

EVALUATION OF PHYTOTOXICITY OF COMPOST DURING COMPOSTING PROCESS

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Abstract: The phytotoxicity of compost as germination index (GI) was evaluated during composting period. This study suggested that $\text{NH}_4^+\text{-N}$ and heavy metals in the organic wastes were major compounds inhibiting seed germination and root elongation. The increase in GI of cress plant during composting coincided with the decrease in $\text{NH}_4^+\text{-N}$, and water extractable Zn and Cu content to very low levels, and with the increase in $\text{NO}_3^-\text{-N}$ and water extractable P and K content. Negative correlation was observed between GI of cress with $\text{NH}_4^+\text{-N}$, Cu, and Zn content, while positive correlation was observed with $\text{NO}_3^-\text{-N}$, P and K content. $\text{NH}_4^+/\text{NO}_3^-$ ratio has been used as an index of compost maturity where values were decreased as composting progresses.

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1. Introduction

Phytotoxicity is one of the most important criteria for evaluating the suitability of compost for agricultural purposes and to avoid environmental risks before these composts can be recycled back to agricultural land. [Tiquia *et al.*, 1996; Brewer & Sullivan, 2003 and Cooperband *et al.*, 2003]. Previous research work has demonstrated that application of immature compost onto the soil causes negative effects on seed germination, plant growth and development. These effects occur because an immature compost induces high microbial activity (which reduce oxygen concentration in the soil), blocks the existing soil available nitrogen [Zucconi *et al.*, 1981a]. Immature compost also introduce phytotoxic compounds such as heavy metals [Tam and Tiquia, 1994]. Phenolic compounds [Wong, 1985], ethylene and ammonia [Tam and Tiquia, 1994], excess accumulation of salts [Tam and Tiquia, 1994], and organic acids [Manios *et al.*, 1989] which could retard seed germination and plant growth.

Acetic acid is probably the most damaging organic acid released from immature compost, but there are also other compounds that contribute to the phytotoxic effect [Ozores-Hampton, 1998].

In fact, application on soil of no stabilized organic materials could affect both crops and the environment because of the presence of phytotoxic compounds [Butler *et al.*, 2001]. High concentrations of salt and the release of organic acids into the composts are also correlated to inhibition of germination and growth. Phytotoxicity is often best evaluated by conducting germination or growth tests [Gariglio *et al.*, 2002 and Brewer and Sullivan, 2003], but the test plants have to be chosen with care [Emino and Warman, 2004].

Germination Index (GI) is the best way to test the phytotoxicity of compost to plant growth because the results of it are quite straightforward and reliable. Germination bioassays are widely used to test for salinity, soil pathogens, toxic substances and some other physical and chemical properties of compost [Zucconi *et al.*, 1985

and Gajdos 1997], which could be the major potential reasons of phytotoxicity. Several investigators reported that phytotoxic compounds are gradually eliminated during the composting process, which could explain the GI increases with composting time.

The Germination index (GI), which combines measures of relative seed germination (G%) and relative root elongation (L%), has been used to evaluate the toxicity of compost. [Tam and Tiquia, 1994, Tiquia *et al.*, 1996 and Wong *et al.*, 2001]. It has been noted that a GI value of 80% indicated the disappearance of phytotoxins in composts [Zucconi *et al.*, 1981b]. [Tiquia *et al.*, 1996] used this value not only as indication of the disappearance of phytotoxicity but also as an indication of the maturity of compost.

The germination index is a maturity test based on seed germination and initial plant growth using a liquid extract from the compost [Zucconi, *et al.*, 1981b]. It reflects the phytotoxicity of the compost extracts at different stages of composting. The compost is considered mature when the germination index is higher than 60 %, compared to the control with distilled water [Zucconi and De Bertoldi, 1987].

The most popular germination test used by researchers is from cress test [Zucconi *et al.*, 1981a and Erhart and Burian, 1997]. In their opinion, the compost is non-toxic when the germination rate is more than 85% or the plant seedling weights are more than 90%. Beside, composting scientists found that GI at each composting time did not show any significant changes with the dilutions of the extract, or even when the extract was diluted up to 75% with distilled water. They conducted that ammonia and low molecular weight organic acids were two phytotoxic substances proposed and plant growth increased as these disappeared. [Wei *et al.*, 2000] reported that when the GI is more than 80 % Compost is considered mature and practically free of phytotoxic substances.

An increased GI is indicative of decreased phytotoxicity and thus of a more mature product [Tiquia *et al.*, 1996; Bernal *et al.*, 1998; Tiquia and Tam, 1998 and

Wong *et al.*, 2001]. However, results obtained using the GI index should be interpreted with care because they are affected by the type of seed used and the source of compost [Bernal *et al.*, 1998; Tiquia and Tam, 1998; and Brewer and Sullivan, 2003]. Application of undecomposed wastes or non-stabilized compost to land may lead to immobilization of plant nutrients and cause phytotoxicity [Butler *et al.*, 2001 and Cambardella *et al.*, 2003].

In fact, germination index is the most sensitive parameter used to evaluate toxicity of compost to seedlings, and to test if compost is mature [Zucconi *et al.*, 1981a; Wong *et al.*, 2001 and Meunchang *et al.*, 2005].

Tang *et al.* (2006) reported that extraction rate is a very important factor that influences GI. They conducted that a 10:1 extraction rate is suitable for evaluating the GI change during the maturing of cattle manure compost. They also declared that different extraction rates gave different changing patterns of GI during the maturing process.

2. MATERIAL AND METHODS

2.1. Agricultural wastes:

Agricultural residues (Corn stalks, cotton stalks and bagasse) were collected from, Qalubia Governorate, to be used for compost production. The used materials along with their chemical analyses are presented in Table (1).

2.2. Organic accelerators:

Cattle manure and poultry manure were collected from Qalubia Governorate. Mature compost was provided from the Egyptian Company for Agricultural Residues Utilization (ECARU). Cairo, and used as an additional source of microorganisms.

2.3. Cultivar:

Cress seeds (*lepidum sativum*) were provided from the Crops Research Institute, Agricultural Research Center, Giza, were used in this investigation.

2.4. Compost preparation:

Six heaps, 100 Kg dried weight each, were prepared from air dried and shredded wastes (corn stalks, cotton stalks and bagasse) using either of six different treatments including: cattle manure (Heap1) and treated with 1% mature compost (Heap 2), poultry manure (Heap3) and treated with 1% mature compost (Heap4), sewage sludge (Heap5) and treated with 1 % mature compost (Heap6). The weights of the raw materials used for particular compost were calculated according to [Christian *et al.* 1997] that give a C/N ratio 30:1, the moisture content of the composting mass was kept at 60%. The heaps were covered with black plastic sheets to avoid heat and water loss. The heaps were turned and mixed every 5 days to provide aeration. Moisture content was adjusted at each turning time and maintained by addition of water.

2.5. Compost water extracts:

A water extract of each compost was prepared by shaking the samples with distilled water at 1:10 w/v ratio for 1 hour, and then filtered [Zucconi *et al.*, 1981b].

2.6. Phytotoxicity evaluation:

The phytotoxicity of compost extracts was evaluated by the seed germination technique [Zucconi *et al.* 1981b, Tam and Tiquia, 1994 and Tiquia *et al.*, 1996]. Cress seeds (*lepidum sativum*) were surface sterilized by immersion in 75% alcohol for three minutes followed by transferring in 0.001 HgCl₂ solution for two minutes with periodical agitation and finally thoroughly washed with sterilized distilled water to get rid of toxic chemicals [Rovira, 1956]. 10 ml of water compost extract was applied to filter paper in a Petri dish and 20 seeds were then placed on the filter paper. All experiments were run in triplicate. The Petri dishes were sealed with tape to minimize water loss while allowing air penetration and then were incubated in the dark for 72 hours at room temperature, the seed germination percentage and root length of the plants in the extracts were determined. The seed germination in distilled water was used as control. The percentage of seed germination, root elongation and germination index (GI) was calculated according to [Zucconi *et al.*, 1981b] as follows:

Seed germination (%)

$$= \frac{\text{No. of seeds germinated in compost extract}}{\text{No. of seeds germinated in control}} \times 100$$

Root elongation (%)

$$= \frac{\text{Mean root length in compost extract}}{\text{Mean root length in control}} \times 100$$

Germination Index

$$= \frac{\text{Seed Germination (\%)} \times \text{Root elongation (\%)}}{100}$$

The germination index was able to account for both low toxicity, which affects root growth and high toxicity, which affects germination. Coefficient of correlation (R²) was also made to determine the possible relationship between bioassay results and the chemical properties of compost as follows:

$$R^2 = \frac{\sum (x_i - \bar{x})^2}{\sum (y_i - \bar{y})^2}$$

2.7. Chemical determinations:

Nitrate-N and ammonical-N were determined according to the method described by [Page *et al.*, 1982]. Available phosphorus was extracted with 0.5M NaHCO₃ and determined calorimetrically in sulphomolybdic acid system as described by [Jackson, 1973]. Available potassium was extracted in boiling HNO₃ acid solution and flame-photometrically determined according to [Page *et al.*, 1982]. Water-extractable 1:10 (W/V) Cu and Zn were determined by atomic absorption spectrometry method [page *et al.*, 1982]. Statistical analysis of the obtained data was carried out according to [Snedecor and Cochran, 1967].

3. RESULTS AND DISCUSSION

The responses of cress plant (*lepidum sativum*) to the toxicity of the compost water extracts during the composting period in term of the relative seed germination and relative root elongation percentages were illustrated in Figures (1 and 2).

At the beginning of composting, the GI of cress plants was very low with initial values of 18.76 – 20.53 % compared to the control using distilled water, probably due to the phytotoxic effects of ammonia and low molecular weight organic acids [Zucconi *et al.*, 1981b and Wong *et al.*, 2001], but as composting proceeded, the GI value increased. At day 80 in this study the GI values were higher for inoculated heaps than uninoculated heaps. However, after composting proceeded, the GI value increased to greater than 80% (81.88 – 97.46) revealing that the phytotoxicity in these heaps was eliminated after composting. The elimination of phytotoxicity has also been widely used as a measure of compost maturity [Wei *et al.*, 2000; Wu and Ma, 2001; Butler *et al.*, 2001 Cambaradella *et al.*, 2003 and Menunchang *et al.* 2005].

Data in Table (2) clearly show that ammonium nitrogen $\text{NH}_4^+\text{-N}$ levels increased rapidly in the first few days due to the microbial degradation of nitrogen containing compounds (ammonification process) which converted a fraction of the organic N to NH_3 and NH_4^+ ions. Then the $\text{NH}_4^+\text{-N}$ content of all heaps decreased gradually. The inoculated heaps had lower $\text{NH}_4^+\text{-N}$ concentrations than uninoculated heaps by the end of composting process, indicating a much more dynamic N transformation during composting. It has been noted that the absence or decrease in $\text{NH}_4^+\text{-N}$ is an indication both for good composting and maturation process [Riffaldi *et al.*, 1986 and Tiquia *et al.* 1996]. Decreases in $\text{NH}_4^+\text{-N}$ content in all composts were observed which was in parallel with the increases in the content of $\text{NO}_3^-\text{-N}$ during the composting process. The $\text{NO}_3^-\text{-N}$ contents of all heaps increased dramatically during composting time. The decreasing in $\text{NH}_4^+\text{-N}$ and increasing in $\text{NO}_3^-\text{-N}$ contents were attributed due to the rapid conversion of NH_4^+ to NO_3^- through nitrification process by the microorganisms during composting [Tiquia *et al.*, 1996 and Tiquia, 2003].

$\text{NH}_4^+ / \text{NO}_3^-$ ratio has been used as an index of compost maturity where values decreasing as composting progresses due to nitrification which converts NH_4^+ to NO_3^- [Forster *et al.*, 1993 and Bernal *et al.*, 1998]. When the NH_4^+ concentration decreases and NO_3^- appeared in the compost material it is considered ready to be as mature compost [Finstein and Miller, 1985]. [Bernal *et al.*, 1998] showed that a high $\text{NH}_4^+ / \text{NO}_3^-$ ratio founded out that there is unstabilized materials during the process.

They reported $\text{NH}_4^+ / \text{NO}_3^-$ ratio values <1 at the end of active composting. It is clear from data presented in Table (3), that the initial ratio of $\text{NH}_4^+ / \text{NO}_3^-$ ranged from 6.10 to 29.78, and decreased to 0.05 and 0.43 at the end of composting period. It was conducted by [Bernal *et al.*, 1998 and Meunchang *et al.*, 2005] that mature compost is expected to have reduced ratio of $\text{NH}_4^+ / \text{NO}_3^-$.

During the initial stage of composting, the water extractable P and K of compost heaps ranged from 3.96 to

6.28 mg/g and 5.59 to 9.30 mg/g, respectively. At mature stage, these levels increased to 10.62 and 17.75 mg/g for extractable P and 15.29 and 29.36 mg/g for extractable K (Table, 4). [Georgacakis *et al.*, 1996] also founded an increase in extractable phosphorus in the final compost product, which support the results found in this study.

As reported in Table (5) the initial concentrations of water-extractable Cu and Zn of all heaps ranged from 6.20 to 28.68 ppm and 21.47 to 69.22 ppm respectively. At mature stage these levels both decreased to between 1.64 to 5.90 ppm for water extractable Cu and 6.01 to 12.22 ppm for water extractable Zn. As composting progressed, the water-extractable Cu and Zn decreased. However, in the literature these parameters have never been considered as indices of compost maturity. Rather, they are often used to assess the quality of compost in terms of potential environmental contamination [Tiquia and Tam, 1998].

The content of the water extractable form of the heavy metals is more important than total metal concentration [Tiquia *et al.*, 1996]. The water-extractable Cu and Zn concentrations decreased during composting because they bind with the humic substances [Ciavatta *et al.*, 1993]. The decline in water extractable Cu and Zn was due to the formation of complexes of these metals with chelating organic compound formed (a metal-humus complex), thus making them not water-extractable and biologically unavailable [Leita *et al.*, 1999].

As shown in Figure (3) the germination index of cress showed a significant negative correlation with $\text{NH}_4^+\text{-N}$ content ($R^2=0.95$). A significant positive correlation was observed between GI of cress with $\text{NO}_3^-\text{-N}$ content ($R^2=0.85$). Negative correlations were also observed between GI of cress with water extractable Zn and Cu as shown in Figure (4), $R^2=0.70$ and 0.58 for Zn and Cu respectively. Significant positive correlation were observed between GI of cress with water extractable P ($R^2=0.75$) and K ($R^2=0.86$) as shown in Figure (5). This study suggested that $\text{NH}_4^+\text{-N}$ and heavy metals in the organic wastes were major compounds inhibiting seed germination and root elongation. The increase in GI of cress plant during composting coincided with the decrease in $\text{NH}_4^+\text{-N}$, and water extractable Zn and Cu content to very low levels, and with the increase in $\text{NO}_3^-\text{-N}$ and water extractable P and K content. The decrease in $\text{NH}_4^+\text{-N}$ content to low levels was associated with the accumulation of $\text{NO}_3^-\text{-N}$ via the nitrification process.

This result is in agreement with that obtained by [Tiquia *et al.*, 1996], which reported that $\text{NH}_4^+\text{-N}$ was the most important factor inhibiting seed germination and root elongation of all species examined. [Meggie *et al.*, 1967 and Wong *et al.*, 1983] reported that ammonia generated from decomposing organic compounds and animal manure is detrimental to seed germination and seedling growth.

4. CONCLUSION

The sum of the above results showed that, un-mature compost (high levels of NH_4^+ to NO_3^- and heavy metals) inhibiting seed germination and root elongation..

Table 1. Chemical characters of organic wastes used for composting.

Character	Corn stalks	Cotton stalks	Bagasse	Cattle manure	Poultry manure	Mature compost
Total Carbon %	50.59	54.00	56.47	41.96	29.00	40.02
Total Nitrogen %	0.61	0.70	0.42	1.88	3.90	2.03
C:N Ratio	83.63	77.15	134.45	22.32	7.44	19.68

Table 2 . Changes in N-NH₄⁺ and N-NO₃⁻ concentration during the composting process of different heaps

Time (days)	Heap 1	Heap 2	Heap 3	Heap 4	Heap 5	Heap 6	LSD 0.05
N-NH₄⁺ (mg/Kg)							
0	382	377	581	573	167	165	149
5	535	565	814	860	225	247	221
10	508	537	773	817	214	235	210
20	325	344	495	523	137	150	134
30	260	275	396	418	109	120	107
40	208	192	317	293	88	84	80
60	133	123	203	187	56	54	51
80	85	60	130	92	31	26	30
100	55	39	83	59	20	17	19
120	44	31	66	47	16	14	15
LSD 0.05	170	188	259	286	73	82	
N-NO₃⁻ (mg/Kg)							
0	41	43	20	22	24	27	7
5	82	86	39	44	49	54	15
10	73	78	35	40	44	49	13
20	88	93	42	48	53	58	16
30	106	112	51	57	63	70	19
40	127	134	61	74	76	91	23
60	183	193	87	125	110	142	33
80	241	255	115	165	145	204	46
100	292	308	140	218	175	247	55
120	322	340	154	240	193	273	61
LSD 0.05	96	101	46	78	57	87	

Table 3. Changes in NH₄⁺ / NO₃⁻ ratio during the composting process of different heaps.

Time (days)	Heap 1	Heap 2	Heap3	Heap4	Heap 5	Heap 6	LSD 0.05
NH₄⁺ / NO₃⁻ ratio							
0.00	9.36	8.74	29.78	25.93	6.82	6.10	8.12
5.00	6.55	6.55	20.85	19.45	4.60	4.57	5.87
10.00	6.92	6.92	22.00	20.53	4.86	4.83	6.19
20.00	3.69	3.69	11.74	10.95	2.59	2.58	3.28
30.00	2.46	2.46	7.82	7.30	1.73	1.72	2.18
40.00	1.64	1.43	5.22	3.93	1.15	0.92	1.37
60.00	0.73	0.64	2.32	1.50	0.51	0.38	0.59
80.00	0.35	0.24	1.12	0.56	0.22	0.13	0.28
100.00	0.19	0.13	0.59	0.27	0.11	0.07	0.15
120.00	0.14	0.09	0.43	0.20	0.08	0.05	0.11
LSD 0.05	3.04	2.98	9.66	8.93	2.18	2.10	

Table 4. Changes in water extractable P and K concentrations during the composting process in different heaps.

Time (days)	Heap1	Heap 2	Heap 3	Heap 4	Heap 5	Heap 6	LSD 0.05
Extractable P (mg/g)							
0	4.80	4.77	3.97	3.96	6.28	6.23	0.84
5	4.99	5.20	4.09	4.31	6.53	6.79	0.90
10	5.67	6.13	4.60	5.13	7.35	7.93	1.04
20	7.31	8.50	5.94	7.14	9.31	10.85	1.46
30	8.33	9.77	6.77	8.21	10.66	12.48	1.70
40	9.33	10.65	7.59	8.94	11.99	13.66	1.84
60	10.69	11.74	8.69	10.45	13.79	15.11	1.94
80	11.93	12.72	9.13	10.89	14.63	15.58	2.05
100	12.88	13.59	9.91	11.66	16.02	16.70	2.11
120	14.46	15.17	10.62	13.00	17.16	17.75	2.19
LSD 0.05	3.19	3.34	2.30	2.89	3.75	3.87	
Extractable K (mg/g)							
0	9.30	9.24	7.75	7.71	5.59	5.59	1.27
5	9.67	10.07	7.98	8.40	5.82	6.09	1.37
10	10.99	11.86	8.98	9.99	6.55	7.11	1.66
20	14.17	16.45	11.58	13.91	8.29	9.73	2.57
30	16.16	18.91	13.20	15.99	9.49	11.19	2.99
40	18.10	20.62	14.80	17.42	10.68	12.25	3.20
60	20.72	22.73	16.96	20.34	12.29	13.54	3.47
80	23.13	24.63	17.82	21.20	13.03	13.96	4.01
100	24.98	26.32	19.33	22.70	14.27	14.97	4.18
120	28.04	29.36	20.72	25.32	15.29	15.92	4.97
LSD 0.05	6.19	6.46	4.48	5.62	3.34	3.47	

Table 5. Changes in water extractable Zn and Cu concentrations during the composting process in different heaps.

Time (days)	Heap 1	Heap 2	Heap 3	Heap 4	Heap 5	Heap 6	
Extractable Zn (mg/g)							
0	30.79	30.88	21.47	21.69	69.22	68.71	18.04
5	40.56	42.63	28.01	29.95	91.18	94.87	24.37
10	33.94	37.02	23.22	26.25	75.58	70.75	18.45
20	27.37	32.08	18.72	22.84	41.89	48.86	9.25
30	18.72	22.13	12.81	15.76	35.63	41.74	9.27
40	17.47	20.10	11.96	14.30	31.61	36.04	7.75
60	16.00	17.73	10.97	12.58	26.60	29.15	5.90
80	14.71	15.83	10.08	11.47	23.52	25.05	4.91
100	12.86	13.69	8.82	9.94	14.54	15.17	2.34
120	8.66	9.16	5.95	6.65	14.71	15.23	3.15
LSD 0.05	9.65	10.41	6.66	7.27	24.72	25.06	
Extractable Cu (mg/g)							
0	13.96	13.82	6.23	6.20	28.68	28.31	8.19
5	18.15	18.83	8.02	8.45	37.28	38.57	10.91
10	14.43	15.54	6.73	7.51	29.36	29.43	8.18
20	11.64	13.46	5.09	6.12	17.20	21.57	5.09
30	8.22	9.60	3.60	4.37	15.44	16.12	4.36
40	7.43	8.44	3.25	3.84	12.28	14.26	3.60
60	6.80	7.44	2.98	3.37	11.02	12.01	3.03
80	6.61	7.02	2.90	3.25	7.67	9.29	2.04
100	5.47	5.74	2.40	2.67	6.02	5.47	1.33
120	3.87	4.04	1.70	1.87	5.33	5.88	1.38
LSD 0.05	4.14	4.35	1.87	1.99	10.06	10.07	

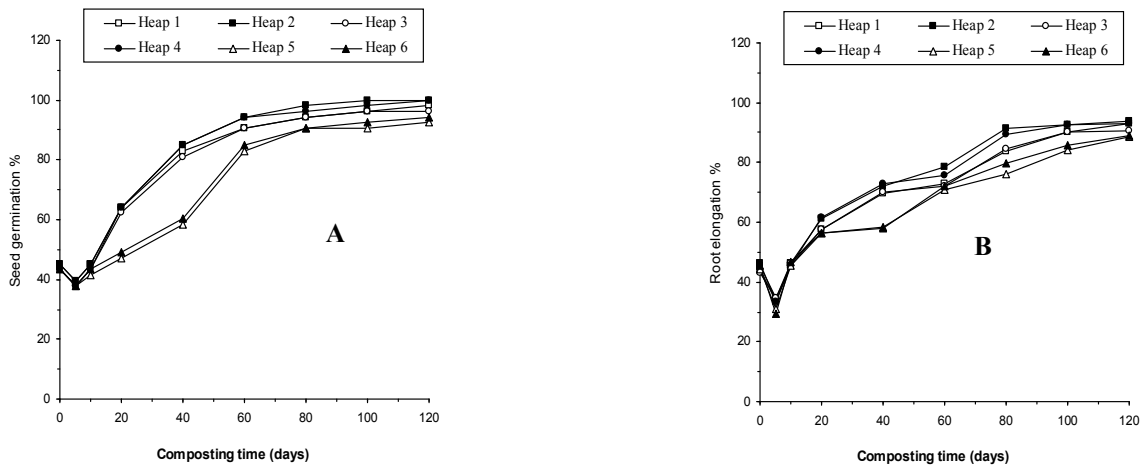


Fig. 1. Seed germination (A) and root elongation (B) % of cress grown on compost extracts of different heaps during composting period.

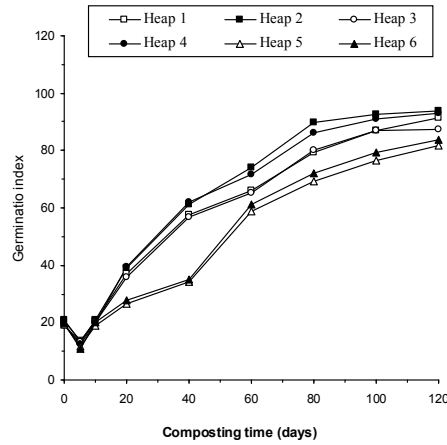


Fig. 2. Germination index (GI) of cress seeds grown on compost extracts of different heaps during composting period.

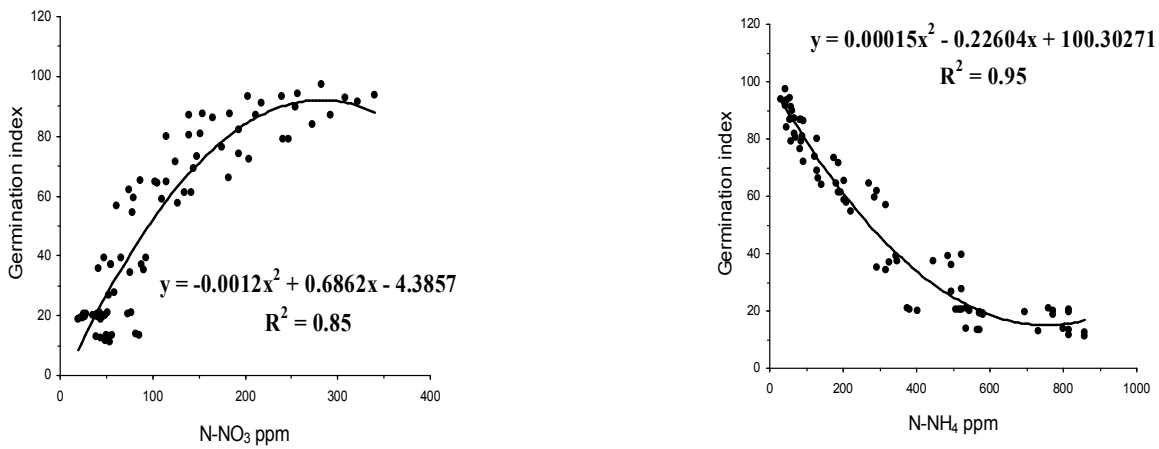


Fig. 3. Relationship between germination index (GI) and each of $N-NO_3^-$ and $N-NH_4^+$ concentrations in the compost extracts during the composting period of different heaps.

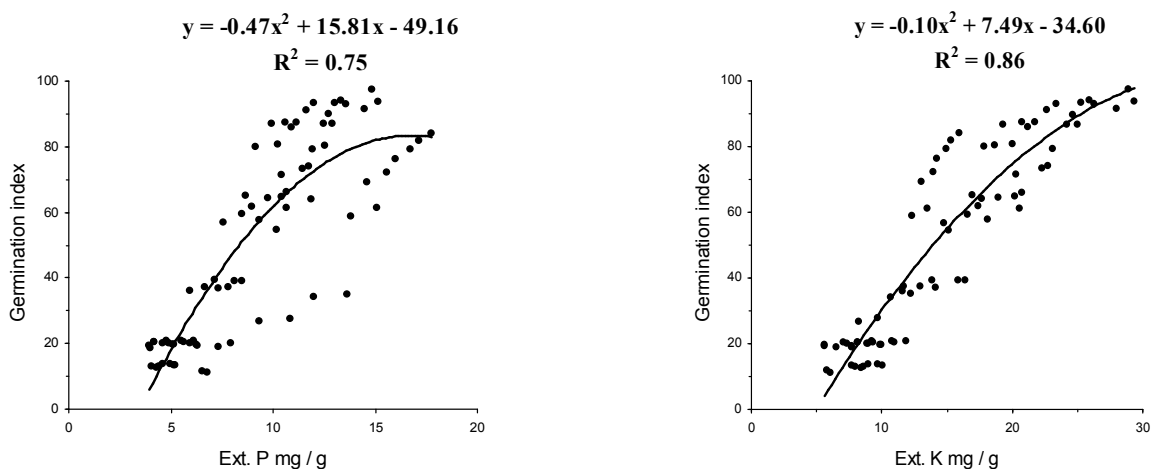


Fig. 4. Relationship between germination index (GI) and each of water ext. P and water ext. K concentrations in the compost extracts during the composting period of different heaps.

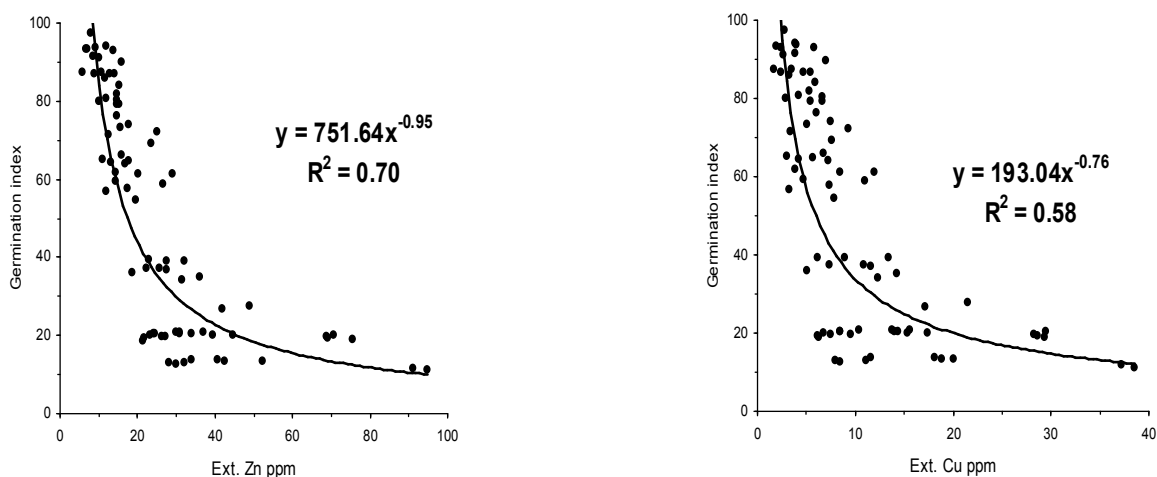


Fig. 5. Relationship between germination index (GI) and each of water ext. Zn and water ext. Cu concentrations in the compost extracts during the composting period in different heaps.

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