Characterization of natural organic matter (NOM) derived from sewage sludge compost. Part 1: chemical and spectroscopic properties

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Abstract

In this study changes in the properties of natural organic matter (NOM) were studied during composting of sewage sludge in a laboratory experiment using the pile method. Typical physicochemical parameters were measured during 53 days of composting including humic fractions. The effects of humification on the molecular properties of humic acids (HA) were investigated by 13C CP/MAS NMR spectroscopy.

On the basis of chemical analyses, 53 days of composting sewage sludge with structural material can be divided into three phases: (i) domination of rapid decomposition of non-humic, easily biodegradable organic matter (two to three weeks), (ii) domination of organic matter humification and formation of polycondensed, humic-like substances (the next two weeks), (iii) stabilization of transformed organic material and weak microbial activity. Spectroscopic characterization (13C NMR) of compost humic acids reveals changes in their structures during maturation. The changes are highly correlated with the processes taking place in bulk compost.

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Keywords: Sewage sludge; Compost; Humic matter; Spectroscopic properties

1. Introduction

Sewage sludge recycling is a preferred concept in sludge management in Europe. Due to its hygienic instability and immaturity of organic matter, sewage sludge should not be introduced into the soil environment directly. Hence, composting, i.e. controlled microbial transformations of organic matter with structural material, is a preferred strategy of utilizing the sewage treatment byproduct (Aubain et al., 2002).

During composting, part of the organic matter is mineralized to carbon dioxide, ammonia and water, whereas the other part is transformed into humic substances which are structurally very similar to those present in soils (Hernanez-Apaolaza et al., 2000).

The exogenous natural organic matter, e.g. of the compost origin, introduced to soils may strongly influence the behavior of organic xenobiotics (Iglesias-Imenez et al., 1997). The sorption capacity of hydrophobic pollutants (pesticides) by soils amended with exogenous organic matter containing humic-type compounds increases with the degree of evolution (humification) of amendments. In addition, non-composted or immature compost or other manure applied to agricultural soils may also cause phytotoxicity to plants, nitrogen and oxygen deficiency and adversely affect the environment (Garcia et al., 1992; Veeken et al., 2000).

Many tests have been proposed to assess compost maturity and stability. The organic matter transformation during composting can be predicted with the C/N ratio, concentration of water soluble fraction of organic carbon, humification indices and spectroscopic measurements including UV–Vis, FTIR and NMR (Drozd et al., 1997; Sapek and Sapek, 1999; Hsu and Lo, 1999).
It has been also proposed that the amounts of humic substances and their fractions (fulvic acids, humic acids, humins) can be used as indices of compost maturity (Senesi, 1989). However, the extraction and fractionation techniques of humic substances are non-specific, and many non-humic substances may be extracted during alkali treatment of soils or compost. The compost organic matter is very rich in non-humic substances which are also soluble in alkali solutions. Hence, a simple and common extraction procedure based on NaOH solution without any pre-extraction steps may lead to serious errors in compost maturity assessment and structure investigations of humic macromolecules (Adani et al., 1995).

The objective of this work was to study chemical and spectroscopic changes in organic matter fractions during composting of sewage sludge compost paying special attention to the molecular properties of humic acids formed during maturation.

2. Methods

2.1. Composting of sewage sludge

The compost was prepared in the Municipal Wastewater Treatment Plant in Toruń (Poland) by mixing fermented sewage sludge with straw and wooden cuttings at a ratio of 2:1:1. About 200 l of the compost was transferred to the laboratory and two piles were formed. Each compost pile was turned over, mixed and sampled after 8, 19, 28, 36, 43 and 53 days of composting. Before analysis compost samples were first air-dried and after 8, 19, 28, 36, 43 and 53 days of composting. Before analysis compost samples were first air-dried and ground to below 0.25 mm. The remnant of humidity was determined by drying at 105 °C.

2.2. Chemical analysis

The organic matter (OM) content was determined by the loss on ignition of the dry mass at 550 °C, and the total nitrogen (N) by the Kjeldahl method. The organic carbon content (C) was determined using a TOC-SSM-5000A (Shimadzu, Kyoto, Japan) analyzer.

2.3. Extractable organic carbon

One gram of each compost sample was weighted into a 250-ml polyethylene flask and extracted with 50 ml of 0.5 M NaOH by shaking for 2 h. After that the flask was left overnight. Next day the suspension was centrifuged at 3000 rpm for 25 min and the absorbance (A) of the supernatant was measured at $\lambda = 280$ nm ($A_{280}$), 472 nm ($A_{472}$) and 664 nm ($A_{664}$) (Sapek and Sapek, 1999). The following absorbance ratios indicating the degree of humification were calculated: $Q_{280} = A_{280}/A_{664}$, $Q_{472} = A_{472}/A_{664}$, $Q_{472} = A_{280}/A_{472}$. The $Q_{280}$ reflects the proportion between the lignins and other materials at the beginning of humification, and the content of materials at the beginning of transformation. The $Q_{472}$ denotes the relation between non-humified and strongly humified material. The $Q_{472}$ is often called the humification index and is the most often calculated ratio. Typical values of the $Q_{472}$ ratio. Typical values of the $Q_{472}$ ratio for humified material are usually <5 (Gieguzyńska et al., 1998).

2.4. UV–Vis spectroscopy of compost samples

One gram of each compost sample was weighted into a 250-ml polyethylene flask and extracted with 50 ml of 0.5 M NaOH by shaking for 2 h. After that the flask was left overnight. Next day the suspension was centrifuged at 3000 rpm for 25 min and the absorbance (A) of the supernatant was measured at $\lambda = 280$ nm ($A_{280}$), 472 nm ($A_{472}$) and 664 nm ($A_{664}$) (Sapek and Sapek, 1999). The following absorbance ratios indicating the degree of humification were calculated: $Q_{280} = A_{280}/A_{664}$, $Q_{472} = A_{472}/A_{664}$, $Q_{472} = A_{280}/A_{472}$. The $Q_{280}$ reflects the proportion between the lignins and other materials at the beginning of humification, and the content of materials at the beginning of transformation. The $Q_{472}$ denotes the relation between non-humified and strongly humified material. The $Q_{472}$ is often called the humification index and is the most often calculated ratio. Typical values of the $Q_{472}$ ratio. Typical values of the $Q_{472}$ ratio for humified material are usually <5 (Gieguzyńska et al., 1998).

2.5. Fractionation of humic substances and extraction of pure humic acids

For the studies on the amount of humic fractions in compost a procedure based on the Kononova–Bieczikova method was applied (Kononova, 1968). One gram of compost was extracted with 100 ml of a mixture of 0.1 M Na2P2O7 + 0.1 M NaOH for 24 h. The supernatant solution was separated by centrifugation (3500 rpm, 30 min) and the carbon content was determined in the solution (corresponding to fulvic—FA and humic acids—HA). Fifty milliliters of the supernatant solution was acidified to pH below 2 with 6 M HCl, heated to 80 °C for 2 h and allowed standing at room temperature overnight. Soluble fulvic acids (FA) were then separated from the precipitated humic acids (HA) by centrifugation (3500 rpm, 30 min). The organic carbon that corresponds to fulvic acids carbon (FA) was determined in the supernatant. The content of humic acids was calculated as (HA + FA) – (FA) = (HA).

To obtain pure samples of humic acids (HA) from compost an original method was applied. A compost sample (about 50 g) was extracted in a Soxhlet extractor with 250 ml of n-hexane for 24 h and then with 250 ml of chloroform also for 24 h to remove non-humic large molecules (bitumins, waxes, lipids, lignins, tannins). The residual compost was shaken overnight with 0.1 M HCl (1:10 soil to solution ratio) to extract small molecules and movable fraction of fulvic acids. The residual solid was shaken with 0.1 M NaOH under N2 for 24 h (1:10 soil to solution ratio) and centrifuged (3500 rpm, 30 min). The supernatant solution was acidified with 6 M HCl to pH below 2, heated to 80 °C for 2 h and allowed standing in at room temperature overnight. Soluble
Fulvic acids (FA) were then separated from the humic acids (HA) by centrifugation (3500 rpm, 30 min). The precipitated humic acids were cleaned from clay fraction by shaking for 24 h with 100 ml of dilute HF–HCl solution (5 ml 52% HF + 5 ml conc. HCl + 990 ml H2O). The cleaning procedure was repeated twice. The HA sample was also washed to completely remove Cl−, and air-dried.

2.6. 13C CP/MAS NMR spectroscopy

The solid state 13C CP/MAS NMR analysis of humic acids (HA) extracted from compost at six stages of the process was carried out using a Brüker spectrometer (Germany) at 75.47 MHz, with a spin rate of 7 kHz, 90° pulse width of a 4.5 µs. The contact time was set to 1 ms. The acquisition time was 0.13 ms. The above-mentioned parameters of analysis were chosen according to the following publications: Wandruszka et al. (1998), Shin et al. (1999), Keeler and Maciel (2000). The 13C CP/MAS NMR spectrum was divided into seven spectral areas as follows: δ = 0–45 ppm corresponds to aliphatic carbons (–CH3, –CH2–), δ = 45–65 ppm, methoxyl (–OCH3), δ = 65–95 ppm, C–O resonances of carbohydrate-type compounds mainly due to alcohol moieties, δ = 95–110 ppm, anomeric carbon of polysaccharides, δ = 110–140 ppm, unsubstituted and alkyl-substituted aromatic carbons, δ = 140–160 ppm, lignins, phenols, aromatic ethers or amines moieties, δ = 160–190 ppm, carboxyl carbon (aliphatic and aromatic) and C in amicid groups (Peuravuori and Pihlaja, 1998; Machieu, 1999).

2.7. Statistical analysis

The values based on chemical analysis are presented as means for two replications of the two composting devices. The data for each pile is the mean for three repetitions. Spectroscopic analyses were made on mixed samples. There were no statistically significant differences (95% confidence level) between the two composting piles. All the calculations were done using the STATISTICA 6.0 PL and MS Excel 2000 software.

3. Results and discussion

3.1. Composting of sewage sludge

The temperature variation during composting followed a typical pattern exhibited by many composting systems. Three phases were observed: (i) the thermophilic phase that lasted until day 22, during which the temperature rose from 22 to about 48 °C on the first 5 days, (ii) the cooling phase, in which the temperature decreased from 48 to about 16–18 °C, (iii) and the stable phase with no measurable temperature changes.

During the composting period the C/N ratio was growing up to 32 (Fig. 1). However, the amounts of carbon and total nitrogen were consequently decreasing in this study. The mineralization of carbon was slower than expected. Simultaneously, the nitrogen transformation was very weak and most of it probably volatilized as ammonia (NH3).

There is no general agreement which value of the C/N indicates maturation of organic matter and compost. Hortalstine and Rothwell (1973) claim that the value of about 15 expresses stabilization of composting mass. In the light of the studies by Iglesias-Imenez and Perez-Garcia (1992), the values below 12 indicate a high degree of compost maturity. According to Kosobucki et al. (2000), the sewage sludge compost possesses a high soil-forming value when the C/N values oscillates between 20 and 30 after maturation. The compost then may serve as a good nitrogen source for plants. All the discussion about the C/N suggests that the ratio does not make a reliable maturity index, and indicates the need for more sophisticated ones.

3.2. Water and CaCl2 extractable fractions of organic carbon

The water extract from compost is the source of the most readily biologically active organic carbon (Chefetz et al., 1998). The water soluble organic C level increased from 69.09 mg l−1 in the compost on day 8 to 105.87 mg l−1 on day 19, and then gradually but with a little variation decreased to 69.83 mg l−1 at the end of the process (Fig. 2). The increasing phase of the water soluble carbon content on the first 19 days of composting was probably caused by the appearance of soluble organic matter (e.g. sugars, proteins, hemicellulose, organic and amino acids) due to microbial-stimulated transformations of compost organic matter. The organic matter was rapidly and easily biologically degraded.
Humic-like substances of higher molecular weight during composting may be transformed from low molecular organics to a water extractable carbon, which is measured as the amount of carbon found in water extracts (Fig. 2). This transformation implies the transformation of low molecular organics to substances with limited ability to dissolve in water, thereby decreasing the water soluble organic C during composting.

During the first period, the decomposition and breakdown of organic matter was continuous, resulting in an increase in water-soluble organic carbon. From day 19, the amount of organic carbon in the water extract was decreasing probably as the result of reducing activity of microbes and the beginning of organic matter humification. A similar trend concerning water soluble organic C during composting was observed during composting pig manure (Hsu and Lo, 1999). However, in other investigations on sewage sludge, a continuous decline in the content of water soluble organic C during composting was reported (Garcia et al., 1991).

A decline in water soluble organic carbon is often used as an indicator of compost maturity. Organic material of fresh compost consists of many easily degradable and water soluble substances, whereas mature compost is rich in stable, decomposition-resistant and characterized by high molecular mass humic-like substances which have limited ability to dissolve in water.

Humic substances formed during composting show colloidal properties and may cause aggregation of organic and inorganic matter. This is a very desirable feature of organic matter because it influences many physicochemical properties of compost and soils. To investigate the colloidal properties of compost organic matter, a 0.01 M CaCl$_2$ solution was used instead of water in the same way as in the study on water extractable fractions. The aggregation of humus origin substances takes place in the presence of polyvalent cations like Ca$^{2+}$. In this study during the composting time, the amount of extractable carbon found in the 0.01 M CaCl$_2$ solution was decreasing faster in relation to the amount of carbon found in water extracts (Fig. 2). This implies transformation of low molecular organics to humic-like substances of higher molecular weight during composting. The ratio between water extractable organic carbon and solution extractable organic carbon (WEOC/SEOC) slowly decreased during the maturation time. The aggregation ability of compost organic matter was increasing during the process. The ratio of water extractable carbon to 0.01 M CaCl$_2$ solution extractable carbon may be treated as another maturity index reflecting the properties of humic substances formed during composting.

### 3.3. UV–Vis spectroscopic properties of compost organic matter

The UV–Vis absorption spectrum of alkali-soluble humic substances (humic or fulvic acids) is generally non-specific. The absorbance monotonically decreases with an increasing wavelength at a low absorption of above 500 nm. Most of the solar energy absorbed by humic materials is between 300 and 500 nm (Gaffney et al., 1996). Despite no direct information can be extracted from the humic UV–Vis spectrum, the technique is widely used for determining the molecular properties of humic material (Gieguzyńska et al., 1998). There are three important regions in the spectrum where exact absorbance is measured: about $\lambda = 280$, 400–500 nm and above 600 nm. UV–Vis analysis of alkali extracts is generally based on the assumption that the absorbance at 260–280 nm is due to lignin and quinone moieties, i.e., the material at the very beginning of transformation. The absorbance at 460–480 nm reflects the organic material at the beginning of humification and the absorbance at 600–700 nm is said to be indicative of strongly humified material with a high degree of aromatic, condensed groups (Kononova, 1968; Gieguzyńska et al., 1998). The following absorbance ratios may be then calculated: $Q_{2/6} = A_{280}/A_{664}$, $Q_{4/6} = A_{472}/A_{664}$, $Q_{2/4} = A_{280}/A_{472}$. On the basis of these absorbance ratios, the degree of maturation (humification) of organic matter may be determined.

The absorbance ratios $(Q_{2/6}, Q_{4/6}, Q_{2/4})$ of 0.5 M NaOH extracts reflect the degree of organic matter maturity in bulk compost (Sapek and Sapek, 1999). In this study all the ratios decreased and reached their minimum on day 19 ($Q_{2/4}$) or 28 ($Q_{2/6}, Q_{4/6}$), then slightly increased and stabilized on day 43 (Fig. 3). Low $Q_{2/6}$ or $Q_{4/6}$ ratios reflect a high degree of aromatic condensation and indicate a higher level of organic material humification. The first 19–28 days are the phase where rapid decomposition of living cells, lignins, quinines moieties takes place. Hence all the ratios decreased. The above-mentioned non-humic compounds generally absorb in the near UV ($\lambda = 280$ nm). After about one month of composting organic resynthesis became the dominant process. A slight increase (on day 43) in the $Q_{2/6}, Q_{4/6}$ ratios indicates a relative increase in compounds with phenolic and benzene-carboxylic groups in the structure of humic substances (Stevenson, 1994; Veeken et al., 2000). At the end of composting the humification index $Q_{4/6}$ exceeded 5, which is character-

![Fig. 2. The soluble organic carbon fractions of sewage sludge compost.](image-url)
istic of matured humic acids, and reached 8.8. This may suggest that humification is not complete, and that compost organic matter in stable and matured compost differs from natural organic matter occurring in soils (Gieguzyńska et al., 1998; Zbytniewski et al., 2002).

3.4. Humic substances content

Humic substances constitute the most important fraction of compost organic matter. Mature and well humified compost applied to a poor sandy soil may greatly improve its properties, increasing fertility and crop productivity. Moreover, humic substances strongly interact with pesticides to speed up their biodegradation, and with metal ions influencing their transport and plant accessibility. They also have ability to buffer pH and to act as a potential source of nutrients for plants. The levels of humic fractions (HA, FA, HM) in compost represent the degree of maturity.

The sum of HA and FA fractions in this study did not change considerably during the composting process (Fig. 4). However, a small increasing trend concerning the humic content was observed, which is in agreement with other studies dealing with composting of sewage sludge and municipal solid wastes (Ciavatta et al., 1993; Chefez et al., 1996). The authors did not report any significant changes in the amounts of humic substances during composting. However, Hsu and Lo (1999) reported a sharp increase in the humic matter content, from 28% to 44% on day 33 and stabilization at this level thereafter during composting of pig manure. The HA level gradually increased during the process, from 8.94% of C to 13.90% of C. The FA level gradually decreased from 11.56% of organic carbon (C_t) to 8.38% of total C_t at the end of composting. Although a wide variation may be observed in the levels of humic fractions, the trends clearly indicate that fulvic acids decrease and humic acids increase during composting. The variations observed in the amounts of humic and fulvic acids as a function of composting time, and especially the substantial decrease of HA during the first 19 days, need to be explained. The increasing level of HA represents the degree of humification and maturity of compost (Veeken et al., 2000). In general fresh composts contain low levels of HA and higher levels of FA. As composting proceeds the HA content increases and the FA level tends to decreases (Drozd et al., 1997). It must be mentioned here that extraction techniques for humic substances are in general non-specific and non-humic, easily biodegradable material (lipids, waxes, tannins, cellulose, sugars, peptides) is isolated during extraction with alkali solutions. It precipitates during acidification and precipitation of humic acids (Adani et al., 1995). However, the amount of non-humics decreases during maturation. In this study the humic fraction of fresh compost must have contained a relatively high level of non-humic, easily biodegradable organic matter which was decomposed during composting. This influenced the humic acid fraction, lowering its level before day 19. From that day the synthesis of humic acids became the dominant process, and its level started to increase.

3.5. $^{13}$C CP/MAS NMR spectroscopy

The spectrum of $^{13}$C CP/MAS NMR of humic acids reflects structural changes of humic acids (HA). On the basis of the studies conducted by other authors, the $^{13}$C NMR spectra were divided into resonance ranges reflecting different types of chemical structures and the abundance of regions in the spectrum was calculated (Table 1) (Peuravuori and Pihlaja, 1998; Machieu, 1999; Smernik and Oades, 2000).

The $\delta = 0\text{–}45$ ppm region which corresponds to aliphatic carbons ($-\text{CH}_3$, $-\text{CH}_2$) reveals a growing contribution in the spectrum during the first 28 days. After that time the spectrum intensity in the region decreases rapidly on day 36 and then shows a growing tendency until the end of composting. The contribution of methoxyl carbon region in the spectrum $\delta = 45\text{–}65$ ppm.
and in the region $\delta = 65–95$ ppm (C–O resonance of carbohydrate-type compounds mainly due to alcohol moieties) generally decreases during composting. Although strong variations may be observed in the amounts of carbohydrate-type compounds on the first 36 days of maturation the trend of the changes shows their reducing contribution in the spectrum. This direction of changes is in agreement with many studies demonstrating that the content of methoxyl and alcohol groups in the structure of immature, fresh humic acids is much higher than in matured acids (Ramini et al., 1994). A high content of –OCH$_3$ group is characteristic of young humic acids. The contribution of anomeric carbons of polysaccharides in the structure of humic acids (region $\delta = 95–110$ ppm) reveals to be a well-resolved peak only in the sample extracted from compost matured for 19 days (Fig. 5). The increase in the contribution of carbohydrate-type and polysaccharides carbon in the spectrum at the first stage of composting may suggest that the formation of humic acids may go according to the sugar–amine condensation pathway (Stevenson, 1994). According to this concept, reducing sugars and amino acids, formed as by-products of microbial metabolism, undergo non-enzymatic polymerization to form brown nitrogenous polymers of the type produced during dehydration of certain food products at moderate temperatures. The $\delta = 110–140$ ppm region corresponds to aromatic carbon in the structure of humic acids. Its contribution in the spectrum generally slowly increases from day 28, which shows growing aromaticity of HA during composting. The contribution of the $\delta = 140–160$ ppm region (lignins, phenols, aromatic ethers or amines moieties) increases from day 28 (after the stage of biodegradation of lignin moieties). The $\delta = 160–190$ ppm region represents largely resonance due to carboxyl carbon (aliphatic) and C in amidic groups. Its contribution in the HA spectrum is the highest on day 28 of composting, i.e. at the end of the biodegradation phase.

To compare the humic acids formed during composting with standard humic acids, the $^{13}$C NMR spectrum of commercially available humic acids (from Fluka) was obtained. It can be easily noticed (Fig. 5) that the spectrum of Fluka humic acids is quite different from any of those obtained for HA from compost. Only three well-resolved signals may be identified in the spectrum.

### Table 1

<table>
<thead>
<tr>
<th>Days of composting</th>
<th>Relative abundance for different types of carbons of compost humic acids</th>
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<tr>
<td></td>
<td>Alkyls</td>
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<td>8</td>
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<td>28</td>
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<td>43</td>
<td>33.05</td>
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<td>53</td>
<td>34.6</td>
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Fig. 5. $^{13}$C CP/MAS NMR spectra of humic acids extracted from comports at various stages of humification and of standard humic acids from Fluka.
of standard HA: $\delta = 0$–50 ppm (aliphatic region), $\delta = 110$–140 ppm (aromatic region) and $\delta = 165$–190 ppm (carboxyl groups region). While the abundances of the aliphatic and aromatic regions are higher than in the spectrum of HA from composts, the carboxylic region reveals lower abundance in the spectrum than reported for HA from composts. In general, in matured humic acids the carboxylic regions are bound to the aromatic core rather than to aliphatic chains.

The finding that compost humic acids differ from those that exist in other natural environments is in agreement with other studies. Garcia et al. (1991) found that humic acids extracted from composted materials have molecular sizes smaller than those extracted from fossil or soil samples. Time-limited composting does not allow the formation of highly condensed molecules of high molecular weight.

To sum up, the $^{13}$C NMR analysis shows that humic acids formed during composting have the strongest aliphatic properties (see alcoholic, aromatic, phenolic and carbonyl regions) between days 19 and 28 of maturation of sewage sludge compost. This may be due to intense phase transformation of easily biodegradable, non-humic aromatic substrates which are mineralized by microorganisms at the first stage of composting.

4. Conclusions

The concentration of compost organic matter fractions generally decreased during the composting time while the C/N ratio exhibited a slow increase during the first 36 days and stabled after another 30 days. Water and solution (0.01 M CaCl$_2$) soluble organic carbon concentrations rapidly increased to a maximum on day 19 and declined thereafter. All absorbance ratios ($Q_{24/6}^0$, $Q_{16/6}$, $Q_{23/8}$) decreased to a minimum between 19 to 28 days of composting and then slightly increased. The changes in the humic fractions reveal that the amount of humic acids decreased during the first 19 days and increased thereafter. Reverse trends were observed in the case of fulvic acids. The amounts of FA reached the maximum value on day 19 and declined thereafter. The $^{13}$C NMR analysis of purified humic acid samples at different stages of maturation reflects all the changes in compost maturity indices. The molecular structure of humic acids also differs from that of standard humic acids to a great extent.

References


The Study of Soil Organic Matter—the Methodical Guide.
Warszawa, Poland (in polish).
Environ. 81/82, 521–542.
Shin, H.-S., Monsallier, J.M., Choppin, G.R., 1999. Spectroscopic and 
chemical characterization of molecular size fractionated humic acid. Talanta 50, 641–647.
determining quantitation in \(^{13}\)C NMR spectra of natural organic matter. 1. Model systems and the effects of paramagnetic impurities. Geoderma 96, 101–129.

Stevenson, F.J., 1994. Humic Chemistry: Genesis, Composition, 
tion of NaOH-extracted humic acids during composting of a 
Zbytniewski, R., Kosobucki, P., Kowalkowski, T., Buszewski, B., 
2002. The comparison study of compost to natural organic matter 