminated. This result also indicated that the spent litter reached maturity by day 60.

Despite the differences in moisture content, all piles showed similar trends of change throughout the composting process. The results of the seed germination showed that all piles were similar and almost overlapped during the whole composting process (Fig. 1). On the contrary, the results of the root elongation and GI values showed that pile C, the pile adjusted to 70%

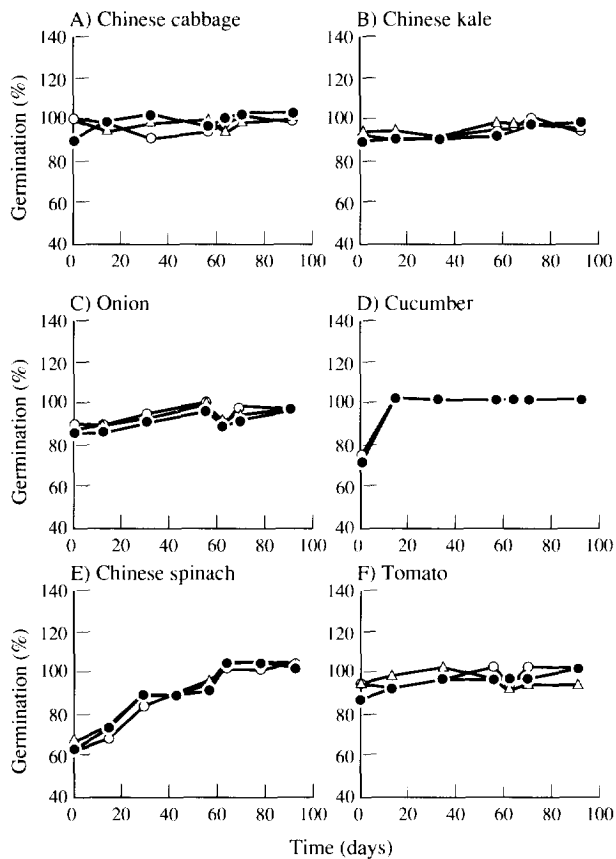


Fig. 1. Seed germination percentages of six plant species in the spent litter water-extracts during the composting process (○ = Pile A, 50% moisture; ● = Pile B, 60% moisture; △ = Pile C, 60% moisture).

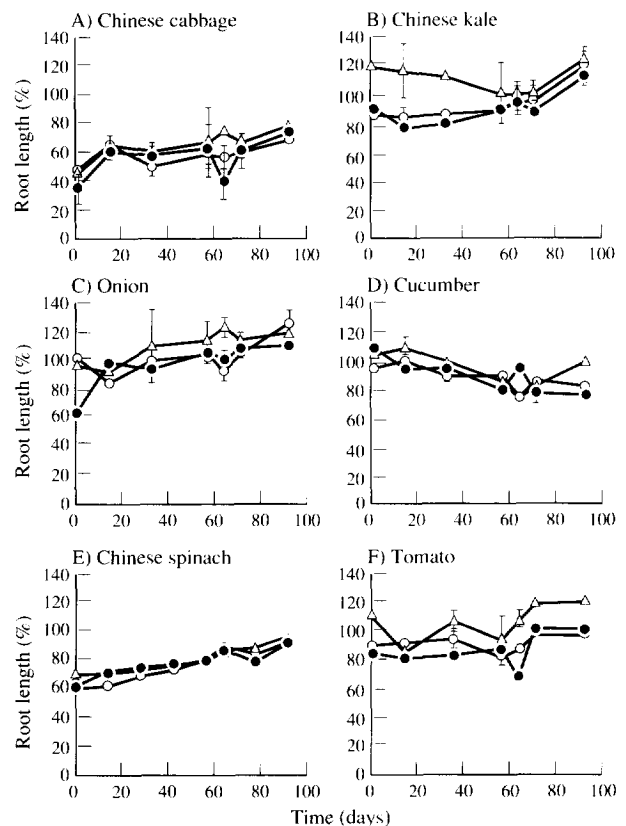


Fig. 2. Root elongation of six plant species in the spent litter water-extracts during the composting process (same symbols as Fig. 1).

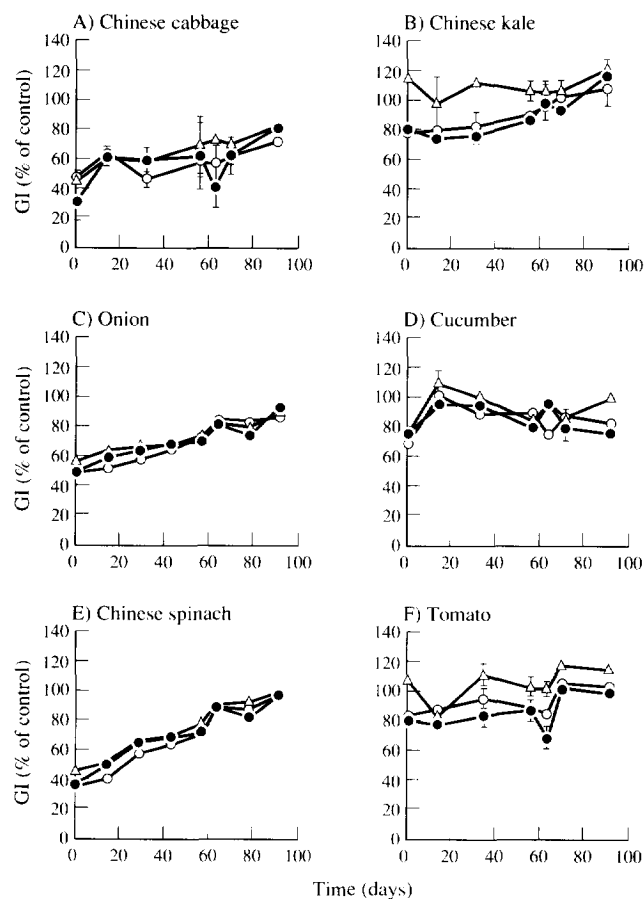


Fig. 3. Germination indices of spent litter water-extract on six plant species during the composting process (same symbols as Fig. 1).

moisture, was different from piles A (50% moisture) and B (60% moisture) on Chinese kale alone, and the difference was observed only during the first 30 days of composting (Figs 2(B) and 3(B)).

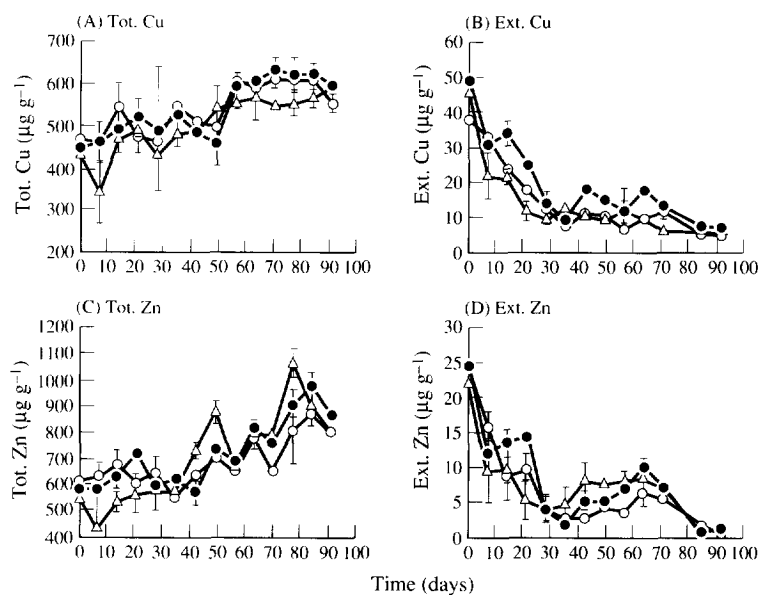


Fig. 4. Changes in total and water-extractable Cu and Zn of the spent litter during the composting process (same symbols as Fig. 1).

Chemical properties of the spent litter

The concentrations of total Cu in all piles increased gradually with time, from an initial $420 \mu\text{g g}^{-1}$ to $500 \mu\text{g g}^{-1}$ for the first 49 days of composting (Fig. 4(A)). The total Cu content of all piles increased to the maximum values ($557\text{--}610 \mu\text{g g}^{-1}$) at day 56 and was maintained at these levels until the end of the composting period. The concentrations of water-extractable Cu of all piles, on the other hand, decreased dramatically from an initial $40 \mu\text{g g}^{-1}$ to $10 \mu\text{g g}^{-1}$ during the first 35 days of composting (Fig. 4(B)). From there onwards, the concentrations of water-extractable Cu of all piles declined slowly, and the final extractable Cu concentration was less than $7 \mu\text{g g}^{-1}$. No significant differences occurred among the three piles in terms of either total or extractable Cu content.

The total Zn content of all piles fluctuated at about $600 \mu\text{g g}^{-1}$ during the first 43 days (Fig. 4(C)). Thereafter, the total Zn content of all piles increased gradually to a level higher than $800 \mu\text{g g}^{-1}$ towards the end of the study. On the contrary, the water-extractable Zn of all piles dropped rapidly from $24 \mu\text{g g}^{-1}$ to less than $5 \mu\text{g g}^{-1}$ for the first 35 days of composting (Fig. 4(D)). The readings increased slightly to about $7 \mu\text{g g}^{-1}$, and fluctuated at this level ($\sim 3\text{--}9 \mu\text{g g}^{-1}$), then declined to a level of less than $1 \mu\text{g g}^{-1}$ at the end of the composting period.

The $\text{NH}_4^+\text{-N}$ content of the spent litter was very high at the beginning of the composting process ($3.88\text{--}3.32 \text{ mg g}^{-1}$) (Fig. 5(A)). The drop in $\text{NH}_4^+\text{-N}$ content of piles A and B was very slow in the first 63 days of composting but declined dramatically from day 63 onwards and reached a level of $0.58\text{--}1.06 \text{ mg g}^{-1}$ at the end of the composting period (Fig. 5(A)). The $\text{NH}_4^+\text{-N}$ content in pile C dropped rapidly as soon as the composting proceeded, from an initial 3.88 mg g^{-1} to 2.04 mg g^{-1} by day 28, was maintained from 1.72 to 2.0 mg g^{-1} between day 35 and day 42, then dropped

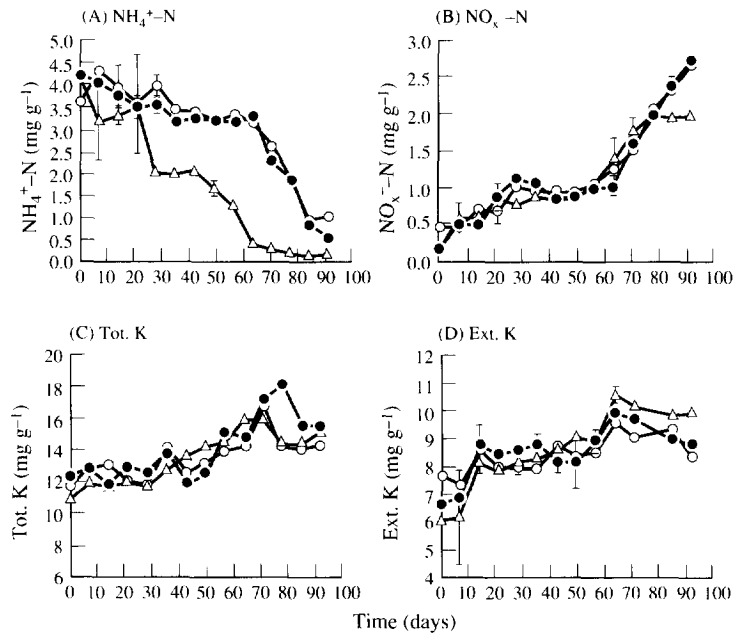


Fig. 5. Changes in concentrations of NH_4^+ -N, NO_x^- -N, and total and water-extractable K during the composting process (same symbols as Fig. 1).

continuously (more rapidly from day 42 to day 63) and reached a level of 0.18 mg g^{-1} by day 91. The decrease in NH_4^+ -N content led to an increase in the ($\text{NO}_2^- + \text{NO}_3^-$)-N content of the spent litter (Fig. 5(B)). Most of the NO_x^- -N was in the form of NO_3^- -N and the NO_2^- -N content was always less than $1 \mu\text{g g}^{-1}$.

Figure 5(C) reveals the changes in concentration of total K in the spent litter during the composting period. The total K content of piles A, B and C was around 11.5 mg g^{-1} at the beginning of composting. From day 28 onwards, the total K concentrations of all piles increased dramatically, reached peak values of up to $16\text{--}17.3 \text{ mg g}^{-1}$ at day 70, then dropped rapidly, and were maintained at levels around 15 mg g^{-1} at day 91. The water-extractable K of all three piles ranged from 6.09 to 7.72 mg g^{-1} at the beginning of the composting (Fig. 5(D)), increased to about 8 mg g^{-1} at day 14, and remained at that level until day 56. The water-extractable K of piles A, B and C then increased slightly to 10 mg g^{-1} and were at this level until the end of the composting.

Relationship between phytotoxicity assays and chemical properties of the spent litter

The relative seed germination of Chinese cabbage and Chinese kale showed significant negative correlations with NH_4^+ -N concentration (Table 2). Apart from NH_4^+ -N, significant negative correlations were also observed between relative seed germination of cucumber, spinach and tomato with water-extractable Cu and Zn. The root lengths were mostly negatively correlated with NH_4^+ -N (tomato being the exception) and water-extractable Cu content (Chinese cabbage and Chinese kale being the exceptions), and the GIs were mostly negatively correlated with NH_4^+ -N content (cucumber

being the exception). Other isolated examples of negative correlations also appeared. The multiple regression analysis revealed that amongst the 5 chemical parameters examined (NH_4^+ -N, NO_x^- -N, and water-extractable Cu, Zn and K), the concentration of NH_4^+ -N was the most important factor inhibiting seed germination and root elongation of Chinese cabbage, Chinese kale and Chinese spinach (Table 3). These results indicated that high levels of NH_4^+ -N would be injurious to the growth and development of Chinese cabbage, Chinese kale, and Chinese spinach. Among these three plant species, Chinese spinach and Chinese cabbage were found to be the most sensitive species. Tomato, cucumber and onion were less sensitive and none of the chemical properties of the spent litter tested could explain the inhibition of relative seed germination or relative root elongation of cucumber.

DISCUSSION

The seed germination and root elongation technique has been devised to evaluate the damaging effects and toxicity of compost (Wong, 1985). The GI has also been used and has proven to be a very sensitive parameter, since it combines germination and root growth (Tam & Tiquia, 1994). The results of the seed germination and root elongation test showed that the spent litter disposed from the POL system exhibited phytotoxic effects during the early stage (first 14 days) of composting, but these gradually disappeared as composting proceeded and as the GIs of the plant species tested increased to over 80% at day 60. The increase in seed germination and root elongation of the selected plant species during composting coincided with the decrease in NH_4^+ -N

(Fig. 5), and water-extractable Cu (Fig. 2) and Zn (Fig. 4) content to very low levels. The decrease in NH_4^+ -N content to low levels was associated with the accumulation of NO_x^- -N via the nitrification process. The decline in water-extractable Cu and Zn was due to the formation of complexes of these metals with chelating organic compound formed, thus making them not water-extractable and biologically unavailable. Ciavatta *et al.* (1993) reported that during the stabilization of organic matter, heavy metals showed a decrease in solubility.

Similar findings were also reported by Inbar *et al.* (1990) and Zucconi *et al.* (1981a,b). They found that the presence of phytotoxins in the decomposing organic matter represents a transient stage, and as the organic matter has become completely stabilized, the phytotoxins disappear. A GI value greater than 80 to 85% indicated the disappearance of this phytotoxicity in the compost. Therefore, the disappearance of the phytotoxicity from the spent litter could be used as an indicator of compost maturity.

Table 2. Simple correlation coefficient values between phytotoxicity assays and chemical properties of the spent litter. (Correlations were based on 15 observations except water-extractable Cu and K where 14 observations were used; *, ** and * indicate correlation was significant at 0.05, 0.01 and 0.001 probability levels, respectively.)**

Plant species	Ext. Cu	Ext. Zn	Parameters NH_4^+ -N	NO_x^- -N	Ext. K
<i>Seed germination</i>					
Chinese cabbage	-0.51	-0.51	-0.87*	0.89**	0.51
Chinese kale	-0.64	-0.47	-0.79*	0.75	0.85*
Onion	-0.72	-0.67	-0.68	0.65	0.48
Cucumber	-0.88**	-0.90**	-0.56	0.56	0.82*
Chinese spinach	-0.91**	-0.79*	0.87**	0.85**	0.83*
Tomato	-0.86*	-0.88**	-0.75	0.74	0.44
<i>Root elongation</i>					
Chinese cabbage	-0.75	-0.81*	-0.79*	0.83*	0.64
Chinese kale	-0.44	-0.42	-0.87*	0.88**	0.37
Onion	-0.88**	-0.81**	-0.97***	0.95**	-0.72
Cucumber	-0.85*	0.70	-0.82*	0.76*	0.79*
Chinese spinach	-0.83*	-0.74	-0.94***	0.92**	0.80*
Tomato	-0.86*	-0.88**	-0.75	0.74	0.44
<i>Germination index</i>					
Chinese cabbage	-0.75	-0.80*	-0.84*	0.87*	0.63
Chinese kale	-0.48	-0.41	-0.90**	0.89**	0.42
Onion	-0.87**	-0.79*	-0.96***	0.93**	0.72
Cucumber	-0.35	-0.52	-0.04	0.01	0.32
Chinese spinach	-0.85*	-0.74	-0.93***	0.90**	0.83*
Tomato	-0.53	-0.50	-0.76*	0.75	0.26

(Negative correlation means germination goes down as concentration of the chemical parameters goes up.)

Table 3. Multiple regression analysis of phytotoxicity assays and chemical properties of the spent litter extract. (Regression equation was calculated based on 5 chemical parameters, with STEPWISE METHOD and PIN (Probability to F-enter) = 0.050 limit; NS, not significant at PIN = 0.50 limit.)

Plant species	Multiple regression equation	Multiple R value	Adjusted R^2 value	F value	Significance of F
<i>Seed germination</i>					
Cabbage	%GERM = 103.3 - (1.69*NH ₄)	0.87	0.70	15.2	0.0114
Kale	%GERM = 101.8 - (2.51*NH ₄)	0.80	0.55	8.4	0.0337
Onion	NS	—	—	NS	—
Cucumber	NS	—	—	NS	—
Spinach	%GERM = 117.9 - (5.14*NH ₄) - (1.89*Ext.Cu) + (2.2*Ext.Zn)	0.99	0.96	52.9	0.0043
Tomato	NS	—	—	NS	—
<i>Root elongation</i>					
Cabbage	% ROOT = 78.6 - (6.91*NH ₄)	0.80	0.56	8.6	0.0328
Kale	% ROOT = 123.9 - (14.8*NH ₄) + (0.59*Ext.Cu)	0.96	0.88	24.0	0.0059
Onion	% ROOT = 127.1 - (7.30*NH ₄) - (0.24*Ext.Cu)	0.99	0.97	83.7	0.0005
Cucumber	NS	—	—	NS	—
Spinach	% ROOT = 66.2 - (7.25*NH ₄) + (3.67*Ext.Cu)	0.99	0.97	104.7	0.0004
Tomato	NS	—	—	NS	—
<i>Germination index</i>					
Cabbage	% GI = 81.0 - (7.96*NH ₄)	0.84	0.64	11.8	0.0170
Kale	% GI = 120.7 - (12.0*NH ₄) + (0.46*Ext.Cu)	0.97	0.92	36.6	0.0027
Onion	% GI = 128. - (12.00*NH ₄)	0.96	0.90	55.9	0.0007
Cucumber	NS	—	—	NS	—
Spinach	% GI = 114.1 - (12.20*NH ₄) - (0.58*Ext.Cu)	0.96	0.87	21.6	0.0072
Tomato	% GI = 110.4 - (6.60*NH ₄)	0.76	0.49	6.7	0.0490

Pig manure often contains high concentrations of Cu and Zn compared to other animal manures, since Cu and Zn are normally added to pig rations. Copper supplements are normally being added to pig rations at concentrations up to $230 \mu\text{g g}^{-1}$ to accelerate weight gain and increase feed conversion rates of fattening pigs. Moreover, Zn is also added to pig diets to counteract any toxicity which might be caused by the high Cu content (Hanharam & O'Frady, 1968). Similar practice has been carried out in Hong Kong. In addition, most of the dietary Cu and Zn is not absorbed but voided in the pig faeces (only 5 to 10% of dietary Cu and Zn is absorbed by the pig). This explains why the spent litter contained remarkably high levels of Cu and Zn (Fig. 4). The effect of individual heavy metals on plant growth has been studied by various authors. McNelly and Bradshaw (1968) observed that Cu inhibited root growth of *Agrostis tenuis* (colonial bent grass) at a $0.25 \text{ mg litre}^{-1}$ concentration. On the other hand, Polson and Adams (1970) concluded that Zn inhibited root growth of *Phaseolus vulgaris* (garden bean) at 5 mg litre^{-1} concentrations. The concentrations of total Cu and Zn seemed to increase in the spent litter during composting but the rate of increase was slower than the pig sawdust litter under the POL (Tam & Tiquia, 1994). The increase in heavy metals was probably due to losses of organic C, H and O from the pile as CO_2 and H_2O during composting, leaving behind Cu and Zn and consequently giving a relative increase in concentrations of these metals. Increases in heavy metals were also observed by Ciavatta *et al.* (1993) during composting of municipal solid wastes.

Ammonia inhibits seed germination and seedling growth (Okuda & Takahasi, 1961). Ammonia generated from decomposing organic compounds and animal manure is detrimental to seed germination and seedling growth (Meggie *et al.*, 1967; Wong *et al.*, 1983). The initial $\text{NH}_4^+\text{-N}$ concentrations of the spent litter in the present study were high (Fig. 2(A)). Such a high initial $\text{NH}_4^+\text{-N}$ content was due to the presence of large amounts of partially decomposed faeces and urine in the spent litter. The multiple regression analysis showed that $\text{NH}_4^+\text{-N}$ content was the major factor affecting relative seed germination and relative root elongation of selected plant species. Molina *et al.* (1971) reported that the toxicity towards soybean seed germination was reduced with decreasing concentration of ammonia in digested sludge. Therefore, reducing the amounts of ammonia by further composting would be a possible way to eliminate ammonia toxicity of the spent litter.

Excess salts may be another factor causing phytotoxicity of compost (Chanyasak *et al.*, 1982). Tam and Tiquia (1994) found that root lengths of all species tested were negatively correlated with total and water-extractable K of pig manure and considered potassium as an important factor affecting plant growth. However, the total and water-extractable K in this study were at low levels and did not appear to be an important factor affecting seed germination and root growth. The total K observed in this study were not all water-extractable

(Figs 5(C) and (D)). It is possible that some of the K become temporarily fixed in the interlayer space of the spent litter and they were not extracted by water. Wild (1988) pointed out that when K is added in a soil solution, as by fertilizer addition, some K ions will become fixed in the interlayer space, and they are not extracted by an ammonium salt under defined conditions, but may nevertheless be used by plants. Similar reactions could have occurred in the present study.

Chinese cabbage and Chinese spinach were the most sensitive species, while tomato and cucumber were the least sensitive ones among the six plant species examined. The sensitivity of a plant species to toxicity depends on the quantity of its food reserves (Cheung *et al.*, 1989). Cheung *et al.* (1989) reported that seeds of root crops, cereals and legumes, containing high quantities of food reserves, would have lower sensitivity to toxicity than seeds of leafy plants with lower food reserves. Chinese cabbage and Chinese spinach seeds were the most sensitive species likely because their seeds are very small. In the study of Cheung *et al.* (1989), Chinese cabbage was the most sensitive species to metal toxicity and was recommended as test species for the assessment of heavy metal toxicity. Cucumber seeds are quite big and have a large quantity of food reserve. This reserve explains why cucumber was not sensitive to toxicity. Moreover, the sensitivity of a plant species could also depend on its growth requirement and tolerance to toxicity. Tomato seeds are quite small and have small quantity of food reserve, but tomato was not found to be sensitive. This finding suggests that tomato had a wider range of tolerance to ammonia, Cu, and Zn toxicity compared to other species examined in this study.

In general, moisture had no effect in reducing the phytotoxicity of the spent litter during composting. Moisture content might be a significant factor in the rate of gas exchange and the transport of nutrients for the metabolic demands of the microorganisms during composting, but not in the elimination of biological toxicity from the spent litter. Other factors, such as aeration, could be a significant factor in the destruction of lethal levels of toxic compounds and, in particular, of ammonia toxicity. Zucconi *et al.* (1985) reported that aeration (such as turning) is the most important factor in the metabolic destruction of organic phytotoxins. In the present study, all piles had similar turning frequency (every 4 days). This could be the reason why all piles had similar trend of changes despite of their differences in moisture content.

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