Towards understanding the stabilization process in vermicomposting of excess sludge under copper oxide nanoparticles stress using PARAFAC analysis of fluorescence spectra

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Abstract. In this study, fluorescence excitation-emission matrix (EEM) combined with parallel factor analysis (PARAFAC) was employed to trace the behaviour of dissolved organic matter during vermicomposting of excess sludge under four dosages (0, 400, 800, 1600 mg/kg-TSS) of copper oxide nanoparticles (CuO NPs) stress. Determination of dissolved organic carbon (DOC) and total content of organic carbon (TOC), available nitrogen and available phosphorous indicated the presence of CuO NPs decrease the sludge stabilization by vermicomposting, but the organics degradation efficiency was not significantly inhibited with the increase of CuO NPs concentration. Fluorescence spectra results of dissolved organic matter (DOM) during the four groups of vermicomposting indicated CuO NPs led to the reduce of humic acid-like materials in vermicomposting. The positive relationship of protein-like component 1 and negative relationship of humic-like component 2 with CuO NPs concentrations in the substrates reflected by PARAFAC analysis suggested CuO NPs inhibit transformation of humic-like maters during the vermicomposting.

Key words: vermicomposting, copper oxide nanoparticles (CuO NPs), fluorescence excitation-emission matrix (EEM), dissolved organic matter (DOM), Parallel factor analysis (PARAFAC analysis).

1. Introduction
Nanotechnology is a double-edged sword. On one hand, it has promoted social development. On the other hand, it has triggered severe environmental pollution and ecological hazards. Take copper oxide nanoparticles (CuO NPs) for example, its magnetic, thermal resistance, chemical activity, antimicrobial and photo catalytic characteristics have enabled it to find wide applications in the field of new material preparation, antimicrobial layer and organic pollution catalysis [1]. However, during the process of production, transportation, application and disposal, it has been inevitably dis-charged into the environment through various channels. As the last screen to sewage discharge into the ambient waters,
waste water treatment plants (WWTPs) have become the final destination of nanocrystalline metal oxides in sewage, which even settle down on excess sludge through the effect of absorption, agglomeration and sedimentation [2]. As a result, conventional excess sludge treatment processing are effected due to the presence of CuO NPs. As an ecological treatment method of excess sludge, vermicomposting can not only realize organic degradation in sludge, but also efficiently increase the content of available nitrogen, available phosphorus, and humus in the compost products. After vermicomposting, the products can be adopted as organic fertilizers to resource utilization of land. However, there has not yet been any report in whether excess sludge, polluted by CuO NPs, can be achieved via vermicomposting, and how CuO NPs will influence organic decomposition in the process of composting.

The size effect and Cu$^{2+}$ released from CuO NPs can, to some extent, do harm to the earthworms and microorganisms in vermicomposting, and may affect the organics degradation and humification. Dissolved organics matters (DOM) is the most biological and chemical active fraction in organic waste [3]. The analysis and characterization of DOM can provide useful information for evaluating the effects of earthworm on the substrates or the microbial activity of the organic matters during vermicomposting. Fluorescence excitation-emission matrix (EEM) is a powerful tool for characterizing heterogeneous DOM by providing an overall view of the composition and properties of DOM in a selected spectral range [4]. Parallel factor analysis (PARAFAC) is a multivariate chemometric method that can decompose EEMs into various individual fluorescent components, thereby reducing the interference among fluorescent components, thereby reducing the interference among fluorescent compounds [5]. The combination of EEM and PARAFAC can provide a more accurate quantification of the composition and behavior of DOM. Therefore, fluorescence EEM spectra and PARAFAC analysis were applied to research the characteristics of DOM in vermicomposting under the stress of different CuO NPs concentrations in order to recover the influence of CuO NPs on sludge stabilization by vermicomposting.

2. Materials and methods

2.1. Preparation, vermicomposting process and sampling

Excess sludge was obtained from the Second municipal wastewater treatment plant in Jinan, Shandong Province, China. Cattle dung was purchased from a farm in Changqing District, Jinan. Earthworms (Eisenia foetida) used in this experiment were purchased from a farm in Fuyang, Anhui Province. The substrate was mixed with sludge and cattle dung at the ratio of 1:1 (dry weigh) and naturally dried under sunlight for 1 week in order to avoid damages to earthworms as a result of the high moisture content and anaerobic fermentation.

CuO NPs (a particle size of 100-200 nm, purity>99.5%) was purchased from Macleans Co, Ltd. In this experiment, CuO NPs stock suspension was produced by adding 2.0g CuO NPs to 1.0L distilled water, and 1 h sonication (25℃, 500W, 40kHz) in bath sonicator was applied to make stock suspensions homogeneous before use.

Four groups of plastic containers (30 cm length × 20 cm width × 20 cm depth) filled with 250 g substrate (dry basis) containing four dosages (0, 400, 800, 1600 mg/kg-TSS) of CuO NPs were used to investigate the impact of CuO NPs on vermicomposting. In addition, sawdust (20 g dry weight) was added to the substrates as a bulking material. The substrates were manually turned over every day for 2 week to avoid the damage of volatile toxic sub-stance to earthworms. Subsequently, 60 nonclitellated earthworms with an average weigh 100-150 mg per individual were inoculated into each container. Three replicates were con-ducted for each treatment. The moisture content was maintained at 65±5% by periodic sprinkling of distilled water during the whole process. The four vermicompostings were labeled as V-0, V-400, V-800 and V-1600, respectively. All the containers were covered with perforated lids and placed in constant-temperature incubator with the temperature of 25±1 ℃ for 8 weeks.

Homogenized samples (about 20 g wet basis) were acquired at 0, 2, 4 and 8 week from each reactor. Subsamples were freeze-dried, and ground to run through an 80-mesh sieve and then stored in desiccator prior to the next analysis.
2.2. Fluorescence spectra analysis
Fluorescence EEM spectra were recorded with an F-7600 (Hitachi, Japan) fluorescence spectrophotometer in a clear quartz cuvette. Emission and excitation slits were set at 5 nm band width, and the scan speed was fixed at 12000 nm min\(^{-1}\). The EEM spectra were collected by scanning the emission wavelength over the range 250-600 nm in 5 nm increments, while the excitation wavelength increased gradually from 200 to 500 nm in 5 nm increments. A total of 48 EEMs were used to generate the dataset of PARAFAC analysis.

2.3. Statistical analysis
The PARAFAC analysis was performed using MATLAB 2008a using the parafac modeling toolbox (V2.0). Analysis of variance (ANOVA) was applied to evaluate the significance of results and \(p<0.05\) was considered to be statistically significant. Statistical analysis was performed using the software SPSS 17.0.

3. Results and discussion

3.1. Chemical variations during vermicomposting.
The chemical variations during vermicomposting under different CuO NPs concentrations are shown in Figure 1.

**Figure 1.** Changes in the chemical characteristics of the substrates for V-0, V-400, V-800 and V-1600.

After 8 weeks the dissolved organic carbon (DOC) in the four groups of vermicompostings (V-0, V-400, V-800 and V-1600) showed an obvious decline compared with the initial substrate. Their degradation rate were 79.7%, 71.5%, 67.7%, and 72.6%, respectively. This suggested that microorganisms could still effectively degradable organics in the substrates under the stress of CuO NPs. The degradation of water-soluble organics could also lead to a significant reduction in the total content of organic carbon (TOC) in the substrates. Compared with the initial substrate, the degradation rate of the TOC in V-0, V-400, V-800 and V-1600 was 55.3%, 47.0%, 43.5% and 45.6%, respectively. This could meet the requirement that the decomposition rate of organics by the surplus sludge processing method should be higher than 40%. At the end of vermicomposting, the content of available nitrogen and available phosphorous also demonstrated an obvious increase. This was caused by mineralization of organics including nitrogen and phosphorus in the substrates. Seen from Figure 1, it is noticeable that the DOC and TOC were lower in V-0 than those in V-400, V-800 and V-1600, which indicated the presence of CuO NPs affected the biodegradation of organics during the vermicomposting. However, the degradation rate of DOC and TOC did not significant decrease with the increase of CuO NPs concentration. It is generally considered that the toxicity of metal oxide nanoparticles is mainly decided...
by their metal ions. In the vermicomposting system, the free Cu^{2+} did not remarkably increase along with the rising of CuO NPs concentration. Therefore, the increase of the CuO NPs concentration would not significantly inhibit degradation of organics during vermicomposting.

3.2. **Fluorescence EEM spectra of water extract material.**

Fluorescence EEM spectra of the water-soluble organic matter (WEOM) fractions of the vermicomposting in the four dosages of CuO NPs are depicted in Figure 2. The excitation/emission (Ex/Em) wavelength pairs and the specific intensity of fluorescence peaks are qualitatively listed in Table 1.

**Figure 2.** Fluorescence excitation-emission matrix spectra of WEOM from four vermivompostings.

Two peaks were detected from the WEOM in the initial substrate. Peak A was characterized by an Ex/Em wavelength pair of 280/315 nm, which fell in the region related to the soluble microbial by-product-like region, like tryptophan9. Peak B represented an Ex/Em wavelength pair of 325/415 nm and associated with the humic acid-like materials [6]. After vermicomposting, the peaks of the four substrates (V-0, V-400, V-800 and V-1600) showed difference with the initial substrate. Peaks A and B were still observed in the four vermicompostings. Compared to the initial substrate, the SFI of Peak A in the four vermicompostings significantly decreased, which could attributed to the bio-degradation of organic matters by the microbial and earthworm activities. The higher SFI of Peak B in the substrates of V-0, V-400, V-800 and V-1600 indicated the generation of humic acid-like materials during vermicomposting. Specially, Peak C was detected only in the V-0 without CuO NPs. The intensity of Peak B in V-400, V-800 and V-1600 were lower than that in V-0, suggested the lower concentration of humic acid-like materials in V-400, V-800 and V-1600 due to the presence of CuO NPs. On one hand, CuO NPs might cause adverse effects to the microorganisms and earthworms during the vermicomposting, and resulted in the decrease of humification degree. On the other hand, the humic acid-like materials might to complexing with Cu^{2+} released from CuO NPs, and led to the decrease of intensity of Peak B. Moreover, the position of Peak B in V2, V3 and V4 were at the Ex/Em wavelength pairs of 350/440 nm, 340/425 nm and 340/410 nm, respectively, were considered as blue-shift compared to the Peak B in V1 with the Ex/Em wavelength pair of 350/450 nm. The shorter Ex/Em wavelength indicated the lower condensation degree of the fractions decreased and the simpler structure of the humic acid-like material [7]. Therefore, the increasing of CuO NPs concentration was harmful to the humification during the vermicomposting.
Table 1. Excitation-emission matrix maxima of WEOM from the initial substrate and vermicompostings from V-0, V-400, V-800, V-1600.

| Origin of WEOM | Peak A |       | Peak B |       | Peak C |       |
|----------------|--------|-------|--------|-------|--------|-------|
|                | Ex/Em  | SFI   | Ex/Em  | SFI   | Ex/Em  | SFI   |
| Initial        | 280/315| 217.5 | 325/415| 82.6  |        |       |
| V-0            | 285/365| 34.7  | 350/450| 229.2 | 275/450| 91.3  |
| V-400          | 280/360| 75.2  | 350/440| 175.4 |        |       |
| V-800          | 270/330| 163.2 | 340/425| 157.7 |        |       |
| V-1600         | 270/325| 144.6 | 340/410| 162.3 |        |       |

3.3. EEM-PARAFAC analysis
The EEM spectra of WEOM samples extracted from the four treatments at different times (0 weeks, 2 weeks, 4 weeks and 8 weeks) were analyzed using PARAFAC. All the fluorescence EEMs collected could be successfully decomposed by PARAFAC into a two-component model and the EEM contours of the two components are shown in Figure 3.

Component 1 had one fluorescence peak at an Ex/Em wavelength pair of 360/460 nm, of which was similar to the terrestrial-like humic fluorophpre. Component 2 had a dominant peak with maximum excitation and emission value of 280/370 nm and a second smaller peak at Ex/Em=240/370 nm. Different from Component 1, Component 2 was composed of protein-like containing tryptophan and aromatic protein. This type of fluorescence signal was often associated with immature compost and low stability of landfilled organic wastes [8].

Figure 4. Distribution of the two PARAFAC-derived components in the four vermicompostings (V1, V2, V3 and V4)

PARAFAC analysis can also provide additional quantitative information on the contribution of each component in studied samples. As shown in Figure 4, the relative distribution of the two components showed significant difference in the initial substrate and the four treatment substrates. The initial substrate had the higher percentage of the protein-like component 2 and lower percentage of humic-like
component 1 than those in the four vermicompostings. Moreover, the positive relationship of component 1 and negative relationship of component 2 with CuO NPs concentrations in the substrates were also observed. This results also indicated the CuO NPs had harmful effects on the formation of humic-like materials during the vermicomposting.

4. Conclusion
CuO NPs in excess sludge affected the transformation of organics during vermicomposting. Although the organic degradation efficiency did not decrease under the stress of CuO NPs, the fluorescence EEM spectra characteristics of DOM showed significant difference due to the presence of CuO NPs. Compared to the control vermicomposting (V-0, without CuO NPs), The lower intensity and the blue-shift of Peak B in the vermicompost substrates polluted by CuO NPs (V-400, V-800, V-1600) relative with humic acid-like materials declared the CuO NPs resulted in the decrease of humification degree during vermicomposting. The positive relationship of protein-like component 1 and negative relationship of humic-like component 2 with CuO NPs concentrations in the substrates reflected by PARAFAC analysis indicated the CuO NPs inhibit transformation of humic-like matters during the vermicomposting.

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References
[1] J. Huo, H. Zeng, Copper nanoparticles embedded in the triphenylamine functionalized bithiazole–metal complex as active photocatalysts for visible light-driven hydrogen evolution. Journal of Materials Chemistry A, 3 (2015) 17201-17208.
[2] M.A. Kiser, P. Westerhoff, T. Benn, Y. Wang, J. Pérez-Rivera, K. Hristovski, Titanium nanomaterial removal and release from wastewater treatment plants. Environmental Science & Technology, 43 (2009) 6757-6763.
[3] X.S. He, B.D. Xi, Z.M. Wei, Y.H. Jiang, C.M. Geng, Y. Yang, Y. Yuan, H.L. Liu, Physicochemical and spectroscopic characteristics of dissolved organic matter extracted from municipal solid waste (MSW) and their influence on the landfill biological stability. Bioresource Technology, 102 (2011) 2322-2327.
[4] B. Lv, M. Xing, Y. Jian, W. Qi, Y. Lu, Chemical and spectroscopic characterization of water extractable organic matter during vermicomposting of cattle dung. Bioresource Technology, 132 (2013) 320-326.
[5] W. Jun, Z. Hua, H. Pin-Jing, S. Li-Ming, Insight into the heavy metal binding potential of dissolved organic matter in MSW leachate using EEM quenching combined with PARAFAC analysis. Water Research, 45 (2011) 1711-1719.
[6] C. Wen, W. Paul, J.A. Leenheer, B. Karl, Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. Environmental Science & Technology, 37 (2015) 5701-5710.
[7] X. Xiong, Y. Li, Y. Ming, F. Zhang, L. Wei, Increase in complexation ability of humic acids with the addition of ligneous bulking agents during sewage sludge composting. Bioresource Technology, 101 (2010) 9650-9653.
[8] C.A. Stedmon, R. Bro, Characterizing Dissolved Organic Matter Fluorescence with Parallel Factor Analysis: A Tutorial. Limnology & Oceanography Methods, 6 (2008) 572-579.