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Assessing toxicity of spent pig litter using a seed germination technique

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ABSTRACT

The phytotoxicity of spent litter collected from pig pens employing the 'pig-on-litter'system at various times was evaluated using seed germination and root elongation techniques. The percentage seed germination of four plant species (lettuce, Chinese cabbage, tomato and green beans) was not affected by the water extracts of spent litter samples collected in the first 30 weeks of production. Seed germination was significantly retarded by litter extracts from 34 weeks onwards. The percentages of seed germination at the end of the 45 weeks study were 1% for lettuce, 16% for cabbage, 21% for tomato, and 44% for green beans. Compared with seed germination, root elongation was more sensitive to the toxicity of the spent litter. The root lengths of all seedlings except green beans were less than 50% of the control (deionized water) throughout the experiment. The inhibitory effects of spent litter on root elongation increased with the age of the litter. The final root lengths of lettuce, Chinese cabbage and tomato seedlings were 14%, 24% and 28% of the control, respectively. Green beans behaved very differently from the other species; spent litter extracts stimulated root growth throughout the study, except the last week. The elevated concentrations of heavy metals (in particular extractable Cu) and nutrients (especially NH_4^+-N) present in spent litter were the main factors responsible for the phytotoxicity of the spent litter. The aged spent litter had accumulated more salts, nutrients and heavy metals and imposed more toxic effects on seed germination than did the young spent litter.

INTRODUCTION

Indiscriminate discharge of livestock waste into surrounding waters has caused considerable damage to the aquatic environment. A 'pig-on-litter' (POL) system, also known as in situ composting, has been developed as one of the recommended methods for pig waste disposal in Hong Kong (EPD, 1989) and other countries. The system utilizes a mixture of sawdust and a commercial bacterial product as the bedding material on which pigs are raised, and the pig excreta are decomposed within the bedding material (EPD, 1990). Under this system, neither wastewater nor solid manure requires disposal, the only waste product is the spent litter (a mixture of sawdust, bacterial products and decomposed pig excreta). This spent litter has properties compara-

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ble to conventional manure compost, with high concentrations of organic matter, nitrogen, phosphorus, potassium and trace elements (Tam and Vrijmoed, 1990, 1993). Like animal compost, spent litter 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 (Tam and Wong, 1993). However, high contents of salts and heavy metals (in particular, Cu and Zn) are frequently reported in pig manure (Cheung and Wong, 1983; Jeffery and Uren, 1979; Wong, 1985) and spent litter (Tam and Vrijmoed, 1990, 1993). These elements, at high concentrations, can impose detrimental effects on seed germination, development of young seedlings, maturation, root and shoot growth (Jeffery and Uren, 1979). The amounts of salts and heavy metals accumulated in spent litter seem to be related to the duration of the litter being used under the POL system before disposal; the longer the litter is kept in the pig pen, the more the salts and heavy metals will be retained. It appears that aged spent litter is more toxic to plant growth than young spent litter. In order to guarantee that spent litter can be re-cycled back to agricultural land without causing any environmental risk, a quick method to evaluate the toxicity of spent litter is required.

Plant seed germination and root elongation tests have been used as simple and sensitive techniques for detection of the toxicity of various environmental pollutants such as heavy metals (Wong and Bradshaw, 1982), phenolic compounds (Wong, 1985), refuse compost (Wong, 1985), industrial effluents from heavy machinery, agricultural product utilization and specialist chemical industries (Wang and Keturi, 1990). Many plant species, including cabbage, lettuce, carrot, cucumber, tomato and oats, have been recommended for seed germination and root elongation tests (FDA, 1987; USEPA, 1982). In the present study, seed germination and root elongation of four plant species were used to evaluate the toxicity of the spent litter. The toxicity of spent litter at different ages was examined. An attempt was also made to determine the possible relationship between bioassay results and the chemical properties of spent litter.

MATERIALS AND METHODS

Collection of spent litter samples

Four pig pens employing the POL system were set up in Ta Kwu Ling Breeding Centre, Hong Kong. Each pig pen at the beginning of the study had a bedding material made up of sawdust (20–35 cm deep) and a commercial bacterial product known as 'Elimexal'. Four piglets were then raised, their excreta being mixed with the bedding material which underwent rapid in-situ decomposition. The spent litter was raked and turned over manually once or twice daily. Composite samples were collected at five locations (four corners and the centre) within each pig pen at weekly intervals for 45 weeks. During the experimental period, three successive batches of pigs were raised, each batch lasting for 10–13 weeks dependent on the growth rate of the pigs. In between two successive batches, an idle period of 3–6 weeks was maintained.

Litter extract preparation and seed germination test

The spent litter extract was prepared by shaking the sample with deionized water at 1 : 10 w/v ratio in a stomacher (laboratory blender) for 20 min, which was then filtered. The phytotoxicity was monitored by seed germination and root elongation techniques (Wang and Keturi, 1990). The test procedure is summarized in Table 1. Four plant species, Chinese cabbage (*Brassica parachinensis*), lettuce (*Lactuca sativa*), green beans (*Azukia mungo*) and tomato (*Lycopersicon esculentum*) were chosen. After 5 days incubation in the dark, the seed germination, root length and germination index (GI) were determined. A 5-mm primary root was used as the operational definition of germination (USEPA, 1982).

Chemical analysis on spent litter and litter extract

The litter extract was measured for pH, electrical conductivity, concentrations of NH_4^+-N, $(NO_2^- + NO_3^-)$-N (Keeney and Nelson, 1982), K (flame photometry) and heavy metals, including Cu, Zn and Mn (flame atomic absorption spectrophotometry). The ash content was measured by igniting a spent litter sample at 550°C for 4 h; the ash was then dissolved in 6 M HCl

TABLE 1

Seed germination test conditions

Test type	Static
Pretreatment	Soaked in distilled water overnight
Temperature	22 ± 3°C
Light	No
Test vessel	100 × 10 mm culture dish plus Whatman No. 1 filter paper
Test volume	10 ml per dish
Number of seeds	15–30 per dish (depends on the size of the seeds); Cabbage, 30 seeds; Lettuce, 20 seeds; Green beans, 15 seeds; Tomato, 20 seeds.
Replicates	
Control	Distilled and deionized water
Test duration	5 days
End point	Germination; primary root equal to or greater than 5 mm
Measurement and calculation	$\% \text{ seed germination} = \frac{\text{germination \% in litter extract}}{\text{germination \% in control}} \times 100\%$
	$\% \text{ root growth} = \frac{\text{mean root length in litter extract}}{\text{mean root length in control}} \times 100\%$
	$\text{Germination index} = \frac{\% \text{ seed germination} \times \% \text{ root growth}}{100}$
	(Control: Germination Index = 100)

for the determination of total heavy metals (Cu, Zn and Mn). The amounts of total K and total N were determined by Micro-Kjeldahl digestion and Markham distillation techniques (Allen et al., 1974).

Statistical analysis

The mean and standard deviation of the four replicates of each determinant were calculated. The chemical properties were calculated on 105°C dry weight basis. Product-moment correlation coefficients were computed to show the relationship between chemical properties of the spent litter and seed germination or root elongation. A stepwise multiple regression analysis was performed to determine the most important chemical factors retarding seed germination and root elongation. All statistical analyses were carried out using the SPSS-PC statistical package.

RESULTS

Seed germination and root elongation

The spent litter collected in the first 30 weeks of the study did not affect seed germination of the four plant species and the germination percentages were generally similar to those found in the control (deionized water). Thereafter, a slight inhibition of seed germination was recorded (Fig. 1). Towards the end of the study, a rapid decline in germination was found in cabbage and lettuce seeds. The germination percentages (with reference to the control) of cabbage dropped from 85% (in Week 34) to 52% (in Week 45) while the decrease in lettuce was from 91% to 5% (Table 2). The germination of tomato and green beans was maintained at 82% even at the end of the study (Table 2). These results indicated that the phytotoxicity of the spent litter depended both on the age of the spent litter and the plant species. The spent litter collected in the first 30 weeks (used for raising two batches of pigs) did not show any detrimental effect on seed germination. However, if the spent litter was used longer than this period before disposal, it inhibited seed germination of sensitive plant species such as cabbage and lettuce.

With respect to root development, the root elongation of all plant species except green beans was significantly retarded by spent litter throughout the experiment (Fig. 2A). The root lengths of cabbage, lettuce and tomato seedlings in spent litter extracts were only 50% of the control. More severe inhibition of root development was found towards the end of the study. The root growth of cabbage, lettuce and tomato seedlings in Week 45 was 24%, 14% and 28% of the control, respectively (Table 2). By contrast, the spent litter extract stimulated root elongation of green beans; their root lengths were similar to or even longer than the control except at the last week (Fig. 2A). Only in Week 45, a 50% reduction on root length of green beans by spent litter extract was recorded. It appeared that root elongation was a more sensitive

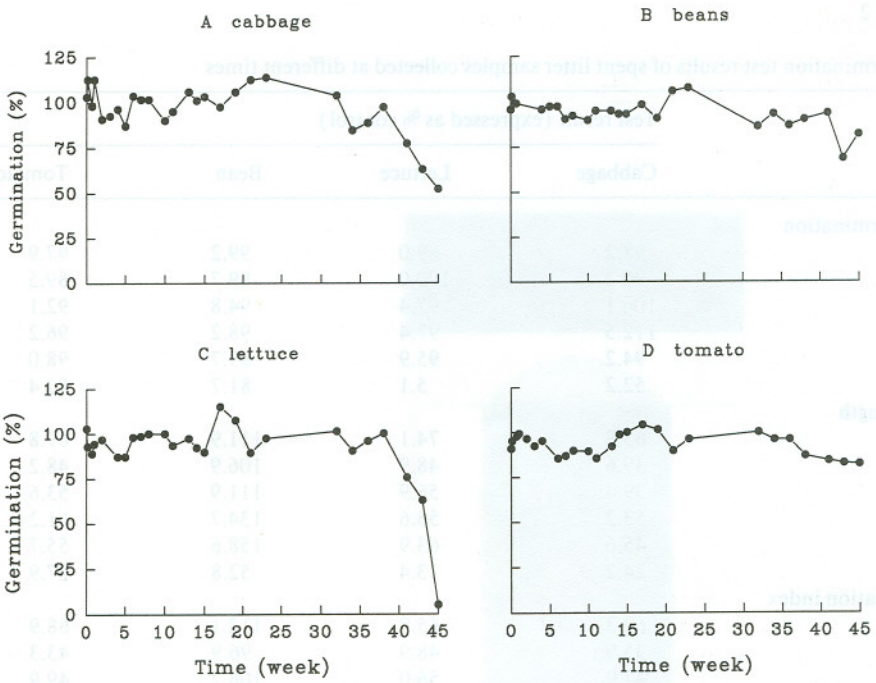

Fig. 1. Percentage of seed germination of four plant species in spent litter extracts of different ages.

indicator than seed germination for evaluating the inhibitory effects of spent litter on plant growth. Root development was found to be more susceptible to stresses such as metal toxicity than was seed germination (Cheung et al., 1989; Wong and Bradshaw, 1982).

In the first 38 weeks GI values were generally between 50% and 70% in all plant species except green beans (Fig. 2B). Zucconi and his co-workers (1981a,b) reported that GI's above 80–85% indicated the disappearance of phytotoxicity in compost, and no injury on olive plant growth was found when GI is above 50–60%. This suggested that spent litter collected in the first 38 weeks might not cause any significant injury to plant growth. However, GI declined significantly towards the end of the study in most plant species, with final values less than 20 (Table 2). This indicated that both seed germination and root elongation were inhibited by the aged spent litter. Among the four plant species, green beans seemed to be the most resistant species and its seed development was little retarded by spent litter.

Chemical properties of spent litter

Figure 3 reveals that the conductivity, concentrations of ash, nitrogen and potassium of spent litter increased significantly with its age (i.e. sampling

TABLE 2

Seed germination test results of spent litter samples collected at different times

Week	Test result (expressed as % control)			
	Cabbage	Lettuce	Bean	Tomato
Seed germination				
1	98.2	89.0	99.2	97.9
10	90.2	100.0	89.7	89.5
13	106.1	97.4	94.8	92.1
26	112.5	97.4	98.2	96.2
33	94.2	95.9	89.7	98.0
45	52.2	5.1	81.7	82.4
Root length				
1	63.8	74.1	111.9	69.8
10	39.6	48.9	106.9	48.2
13	39.4	56.9	111.9	53.6
26	53.2	56.6	134.7	61.2
33	45.6	63.9	158.6	55.7
45	24.2	13.4	52.8	27.9
Germination index				
1	62.3	65.9	112.1	68.9
10	35.9	48.9	96.9	43.3
13	42.9	56.0	106.2	49.9
26	67.3	55.1	132.3	58.9
33	42.9	61.3	142.3	54.6
45	15.9	0.9	43.5	21.9

Week 1; Start of the experiment; Weeks 10, 26 and 45; end of the 1st, 2nd and 3rd batch of pig raising, respectively; Weeks 13 and 33; end of the first and second idle periods, respectively. GI, germination index.

time). The accumulation of salts and nutrients was more obvious in the first 10 weeks, with conductivity raised from an initial 0.8 to 3 mS cm^{-1} (Fig. 3A), total N from 0.3% to 3.6% (Fig. 3C) and total K from 0.22% to 1.72% (Fig. 3D). The NH_4^+-N content of spent litter also increased from an initial 0.11 to 4.8 mg g^{-1} after rearing the first batch of pigs and reached a level of 7.1 mg g^{-1} at the end of the study (Fig. 3C). Such high concentrations of NH_4^+-N might be toxic to plants. Therefore the spent litter collected at the latter part of the study would produce more severe damage to plant growth, especially root elongation, than young spent litter.

The concentrations of total Cu and Mn increased dramatically in the first 10 weeks (about 1000 $\mu g\ g^{-1}$ Cu and 400 $\mu g\ g^{-1}$ Mn), declined during the first idle period and raised again, with final Cu and Mn values of 1580 and 633 $\mu g\ g^{-1}$, respectively (Fig. 4A and C). An initial increase in Zn level was recorded in the first 10 weeks, which then fluctuated at over 1000 $\mu g\ g^{-1}$ throughout the rest of the experimental period (Fig. 4E). Such accumulation

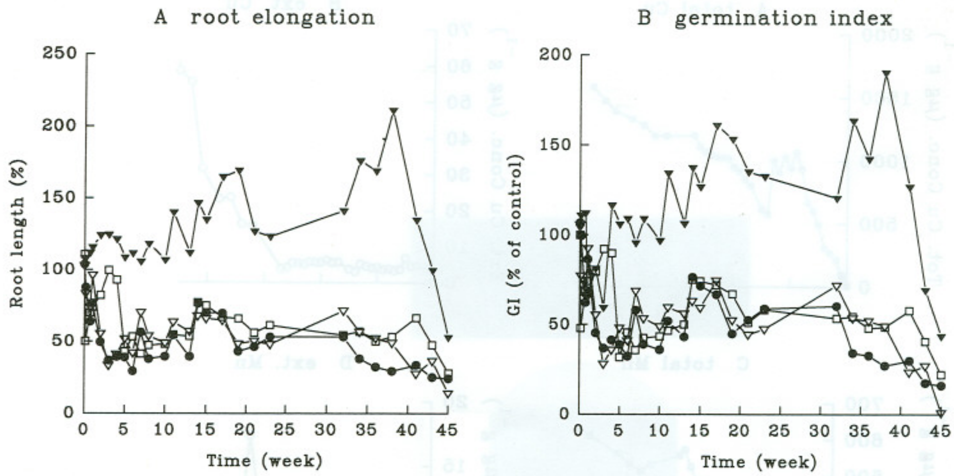

Fig. 2. Root elongation and germination index (GI) of four plant species in spent litter extracts of different ages. (●, Chinese cabbage; ▽, lettuce; ▼, green beans; □, tomato)

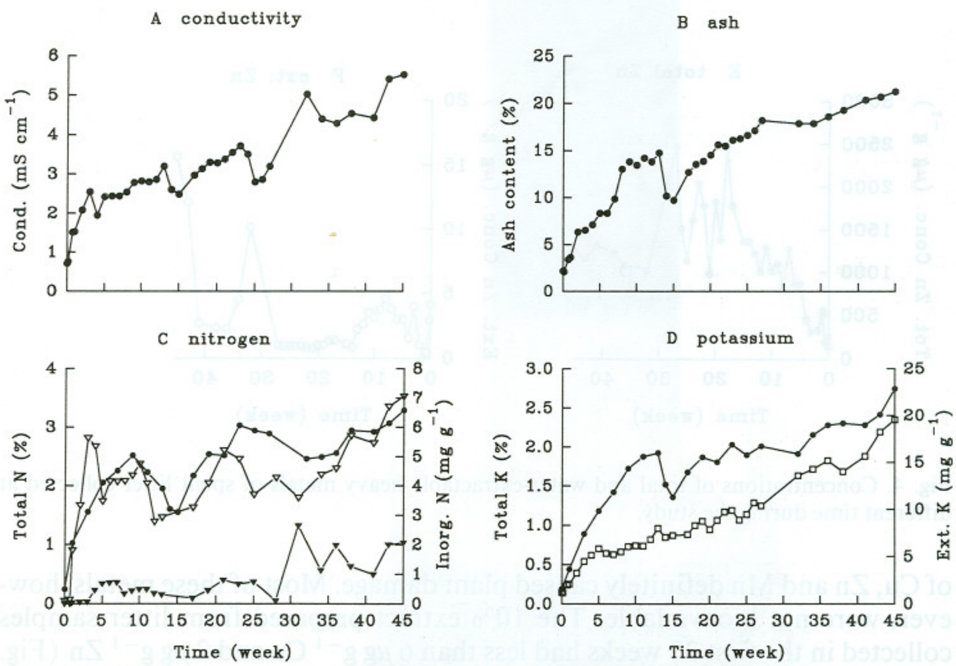

Fig. 3. Conductivity, concentrations of ash, nitrogen and potassium of spent litter collected at different time during the study. In Fig. 3C: ●, total N, ▽, NH_4^+-N; ▼, NO_3^--N. In Fig. 3D: ●, total K; □, Ext. K.

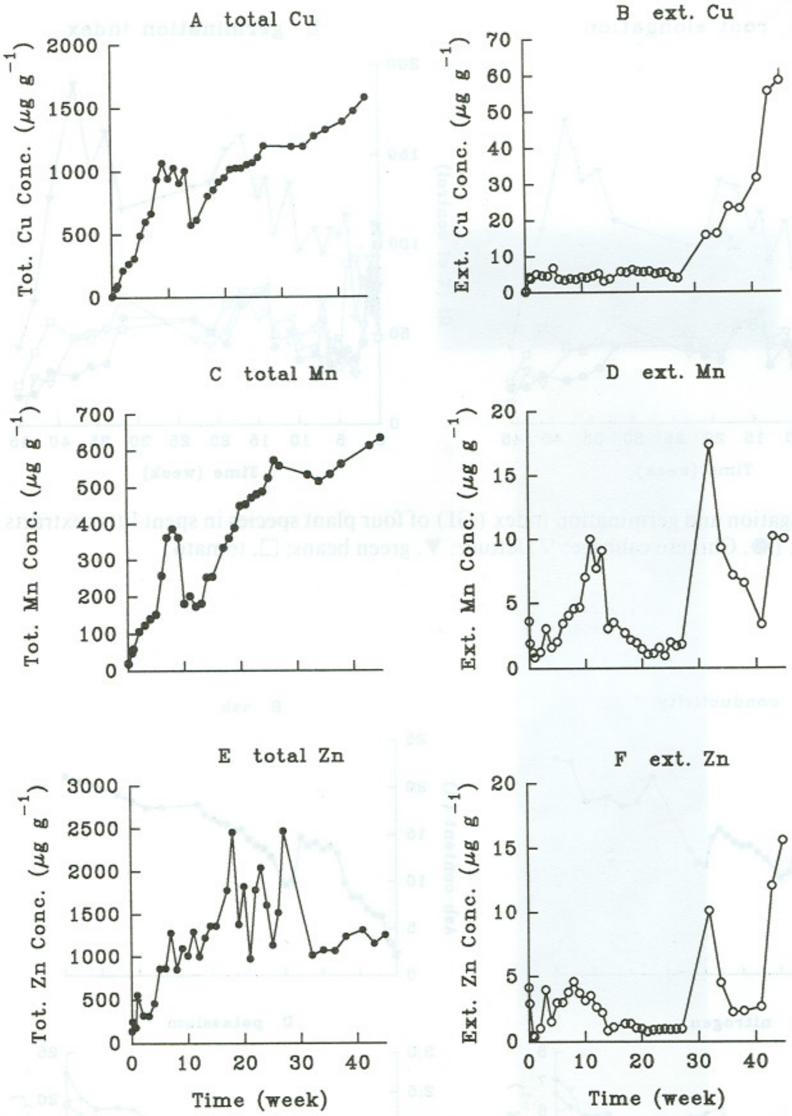

Fig. 4. Concentrations of total and water-extractable heavy metals of spent litter collected at different time during the study.

of Cu, Zn and Mn definitely caused plant damage. Most of these metals, however, were not bioavailable. The 10% extract prepared from litter samples collected in the first 30 weeks had less than 6 $\mu g\ g^{-1}$ Cu and 2 $\mu g\ g^{-1}$ Zn (Fig. 4), i.e. less than 1% metal was water-soluble. Lustenhouwer and Hin (1993) reported that the directly available fraction of heavy metals was low and in general less than 1% of the total content in compost. After 30 weeks, the ex-

TABLE 3

Product-moment correlation coefficient (r) values between seed germination, root elongation and chemical properties of spent litter

	Cond	Ash	Ext-K	Tot-K	NH_4^+	$(NO_2^- + NO_3^-)$	Inorg N	Ext-Cu	Tot-Cu	Ext-Zn	Tot-Zn	Ext-Mn	Tot-Mn
Seed germination													
Cabbage	−0.55*	−0.45*	−0.67**	−0.51*	−0.52*	−0.49*	−0.57**	−0.85***	−0.46*	−0.71***	−0.02	−0.37	−0.42*
Lettuce	−0.47*	−0.35	−0.56*	−0.40	−0.52**	−0.43*	−0.55**	−0.78***	−0.36	−0.73***	−0.01	−0.24	−0.36
Green bean	−0.002	−0.19	−0.06	−0.29	−0.14	−0.009	−0.11	−0.21	−0.21	−0.23	−0.38	−0.05	−0.16
Tomato	−0.17	−0.19	−0.17	−0.14	−0.38	−0.20	−0.36	−0.37	−0.26	−0.37	−0.001	−0.10	−0.15
Root elongation													
Cabbage	−0.65***	−0.62***	−0.72***	−0.78***	−0.81***	−0.49*	−0.79***	−0.53**	−0.63***	−0.33	−0.25	−0.27	−0.56**
Lettuce	−0.59**	−0.58**	−0.67**	−0.72***	−0.86***	−0.37	−0.79***	−0.57**	−0.57**	−0.36	−0.29	−0.09	−0.53**
Green bean	−0.18	−0.24	−0.09	−0.16	−0.01	−0.05	−0.03	−0.18	0.19	−0.44*	0.25	0.03	0.24
Tomato	−0.59**	−0.65***	−0.56*	−0.71***	−0.43*	−0.56**	−0.52**	−0.44*	−0.69***	−0.41*	−0.58**	−0.44*	−0.59**

Cond, conductivity; Inorg N, total inorganic nitrogen; Ext, water-extractable content; Tot, total content, obtained from acid digestion.
Correlations were based on 24 samples except K data where 19 cases were used; *, ** and *** indicate significance at 0.05, 0.01 and 0.001 probability levels, respectively.

tractable Cu and Zn increased rapidly and reached the final concentrations of 59 and 16 $\mu g\ g^{-1}$, respectively (Fig. 4B and F). This suggested that if spent litter was disposed within two batches of pig raising, it would not affect plant development but it would be dangerous to re-use spent litter older than 7 months as large amounts of Cu and Zn would become available which could cause damage to plants.

Relationship between germination performance and chemical properties

The seed germination percentages of both cabbage and lettuce were inversely related to the concentrations of extractable Cu and Zn, with r (correlation coefficients) values greater than 0.7 (Table 3). Besides Cu and Zn, significant negative values of r were also found between seed germination and contents of inorganic N, NH_4^+-N, and extractable K in these two species. However, the seed germination of green beans and tomato was not related to any chemical property determined. A slightly different picture was obtained when root length was considered. Root lengths of all plant species except green beans were negatively associated with many chemical parameters of the spent litter, including total and extractable Cu, inorganic and NH_4^+-N, total and extractable K, conductivity and ash content (at $P<0.01$ level, Table 3). These

TABLE 4

Multiple regression analysis of seed germination bioassays and chemical properties of spent litter extract

Plant species	Regression equation*	*r*	Adjusted *r*	*f*	Significance of *f*
Seed germination					
Cabbage	−1.06 Ext-Cu + 0.03 Tot-Mn + 99.4	0.89	0.77	31.6	<0.0001
Lettuce	−1.09 Ext-Cu + 105.2	0.79	0.61	29.3	<0.0001
Green bean	−0.02 Tot-Zn + 67.8	0.50	0.21	5.8	0.0281
Tomato		NS			
Root elongation					
Cabbage	−10.41 NH_4 + 91.9	0.87	0.74	51.5	<0.0001
Lettuce	−11.80 NH_4 + 105.8	0.91	0.81	79.1	<0.0001
Green bean	−7.65 Ext-Zn + 0.018 Cond + 101.9	0.72	0.46	8.5	0.003
Tomato	−20.67 Tot-K + 95.6	0.71	0.48	17.5	0.0006
Germination index					
Cabbage	−12.19 NH_4 + 99.9	0.87	0.76	57.9	<0.0001
Lettuce	−13.56 NH_4 + 111.3	0.92	0.83	90.0	<0.0001
Green bean	−6.61 Ext-Zn + 6.45 Ash − 11.44 NH_4 + 101.9	0.82	0.61	10.5	0.0006
Tomato	−19.79 Tot-K + 89.5	0.68	0.43	14.4	0.0015

*Regression equation was calculated based on 13 chemical parameters, using SPSS-PC statistical package with STEPWISE METHOD and PIN (probability of *f*-to-enter) = 0.05 limit.
NS, not significant at PIN = 0.05 limit.

results implied that among the four plant species examined, green beans was relatively unaffected by the potentially toxic elements presented in the spent litter.

The multiple regression analyses demonstrated that among the 13 chemical parameters of spent litter being examined, concentration of extractable Cu was the most important single factor inhibiting seed germination while NH_4^+-N content appeared to be the most significant parameter retarding root growth in cabbage and lettuce (Table 4). It can be concluded that high concentrations of Cu and NH_4^+-N in spent litter were most detrimental to the growth and development of cabbage and lettuce. However, tomato and green beans were less sensitive, and no single chemical property of spent litter could satisfactorily explain the inhibition of seed germination in tomato. It is generally agreed that seeds of legumes and root crops, containing higher quantities of food reserves, were less sensitive to toxicity than leafy plants (Cheung et al., 1989).

DISCUSSION

A seed germination test has been used as a rapid, simple, reliable and reproducible technique to indicate the damaging effects of land application of animal manure, compost or sewage sludge on plant growth (Baca et al., 1990). Root elongation was found to be a more sensitive parameter than seed germination, as a reduction in root growth was observed throughout the study while inhibition of seed germination was found only towards the end of the experiment. Ratsch (1983) concluded that inhibition of root elongation was a valid and sensitive indicator of toxicity. Germination index (GI) which combines germination and root growth, has proved to be a very sensitive parameter. GI is able to account both for low toxicity, which affects root growth and increased toxicity which affects germination (Zucconi et al., 1981a). The present study revealed that young spent litter, being used in the POL system for a period less than 30 weeks, had low toxicity. Only root elongation of cabbage, lettuce and tomato was retarded, while seed germination of all plant species was somewhat indifferent to the spent litter extract (Figs. 1 and 2). In contrast, root elongation of green beans was stimulated by young spent litter extract. Zucconi and his coworkers (1981b) found that the mature compost extracts had stimulatory effects on plant growth. When spent litter was collected after Week 30, severe retardation of both seed germination and root elongation was recorded for all plant species (Figs. 1 and 2), indicating that severe toxicity occurred in aged spent litter.

It has been suggested that inhibition of plant growth is directly related to the presence of water soluble phytotoxic substances present in compost (Inbar et al., 1990). Gupta and Doherty (1990) concluded that the toxicity of poultry litter extract resulted from the presence of NO_3, NH_3, metals, organic and inorganic salts. The phytotoxicity of organic wastes was also due to high electrical conductivity, an excess of ammonia, phenolic substances, organic acids of low molecular weight and other phytotoxic organic metabolites (Garcia et al., 1992a). In the present study, the accumulated concentrations of salts, nitrogen, Cu and Zn, especially their water-soluble counterparts, were the major problems associated with aged spent litter. The presence of so many complex chemicals (including metal ions and organic pollutants) in the extract caused toxicological interactions varying from synergistic to anatogonistic (Gupta and Kelly, 1990), and made it difficult to identify which was the most significant factor causing plant damage in the bioassay. The multiple regression analyses showed that the elevated concentrations of extractable Cu were the most significant factor inhibiting seed germination, while NH_4^+-N was the main agent causing injury to root development. It has been reported that low concentrations of some trace metals retard root elongation and delay seed germination in a large range of plants (Wong and Bradshaw, 1982; Wong and Lau, 1983). Ammonia produced upon decay of animal manure can also inhibit seed germination and seedling growth of *B. parachinensis* (Ellis et al., 1991; Wong et al., 1983).

Pig manure often contains high concentrations of copper compared with other animal manures, because Cu supplements are normally added to pig rations at concentrations up to 250 $\mu g\ g^{-1}$ to accelerate weight gain and increase food conversion rates of fattening pigs. In addition, Zn is also added to pig diets to counteract any toxicity which might be caused by the high Cu content (Hanraham and O'Grady, 1968). 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. This explains why spent litter contained extremely high levels of Cu and Zn, and their contents increased proportionally with the age of the spent litter (Fig. 4). Reducing the amounts of Cu and Zn added to the pig diet might be one possible way to alleviate the heavy metal toxicity of spent litter.

The spent litter used in this study was removed directly from the POL system and the decomposition might be incomplete, the litter could be viewed as an immature compost. The phytotoxicity of immature compost was much higher than the mature compost (Baca et al., 1990; Garcia et al., 1992b; Zucconi et al., 1981a,b) because of the presence of toxins in decomposing organic material, NH_3, high C:N ratio and the availability of heavy metals (Inbar et al., 1990). 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 (Inbar et al., 1990; Zucconi et al., 1981a, b). This suggests that the toxicity of aged spent litter could be ameliorated if it is left for further composting and maturation before re-utilization as a soil amendment.

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