

Extracellular enzyme profiles during co-composting of poultry manure and yard trimmings

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Abstract

APZYTM assays were used to detect the presence of enzyme activity from 19 different enzymes, including three phosphatases, three esterases, three amino-peptidases, and eight glycosyl-hydrolases during co-composting of poultry manure and yard trimmings (poultry + yard trimmings). Results of this study have shown that the poultry + yard trimmings compost went through physico-chemical and biochemical changes during composting. These changes included self-heating of the compost mass, relative increases in enzyme activities, decreases in water-soluble components (i.e. water-extractable C, inorganic N, and heavy metal contents), and elimination of phytotoxicity. An overall increase in enzyme activity was observed over the course of the present experiment. Alkaline and acid phosphatase, and leucine amino-peptidase activities were high, while lipase, esterase, and esterase activities were moderate at the beginning of composting. Cystine amino peptidase, chymotrypsin, and trypsin showed no evidence of activity during the entire period of composting. Of the eight glycosyl-hydrolases, only α -galactosidase, β -glucosidase, and *N*-acetyl- β glucosaminidase showed any significant activity, fluctuating between low and moderate activity. The activity of α -mannosidase was low while β -galactosidase, β -glucuronidase, and α -fucosidase remained undetected during the entire testing period). Although this enzyme test is rather preliminary, the results of this study seem to show potential usefulness of enzyme activity measurements as indices of the course of the actual composting. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Manure composting; Enzyme activities; Phytotoxicity; Compost maturity; β -glucosidase

1. Introduction

The composting reaction is a biochemical decomposition of organic matter of the starting material by microorganisms [1–3]. During composting, the starting material is modified by decomposition and humification through a wide variety of biological and biochemical processes. Enzymes play a key role in these transformations [4,5]. For instance, the mineralization of organic N, which involves the release of N from nonpeptide C–N bonds in amino acids and urea is mediated by enzymes such as amidohydrolases and dehydrogenases [6]. Alkaline and acid phosphatases are important en-

zymes in organic P mineralization and plant nutrition [7]. Enzymes in composts can be classified as intracellular (enzymes inside viable cells) or extracellular (enzymes outside viable cells) [8]. Intracellular enzymes are enzymes that catalyze biochemical reactions occurring within cells [9]. Many of these enzymes can be found in soil/compost apart from cells, and are thought to be released by cell lysis. Extracellular enzymes are enzymes purposely released exterior to cells, generally to catalyze the degradation of a polymeric substance too large to cross the cellular membrane [9]. In N transformation, extracellular enzymes depolymerize proteins, aminopolysaccharides (microbial cell wall), and nucleic acids and hydrolyze urea [5,10]. The production of ammonium, on the other hand, occurs within microbial cells through the action of intracellular enzymes [11]. Intracellular and extracellular enzymes cannot be dis-

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tinguished in compost suspensions. However, after a brief incubation period, the extracellular groups of enzymes can be assigned more readily to which a large portion of enzymes in soils and composts belong [8,12].

Microbes in the compost pile cannot directly metabolize the insoluble particles of organic matter [13–15]. Rather, they produce hydrolytic extracellular enzymes to depolymerize the larger compounds to smaller fragments that are water-soluble. The water-soluble components dissolve in water and are assimilated by microorganisms in the compost. The insoluble components on the other hand, are decomposed by secreted microbial enzymes (extracellular enzymes) into water-soluble matter and subsequently absorbed into the microbial cells [4]. Consequently, by detecting the change in components in the water extracts of compost during composting, the composting process can be better understood, and parameters can be sought that can give a measure of compost maturity. In this paper, the extracellular enzyme profiles of poultry manure and yard trimmings (poultry + yard trimmings) were evaluated using APYZYM™ strips to evaluate the extracellular enzyme activities at various stages of composting. The strips allowed the systematic and rapid study of 19 enzymic reactions in the poultry + yard trimmings extracts. Since the metabolism of microorganisms occurs in the water soluble fraction of the compost, the quantitative changes in chemical components of the poultry + yard trimmings water extracts were also determined during composting.

2. Materials and methods

2.1. Composting piles

Poultry litter (a mixture of poultry manure, waste feed, wood shavings, and feathers) and yard trimmings were co-composted in static piles [16,17] at a ratio of 1:2 (poultry litter:yard trimmings; v/v). The piles were built on perforated pipes connected to an air pump (Regenair R1102). To insulate the piles and minimize water loss, the piles were covered with 5-cm layer of mature compost. The cover also acted as a biofilter to minimize odour emission. During composting, the air was pumped at a rate of 634 l min⁻¹ for 24 h once a week. The water content of the compost mixture was adjusted to 65% (w/v) at the beginning, and then weekly during the composting trial, which lasted for 91 days.

2.2. Sampling and temperature measurements

Temperature and poultry + yard trimmings samples were taken from three different locations of the compost piles: top (85 cm from the base of the pile), middle

(50 cm from the base of the pile) and bottom (30 cm from the base of the pile). Subsamples collected from these three locations were mixed homogeneously to generate one composite sample. Nine composite samples (3 locations × 3 piles; ~ 1 kg each) were collected at day 0 and then weekly until day 91.

2.3. Extracellular enzyme assay

Enzyme extracts were prepared by mixing 5 g from each sample with 50 ml of phosphate buffered water. The solution was shaken for 10 min using a stomacher (a shaker), allowed to settle for 10 min, and the supernatant was used for enzyme analysis. APYZYM™ strips (API Analytab Products, New York) were used to evaluate the extracellular enzyme profiles at different stages of composting. APYZYM™ is a commercial semi-quantitative micro-method designed for systematic and rapid study of 19 enzymatic reactions. It consists of a series of microcupules containing dehydrated chromogenic substrates of 19 different enzymes and one control (a microcupule containing no enzyme substrate). Two drops from each extract supernate were dispensed into each of the 20 microcupules. The strips were then covered and incubated at 37°C for 24 h. After incubation, one drop each of Reagent A and Reagent B (API Analytab Products, New York) were added to all microcupules. Following a waiting period for colour development of ~ 5 min, a numerical value of 1–5 was assigned to each microcupule according to the colour chart provided by the manufacturer. For the purposes of this study, the results were reported as reactions of low intensity (value of 1), moderate intensity (values of 2–4), and high intensity (value of 5).

2.4. Analysis of water-soluble fraction of poultry litter and yard trimmings compost

Water extracts of the poultry + yard trimmings were prepared by shaking the fresh sample with distilled water at 1:10 w/v using a horizontal shaker for an hour, and then filtered. The water extracts were measured for pH using a pH electrode; electrical conductivity (1:5 w/v compost:water extract) using an electrical conductivity electrode; concentrations of water-extractable C (total organic carbon analyser, Shimadzu, TOC-500); NH₄⁺-N and NO₃⁻-N by colourimetric method [18]; soluble phenol, expressed as vanillic acid was determined by Folin-Ciocalteu method [19]; and water-extractable Cu and Zn by atomic absorption spectrophotometry.

2.5. Phytotoxicity assay

The phytotoxicity of poultry + yard trimmings extracts on Chinese cabbage (*Brassica parachinensis*) was

monitored using the seed germination technique [20]. After 5 days of incubation in the dark, the seed germination percentage and root length of *B. parachinensis* in the water extracts were determined. The seed germination percentage and root elongation of *B. parachinensis* in deionized water were also measured and used as control. Finally, the germination index (GI) of the water extract was calculated based on relative seed germination percentage and relative root elongation.

2.6. Statistical analysis

Pearson product-moment correlation coefficients were computed to show relationship between different extracellular enzymes and chemical properties of poultry + yard trimmings compost. Statistical analysis was performed using a SYSTAT statistical computing package (SYSTAT Version 9.0).

3. Results

3.1. Temperature characteristics

The temperature characteristics of poultry + yard trimmings at different locations of the forced-aeration piles are shown in Table 1. The compost mass self-heated to 55°C in the first 2 days of composting, and began to peak by day 4. Peak temperature at the bottom location of the forced-aeration piles was lower (60°C) than the top and middle locations (71 and 70°C, respectively). Thermophilic temperatures (> 55°C) persisted for 17 days at the top and middle locations of the forced-aeration piles. In the bottom location, thermophilic temperatures were sustained for 13 days. After peaking temperatures in all three locations of the forced-aeration piles began to decline to ambient level. The temperature at the bottom location of the piles took only 42 days to reach ambient level,

whereas the top and middle locations took much longer (60 days, Table 1).

3.2. Extracellular enzyme profiles at different stages of composting

At each sampling period, the presence and activity of 19 extracellular enzymes were detected (Table 2). These enzymes include three phosphatases (alkaline phosphatase, acid phosphatase, and phosphohydrolase), three esterases (lipase, esterase-lipase, and esterase), three amino-peptidases (leucine amino-peptidase, valine amino-peptidase, and cystine amino-peptidase), two proteases (chymotrypsin and trypsin), and eight glycosyl-hydrolases (β -galactosidase, β -glucosidase, *N*-acetyl- β glucosaminidase, α -glucosidase, α -galactosidase, β -glucuronidase, α -mannosidase, and α -fucosidase).

Both alkaline and acid phosphatase activities were high from the beginning of the test reaching maximum activity by the end of the testing period (Table 2). Phosphohydrolase activity was moderate at the onset of composting, but had declined to low level by the end of composting. Overall, moderate level of lipase, esterase, and esterase activity was observed at the beginning of composting. The activity of esterase-lipase and esterase increased as composting proceeded, while lipase activity declined to low level by the end of the testing period. Leucine-amino peptidase activity was moderate by day 0, reaching maximum activity by day 14, and maintaining this level throughout the composting trial. Cystine amino peptidase, chymotrypsin, and trypsin showed no evidence of activity during the entire period of composting (Table 2). Of the eight glycosyl-hydrolases, only α -galactosidase, β -glucosidase, and *N*-acetyl- β glucosaminidase showed any significant activity, fluctuating between low and moderate activity. The activity of α -mannosidase was low while β -galactosidase, β -glucuronidase, and β -fucosidase remained undetected during the entire testing period (Table 2).

3.3. Chemical properties of water extracts

The chemical characterization of poultry + yard trimmings is illustrated in Fig. 1. Initial pH (Fig. 1A) was measured as 6.8, but rose quickly to 8.3 by day 14. On day 21, the pH began a gradual descent to ~7.0–7.2, where it remained for the rest of the composting period. The electrical conductivity fluctuated between 1550 and 3145 $\mu\text{S cm}^{-1}$ during composting (Fig. 1B). Concentration of water-extractable C decreased sharply from an initial value of 63.9–86.5 g kg^{-1} to very low levels (3.0–4.5 g kg^{-1}) by day 63. This level was maintained until the end of composting (Fig. 1C). As shown in Fig. 1D, the concentration of NH_4^+ -N decreased with composting time from an ini-

Table 1
Temperature characteristics at different locations of the forced-aeration piles

Pile temperature characteristics	Pile locations		
	Top	Middle	Bottom
Initial temperature	30°C	30°C	30°C
Time to reach 55°C	2 days	2 days	2 days
Time to reach >55°C	3 days	3 days	4 days
Peak temperature	71°C	70°C	60°C
Time to reach peak temperature	4 days	4 days	4 days
Duration of thermophilic stage (>55°C)	17 days	17 days	13 days
Time to drop at ambient temperature (35°C)	60 days	60 days	42 days

Table 2
Relative activity of extracellular enzymes extracted from poultry + yard trimmings at different stages of composting^a

Enzyme	Day 0			Day 7			Day 14			Day 63			Day 91		
	T	M	B	T	M	B	T	M	B	T	M	B	T	M	B
Phosphatases															
Alkaline phosphatase	4	4	4	4	4	4	5	5	5	4	4	4	5	5	5
Acid phosphatase	4	4	4	4	4	4	4	4	5	4	4	5	5	5	5
Phosphohydrolase	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3
Esterases															
Lipase	3	2	2	3	3	3	4	4	4	2	3	3	1	2	2
Esterase-lipase	2	2	2	3	3	3	4	4	4	4	3	4	4	4	4
Esterase	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3
Amino-peptidases															
Leucine amino-peptidase	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Valine amino-peptidase	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2
Cystine amino-peptidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proteases															
Chymotrypsin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trypsin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycosyl-hydrolases															
α -galactosidase	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3
β -glucosidase	3	3	3	1	1	1	1	1	1	3	3	3	3	3	3
<i>N</i> -acetyl- α -glucosaminidase	1	1	1	1	1	1	1	1	1	1	1	1	2	1	2
α -glucosidase	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
β -galactosidase	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
β -glucuronidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α -mannosidase	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2
α -fucosidase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

^a T, top; M, middle; B, bottom.

tial value of 2.6–4.4 g kg⁻¹ to low levels (0.07–0.72 g kg⁻¹). The reduction in NH₄⁺-N concentration corresponded to a concomitant increase in (NO₃⁻ + -NO₂⁻)-N concentration of the poultry + yard trimmings compost over the course of the study (Fig. 1E). Extractable P concentrations decreased gradually as composting progressed from an initial level of 4.18–5.40 g kg⁻¹ to a final value of 1.83–2.58 g kg⁻¹ (Fig. 1F), whereas concentrations of water-extractable K fluctuated between 9.49 and 13.30 g kg⁻¹ during composting (Fig. 1G). The contents of water-soluble phenols, and water-extractable Cu and Zn increased by day 14 and then declined gradually towards the end of the composting trial (Fig. 1H–1J).

3.4. Phytotoxicity test

The germination index (GI), was calculated from percentage of seed germination and root elongation, was used to indicate the disappearance of phytotoxicity in the present study. The initial readings of GI in all piles were lower than 80 in the present study but a strong increase in GI was found from day 7 onwards, with the GI values over 100 (Table 2). The GI values greater than 100 suggested that the water-extract had a stimulatory effect on plant growth.

4. Discussion

Results of this study have shown that the poultry + yard trimmings compost went through physico-chemical and biochemical changes similar to other composting systems [5,12,21–23]. The changes included self-heating of the compost mass, relative increases in enzyme activities, decreases in water-soluble components (i.e. water-extractable C, inorganic N, and heavy metal contents), and elimination of phytotoxicity.

4.1. Temperature and extracellular enzyme activities

The temperature data showed the rapid self-heating from ambient temperature of 30°C to a temperature of 55°C in the first 2 days of composting (Table 1). Temperature has been widely recognized as the single most important parameter in the composting process [24,25]. McKinley and Vestal [24] have shown that temperature has the greatest effect on both microbial biomass and microbial activity during composting of municipal sludge, with an optimal temperature being 30–50°C. Temperatures above 55°C exhibited marked decline on activity [24,26]. Strom [25] found that temperatures above 60°C decreased bacterial diversity,

whereas species diversity was similar in tests at 49, 50, and 55°C. In the present study, temperature peaked at temperatures between 60 and 71°C (Table 1), but this did not affect the activities of enzymes on days 7 and 14, with the exception of β-glucosidase and β-galactosidase (Table 3). β-glucosidase declined from a mod-

erate level to a low level of activity by day 7. That of β-galactosidase declined to a non-detectable level by day 7 until the end of composting (Table 3). The decline in enzyme activity was also observed by Hankin et al. [4] during the thermophilic stage of composting.

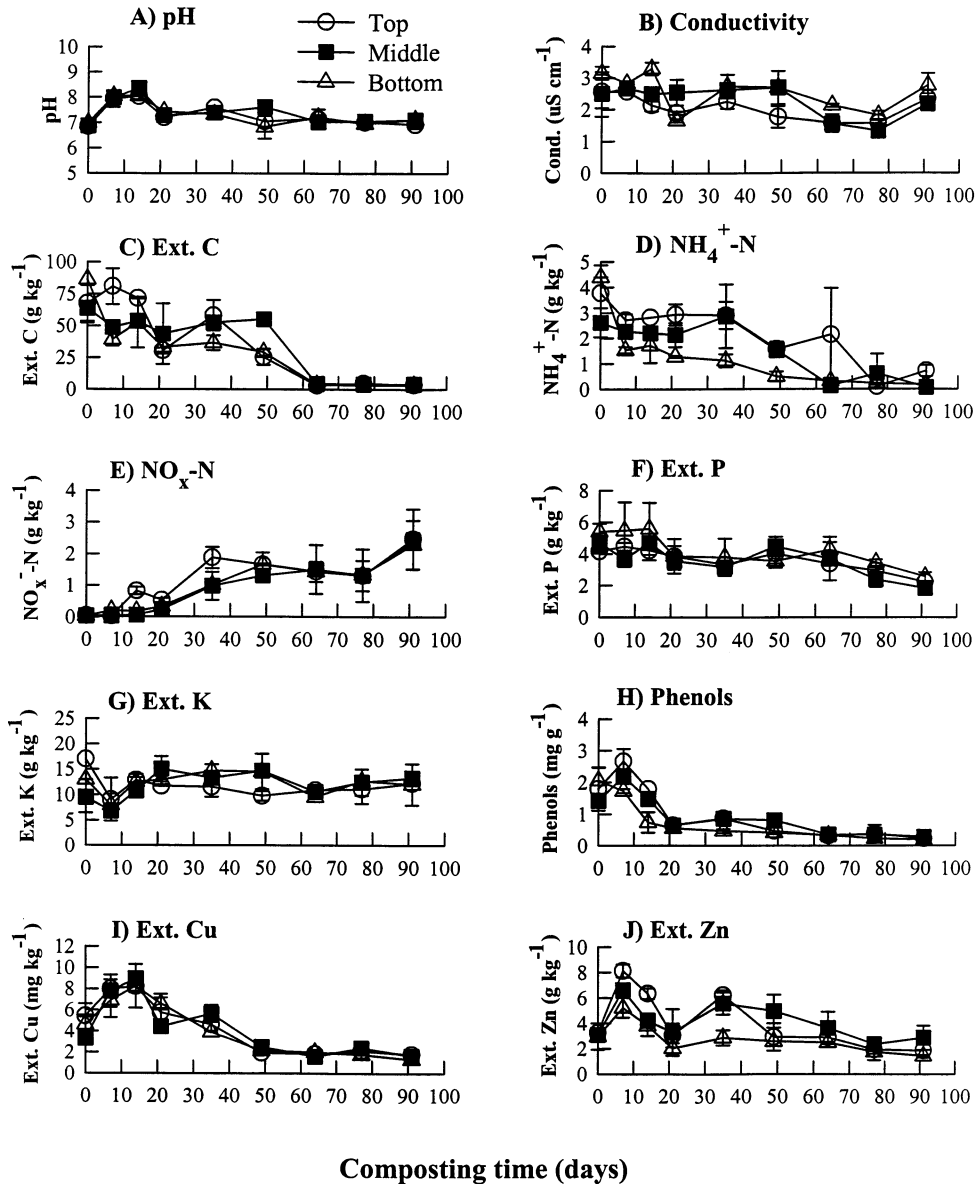


Fig. 1. Changes in water-extractable components of poultry + yard trimmings.

Table 3 Germination Indices in compost samples collected from positions top, middle, and bottom positions during co-composting process

Sampling position	Composting time (days)				
	0	7	14	49	91
Top	73.84 ± 20.10	88.52 ± 12.98	91.64 ± 21.32	148.78 ± 21.71	123.44 ± 15.83
Middle	49.07 ± 36.78	119.35 ± 9.56	124.69 ± 4.50	132.98 ± 8.85	128.47 ± 4.39
Bottom	28.62 ± 21.28	124.53 ± 5.48	135.71 ± 17.12	166.82 ± 17.44	134.24 ± 11.57

Table 4
Coefficient of determination (r^2) for the relationship between extracellular enzyme activities and chemical properties of the water extracts^a

Enzyme	pH	Cond ^b	Ext. C	NH ₄ ⁺ -N	NO _x ⁻ -N	Ext. P	Ext. K	Ext. Cu	Ext. Zn	Phenols
Phosphatases										
Alkaline phosphatase	0.58*	0.28	-0.10	-0.33	0.33	-0.33	0.14	0.34	0.14	-0.22
Acid phosphatase	-0.04	0.24	-0.56	-0.69*	0.62*	-0.44	-0.03	-0.13	-0.54	-0.68*
Phosphohydrolase	-0.63*	-0.06	-0.18	-0.09	0.32	-0.21	0.20	-0.36	-0.24	-0.17
Esterases										
Lipase	0.81**	0.12	0.41	0.19	-0.54	0.50*	0.05	0.77**	0.77**	0.34
Esterase-lipase	0.52	-0.24	-0.59*	-0.63*	0.58*	-0.42	-0.27	0.04	0.01	-0.65*
Esterase	-0.43	-0.60*	-0.97***	-0.82**	0.91***	-0.78**	-0.30	-0.76**	-0.61*	-0.89***
Amino-peptidases										
Leucine amino-peptidase	0.28	-0.11	-0.31	-0.42	0.32	-0.07	-0.76**	-0.04	-0.06	-0.39
Valine amino-peptidase	-0.43	-0.60*	-0.97***	-0.82**	-0.91***	-0.78**	-0.30	-0.76**	-0.30	-0.89**
Cystine amino-peptidase	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Proteases										
Chymotrypsin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trypsin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycosyl-hydrolases										
α -galactosidase	-0.47	0.40	-0.68*	0.74**	-0.62*	0.43	0.33	0.01	-0.04	0.72**
β -glucosidase	-0.97***	-0.29	-0.44	-0.21	0.43	-0.47	-0.01	-0.87***	-0.74**	-0.31
<i>N</i> -acetyl- β -glucosaminidase	-0.25	0.14	-0.44	-0.41	0.62*	-0.58*	0.03	-0.38	-0.60*	-0.45
α -glucosidase	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
β -galactosidase	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
β -glucuronidase	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
α -mannosidase	-0.18	-0.07	-0.43	-0.51	0.61*	-0.66*	0.15	-0.36	-0.41	-0.42
α -fucosidase	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

^a ***, **, and * indicate correlations significant at 0.001, 0.01, and 0.05 probability levels, respectively.

^b Cond, electrical conductivity.

APYZYM™ testing of poultry + yard trimmings in the forced-aeration piles showed an overall increase in enzyme activity over the course of the present experiment (Table 3). Alkaline and acid phosphatase, and leucine amino-peptidase activities were high at the beginning of composting. The high activity of these enzymes was probably due to high organic matter and a large quantity of nutrients in poultry + yard trimmings compost [27], which stimulated growth of total aerobic bacteria and subsequent phosphatase and peptidase synthesis. Nannipieri et al. [28] found that addition of organic matter in soil had a stimulatory effect on enzymic activities. Higher values of β -glucosidase activ-

ity were obtained at the end of composting (Table 3). Hayes [29] has found fungi to be the primary source of β -glucosidase in soil. Tiquia et al. [27] reported that β -glucosidase, which is involved in the hydrolysis of cellobiose, showed significant positive correlation with fungal ($r = 0.98$) and actinomycete ($r = 0.77$) populations. However, correlation between β -glucosidase and fungi was higher. Fungi and actinomycete populations were also had significant positive correlation with β -galactosidase (enzyme involve in the hydrolysis of lactose) [27]. β -Galactosidase (another enzyme involved in the hydrolysis of lactose) had the most significant positive correlation with microbial populations.

4.2. Relationship between extracellular enzyme activities and chemical properties

Chemical analysis of poultry + yard trimmings showed that composting resulted in loss of C. This result was due to mineralization of organic matter during composting, as shown by the decline in water-extractable C (Fig. 1C). The decrease in NH_4^+ -N content to low levels was associated with the accumulation of NO_x^- -N in the poultry + yard trimmings compost (Fig. 1(D–E)). 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. A previous study on composting of spent pig litter has demonstrated that decreases in water-extractable Cu and Zn coincided with increases in humic substances [30]. The GI values increased as composting progresses, which indicated that the phytotoxicity in the poultry + yard trimmings was gradually eliminated. Increases in GI values corresponded with the decrease in NH_4^+ -N, soluble phenolics, and water-extractable heavy metal contents (Fig. 1(D,H,I,J)). These compounds were found to be the major contributor to phytotoxicity in animal manure and yard trimmings [3,17,20,30].

Enzyme activities correlated with some chemical properties of poultry + yard trimmings (Table 4). Acid phosphatase, esterase-lipase, valine-amino-peptidase, and α -galactosidase, which are involved, respectively in P, N, and C cycles were correlated with water-extractable C and P, NH_4^+ -N, NO_x^- -N, soluble phenolics. These enzymes also correlated with water-extractable Cu and Zn.

Although this enzyme test is rather preliminary, the results of this study seem to show potential usefulness of enzyme activity measurements as indices of the course of the actual composting. The enzymes tested in the present study may also represent a good index of qualitative and quantitative fluctuation of the amount of substrate during composting, since these enzymes are substrate-inducible enzymes.

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