

Phytotoxicity mechanisms of seed germination during co-composting of chicken manure and tobacco powder

Guoying Wang, Jing Yuan, Guoxue Li*

College of Resources and Environmental Science, China Agricultural University, Beijing, 100193, China

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Presenting author email: wanguoying@cau.edu.cn

Abstract: Chicken manure (CM) and tobacco powder (TP) are two highly toxic agricultural solid wastes. This study systematically investigated the phytotoxicity mechanisms of mushroom residue (MR) co-composted with CM and TP on seeds germination. Three mixtures were piled and composted for 35 days. The results showed that the addition of MR reduced the phytotoxicity of CM-TP compost and increased the germination index (GI) by more than 50%. The pH values were a little higher (below 5%) and electrical conductivity (EC) decreased by 5%~20%. Redundancy analysis indicted that the GI was positively correlated with the concentrations of nitrate nitrogen, Fe^{3+} , Na^+ , Cu^{2+} , Zn^{2+} and K^+ of the compost water extraction, while negatively correlated with EC, ammonium nitrogen, Ca^{2+} and Mg^{2+} , in which Fe^{3+} and EC were the most significant. Besides, the enzymes protected system, including peroxidase, catalase, malondialdehyde, which demanded for seed germination had similar correlations with the above physicochemical indicators. Results obtained from this study could provide insights and guidances for the reuse of high phytotoxicity composts of agricultural wastes and thus improve the utilization value of organic compost.

1. Introduction

Poultry production is an important agro-industry worldwide. According to statistics from Food and Agriculture Organization of the United Nations, world production of eggs has increased 24.98% during recent 10 years, and a large volume of chicken manure (CM) is an inevitable side production. Many studies have announced that composting is an effective and sustainable way for poultry manure waste treatment (Zhang and Sun, 2015). Traditionally, composting is a bio oxidative process involving the mineralization and partial maturation of the organic matter, leading to a stabilized final product, free of phytotoxicity and pathogens and with certain mature properties (Zhang et al., 2013). However, as the fertilizer value of CM has been recently recognized, much

*Corresponding author. E-mail: ligx@cau.edu.cn; Tel: +86 01062733498; Fax: +86 01062731016.

more attention has been concentrated on the effect of manure compost application in farmland (L.L. Fialho et al., 2010). Thus, the quality of the compost may be an important criterion for the usability on plant health, the phytotoxicity is an essential factor which has direct influences in seeds germination (X.S. He et al., 2015).

With the increasing generation of organic waste, a great volume of tobacco powder (TP) are produced during the tobacco plants harvest to its processing in the cigarette industry (Liu et al., 2015; Silva et al., 2016a,b). Especially in southwest China, TP is a typical by-product agricultural waste which needs to be disposed. Besides, TP is also a carbon-rich material which could regulate the carbon and nitrogen ratio (C/N) of organic manure in composting (Silva et al., 2014). And co-composting of CM and TP is a normal treatment way in southwest China because CM and TP have complementarity in the conditions of composting. Furthermore, previous researches studied mushroom residue (MR) as a bulking agent in compost could regulate the porosity, adjust the water content, change the degradation kinetics, induce microbes, shorten the composting procedure and improve the compost quality and safety in the end (Blazy et al., 2014; Shao et al., 2014).

Germination index (GI) is commonly used to assess phytotoxicity for complex solid samples, such as waste or compost (Luo et al., 2018). When seeds absorb nutrients from endosperm transformed from compost extract, it is important to concern the effect of compost on seeds germination and growth. Thus GI is correlated with physicochemical parameters compost extract and physiological indexes of seeds, however, the mechanism of these factors are still unclear. And this study investigated the phytotoxicity mechanism of co-compost extract on seeds germination, and the extraction were produced by MR replaced TP in CM-TP compost.

2. Materials and methods

2.1 Composting process

The main raw material of compost is CM, the auxiliary material is TP and MR. CM was obtained from Kangzhuang Chicken Farm, Yanqing County, Beijing. TP was obtained from Guizhou Tobacco Research Institute, Guizhou Province, China. MR was obtained from Mushroom Garden in Zunyi, Guizhou Province, China. Key physicochemical properties of the three materials were shown in Table 2-1.

Table 2-1 Characters of the compost materials

Raw material	Total carbon (g kg ⁻¹)	Total nitrogen (g kg ⁻¹)	Moisture content (%)	pH	Electrical conductivity (MR cm ⁻¹)	C/N
CM	378.9±2.01	23.2±0.02	77.3±1.10	7.89±0.06	7.02±0.02	16.3
TP	389.3±1.11	28.3±0.21	7.9±0.39	6.03±0.05	8.99±0.03	19.1
MR	367.1±1.11	18.7±0.06	12.8±0.89	773±0.02	2.27±0.02	19.6

Composting was conducted at the Shangzhuang Experimental Station at China Agricultural University. The reactor consisted of a cylindrical reaction chamber (Φ 36*60 cm) which had two layers of stainless steel with polystyrene foam embedded in the middle to minimize heat loss and exhaust gas was emitted from the top of the reactor. A perforated plate with 3 mm mesh was positioned at the bottom of the reactor to support compost materials and convenient for aeration. Air was pumped in by an air pump, passed through a ventilation control cabinet and then delivered into the vessel from the bottom air inlet port and through the mesh to the matrix. A temperature sensor was inserted from the top and automatically record the pile temperature. Details of the composting system can also be found elsewhere (Yang et al., 2013; Yuan et al., 2016).

This study investigated three treatments (T1-T3) and each consisted of 80% CM (wet weight), and 20% auxiliary materials, which with three different proportions of TP (20%, 15% and 10%) and MR (0, 5% and 10%), to investigate their effects on the phytotoxicity performance. MR were used as toxic regulator agent to decline phytotoxicity, inoculated microbiological and adjusted the C/N and structure of the compost files. Samples (approximately 100 g) were collected using the multiple sampling method at day 0, 3, 7, 14, 21, 28 and 35 after completely turning the composting matrix.

2.2 Phytotoxicity assay

The phytotoxicity bioassay is evaluated by seeds germination according to the method previously reported by Guo et al. (2012). This method involves extracting aqueous compost by diluting compost samples in distilled water (1:10 w/v ratio) and shaking the mixtures for 0.5 h, then filtering and collecting the supernatant. And incubating seeds with this extract (control with distilled water) at 20±2 °C in dark for 24 hours, then counting the number of germinated seeds (SG), measuring relative root growth (RRG), and calculating GI. For each treatments (including control

(CK)) set three replicated dishes containing 10 seeds. If the seeds did not germinate, their root lengths were considered to be 0 mm. A 5 mm primary root was used as the operational definition of SG. According to Tiquia and Tam (1998), SG, RRG and GI were calculated as follows:

$$SG = \frac{\text{Number of germinated seeds in extract}}{\text{Number of germinated seeds in control}} * 100\%$$

$$RRG = \frac{\text{Root length in extract}}{\text{Root length in control}} * 100\%$$

$$GI = \text{Seed germination} * \text{Relative root growth}/100\%$$

2.3 Measurement of compost extracts and seeds

2.3.1 Physicochemical parameters of compost extracts

The physicochemical parameters included: the pH and electrical conductivity (EC) were measured by using a pH/EC meter (MP-521); Ammonium nitrogen ($\text{NH}_4^+\text{-N}$), nitrate nitrogen ($\text{NO}_3^- \text{-N}$) were analyzed by the Segmented Flow Analyzer (Seal XY-2 Sampler, Australia); The concentration of water-extractable copper (Cu^{2+}), zinc (Zn^{2+}), ferric (Fe^{3+}), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) were measured by atomic absorption spectrophotometry.

2.3.2 Antioxidant enzyme activities of seeds

Physiological parameters of seeds were also measured after seeds germination assay, which including catalase (CAT), peroxidase (POD) and malondialdehyde (MDA). According to Nawaz et al. (2012), 0.4 g of sample were homogenized in 4.5 ml extraction buffer (50 mM phosphate, pH 7.8) and then centrifuged at 4 °C for 25 min at 12000 rpm. The supernatant was used for enzyme activity determination by using a UV–vis double beam spectrophotometer (Systronics, India).

2.4 Statistical analysis

The changes of all physicochemical properties indicators, seed germination characters were analyzed by Origin pro 8.5. Redundancy analysis (RDA) was conducted to analyze relationships between physiological indexes of seed germination and physicochemical properties of compost extract by Canoco for Windows (Version 4.5).

3. Results and discussion

3.1 Elementary chemical analysis

The pH is one of the most important factors affecting the effectiveness of nutrient elements

required for seed germination and crop growth. As shown in Fig. 3-1a, pH were 5.8~6.1 at the initial of each treatments, and rapidly climbed to 8 at the first 7 days which revealed the dramatic activity of microbes in each treatments. And pH gradually raised to about 8.5 at the end of composting. There was no significant difference between each treatments, and all of them meet the appropriate composting condition of weak alkalinity. Generally, seeds need weak alkalinity to grow, so early compost extract may not be suitable for seed germination.

Electrical conductivity (EC) is an indicator of the salinity of the compost and the high salinity (more than $4 \text{ sm}\cdot\text{cm}^{-1}$) extraction is not suitable for seeds germination. The initial EC mean values were 5.9~6.3 $\text{sm}\cdot\text{cm}^{-1}$ for the all trials (T1-T3) and they showed a decreasing pattern during composting process (Fig. 3-1b). After 35 days' composting, the EC values were recorded as 3.8, 3.6 and 3.2 $\text{sm}\cdot\text{cm}^{-1}$ for trials T1-T3, respectively, and they all reached the requirement standard of seed germination. In T1 compost extract, the EC declined range was smaller than the others which could be due to TP with higher phytotoxicity than MR in compost extract.

The nitrogen in compost extracts are mainly in the form of nitrate nitrogen ($\text{NH}_4^+\text{-N}$) and ammoniacal nitrogen ($\text{NO}_3^-\text{-N}$), the change of $\text{NH}_4^+\text{-N}$ concentration in compost extracts during the composting process were illustrated in Fig. 3-1c. At the initial stages, the $\text{NH}_4^+\text{-N}$ content were significantly different in the three treatments, T1 ($813 \text{ mg}\cdot\text{L}^{-1}$) > TP2 ($583 \text{ mg}\cdot\text{L}^{-1}$) > T3 ($448 \text{ mg}\cdot\text{L}^{-1}$), which were owing to differently decomposed degree of organic matter in this process. Then all treatments' EC decreased to around $400 \text{ mg}\cdot\text{L}^{-1}$ on day 3. Day 3~28 was the plateau phrase with a slightly declining of all treatments, then fell sharply again to the minimum ($220\sim 232 \text{ mg}\cdot\text{L}^{-1}$). During the whole process, $\text{NH}_4^+\text{-N}$ concentration of T1 decreased largest than the others, which revealing the addition of MR could reduce the $\text{NH}_4^+\text{-N}$ content in composting especially at the initial phases.

The concentration of $\text{NO}_3^-\text{-N}$ had strongly negative relation to $\text{NH}_4^+\text{-N}$ and the amount of $\text{NO}_3^-\text{-N}$ in compost extracts were depicted in Fig. 3-1d. At the initial 7 days, the concentrations of $\text{NO}_3^-\text{-N}$ in all treatments were nearly to 0, which might due to the high temperature inhibit the nitrifying bacteria's activity. However, there was a continues rising in T1 and T3 during between day 7~14, while T2 increased at first then decreased at the middle time, which might due to the 10% addition of MR had more restraints on producing $\text{NO}_3^-\text{-N}$ compared to 5% at this period. After the day 14, an urgently increasing of T1~T3 were observed and reached to 2.2, 2.1 and 1.1 $\text{mg}\cdot\text{L}^{-1}$, respectively.

Thus the 10% of MR addition was more effective in composting to reduce the concentration of NO_3^- -N.

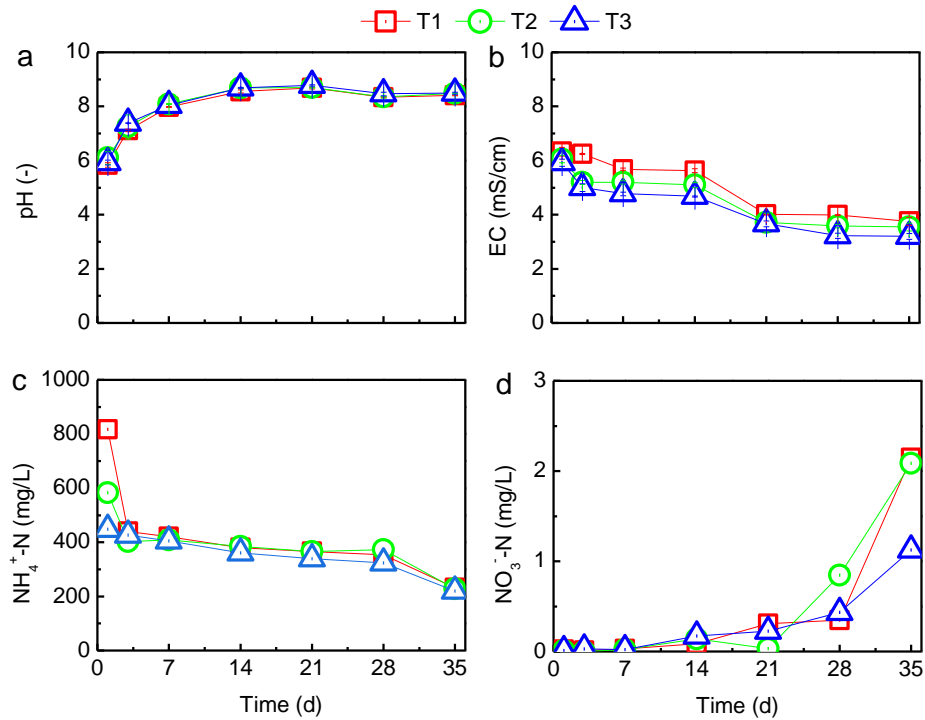


Fig. 3-1. The variation of elementary chemical parameters.

3.2 Elementary nutrients analysis

As shown in Fig. 3-2a, the concentration of Cu^{2+} was lower in the early stages of T1~T3 (0.52, 0.32 and 0.35 $\text{mg}\cdot\text{L}^{-1}$), then T3 declined slightly while T1 and T2 increased in first 3 days, then they all raised with a slowly speed until day 21. And in the next 7 days, T1~T3 witnessed an urgently growing and reached to the peak point (1.38, 1.03, 1.06 $\text{mg}\cdot\text{L}^{-1}$) on day 28, the growth rate of T1 was larger than the others. During the compost maturity periods, the content of Cu^{2+} in T1~T3 all decreased and reached to 0.99, 0.91 and 0.62 $\text{mg}\cdot\text{L}^{-1}$ in the end, however, higher than the initial concentrations. And the decreased speed of T3 was faster and always had lower Cu^{2+} concentration than the others, which indicated that 10% (wet weight) MR replacement of TP had better effect on reducing Cu^{2+} concentration than 0 and 5% (wet weight).

It can be concluded from the variation of the concentrations of Zn^{2+} in T1~T3, which all depicted a firstly rising then slightly decreasing trends in the whole composting periods (Fig. 3-2b). Treatments started with the minimum values of 0.90, 0.78 and 0.55 $\text{mg}\cdot\text{L}^{-1}$ for T1~T3, respectively, and fast increased in 1-7 days, then slowly climbed to the highest points on day 28 (2.05, 1.80 and

1.52 mg·L⁻¹, respectively), then slightly decreased to 1.82, 1.73 and 1.33 mg·L⁻¹ of T1~T3 in the end. The concentrations of Zn²⁺ in all treatments were T1 > T2 > T3, which indicated that the addition of MR replaced the TP in CM compost can reduce the concentration of Zn²⁺ and the 10% (T3) addition reduced more.

Fe³⁺ is one of the essential nutrients for seeds germination, which plays a vital role in the synthesis and transform of matters in plants. Fig. 3-2c depicted the variation of Fe³⁺ concentrations in compost extracts of different periods of composting. In the initial periods (1-3 days), the contents of Fe³⁺ in T1~T3 showed a decreasing trend and reached to the minimum points (1.68, 1.84 and 1.37 mg·L⁻¹, respectively). Then increased quickly to the maximum points on day 28 and slightly decreased in the last 7 days and reached to 7.59, 6.43 and 5.39 mg·L⁻¹, respectively. All treatments had the similar rising speeds in 0-14 days but showed different changes later, especially the rising speed of T3 decreased significantly. The concentration of Fe³⁺ in T3 only increased 2.6 mg·L⁻¹ while T1 and T2 increased 5.2 and 4.1 mg·L⁻¹ in the next 20 days. On the whole sight, Fe³⁺ content of T1 was much higher than T3, which indicating that MR can inhibit the increasing of Fe³⁺ concentration and the ratio of 10% (wet weight) was more effective compared to 5% (wet weight).

The content of Na⁺ also has an effect on the germination of seeds, the proper low concentration can promote seeds germination, but the high content can inhibit it. As you can see from Fig. 3-2d, Na⁺ contents were increased moderately to the highest points (T1-T3 were 94.63, 110.33, 98.03 mg·L⁻¹, respectively) on 28 day and quickly decreased in the next 7 days of all treatments. In the end of the compost extracts, the concentration of Na⁺ of T1~T3 were 5~25 mg·L⁻¹ and higher than the initials (62.65, 80.30, 75.40 mg·L⁻¹, respectively). And MR replaced TP in CM compost had better effect in improving the concentration of Na⁺ by analyzing T1~T3, and the 10% (wet weight) addition of MR in co-compost was more significant than 5% (wet weight).

K⁺ is one of the essential elements and inorganic cations for seeds germination and crops growing. But too much amount of K⁺ is not always benefit for seeds germination. From Fig. 3-2e, the initial values of K⁺ contents in T1~T3 were 932.83, 976.33 and 832.33 mg·L⁻¹, respectively. And the changes were same with other cations, the contents of K⁺ increased with different speeds (T1 > T2 > T3) and decreased quickly after the 28 day of composting. At the end of composting, the K⁺ contents of T1~T3 decreased to 1316.33, 1452.50 and 976.50 mg·L⁻¹, respectively, and higher than the initial concentrations. Because tobacco is a potassium-loving plant, and the K⁺ contents of

the water extracts from the composts were higher than other cations. Apparently, the concentration of K^+ in T3 was lowest due to the 10% (wet weight) MR replacement in CM-TP compost had better reduction effect.

Ca^{2+} is one kind of medium amount elements, which need less but play an important role in the process of seeds germination. The initial Ca^{2+} contents in T1~T3 were 617.83, 522.52 491.42 $mg \cdot L^{-1}$, respectively (Fig. 3-2f), and the concentrations decreased rapidly till the 14 day (90 $mg \cdot L^{-1}$) and then the values basically did not change until the end of composting, and the final concentrations of T1~T3 were 77.72, 76.97, 81.27 $mg \cdot L^{-1}$, respectively. From the whole perspective, T1 always had a higher Ca^{2+} content than T2 and T3, which also indicating the more replacement (10% wet weight) of MR in CM-TP compost had the better reduction effect of Ca^{2+} contents in compost extractions.

Mg^{2+} is another medium amount elements, which also needed and essential in seeds germination. The Mg^{2+} concentration of different stages compost extracts were shown in Figure 3-2g that all treatments had a relatively high values at the beginning of composting, which were 222.92, 220.22, 206.78 $mg \cdot L^{-1}$ of T1, T2 and T3. But the descended rates of Mg^{2+} concentrations were sharply grown in the first 10 days, then got slowly in the next 20 days, finally got a little quicker in last 7 days, and reached to the lowest values for T1~T3 (46.80, 38.47, and 35.71 $mg \cdot L^{-1}$, respectively). Apparently, Mg^{2+} concentrations of all treatments obeyed $T1 > T2 > T3$ during the processes of composting, which indicating that adding MR to replace some TP in CM composts can reduce the Mg^{2+} concentrations in compost extracts.

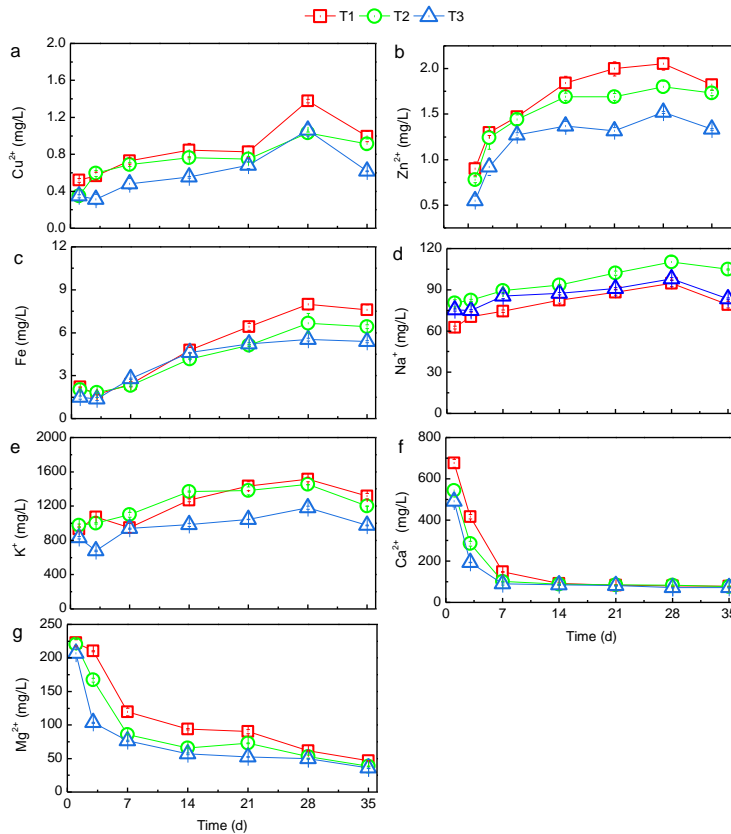


Fig. 3-2. The variation of elementary nutrients.

3.3 Germination parameters analysis

SG refers to the proportion of germinated seeds in the number of total seeds. SG of all treatments increased during composting (Fig. 3-3a), with less than 80% at the initial, which increased significantly in 0-10 days before reaching a plateau phase, which indicating inhibition of seeds germination and decomposition of phytotoxicity in compost. Especially the SG of T1, which was far lower than other two treatments, which indicating higher phytotoxicity in this treatment. While 10 days later, the SG reached to 80%~100%, and there were no significant differences between the three treatments. RRG were different from SG trend, which increased significantly in a linear manner from 0~130% during the whole composting of all treatments (Fig. 3-3b), among which the T1 was slower than the others, and which revealed that the addition of MR has contributions on RRG.

GI is a biological indicator, which can comprehensively reflect the degree of phytotoxicity of compost products. From Fig. 3-3c that the GI values of each treatments showed an upward trend in composting process, but the rates of each periods were different. Before 15 days of composting, GI were less than 50% of all treatments, which indicating that the compost extract during this period

has a significant inhibitory effect on seeds germination. After 15 days, GI of T2 and T3 increased rapidly, and reached over 80% in the next 6 days and to 124.62% and 124.98% in the end. While GI of the T1 reached to 50% on around day 21, and to 80% on day 30, and finally reached to 110.49%. It can be found that the replacement of MR to TP in CM compost can improve GI, which means that the reduction of phytotoxicity of seeds germination in composting.

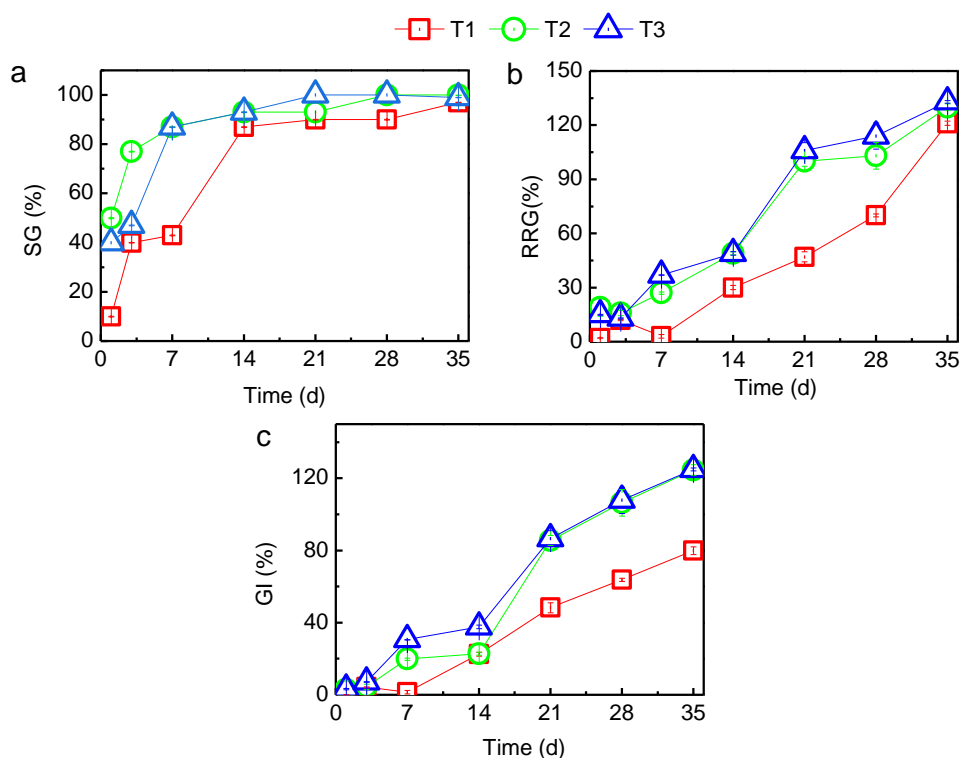


Fig. 3-3. The variation of germination parameters.

3.4 Enzymes of seeds germination analysis

CAT is the main enzyme for scavenging superoxide and hydroxyl free radicals in plants. It inhibits lipid peroxidation and plays a protective role in alleviating membrane damaged. For all treatments (Fig. 3-4a), CAT activities were basically unchanged and closed to zero in first 3 days, but increased urgently in 4~7 days until they had the similar values with CK (The control seeds in germination assay), which indicated the compost extracts of this period inhibited the activities of CAT. After day 7, CAT activities still increased but with relatively lower speeds until the last day of composting. It was worth noting that CAT activities in the mature stage was much higher than CK, which indicated that the longer of composting the higher of CAT activities in compost extracts, which led to the decrease of superoxide and the increase of GI. Moreover, in the last stage of composting, the CAT activities in the order of T3 > T2 > T1, which indicated that the addition of

MR in CM compost could promote the activities of CAT in seeds germination, and the effect of 10% (wet weight) was better than 5% (wet weight).

The activities of POD (Fig. 3-4b) increased during the composting of all treatments, except in thermophilic phases (7~21 days), which due to the high temperature inhibited the POD activities. At the second half of composting period, the activities of POD in all treatments' extract were higher than CK, which indicated the compost extracts in this period could improve the activities of POD because of the mineralization and maturity of composts. Furthermore, the activities of POD were $T3 > T2 > T1$ and indicated the addition of MR to replace the TP in CM compost was conducive to improve the POD activities, and the ratio of 10% (wet weight) was better and 5% (wet weight).

MDA is an important indicator, which reflecting the damage degree of the membrane and the activities of free radical scavenging enzymes in seed cells. It can be seen from the Fig. 3-4c that MDA contents decreased from about $40 \text{ nmol}\cdot\text{g}^{-1}$ to below $20 \text{ nmol}\cdot\text{g}^{-1}$ in the end of composting. Before the day 20, the MDA concentrations of each treatments were higher than CK, which indicating that the lipid peroxidation in seeds cultured with compost extracts was strong during this period, which was not conducive to seeds germination. After day 20, the concentrations of MDA in all treatments were lower than CK, which indicated that the lipid peroxidation was decreased in compost extracts of this period. And this could produce a beneficial environment for the growth of seeds, especially at the end of compost extracts, which was most suited for seeds germination. More than that, MDA activities in T2 and T3 were totally lower than T1 owing to the addition of MR replaced TP in CM compost.

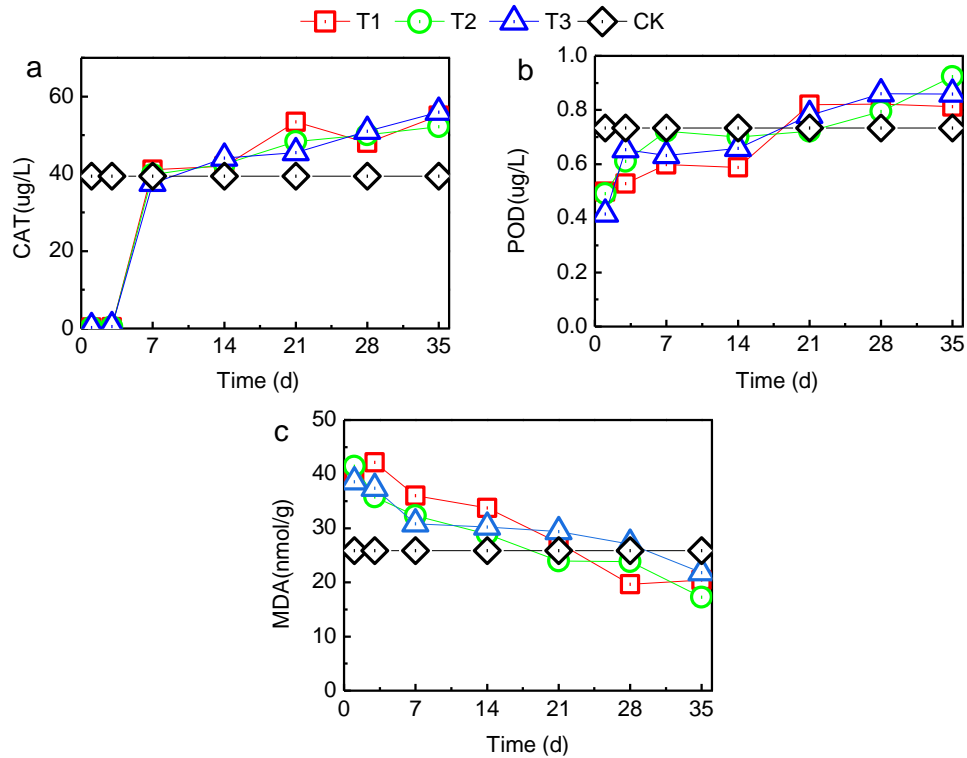


Fig. 3-4. The variation of enzymes of seed germination.

3.5 Redundancy analysis

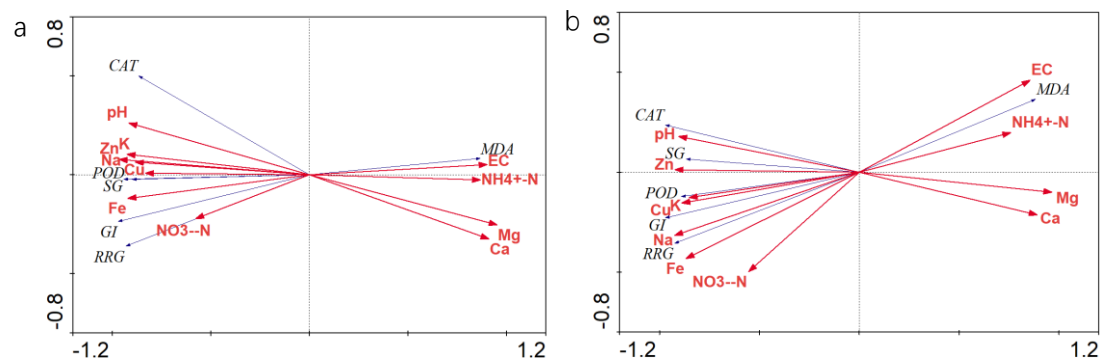
RDA analysis between physiological indexes of seeds germination and physicochemical properties of compost extracts were depicted in Fig. 3-5. The red vectors represented the physicochemical parameters of the compost extracts and the blue vectors represented physiological indexes of seeds germination. RDA can standardized these parameters, the length of the arrows indicated the relation degrees and the angle indicated the correlation between physicochemical parameters and physiology indexes. When the angle is acute, it is positive correlation; when the angle is obtuse, it is negative correlation; when the angle is right, they are not correlated.

All redundancy analysis showed that different seeds physiological parameters were affected differently with the same physicochemical parameters of the compost extracts. T1 redundancy analysis (Fig. 3-5a) showed GI was most affected by NO_3^- -N concentration, followed by EC, Fe^{3+} and Mg^{2+} concentrations had the same influences, and other physicochemical parameters had little influences. As for enzymes protection system, POD and CAT activities had the most negative correlation with Ca^{2+} and Mg^{2+} concentrations, and second negative correlation with EC value and positive correlation with Fe^{3+} concentration. More than that, CAT activity also had much correlation with Zn^{2+} concentration and pH, which can effect in large degree. MDA concentration was mostly

affected by Mg^{2+} concentration and they had positive correlation, then Fe^{3+} , Zn^{2+} concentration and EC had relatively little affection in MDA content. In another way, POD and CAT activities had positive correlation with GI, while had negative correlation with MDA content, so that some parameters had little affection with GI but more with these physiological indexes also could accelerate GI, such as NO_3^- -N、 Mg^{2+} 、 Fe^{3+} concentration and EC. To some degree, this is a way to decrease the phytotoxicity in compost extracts.

T2 redundancy analysis (Fig. 3-5b) showed that all physicochemical parameters had more or little influences with GI, although the biggest factor was different for physiological indexes. GI was most positive correlation with Na^+ and Fe^{3+} concentration, and negative correlation with EC. POD activity was most positive correlation with Na^+ and Fe^{3+} concentration, negative correlation with EC. CAT activity had the same correlation with Na^+ , Fe^{3+} concentration and EC, but it had highest negative correlation with Mg^{2+} concentration. MDA concentration was most negative correlation with EC then with Na^+ and Fe^{3+} concentration. And in T2, GI was most affected by POD activity and MDA concentration, so that Na^+ , Fe^{3+} , Mg^{2+} concentration and EC can all affect GI according to adjust POD activity and MDA concentration.

T3 redundancy analysis (Fig. 3-5c) showed that GI was most negative with EC then with NH_4^+ -N concentration, and positive correlation with Fe^{3+} concentration, POD and CAT activities had the same relation with EC and Fe^{3+} concentration. MDA concentration had negative correlation with Fe^{3+} , Zn^{2+} concentration and pH, and positive correlation with EC and NH_4^+ -N concentration, among which Fe^{3+} concentration and EC were the most influenced factors. Because GI was most affect by MDA concentration and POD activity, so the most influenced parameters were Fe^{3+} , NH_4^+ -N concentration and EC.



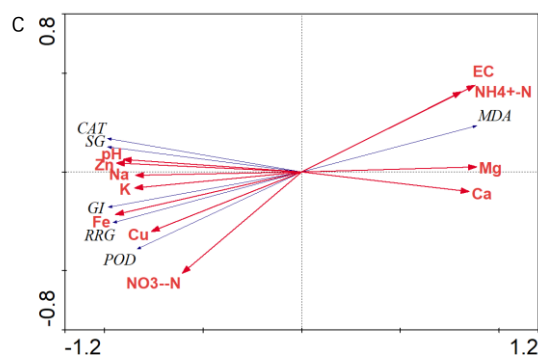


Fig. 3-5. The RDA analysis results between physiological indexes of seeds germination and physicochemical properties of compost extract.

4. Conclusions

According to elementary chemical parameters analysis of extracts of co-compost, the study showed that pH of different treatments' compost extracts were weakly acidic at the beginning of composting, but they changed to weakly alkaline after one week until the end. EC decreased of all treatments and they were in the order of T1 > T2 > T3 during the whole composting process. NH₄⁺-N concentration firstly increased then decreased and NO₃⁻-N increased in all. The concentrations of Cu²⁺, Zn²⁺, Fe³⁺, Na⁺ and K⁺ were increased while Ca²⁺ and Mg²⁺ were decreased during the composting process. And the growing trends were similar, first at a quickly speeds then got slower and reached to the highest points, then decreased until the end of composting. The reducing trends were differently, they all decreased sharply in 0~14 days, but then the concentrations of Ca²⁺ almost did not change while Mg²⁺ still decreased at a slower speed until the end.

The SG of seeds in all treatments increased firstly and didn't change until the end. However, the RRG and GI in different treatments changed a lot, and they had similar changes. The RRG and GI of the compost extracts in T3 (10% MR replacement) were the highest, while in T1 (No MR replacement) were the lowest. The activities of CAT and POD increased during the composting process, but in the early stages, they were lower than CK, then slowly raised and higher than CK at the middle of composting, and largely higher in the end. Because in the different periods of composting, the compost extracts had different stimulate effects in protective enzymes activities in seeds germination. And the 10% addition of MR replaced TP had a better effect in accelerating the seed's enzyme activities. The MDA concentration was high in the early stage of composting, which indicating that the compost extracts in this stage destroyed the antioxidant enzyme system of seeds,

and produced much membrane lipid peroxidation which could inhibit the germination of seeds.

The redundancy analysis showed that the seeds germination of T1 (CM-TP co-compost) were mainly affected by the EC, NO₃⁻-N, Mg²⁺ and Fe³⁺ concentration; T2 (MR replaced 5% TP in co-compost) were positive correlation with Na⁺ and Fe³⁺ concentrations, and negative correlation with EC and Mg²⁺ concentration; T3 (MR replaced 10% TP in co-compost) were positive correlation with Fe³⁺ concentration, negative correlation with EC and NH₄⁺-N concentration. Overall, the three treatments were mainly affected by Fe³⁺ concentration and EC in extracts of compost.

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