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Evaluating Phytotoxicity of Pig Manure from the Pig-on-Litter System

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Abstract

The inhibitory effects of pig manure during in-situ composting in the pig-on-litter (POL) system, and during further composting in windrows were evaluated on seed germination and root elongation of selected plant species. Aqueous pig manure sawdust litter extracts at different stages of in-situ and further composting were incubated with different test seeds for five days in the dark, and the seed germination and root elongation were recorded. The seed germination percentage of the plants was not affected by the extracts during the first 30 weeks of in-situ composting in the pig-on-litter system (POL), but was significantly affected thereafter. The root lengths of the plants were less than 50% of the control (deionized water) throughout the experiment. Phytotoxicity of the extracts increased with age of pig manure because more salts, nutrients, and heavy metals, accumulated and imposed toxic effects on plants. To determine the elimination of phytotoxicity, the spent pig litter (collected after 13 weeks of in-situ composting) was stacked in windrows and composted for 91 days, and was examined for phytotoxicity. Results of the assay revealed that the phytotoxicity of spent litter was evident only during the early stage of composting. By day 60, the germination index (GI) increased to over 80%. This increase corresponded with decreases in concentrations of NH_4^+-N, and water-extractable Cu and Zn of compost, demonstrating that these chemical compounds gradually disappeared during composting due to metabolism to other compounds and immobilization effects. The multiple regression analysis revealed that NH_4^+-N was the most important factors affecting the phytotoxicity of spent pig litter in the present study.

Key Words: composting deep litter system, hog manure, phytotoxicity, seed germination index

Introduction

Animal waste, particularly pig manure, has been used to maintain soil fertility for many years (Lopez-Real and Baptista, 1996). However, in recent years, intensive pig production has resulted in high concentrations of pigs in small areas producing large quantities of waste with insufficient land nearby for application. This has led to environmental concerns regarding water pollution. In Hong Kong, indiscriminate disposal of pig manure (22000 tonnes annually), had been reported to account for most of the organic pollution of waterways (Hodgkiss and Griffiths, 1987). The environmental pollution in the territory of Hong Kong has stimulated research into identifying environmentally sound and economically feasible technologies for pig waste treatment. One of the treatment methods for pig wastes is the pig-on-litter (POL) system. The POL system, also known as in-situ composting, uses sawdust as a bedding material for pigs. The pig manure is partially decomposed within the bedding material, and the pig-manure- sawdust litter remains in the pig pen during the entire period of pig raising (about 10-13 weeks). Thereafter, the spent pig litter is stacked in windrows for further composting and maturation (Tiquia and Tam 1998a and 1999).

The spent pig litter (a mixture of partially decomposed sawdust and pig manure) has properties comparable with the conventional manure compost, with high concentrations of organic matter and nutrients (Tam and Wong, 1995; Tiquia and Tam, 1999a). It can be re-utilized as a resource material such as soil fertilizer, conditioner, or both. However, care must be taken in its use, and it is necessary to understand the characteristics of the end product (composted product) in order to avoid any undesirable effects. One of the problems associated with the use of pig manure is that it often contains high concentrations of heavy metals (Cu and Zn, in particular) (Cheung and Wong, 1983), salts (Tam and Tiquia, 1993; Tiquia and Tam, 2000), and an excess of ammonium (Tiquia and Tam, 1998b; Tiquia et al. 1996a). Other negative effects such as excess accumulation of organic acids, phenolic substances, ethylene, and ammonia are often present in immature composts (Jimenez and Garcia, 1989; Wong et. al. 1983). A process such as composting can contribute to the reduction of these phytotoxic compounds. In order to guarantee that the pig–manure sawdust litter can be re-cycled back to agricultural land without causing any environmental risks, a quick method to evaluate its phytoxicity is essential. The seed germination and root elongation tests have been used as simple, reliable, and reproducible methods to evaluate the damaging effects and the phytoxicity of composts (Wong et al. 1983; Garcia et al. 1992; Grebus et al. 1994; Helfrich et al. 1998; Wan et al. 1998). Many species, including cabbage, lettuce, carrot, cucumber, tomato, oats, rice, lettuce, green beans, and cress have been recommended for the phytotoxicity test (OECD, 1981; Zucconi et al. 1981a, 1981b; USEPA, 1982; FDA, 1987). These species were recommended based on fast growth rate, high sensitivity, regular growth pattern, short germination period, economic importance, and availability of seed sources.

In the present study, the toxicity of the pig litter was examined during in-situ composting in the POL system, and during further composting in windrows. An attempt was also made to correlate some chemical properties of the litter with seed germination and root elongation, and to establish some chemical parameters that can be considered as indicators of phytotoxicity.

Materials and Methods

In-situ composting of pig litter in the POL system

Four pens employing the POL system were set-up at the Ta Kwu Ling Pig Breeding Centre, New Territories of Hong Kong. The floor of each pen was covered with a 30-cm-thick layer of sawdust (bedding material) mixed with a commercially available bacterial inoculum

(Elimexal) to aid decomposition. Four piglets were raised inside the pen. Three successive batches of pigs were raised, each batch raised for 10–13 weeks, depending on the growth rate of the pigs. An idle period of 3–5 weeks was maintained between batches. During in-situ composting, which lasted 45 weeks, pig litter samples were collected weekly at five locations (at the four corners and the centre) in each pen. The five samples were combined and mixed homogeneously to provide a composite sample.

Further composting of the spent pig litter in windrows

To determine the effect of windrow composting on the elimination of phytotoxicity in the spent pig litter, a windrow composting experiment was carried out. The spent pig litter was collected from pigpens employing the POL for 13 weeks with 40 piglets raised inside the pigpen. Two sets of windrow experiments were set-up: one during winter, the other during summer. Duplicate piles were constructed during winter (December–March), and also during summer (June–September). Each pile was triangular in cross-section, about 2 m in width at the base and 1.5 m in height. The piles were turned every four days using a truck and front-end loader until the end of the composting period (91 days). The moisture content of the piles was adjusted to 60% at the beginning of composting and no further adjustment in moisture content was carried out thereafter. Spent pig litter samples were collected at day 0 and then weekly for a period of 91 days. At each sampling period, composite samples were taken at five random locations in each pile. Triplicate composite samples were collected from each pile and brought back to the laboratory for analysis.

Preparation of the pig litter extract and phytotoxicity assay

Aqueous pig litter extracts were prepared from samples collected during in-situ composting in the POL system, and during further composting in windrows. The extracts were made by shaking the samples with distilled water at 1:10 w/v ratio using a horizontal shaker for 1 h, and then filtered using a filter paper (Whatman # 42). The phytotoxicity bioassay was evaluated using the seed germination technique (Zucconi et al. 1981a and 1981b; Tiquia et al. 1996a). This method involves incubating the compost extract with seeds at 22°C for five days in the dark, and then measuring the number of seeds germinated and root growth thereafter. Four plant species, namely Chinese cabbage *(Brassica parachinensis)*, green beans (*Azukia mungo*), lettuce (*Lactuca sativa*) and tomato (*Lycopersicon esculentum*) were chosen to determine the phytotoxicity of pig litter collected during in-situ composting. Two plant species, namely Chinese cabbage and Chinese spinach (*Amaranthus espinosus*) were used to determine the phytotoxicity of spent pig litter collected during windrow composting. These two species were found to be most sensitive to toxicity of sewage sludge (Wong et al. 1981), refuse compost (Wong and Chu, 1985), chicken litter-green manure (Wan et al. 1998), racetrack manure (Warman and Termeer, 1996), spent pig litter (Tiquia et al. 1996a), and spent pig litter and sludge (Tiquia and Tam, 1998b). Details of the test conditions are summarized in Table 1. The number of seeds per dish depended on the size of the seeds. A smaller number of seeds were used for seeds that are bigger in size. The reason for using a different number of seeds was to balance the size of each type of seeds with the space available for them to germinate.

After five days of incubation in the dark, the seed germination and root length of the five plants in the extracts were determined. The seed germination percentage and root elongation of the plants in de-ionized water were also measured and used as the control. A 5-mm primary root was used as the operational definition of seed germination (USEPA, 1982). This means that if the root was ≥ 5 mm, germination was positive. The percentages of relative seed germination,

Table 1. Seed germination test conditions.

1. Test type	Static (Batch)
2. Pre-treatment	Seeds soaked in deionized water overnight
3. Temperature	22 ± 3°C (pre-treatment and germination)
4. Light	None
5. Test vessel	10 x 100 mm Petri dish, Whatman # 42 filter paper
6. Test volume	10 ml litter extract per dish
7. Number of seeds	15–30 depending on the size of the seeds*
8. Replicates	3
9. Control	Deionized water
10. Test duration	5 days
11. End point	Germination, primary root ≥ 5 mm
12. Test species:	
Common name	Latin name
Chinese cabbage	*Brassica parachinensis*
Green beans	*Azukia mungo*
Lettuce	*Lactuca sativa*
Tomato	*Lycopersicon esculantum*
Chinese spinach	*Amaranthus espinosus*

*Chinese cabbage, 30 seeds; Green beans, 15 seeds; Tomato, 20 seeds; Lettuce, 20 seeds; Chinese spinach, 30 seeds.

relative root elongation, and germination index (GI, a factor considering seed germination and root elongation) were calculated as follows:

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

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

$$\text{GI} = \frac{\text{(\% Relative seed germination)} \times \text{(\% Relative root growth)}}{100}$$

Chemical analysis of the pig litter samples

The pig litter samples were analyzed for concentrations of total and water-extractable copper and zinc (atomic absorption spectrophotometry); NH_4^+-N, and $(NO_3^- + NO_2^-)$-N (KCl extract); water-extractable K (atomic absorption spectrophoto-metry); electrical conductivity (EC) (1:5 litter:water extract) using a conductivity electrode; and humic (HA) and fulvic (FA) acids (Schnitzer, 1982). All chemical properties, with the exception of EC, were calculated on 105°C dry weight basis.

Statistical analysis

Pearson Product Moment correlation coefficients were computed to show relationships between phytotoxicity assays and chemical properties of the spent pig litter during in-situ and further (windrow) composting. A stepwise multiple regression analysis was performed to determine the most important chemical factors inhibiting seed germination and root elongation. All statistical analyses were based on the procedures described by Zar (1999).

Results and Discussion

Phytotoxicity of the pig litter during in-situ composting

The pig litter extracts taken during the first 30 weeks of in-situ composting in the POL system did not affect the seed germination of Chinese cabbage, beans, lettuce, and tomato (Figure 1). The germination percentages were similar to those found in the control (de-ionized water). After 30 weeks, a rapid decline in seed germination was found in cabbage and lettuce seeds. Those of the tomato and green beans maintained an 82% germination until the end of the study. With respect to the root elongation, all plant species, with the exception of green beans, were significantly retarded throughout the experiment (Figure 2A), and a more severe inhibition of the root development was observed towards the end of the study. The germination index (GI), which combines seed germination and root growth, is able to account for both low toxicity—which affects root growth, and increased toxicity—which affects seed germination (Zucconi et al 1981a and 1981b). In the present study, the GI was generally between 50 to 70% in all plants during the first 30 weeks of in-situ composting, except green beans (Figure 2B). This result reveals that the pig litter had a lower phytotoxicity during the first 30 weeks of composting compared with weeks 35 to 45. Only the root elongation of the four plants was retarded, and the seed germination was not significantly inhibited by the pig litter extracts (Figure 1 and 2A). After 30 weeks of in-situ composting, both seed germination and root elongation of the four plants were severely retarded, indicating that severe toxicity occurred in the pig litter. The severe retardation was related to the accumulation of Cu, Zn, NH_4^+-N, $(NO_3^-+NO_2^-)$-N, water-extractable K, and EC in the pig litter (Figure 3).

Pig manure often contains high concentrations of Cu compared with other animal manures, since Cu is normally added to pig rations at concentrations up to 250 mg kg^{-1} to

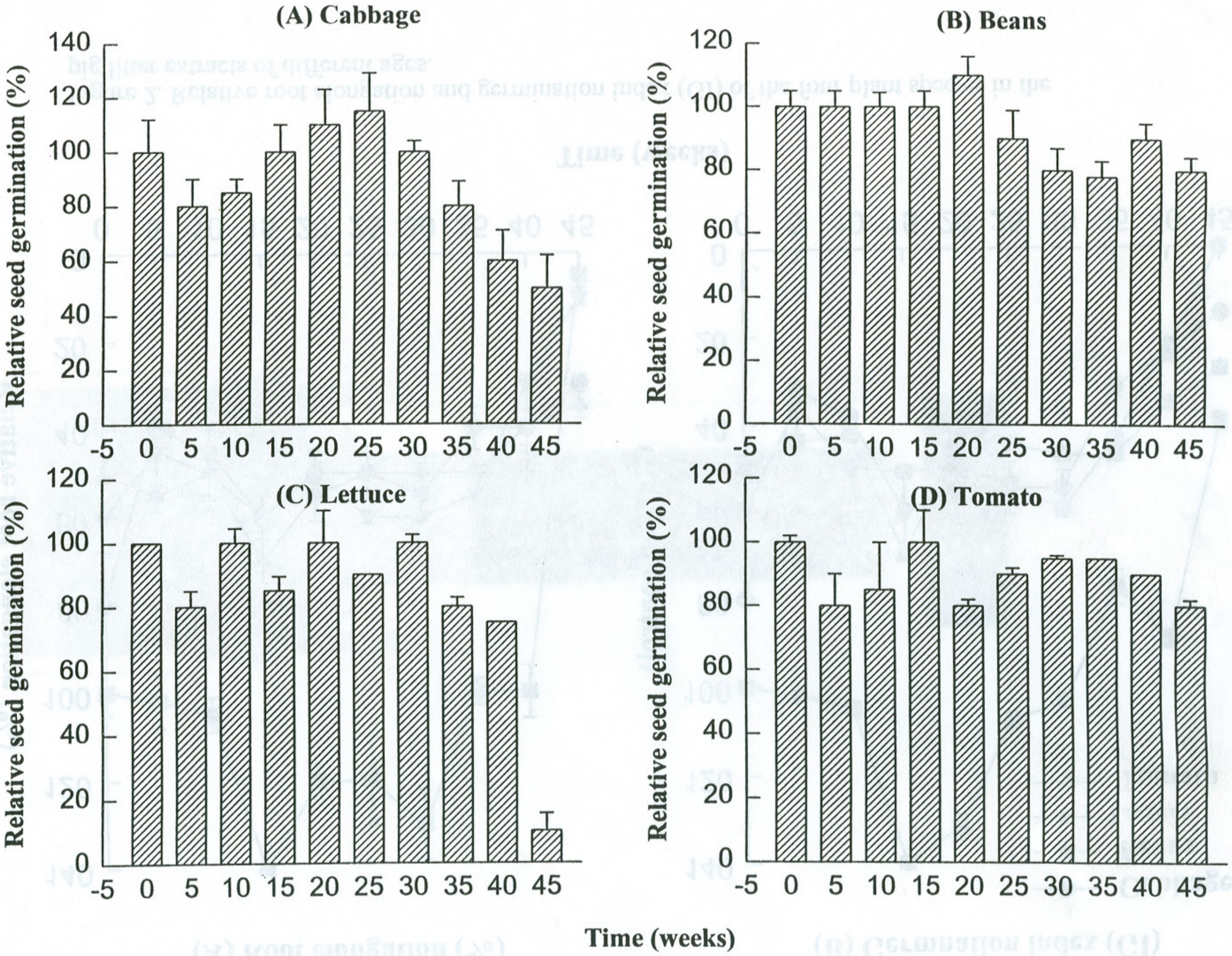

Figure 1. Relative seed germination percentage of four plant species in the pig litter extracts of different ages.

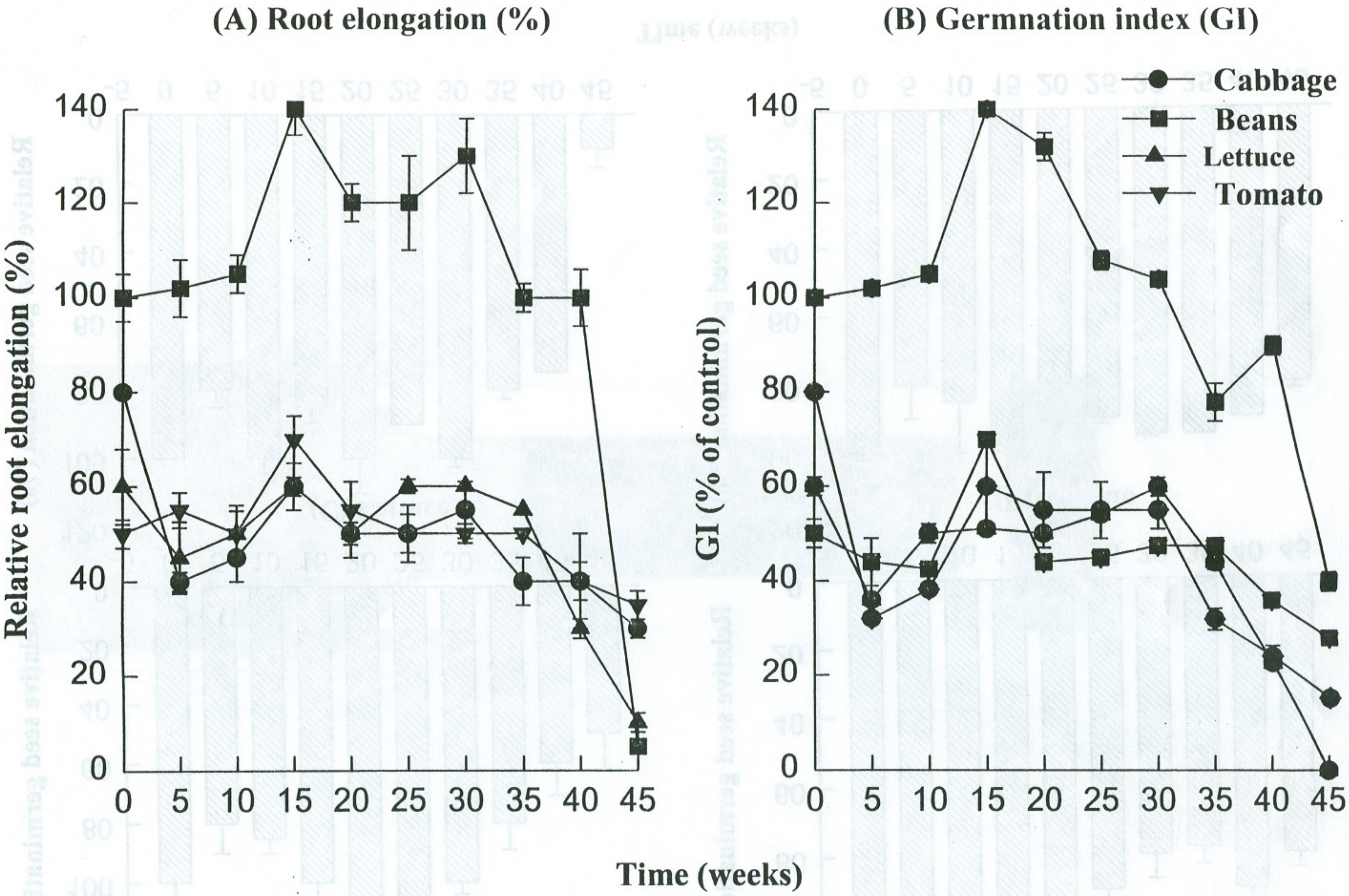

Figure 2. Relative root elongation and germination index (GI) of the four plant species in the pig litter extracts of different ages.

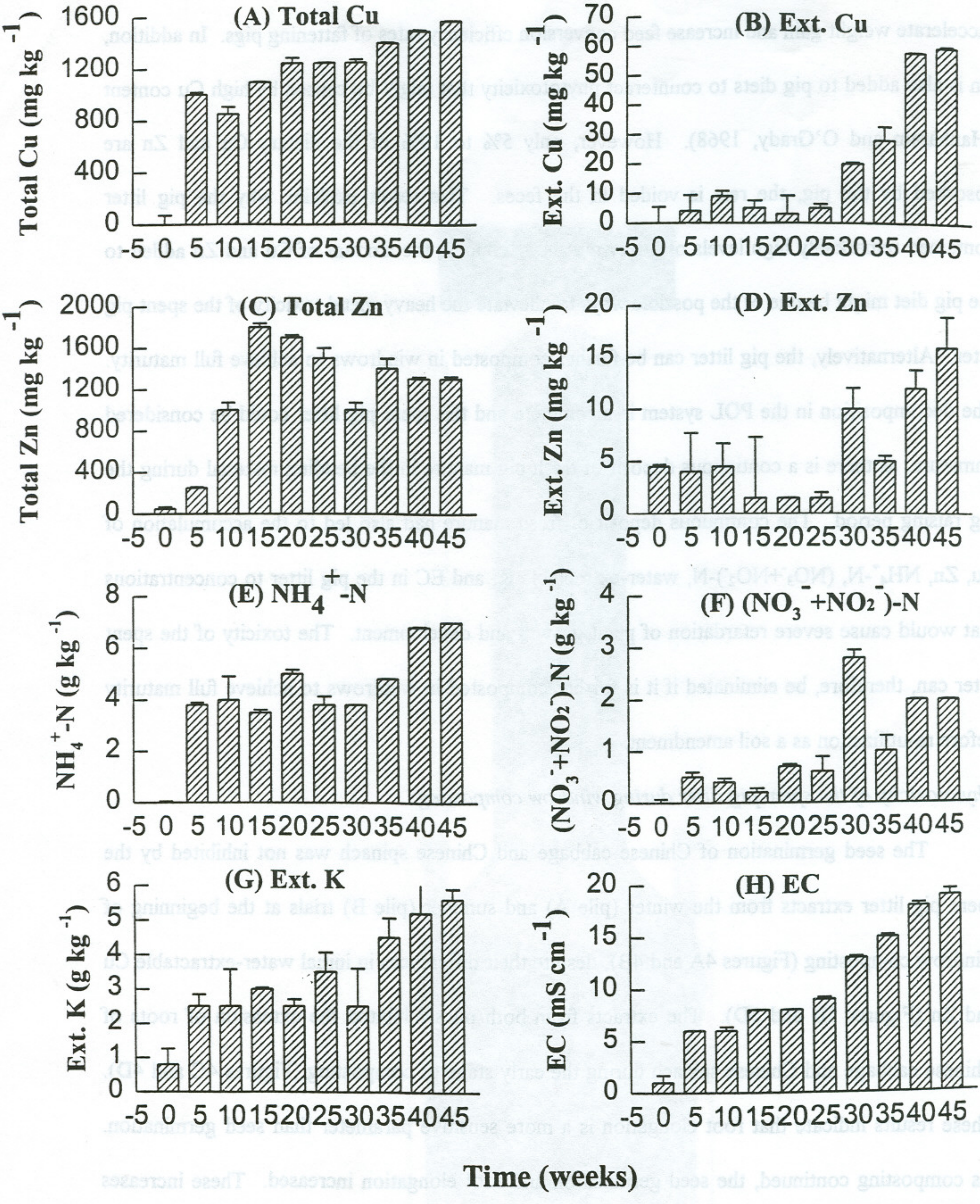

Figure 3. Concentrations of (A) total Cu, (B) water-extractable Cu, (C) total Zn, (D) water-extractable Zn, (E) NH_4^+-N, (F) ($NO3^-$+$NO2^-$)-N, (G) water-extractable K, and (H) electrical conductivity (EC) of the pig litter at different ages.

accelerate weight gain and increase feed conversion efficiency rates of fattening pigs. In addition, Zn is also added to pig diets to counteract phytotoxicity that might be caused by high Cu content (Hanharam and O'Grady, 1968). However, only 5% to 10% of the dietary Cu and Zn are absorbed by the pig, the rest is voided in the feces. This result explains why the pig litter contained remarkably high levels of heavy metals. Reducing the amount of Cu and Zn added to the pig diet might be one of the possible ways to alleviate the heavy metal toxicity of the spent pig litter. Alternatively, the pig litter can be further composted in windrows to achieve full maturity. The decomposition in the POL system is incomplete and the spent pig litter could be considered immature, as there is a continuous deposit of fresh pig manure in the bedding material during the pig raising period. The continuous deposit of fresh manure had also led to the accumulation of Cu, Zn, NH_4^+-N, $(NO_3^-+NO_2^-)$-N, water-extractable K, and EC in the pig litter to concentrations that would cause severe retardation of plant growth and development. The toxicity of the spent litter can, therefore, be eliminated if it is further composted in windrows to achieve full maturity before re-utilization as a soil amendment.

Phytotoxicity of the spent pig litter during windrow composting

The seed germination of Chinese cabbage and Chinese spinach was not inhibited by the spent pig litter extracts from the winter (pile A) and summer (pile B) trials at the beginning of windrow composting (Figures 4A and 4B), despite their differences in initial water-extractable Cu and Zn (Figures 5B and 5D). The extracts from both piles inhibited the extension of roots of Chinese cabbage and Chinese spinach during the early stage of composting (Figures 4C and 4D). These results indicate that root elongation is a more sensitive parameter than seed germination. As composting continued, the seed germination and root elongation increased. These increases coincided with decreases in concentrations of water-extractable Cu and Zn, and NH_4^+-N (Figures

5B, 5D, and 5E). The seed germination, root elongation, and GI of the two plant species were negatively correlated with these three chemical compounds (Table 2). The decrease in NH_4^+-N content to low levels was associated with the accumulation of $(NO_3^- + NO_2^-)$-N (Figure 5F) via nitrification. On the other hand, the decline in water-extractable Cu and Zn was due to the formation of complexes of these metals with chelating organic matter, thus making them non water-extractable and biologically unavailable. Decreases in water-extractable Cu and Zn coincided with increases in humification parameters (HA and FA) (Figures 5B, 5D, and 6). This result reveals the ability of humic substances to form stable complexes with Cu^{2+} and Zn^{2+} in the spent pig litter, due to their high content of oxygen-containing functional groups including COOH, phenolic, alcoholic and enolic-OH, and C=O structures of various types (Chen and Stevenson, 1986).

The total Cu, Zn, and EC contents increased in the spent pig litter during composting (Figures 5A, 5C, and 5H). These increases in heavy metals and EC values were probably due to concentration effect as a result of organic matter mineralization. In this process, the organic C, H, and O had been lost from the piles in the form of CO_2 and H_2O during composting, leaving behind Cu, Zn, and soluble salts, consequently giving a relative increase in concentrations of these metals. It has been noted that the phytotoxicity of compost could also be due to high EC (>4 dS m^{-1}) (Allison, 1973). The EC of the spent pig litter in this study was <4 dS m^{-1} during the entire composting process, and therefore did not cause any retardation of seed germination and root elongation of the two plants.

Relationship between phytotoxicity assay and chemical properties of the spent pig litter

The multiple regression analysis demonstrated that NH_4^+-N was the most important chemical factor affecting phytotoxicity of Chinese cabbage and Chinese spinach (Table 3).

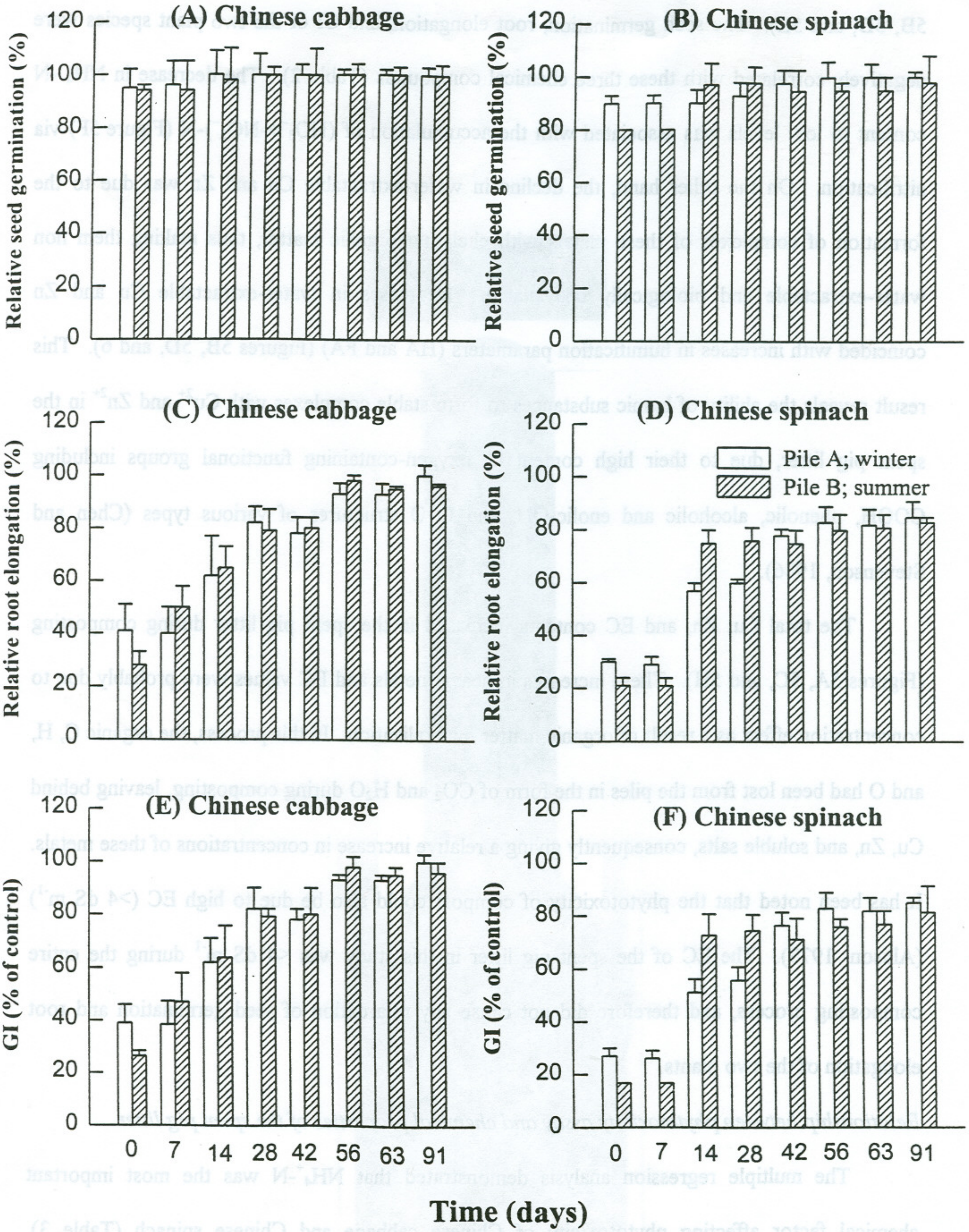

Figure 4. Relative seed germination percentage and relative root elongation of two plant species in spent pig litter extracts at different stages of composting.

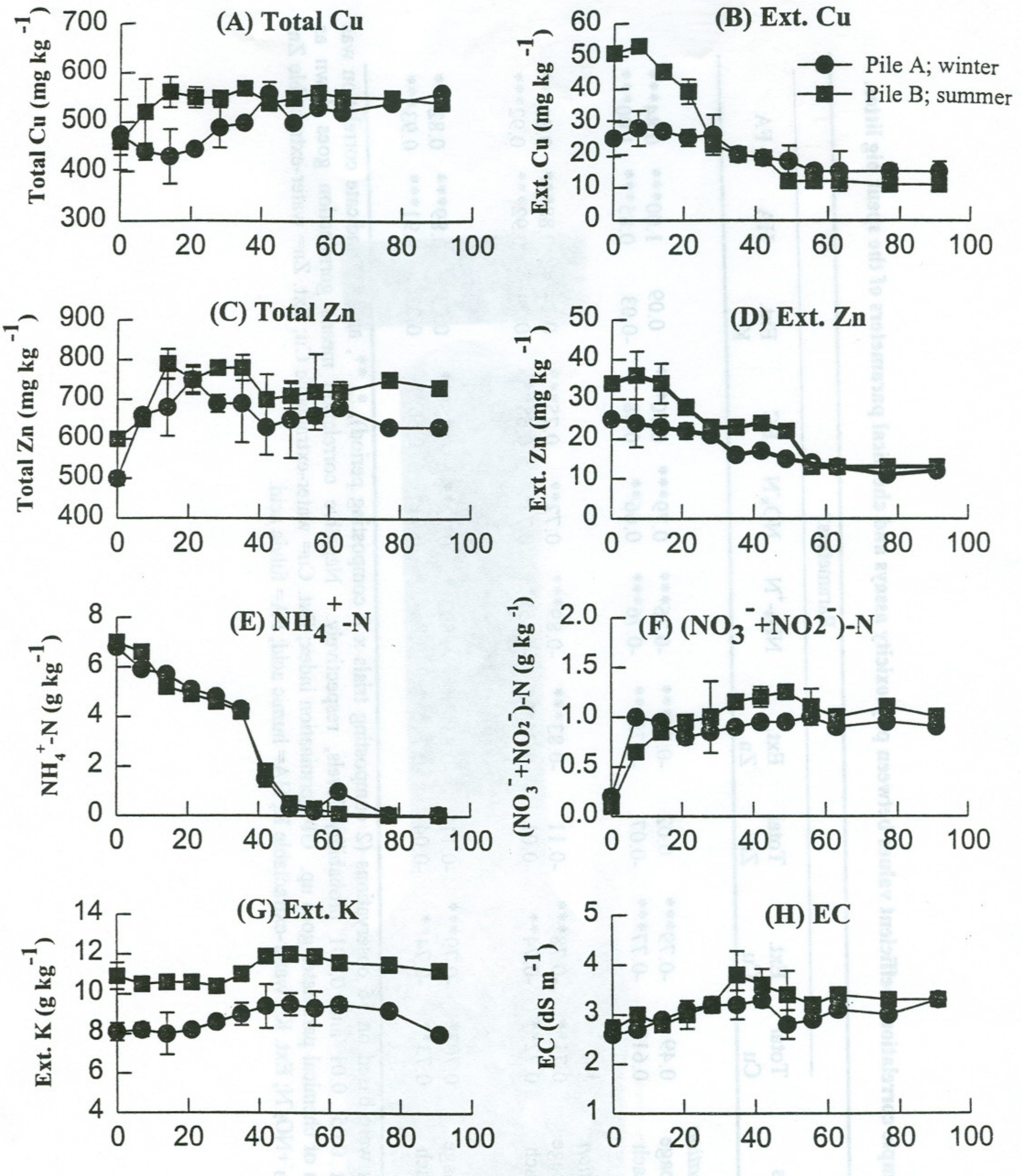

Figure 5. Changes in concentrations of (A) total Cu, (B) water-extractable Cu, (C) total Zn, (D) water-extractable Zn, (E) NH_4^+-N, (F) $(NO_3^-+NO_2^-)$-N, (G) water-extractable K, and (H) electrical conductivity (EC) of the spent pig litter during composting.

Table 2. Simple correlation coefficient values between phytoxicity assays and chemical parameters of the spent pig litter.

Plant species	Parameters†									
	Total Cu	Ext. Cu	Total Zn	Ext. Zn	NH_4-$^+$N	NO_x^-N	EC	Ext. K	HA	FA
Seed germination										
Chinese cabbage	0.49	-0.79***	0.02	-0.76***	-0.76***	0.79***	0.70***	0.09	1.00***	0.84***
Chinese spinach	0.61*	-0.77***	-0.07	-0.75***	-0.76***	0.66**	0.48	-0.03	0.85***	1.00***
Root elongation										
Chinese cabbage	0.71**	-0.78***	-0.11	-0.83***	-0.89***	0.72**	0.75***	0.24	0.88***	0.81***
Chinese spinach	0.72**	-0.74**	-0.01	-0.74***	-0.85***	0.72**	0.68**	0.24	0.92*** 0	0.92***
GI										
Chinese cabbage	0.70**	-0.79***	-0.11	-0.83***	-0.89***	0.72**	0.75***	0.23	0.89***	0.82***
Chinese spinach	0.73**	-0.74**	-0.04	-0.74***	-0.86***	0.71**	0.66**	0.22	0.91***	0.93***

†Correlations were based on 16 observations (2 composting trials x 8 composting period); *, **, and *** indicate correlation was significant at 0.05, 0.01 and 0.001 probability levels, respectively. Negative correlation means germination goes down as concentration of chemical parameter goes up. GI=germination index; Ext. Cu= water-extractable Cu; Ext. Zn= water-extractable Zn; $NO_x^-N = NO_3^- + NO_2^-N$; Ext. K= water-extractable K; HA= humic acid; FA= fulvic acid.

Ammonium has been noted as one of the most important chemical factors retarding the growth, seed germination and root elongation of Chinese cabbage and Chinese spinach in sludge (Tiquia and Tam, 1998b, 2000) and in mixtures of chicken and green manure (Wan et al. 1998) extracts. These authors reported that the toxicity was reduced with decreasing NH_4^+-N concentration during composting. Therefore, reducing the amounts of NH_4^+-N by further composting would be a possible way to eliminate toxicity of the spent litter.

It has been suggested that a GI ≥ 80% indicates the disappearance of phytotoxins in composts (Zucconi et al. 1981a and 1981b; Tiquia et al. 1996a). In the present study, such a value was achieved by day 56. The maturity of compost in terms of the elimination of phytotoxicity has been widely used as a measure of compost maturity (Zucconi et al. 1981a; Baca et al. 1990; Tiquia et al. 1996a; Helfrich et al. 1998). However, these tests have not always supported that low phytotoxicity indicates compost maturity. In properly controlled composting systems, the stage characterized by a strong toxicity (GI lower than 50%) could be well completed before the end of the thermophilic phase (Zucconi et al. 1981a). At this stage, the compost is far from stabilization. The disappearance of phytotoxicity at day 56 in this study corresponded with the stabilization of physico-chemical and microbial properties of the spent litter. Tiquia and Tam (1999) and Tiquia et al. (1996b) reported that the physico-chemical and microbial parameters of spent pig litter stabilized in 60 days (two months) when optimum environmental conditions were met (piles turned every four days and moisture content adjusted to 60%).

Tiquia and Tam (1998b) found a curvilinear relationship between GI and the water-extractable chemical properties of spent pig litter and sludge. They reported that the changes in the GI were strongly dependent on the chemical properties of the compost. In the present study,

Table 3. Multiple regression analysis of the phytotoxicity assays and chemical properties of the spent pig litter.

Plant species	Multiple regression equation†	Multiple R value	Adjusted R^2 value	F value	Significance of F
Seed germination					
Chinese cabbage	NS			NS	
Chinese spinach	% GERM = 107.9-(0.68 * NH_4)	0.97	0.96	179.0	<0.0001
Root elongation					
Chinese cabbage	% ROOT = 120.7-(4.19 * NH_4)- (1.97 * Ext. Zn)	0.99	0.98	185.0	<0.0001
Chinese spinach	% ROOT = 120.2-(1.18 * NH_4) + (1.20* Ext. Cu)	0.82	0.79	27.7	0.0019
GI					
Chinese cabbage	% GI = 119.2-(4.09 * NH_4)- (1.86 * Ext. Zn)	0.99	0.98	220.1	<0.0001
Chinese spinach	% GI = 120.9-(1.19*NH4)- (1.07 * Ext. Cu)	0.96	0.95	141.2	<0.0001

†Regression equation was based on 8 chemical parameters with STEPWISE METHOD (Probability to F-enter) = 0.050 limit; NS= not significant at PIN = 0.05 limit. GI= germination index; Ext. Cu= water-extractable Cu; Ext. Zn= water-extractable Zn; NH_4= NH_4^+-N.

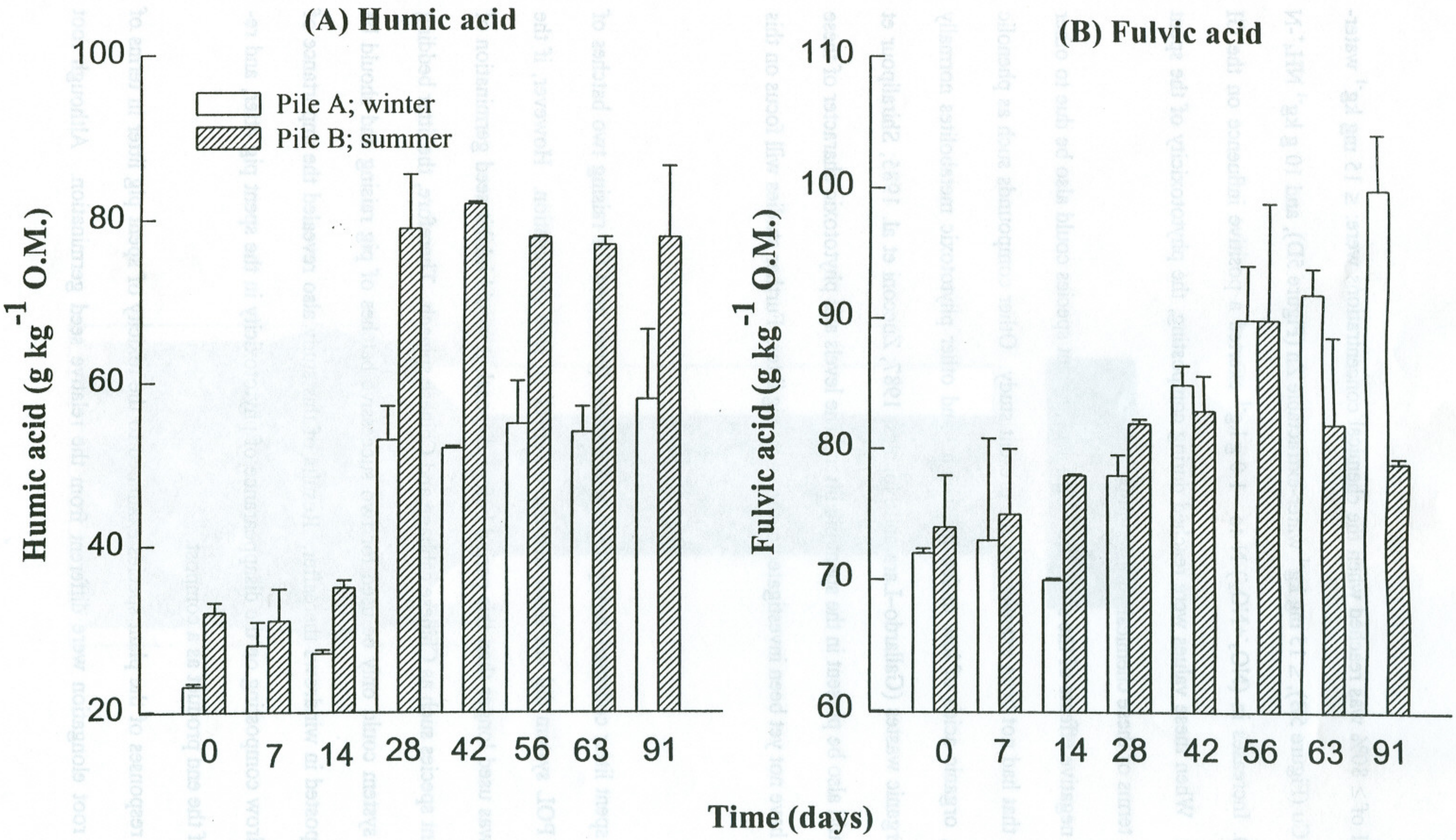

Figure 6. Changes in humic (HA) and fulvic (FA) acid contents of spent pig litter during composting.

a GI of ≥ 80% was reached when the chemical concentrations were: ≤ 15 mg kg^{-1} water-extractable Cu (Figure 5B), ≤ 15 mg kg^{-1} water-extractable Zn (Figure 5D), and 10 g kg^{-1} NH_4^+-N (Figure 5E). Increases in (NO_3^-+NO_2^-)-N to ~1.0 g kg^{-1} exerted a positive influence on the GI (Figure 5F). When these values were reached during composting, the phytotoxicity of the spent pig litter, in terms of these chemicals, was eliminated.

The negative effects of the spent litter on selected plant species could also be due to other phytotoxins that had not been examined in the present study. Other compounds such as phenolic compounds, organic acids of low molecular weight, and other phytotoxic metabolites normally present in organic wastes (Gallardo–Lara and Nogales, 1987; Zucconi et al. 1985; Shiralipour et al. 1997) could also be present in the spent pig litter. The levels and phytotoxic character of these compounds have not yet been investigated in the spent pig litter. Further studies will focus on this aspect.

Summary

The spent litter collected during the first 30 weeks (litter used for raising two batches of pigs) in the POL system did not show detrimental effect on seed germination. However, if the spent litter was used longer than this period before disposal, it would inhibit seed germination of sensitive plant species such as Chinese cabbage and Chinese spinach. Therefore, the litter bedding in the POL system could only be used for two successive batches of pig raising and should be further composted in windrows thereafter. Results of this study also revealed the importance of further windrow composting on the disappearance of phytotoxicity in the spent pig litter, and re-utilization of the end product as a compost.

The responses of the plant species examined to the toxicity of spent pig litter in terms of the relative root elongation were different from the relative seed germination. Although root elongation of the plant species was to a large extent inhibited, seed germination was not affected by the phytotoxic compounds in the spent pig litter during windrow composting. These results suggest that relative root elongation was a more sensitive test than relative seed germination. Therefore, measuring the relative root elongation alone can be used as an index to evaluate the damaging effects and toxicity of compost. This will reduce the time required for determining phytotoxicity of composts.

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