Aim and Scope

The objective of the *Journal of Residuals Science & Technology* (JRS&T) is to provide a forum for technical research on the management and disposal of residuals from pollution control activities. The Journal publishes papers that examine the characteristics, effects, and management principles of various residuals from such sources as wastewater treatment, water treatment, air pollution control, hazardous waste treatment, solid waste, industrial waste treatment, and other pollution control activities. Papers on health and the environmental effects of residuals production, management, and disposal are also welcome.

JOURNAL OF RESIDUALS SCIENCE & TECHNOLOGY—Published quarterly—January, April, July and October by DEStech Publications, Inc., 439 North Duke Street, Lancaster, PA 17602.

Indexed by Chemical Abstracts Service. Indexed/abstracted in Science Citation Index Expanded. Abstracted in Current Contents/Engineering, Computing & Technology. Listed in ISI Master Journal.

Subscriptions: Annual $219 per year. Single copy price $60. Foreign subscriptions add $45 per year for postage.

(ISSN 1544-8053)

DES*tech* Publications, Inc.
439 North Duke Street, Lancaster, PA 17602-4967, U.S.A.

CONTENTS

Research

Comparison of Maturity Indices for Composting Different Organic Waste 55
MD. KAMRUL ISLAM, DONATO MONDELLI, ZIAD AL CHAMI, GIANCARLO MIMIOLA and STEFANO DUMONTET

Molecular Structures and Biofilm Characterization in Compost at Different Maturity Stages Using ^{13}C NMR Spectroscopy and Multiple Fluorescence Labeling Techniques 65
MINJIE WU, XINLAN MEI, QIRONG SHEN and GUANGHUI YU

Soil Texture Effects on Rhizodegradation of Crude Oil Contaminated Soil 73
FARHANA MAQBOOL, YING XU, DONGMEI GAO, ZULFIQAR AHMAD BHATTI and ZHENYU WANG

Inherent Error and Dangers of "Once-in-a-Lifetime" Exposure Limits 81
ROD O'CONNOR and P. "BRENT" DUNCAN

Comparative Analysis of Structural and Socio-cultural Properties of Agricultural Farm Enterprises in Terms of Sludge Application 87
K. YAMAN and E. OLHAN

Comparison of Maturity Indices for Composting Different Organic Waste

MD. KAMRUL ISLAM[1], DONATO MONDELLI[2], ZIAD AL CHAMI[3,*], GIANCARLO MIMIOLA[3] and STEFANO DUMONTET[1]

[1]*Dipartimento di Scienze per l'Ambiente, Università degli Studi di Napoli "Parthenope". Via F. Acton, 38, 80133 Napoli, Italy*
[2]*Dipartimento di Biologia e Chimica Agro-Forestale ed Ambientale, Università degli Studi di Bari "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy*
[3]*Mediterranean Agronomic Institute of Bari – CIHEAM, Via Ceglie 9, 70010 Valenzano, Italy*

ABSTRACT: Among several methods used to assess maturity of compost, determination of diethyl ether and choloroform extractable lipids seems to be a promising technique as lipids are an important portion of all organic wastes. The aim of this paper was to monitor maturity parameters of compost piles made from different organic sources (cow manure, poultry slaughterhouse, and dairy industry) using different methods. Diethyl ether (DEE) and chloroform ($CHCl_3$) extractable lipids have been measured during composting and compared to other parameters commonly utilized to study compost stability and maturity (organic carbon, total and inorganic N, CO_2 evolution rate, microbial biomass C, and phytotoxicity test). Results suggested none of these parameters may exhaustively describe maturity of composts studied.

INTRODUCTION

EVER increasing anthropogenic pressure on arable lands is strictly associated with population growth, technical and institutional innovation, market development, and policy actions [1–4]. The soil quality concept in this context together with a sustainable agriculture approach appears to be of special concern. Within the next 50 years it is expected that there will be an addition to the world population of 2.3 billion people, a growth of global economy around 2.9 percent annually, and a growth in food demand ranging from 2.1 billion to 3 billion tons per year [5]. However, concern is also growing about sustainability of current agricultural practices considering the detrimental effect exerted by conventional agriculture on soil ecosystems which may result in impairment of biological and ecosystem functions [6–9].

The concept of soil quality is strictly intertwined with management of soil organic matter and its enhancement and preservation [10]. Compost is an excellent source of organic matter and it contributes to improvement of soil structure and ameliorates soil carrying capacity resulting in the betterment of crop nutrition [11–13].

Composting organic residues of different origin is consistent to a host of environmental constraints and soil management requirements and is fuelled by a cultural approach which impels re-use of waste and contains elements of agronomic interest. Such an approach is in line with the concept of sustainable development already enunciated by the Brundtland Commission [14] and to a recycling philosophy widely accepted as a major concern for industrialized societies.

Compost maturity and compost stability are significant parameters depicting quality of compost. These definitions are often interchangeably used although they refer to specific properties which partially overlap. Stability refers to a specific state of organic matter of composted organic waste which is related to type of organic compounds remaining and to intensity of biological activity [15]. Maturity may be considered as the index of completeness for composting. It is widely accepted that maturity is better described by an array of compost parameters being any unique synthetic index unable to properly assess this specific feature [16]. Immature and poorly stabilized composts may cause a number of problems during storage, marketing, and use. Active decomposition of these materials into the

*Author to whom correspondence should be addressed.
E-mail: alchami@iamb.it

1544-8053/12/02 055-10

soil matrix or growth media has detrimental effects on plant growth due to reduced oxygen and nitrogen availability or due to presence of phytotoxic compounds.

There are no universally accepted standards for compost stability and/or maturity, terms often used with a unique meaning. Both at a regulatory and at a scientific level a number of scientific and technical publications deal with a host of different tests, or arrays of tests, designated as able to point out completeness of the composting process and absence of harmfulness for crops.

Methods used to assess completeness of the composting process can be roughly divided in three different groups [e.g., (1) physical and chemical properties, (2) microbiological methods, and (3) phytotoxicity index]: (1) pH [17], oxygen consumption of the composting biomass [18], maximum reheating temperature [19], rise in redox potential during the composting process [20], monitoring of oxygen consumption and carbon dioxide production [21–22], humification rate and humification index [23], humic acid to fulvic acid ratio [24], measure of compost fiber content [25], compost C/N ratio [26], losses in OM [27], and monitoring of volatile organic acids and measurement of dietyl ether and choloroform extractable lipids [29–30]; (2) dehydrogenase activity and arginine ammonification [31], nitrification and ammonification [32], and community level physiological profiles which identifies microbial populations shift from capacity of microorganisms to utilize different carbonaceous substrates [33]; and (3) previous association with presence of organic acids in composts [21,34] and germination index as described by Zucconi *et al.* [35] still widely used and being considered a precise and reliable test. Further details have been recently published in an well written review [36].

Some of the physico-chemical and biological parameters mentioned above are proven to be inconsistent [37] while some other procedures require costly equipment's, skilled manpower, and imply high analytical cost. Determination of compost stability on the basis of dietyl ether and choloroform extractable lipids [30] seems to be a promising technique as lipids are an integral part of all organic wastes and this procedure is relatively simple to do and may be done at any most chemical laboratories. The objective of this paper is to monitor maturity parameters for compost piles made from different organic sources (e.g., cow manure, poultry slaughterhouse waste, and dairy industry waste). Diethyl ether (DEE) and chloroform ($CHCl_3$) extractable lipids have been measured during composting and compared to other parameters commonly utilized to study compost stability and maturity (e.g., organic carbon, total and inorganic N, CO_2 evolution rate, microbial biomass C, and phytotoxicity test). There is a possible correlation between these parameters and extractable lipid fractions potentially confirming usefulness as a synthetic index for evaluation of compost maturity.

MATERIALS AND METHODS

Pile Composition and Management

Three windrows of compost were prepared in a store house of the Mediterranean Agronomic Institute of Bari (Apulia region, Southern Italy) by mixing 34% of vineyard pruning waste with 66% cattle manure (P1), 74% of vineyard pruning waste with 26% of poultry slaughterhouse waste (P2), and 35% of vineyard pruning waste with 65% dairy industry waste (P3) (See Table 1). A C/N ratio of 28 (P1 and P2) and 32 (P3) was obtained, respectively.

Piles' temperatures were recorded daily and piles' moisture was analyzed weekly. Pile turning and addition of water were done to maintain pile temperature below 70°C and moisture content between 55 and 65%. Samples were obtained by thoroughly homogenizing individual samples (10–12) from different spots in each pile at a 40–50 cm depth range.

Physico-Chemical and Microbiological Analysis

Temperature has been recorded daily by inserting a digital thermometer in 12 different locations at a depth

Table 1. Starting Composition of Composting Piles.

	f.w. kg	d.w. kg	Org. C g kg^{-1}	Total N g kg^{-1}	C/N
P1					
Cattle manure	1832	365	309	16.8	18
Vineyard pruning	314	191	483	6.1	79
Pile starting composition	2146	556	375	13.1	28
P2					
Poultry slaughterhouse waste	749	181	467	49.7	9
Vineyard pruning	640	509	503	6.1	82
Pile starting composition	1389	690	493	17.6	28
P3					
Dairy industry waste	1250	474	419	18.1	23
Vineyard pruning	314	250	503	6.1	82
Pile starting composition	1564	740	448	13.9	32

f.w.: fresh weight; d.w.: dry weight; org. C: organic carbon; total N: total nitrogen

between 40 and 50 cm for each pile. Moisture was measured using a gravimetric method after sample drying in an oven at 105°C for 48 hours. Pile pH was assessed in a suspension containing 3 g of sample (d.w.) with 50 mL of water using a digital pH-meter. Organic carbon was measured by dry combustion using an automatic analyzer (Thermo Scientific, Flash 2000). Samples were pretreated with 10% HCl to remove inorganic carbon. Total N was determined according to the Kjeldahl method [38] whereas inorganic N forms (NO_3^-, NO_2^- and NH_4^+) were determined according to Mulvaney [39]. CO_2 evolution rate was measured by incubating 50 g of samples on a dry weight basis (i.e., 60% of moisture) in 1,000 mL air tight containers in presence of an alkaline trap (50 mL of 1 M NaOH) at 25°C for 5 days. Trapped CO_2 was determined after addition of $BaCl_2$ and a few drops of phenolphthalein by titration with 0.5 M HCl [40]. Microbial biomass C and N were determined using the fumigation extraction method [41].

Daily respiration was estimated by sampling piles at 25, 94, and 120 days of composting. Samples were incubated as previously described for 10 days. CO_2 evolution was monitored daily and cumulated CO_2 production was calculated. Linear equations were fitted to data and their angular coefficient was assumed as daily CO_2 production. Metabolic quotient (qCO_2) was calculated as amount of C–CO_2 produced by unit of biomass-C per day. Sequential extraction of lipids was firstly done with diethyl ether (DEE) and successively with chloroform ($CHCl_3$) using a Soxhlet apparatus on 5 g (d.w.) of samples.

Phytotoxicity Test

The phytotoxicity test was carried out according to Zucconi *et al.* [42]. Briefly, samples were incubated 2 hours at 85% of moisture, centrifuged at 6000 rpm for 10 min, and then filtered through 0.2 μm cellulose filters. One mL of filtered solution was pipetted into a sterilized plastic petri-dish lined with a Whatman grade 1 filter paper. Five Petri dishes were prepared for each sample. Ten cress seeds (*Lepidium sativum* L.) were placed in each Petri dish and incubated at 23°C in the dark for 48 hours. Treatments were evaluated by counting number of germinated seeds and length of the roots. Germination index (GI) was calculated using the following formula, Equation (1):

$$GI(\%) = \frac{Gs \times Ls}{(Gc \times Lc)} \times 100 \quad (1)$$

Where, Gs = Number of germinated seeds as a sample, Ls = Average root length as a sample, Gc = Number of germinated seeds as a control, and Lc = Average root length as a control.

Statistical analysis involved using the Duncan range test of means carried out using SYSTAT software. Five replicates were analyzed for all parameters.

RESULTS

Temperature

The temperature profile of composting piles is displayed in Table 2. Peak temperature for all composting piles was rather similar even though pile P2 needed more time to reach it. Whereas, pile P3 remained at temperatures between 45 and 60°C for a longer time than piles P1 and P2.

Organic Carbon and Total and Inorganic Nitrogen

In Table 3 evolution of chemical parameters for composting piles is displayed in Table 3. The pH of pile P1 started at a value of 8.1, increased sharply at the beginning of composting, reached the maximum value at day 28 (9.7), and decreased to 9.1 at the end of the process. Pile P2 displayed the most acidic pH value (5.9) which increased during composting reaching a final value of 8.1. Pile P3 maintained a fairly constant pH starting at 7.8 and ending at 7.6. The decrease of

Table 2. Temperature Profiles for Composting Piles.

Piles	Mean Ambient Temperature* (°C)	Pile Temperature After 24h (°C)	Time to Reach 50°C (days)	Peak Temperature (°C)	Time to Reach Peak Temperature (days)	Time at a Temperature Between 45 and 60°C (days)
P1	21	31	8	60	12	17
P2	21	24	10	65	17	19
P3	21	29	7	63	10	21

*Mean of the ambient temperature over the incubation period.

Table 3. Chemical Parameters' Dynamics During the Composting Process.

Sampling Day	pH (H_2O)			Organic Carbon g kg^{-1}			Total Nitrogen g kg^{-1}			C/N			NH_4^+-N g kg^{-1}			NO_3^--N g kg^{-1}		
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
1	8.1	5.9	7.8	374.0	487.9	439.3	13.0	22.0	13.8	29	22	32	0.7	7.4	9.6	0.1	0.1	0.6
7	9.3	6.9	7.8	363.0	485.0	428.0	12.7	24.3	13.2	29	20	32	0.8	3.8	5.6	0.1	0.3	0.5
14	9.4	7.0	7.7	334.0	477.0	423.4	13.2	22.4	12.9	25	21	33	0.3	2.1	3.0	0.1	0.3	0.1
28	9.7	7.5	7.7	293.0	475.3	420.7	17.6	25.5	13.9	17	19	30	0.3	1.2	0.7	0.1	0.2	0.1
45	9.2	8.1	7.8	287.0	467.0	413.5	21.9	30.0	14.3	13	16	29	0.3	0.3	0.5	0.1	0.1	0.1
60	9.1	7.8	7.9	252.0	448.8	407.6	19.1	35.3	16.6	13	13	25	0.3	0.2	0.2	0.1	0.5	0.3
91	8.9	8.0	7.5	241.0	436.0	363.4	23.6	37.2	18.4	10	12	20	0.6	0.1	0.1	1.1	0.5	0.3
120	9.1	8.1	7.6	203.8	425.6	348.1	21.2	37.1	19.2	10	11	18	0.5	0.1	0.1	1.1	0.5	0.3

organic matter at the end of composting was 54, 87, and 79% of starting values in P1, P2, and P3 respectively, whereas total N increased by 63, 68, and 39% in P1, P2, and P3, respectively. Inorganic-N decreased by 8 and 4% in piles P2 and P3, respectively, whereas in pile P1 values remained constant over the composting period.

Sample Respiration

Samples taken from composting piles at days 28, 91, and 120 were incubated for 10 days in order to monitor daily CO_2 production (See Figure 1). All composting piles ended their respiration flush at day 1 when respiration activity ranked as follows (sampling day in brackets):

$$P1(28) > P2(28) = P3(28) = P1(91) > P3(91) > P2(91) > P1(120) = P3(120) > P2(120)$$

All samples irrespective of sampling time displayed from day 3 to 10 of incubation a linear trend of CO_2 evolution. P3 at 28 days of composting displayed the highest post-flush respiration values which accounted for 15.2 mg CO_2–C kg^{-1} organic C (mean of CO_2 evolution from day 3 to day 10). Respiration values of P1 and P2 pooled together produced a 7 days mean of only 2.8 mg CO_2–C kg^{-1} organic C. Respiration flushes lowered in the latest composting time as expected indicating progressive shortage in easily usable organic matter for microorganisms.

Angular coefficients and the R^2 of linear equations fitting cumulated respiration data are reported in Table 4. Angular coefficients were assumed to represent daily respirations for composting piles at sampling times of 28, 91, and 120 days. P2 showed the lowest daily respiration in all sampling times whereas P1 and P3 had no statistically different CO_2 daily evolutions. All values progressively decreased over sampling times.

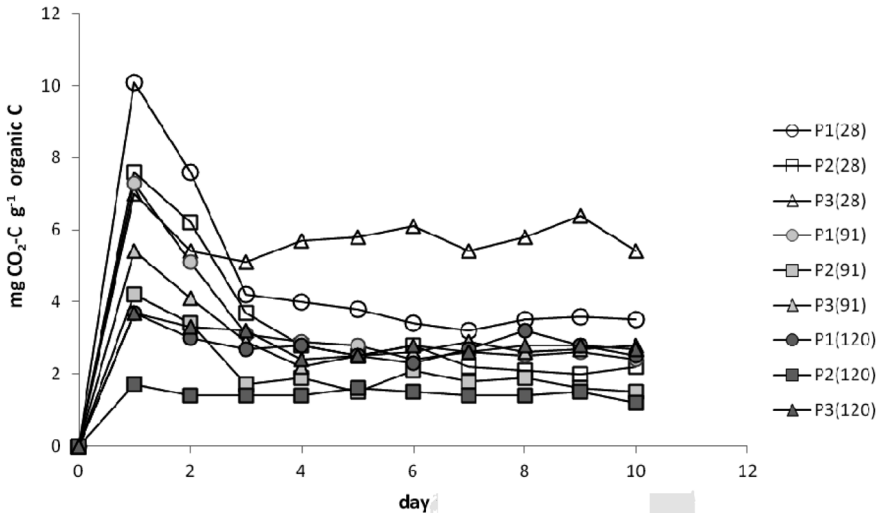


Figure 1. *Respiration of composting piles sampled at 28, 91, and 120 days over a 10 day incubation period.*

Table 4. Daily Respiration at 28, 91, and 120 Days of Composting.

Pile and Sampling Time	mg CO_2-C g^{-1} org. C.	R^2
P1-28	5.18[a]	0.9035
P2-28	3.92[b]	0.8602
P3-28	5.82[a]	0.9994
P1-91	3.72[b]	0.9123
P2-91	2.35[d]	0.9427
P3-91	3.22[b,c]	0.9679
P1-120	2.82[d]	0.9979
P2-120	1.48[e]	0.9992
P3-120	2.94[d]	0.9946

Means with different letters indicate significant difference between values (Duncan test, $P < 0.05$).

Microbial Biomass and Metabolic Index (qCO_2)

Microbial biomass evolution and metabolic quotients during composting are displayed in Table 5. The MBC remained fairly constant for the three sampling dates of 28, 91, and 120 days for Piles P1 and P2. Whereas, the MBC decreased during composting in pile P3 reaching a value at 120 days which was 60% lower than those recorded at 28 days. The metabolic quotients (qCO_2) expressed as unit of CO_2-C respired by unit of MBC were on the contrary rather constant in pile P1, increased by the end of the incubation in pile P2, and decreased in pile P3. Pile P2 displayed the highest qCO_2 values. The pile P2 had an intermediate value and the pile P3 displayed the lowest value.

Lipids

At the starting of the composting process pile P2 displayed the highest content of DEE + $CHCl_3$ extractable lipids (29.7 mg kg^{-1} d.m.) followed by pile P1 (19.5 mg kg^{-1} d.m.) and pile P3 (10 mg kg^{-1} d.m.). After 122 days of incubation these values were reduced by 70% in pile P1 (6.1 g kg^{-1} d.m.), by 93% in pile P2 (2.0 g kg^{-1} d.m.), and by 81% in pile P3 (1.9 g kg^{-1} d.m.). The sharp reduction in lipid content in the composting piles was mainly due to a decrease of DEE extractable lipids as displayed in Figure 2.

The DEE extractable lipids decreased with different behaviors in composting piles for piles P1 and P2 at the 14th day of composting (i.e., 50 and 30% of their starting values, respectively). In pile P3 this parameter was on the contrary slightly higher than that recorded at the beginning of composting. The highest value recorded in pile P2 (26.9 g kg^{-1} d.m.) is due to specific composition of the organic waste (poultry slaughterhouse waste). At 46 days of composting the DEE values in piles P1, P2, and P3 were 39, 25, and 68% of their starting values, respectively. At the end of the composting DEE was reduced to a larger extent in pile P1 (32% of the starting value). Whereas, in piles P2 and P3 the DEE decreased by 6 and 15% of the starting value, respectively.

The $CHCl_3$ extractable lipids displayed a comparable trend for all composting piles as they were reduced by 70% (P1), 82% (P2), and 71% (P3) at the end of composting.

Seen in Figure 3 are the ratios of total extractable lipids TEL/$CHCl_3$ and DEE/$CHCl_3$. It is interesting to highlight that both the TEL/$CHCl_3$ and DEE/$CHCl_3$ ratios for piles P1 and P3 displayed values between 1.8 and 3.2 at the beginning of the composting and they kept a linear trend during the incubation time which was roughly parallel to the x-axis. On the contrary values pertaining to pile P2 started at 10.6 and 9.6 for TEL/$CHCl_3$ ratio and DEE/CHCL3 ratio, respectively. These values dropped to 4.4 and 3.4 at the 14th day and reached 4.0 and 3.0 at the end of composting.

Germination Index

Germination index for the composting piles is reported in Figure 4. The index increased in all the composting piles during the incubation time and reached a steady state set around 40% in piles P1 from day 60 and in pile P3 from day 90 up to the end of the incubation. In pile P2 on the contrary the index increased con-

Table 5. Microbial Biomass C and Metabolic Quotient for Composting Piles.

	Pile 1		Pile 2		Pile 3	
Composting Time (days)	MBC mg C g^{-1} org. C	qCO_2 mg CO_2-C mg^{-1} MBC	MBC mg C g^{-1} org. C	qCO_2 mg CO_2-C mg^{-1} MBC	MBC mg C g^{-1} org. C	qCO_2 mg CO_2-C mg^{-1} MBC
28	21.8[b]	4.2[a]	12.1[b]	3.1[a]	19.5[b]	3.3b
91	17.4a,b	4.7[a]	9,6[a,b]	4.1a,b	9.5[a]	2.9[b]
120	20.1[b]	3.4[a]	9.6[a,b]	6.5[b]	7.8[a]	0.7[a]

Means with different letters indicate significant difference between values (Duncan test, $P < 0.05$).

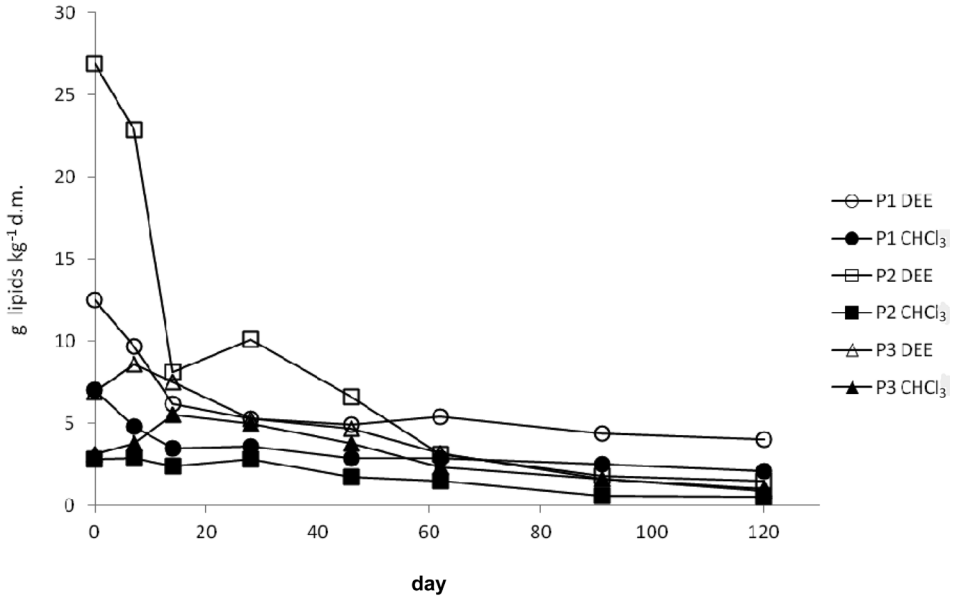


Figure 2. *Yields of DEE- and $CHCl_3$-extract lipids from 3 composting piles during incubation time.*

stantly during incubation reaching the value of 80% at day 120.

DISCUSSION

The evaluation of compost maturity is necessary to predict the effect of compost amendment on soil physical, chemical, and biological properties [43]. Also, a more comprehensive definition of compost maturity suggests an absence of phytotoxicity effect on plant growth [44]. The heterogeneity of starting materials (i.e., all kinds of organic residues may be composted) makes it difficult to identify a unique maturity index. Forster *et al.* [31] observed that only few significant correlations were found between different chemical and microbiological methods they used to assess compost maturity for various organic materials.

Tiquia [45] found that composting parameters (e.g., temperature, microbial indexes, and chemical compounds) changed predictably during composting of pig manure even though composting piles were made and managed in different ways. During this study organic matter of different origin was composted using the same management practice but not all the studied parameters predictably evolved during composting. Starting organic materials were rather different (cattle manure, poultry slaughterhouse, and dairy industry waste) even though they were all mixed with vineyard pruning wastes in order to reach an optimal C/N for all composting piles.

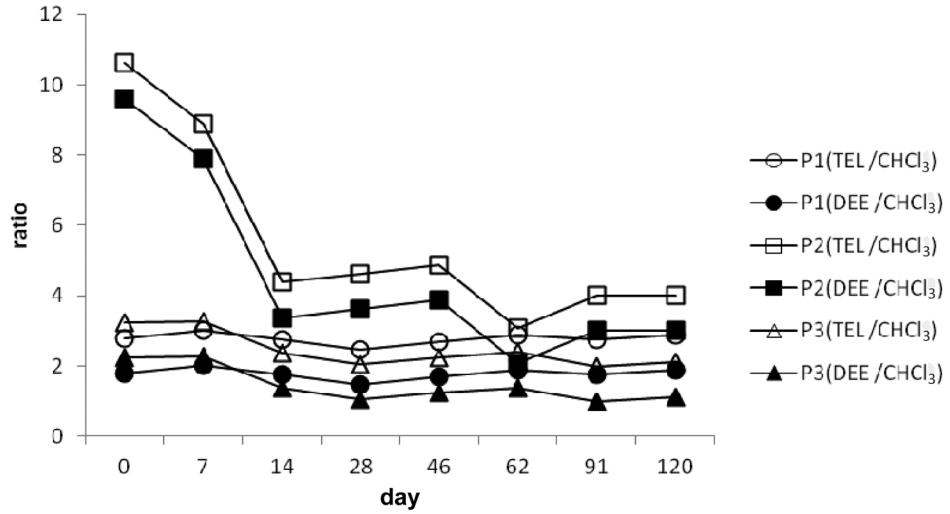


Figure 3. *Ratios of total TEL/$CHCl_3$ extractable lipids and $CHCl_3$/DEE extractable lipids.*

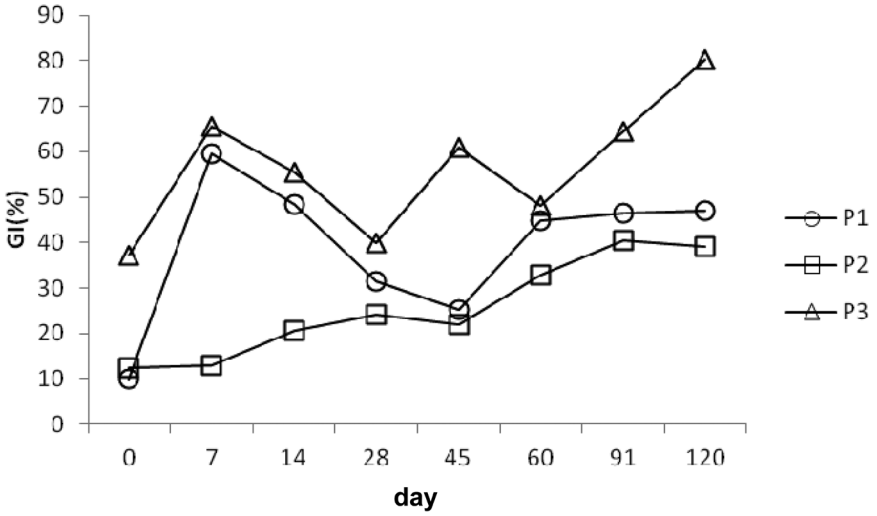


Figure 4. *Germination index of composting piles during the composting time.*

Nevertheless, different composition of starting organic matter was responsible for a few different behaviors. The temperature profiles of the composting piles were different (See Table 2) including the time period at which the piles' temperature, longer for pile P3, was between 45 and 60°C.

In addition, a phenomenon such as the total N increase during composting common to all the piles is likely determined by different reasons. In fact, in pile P1 it could be attributed to an increase of both organic and inorganic N as the latter increased by 65% from day 0 to day 120. In piles P2 and P3 on the contrary the mineral N sharply decreased 92 and 96%, respectively, whereas increase in total N could be attributed to a steady increase in organic N.

Microbial biomass also evolved in a different manner in the composting piles. This parameter was rather stable in piles P1 and P2 whereas it decreased sharply in pile P3 from the incubation starting day to day 91 and remained stable until the end of the incubation period.

Anderson and Domsch [46] theorized the microbial metabolic quotient (qCO_2) as an index able to increase under ecosystem disturbance. This index is considered to be an estimate of "excessive" CO_2 production from microbial biomass indicating diversion of significant quantities of metabolic energy for repair and maintenance activities due to stress. The metabolic quotient was extensively used as tool for assessing ecotoxicological behaviors of xenobiotic compounds towards soil microbial biomass and its activities [47–48]. The quantitative estimation of the C flux through the microbial biomass as measured by the qCO_2 should be able in this context to identify compost maturity as a condition characterized by lowest concentration of bioactive organic compounds and the highest concentration of biostable organic compounds as it integrates compost respiration and microbial biomass C. Such a maturity state should be responsible for the lowering of the qCO_2 as a result of a process culminating in a stabilized ecosystem in which microbial biomass respiration is maintained as low as possible as result of an optimized energy flow.

The metabolic quotient followed different patterns in different piles remaining stable during incubation in pile P1, increasing in pile P2 where it reached a rather high value at day 120, and decreasing sharply in pile P3 where it reached a very low value at the end of incubation. If metabolic quotient is taken into consideration as a potential maturity index, then only the pile P3 could considered mature.

Lipids are organic compounds soluble in organic solvents but sparingly soluble or insoluble in water. Procedures for the extraction of lipids from source material usually involve selective solvent extraction. Solubility of lipids is an important criterion for their extraction from source materials and strongly depends on the type of lipid present and the proportion of nonpolar (principally triacylglycerols) and polar lipids (mainly phospholipids and glycolipids) in the sample. Therefore, several solvent systems have to be considered depending on type of sample and its components.

Sequential lipid extraction with DEE and $CHCl_3$ described by Dinel *et al.* [29–30] yields easily biodegradable lipids (DEE extractable) and more recalcitrant ones ($CHCl_3$ extractable). These same authors demonstrated that the lipid fraction of organic waste became more homogeneous during composting as the

more easily decomposable part is mineralized and the more recalcitrant and stable part resists mineralization.

Even though Dinel *et al.* [29] stated that the ratios of easily biodegradable to bioresistant lipids are generally less than 2.5 at the end of composting, a value of 3 was found in pile P2 likely not subjected to further decrease. Piles P1 and P3 showed rather constant ratios of both TEL/$CHCl_3$ extractable and DEE/$CHCl_3$ extractable lipids without any significant modification during composting. Only pile P2 made of highly proteinaceus and lipidic material (i.e., poultry slaughterhouse waste) typically contains 15.2% of proteins and 10.0% of lipids over total solids [49]. This displayed a bioresistant lipid fraction accounting for 40% of total extractable lipids.

Substantial steadiness of lipid ratios (total versus bioresistant lipids in particular) made it difficult to judge compost maturity in piles P1 and P3 on the basis of this parameter. In pile P2, however, a sharp decrease in both TEL/$CHCl_3$ extractable and DEE/$CHCl_3$ extractable lipids took place at day 14 and these ratios reached their minimum value on day 62. According to Dinel *et al.* [29–30] the composting material in pile P2 should have then reached its maturity at day 62.

Compost phytotoxicity is one of the most important criteria used to discriminate potential environmental risk of composts to be re-used on agricultural lands. Previous studies [50] showed various negative effects on seed germination, plant growth, and development resulting from soil amendment of immature compost. In fact, an immature compost application induces higher microbial activity (i.e., reducing oxygen concentration in the soil) and blocks existing available nitrogen which gives rise to serious N-deficiencies in crops [41]. It also introduces phytotoxic compounds such as phenolic compounds, ethylene and ammonia [51], excess accumulation of salts [52], and organic acids which could retard or prevent seed germination and growth.

Germination index at the end of incubation was rather low for piles P1 and P2 (47 and 39%, respectively) pointing out a potential phytoxicity problem. Pile P3 showed, instead, a germination index of 80% at day 120.

CONCLUSIONS

Composting confers agronomic value to organic waste and it is in line with the recycling philosophy widely acknowledged as a primary concern for industrialized societies. Compost quality has to be consistent with keeping of soil biological functions to the safeguard physico-chemical characteristics and for crop protection. In this regard maturity is an essential parameter to take into consideration in order to prevent detrimental effect on soil and crops when compost is used as a soil conditioner.

In this work the comparison among 3 different organic wastes composted in turned windrows was made. Results demonstrated an inconsistency among some of the most widely used parameters for assessing compost maturity. Temperature profiles were rather different for 3 composting piles for their several parameters such as time to reach 50°C, peak temperatures, and the time phase in which temperature ranged between 45 and 60°C. However, temperature profiles of the three windrows demonstrate that pile management was adequately prepared to facilitate the composting process.

Results revealed that from the beginning till to the end of the composting process organic carbon decreased from 13 to 45% while the total N increased from 39 to 68% in all the composting piles. According to Zucconi and de Bertoldi [32], a NH_4^+-N content < 0.4 g kg^{-1} should be indicative of compost maturity. In this work a value below that threshold was obtained after 45 (P2) and 60 (P3) days of composting in P2. In pile P1 on the contrary the NH_4^+-N content was 0.5 g kg^{-1} at the end of composting. Thus, evolution of NH_4^+ during the composting period for the different composted wastes seems to be inadequate for determining compost maturity.

Daily respiration shows a similarly decreasing trend with increasing composting time. However, values differ consistently from pile to pile with the following use of this parameter as an index of compost maturity. The values of microbial biomass C and metabolic quotient are also not consistent across piles.

Decrease of total lipids varied from 70 to 93% during the composting period for the different piles mainly due to the sharp decrease of DEE extractable lipids while the decrease of $CHCl_3$ extractable lipids followed the same pattern for all composting piles. Data were not in agreement with those of Dinel *et al.* (1996) regarding the TEL/$CHCl_3$ and DEE/$CHCl_3$ ratios pointing out compost maturity for piles P1 and P3 whereas such ratios indicated attainment of maturity at 60 days for the pile P2.

Even the germination index gave contrasting results indicating potential toxicity problems for piles P1 and P2 at the end of the composting process while pile P3 showed a germination index of 80%. Compost maturity should assure suitability of compost for plant growth. The study revealed that none of the single pa-

rameters are applicable to identify compost maturity for the cases of organic wastes used here. Although, the process was managed in the same way for all piles. In order to come to an unambiguous maturity index a careful selection of parameters is necessary based on the characteristics of wastes used.

ACKNOWLEDGEMENT

The authors thankfully acknowledge Mr. Rocco Laricchia (CIHEAM-IAM-Bari) for his role in the management of the three compost piles.

REFERENCES

1. Harvey, M., Pilgrim, S. The new competition for land: Food, energy, and climate change, *Food Policy* Vol. 36, 2011, pp. S40–S51.
2. Kim, J. H. Linking Land Use Planning and Regulation to Economic Development: A Literature Review, *J Plan Lit,* Vol. 26, No. 1, 2011, pp. 35–47.
3. Pacheco, P., Aguilar-Stoen, M., Borner, J., Etter, A., Putzel, L. and Diaz, M. del C. V. Landscape transformation in tropical Latin America: assessing trends and policy implications for REDD^{+}. Forests Vol. 2, No. 1, 2011, pp. 1–29.
4. Tidsskrift, N. G. Responses of rural households to the impacts of population and land-use changes along the Eastern Escarpment of Wello, Ethiopia, *Norw J Geogra,* Vol. 65, No. 1, 2011, pp. 42–53.
5. FAO 2009. Global agriculture towards 2050, High Level Expert Forum-How to Feed the World in 2050. Agricultural Development Economics Division, Rome, Italy, pp 4.
6. Matson, P., Parton, W., Power, A. and Swift, M. Agricultural intensification and ecosystem properties, *Science,* Vol. 277, 1997, pp. 504–509.
7. Roba, H. G. and Oba, G. Efficacy of integrating herder knowledge and ecological methods for monitoring rangeland degradation in northern Kenya, *Hum Ecol,* Vol. 37, No. 5, 2009, pp. 589–612.
8. Darnhofer, I., Fairweather, J., Moller, H. Assessing a farm's sustainability: insights from resilience thinking, *Int J Agr Sustain,* Vol. 3, No. 8, 2010, pp. 186–198.
9. Viglizzo, E. F., Frank, F. C., Carreno, L. V., Jobbagy, E. G., Pereyra, H., Clatt, J., Pincen, D. and Ricard, M.F. Ecological and environmental footprint of 50 years of agricultural expansion in Argentina, Glob Change Biol, Vol. 17, No. 2, 2011, pp. 959–973.
10. Carter, M. R. Soil Quality for Sustainable Land Management: Organic Matter and Aggregation Interactions that Maintain Soil Functions, *Agron J,* Vol. 94, 2002, pp. 38–47.
11. Dumontet, S. and Chaussod, R. The effect of sewage sludge and sludge compost on soil microflora, *Agr Med,* Vol. 127, 1997, pp. 205–219.
12. Dinel, H., Monreal, C. M. and Schnitzer, M. (1998) Extractable lipids and organic matter status in two soils catenas as influenced by tillage, Geoderma, Vol. 86, 1998, pp. 279–293.
13. Senesi, N. (1989) Composted materials as organic fertilizers, *Sci. Total Environ,* Vol. 81, 1989, pp. 521–542.
14. WCED 1987. Our Common Future. The World Commission on Environment and Development, G H Brundtland (ed), Oxford
15. Leege, P. B. and Thompson, W. H. 1997. Test Methods for the Examination of Composting and Compost. The US Composting Council, 4424 Montgomery Avenue, Suite 102, Bethsda, Maryland 20814. USA.
16. Goyal, S., Dhull, S. K. and Kapoor, K. K. Chemical and biological changes during composting of different organic wastes and assessment of compost maturity, Bioresource Technol, Vol. 96, No. 14, 2005, pp. 1584–1591.
17. Sanchez-Monedero, M. A., Roig, A., Paredes, C. and Bernal, M. P. Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures, Bioresour Technol, Vol. 78, No. 3, 2001, pp. 301–308.
18. Haug, R. T. 1993. The practical handbook of compost engineering/Boca Raton: Lewis Publishers, Boca Ratan Florida, pp. 718.
19. Richard, D. and Zimmerman, R. Respiration rate - Reheating potential: A comparison of measures of compost stability, *Compost Sci Util,* Vol. 3, No. 2, 1995, pp. 74–79.
20. Hubbe, M. A., Nazhad, M. and Sánchez, C. (2010) Composting of lignocellulosics. BioResources, Vol. 5, No. 4, 2010, pp. 2808–2854.
21. Iannotti, D. A., Grebus, M. E., Toth, B. L., Madden, L. V. and Hoitink, H. A. J. Oxygen respirometry to assess stability and maturity of composted municipal solid waste, J Environ Qual, Vol. 23, 1994, pp. 1177–1183.
22. Paletski, W. T. and Young, J. C. Stability measurement of biosolids compost by aerobic respirometry, *Compost Sci Util,* Vol. 3, 1995, pp. 16–24.
23. Mathur, S. P., Owen, G., Dinel, H., Schintzer, M. (1993). Determination of compost biomaturity. I: Literature review. II: Optical density of water extracts of composts as a reflection of their maturity, *Biol Agric Hortic,* Vol. 10, No. 2, 1993, pp. 65–124.
24. Bernal, M. P., Navarro, A. F., Roig, A., Cegarra, J. and Garcıa, D. Carbon and nitrogen transformation during composting of sweet sorghum bagasse, *Biol. Fert. Soils,* Vol. 22, 1996, pp. 141–148.
25. Hutchinson, M. and Griffin, T. S. Evaluation of Fiber Content Relative To Other Measures of Compost Stability, *Compost Sci Util,* Vol. 16, No. 1, 2008, pp. 6–11.
26. Poincelot, R. P. 1975. The biochemistry and methodology of composting. The Connecticut Agricultural Experiment Station, New Haven, Bulletin pp 754.
27. Benito, M., Masaguer, T. O., Moliner, T. O., Arrigo, N. and Palmmm, M. Microbiological Chemical and parameters for the characterization of the stability and maturity of pruning waste compost, *Biol. Fert Soils,* Vol. 37, 2003, pp. 184–189.
28. Namkoong, W., Hwang, E., Cheong, J. and Choi, J. (1999) A Comparative Evaluation of Maturity Parameters for Food Waste Composting, *Compost Sci Util.* Vol. 7, No. 2, 1999, pp. 55–62.
29. Dinel, H., Schnitzer, M. and Dumontet, S. Compost maturity: Chemical characteristics of extractable lipids, *Compost Sci Util,* Vol. 4, 1996, pp. 16–25.
30. Dinel, H., Schnitzer, M. and Dumontet, S. Compost maturity: extractable lipids as indicators of organic matter stability, *Compost Sci Util,* Vol. 4, 1996, pp. 6–12.
31. Forster, J. C., Zech, W. and Wurdinger, E. Comparison of chemical and microbiological methods for the characterization of the maturity of composts from contrasting sources, *Biol Fert Soils,* Vol. 16, 1993, pp. 93–99.
32. Zucconi, F. and De Bertoldi, M. 1987. Compost specifications for the production and characterization of compost from municipal solid waste. In: De Bertoldi, M., Ferranti, M. P., Hermite, P. L. and Zucconi, F. (eds) *Compost: Production, Quality and Use.* Elsevier Applied Science: London, pp. 30–50.
33. Mondini, C. and Insam, H. Community level physiological profiling as a tool to evaluate compost maturity: a kinetic approach, *Eur J Soil Biol.* Vol. 39, 2003, pp. 141–148.
34. Herrmann, R. F. and Shann, J. R. Enzyme Activities as Indicators of Municipal Solid Waste Compost Maturity, *Compost Sci Util,* Vol. 1, No. 4, 1993, pp. 54–63.
35. Zucconi, F., Monaco, A., Forte, M. and Bertoldi, M. 1985. Phytotoxins during the stabilization of organic matter. In: Gasser, J. K. R (ed) *Composting of agricultural and other wastes.* Elsevier Applied Science, London and New York. pp. 73–88.
36. Wichuk, K. M., McCartney, D. Compost stability and maturity evaluation—a literature review, *Can J Civil Eng,* Vol. 37, No. 11, 2010, pp. 1505–1523.
37. Itavaara, M., Venelampi, O., Vikman, M. and Kapanen, A. 2002. Compost Maturity Problems Associated with Testing. In: Insam, H., Riddech, N. and Klammer, S. (eds) *Microbiology of Composting,* Springer Verlag, Heidelberg, pp. 373–382.
38. Bremner, J. M., Mulvaney, C. S. 1982. Nitrogen—Total. In: Page, A. L.

and Miller, R. H. (eds) *Methods of Soil Analysis.* Agronomy Monograph 9, Part 2, ASA and SSSA, Madison, USA, pp. 595–624.

39. Mulvaney, R. L. 1996. Nitrogen-inorganic forms. In: Sparks, D. L. (ed) *Methods of Soil Analysis,* Part 3—Chemical Methods, ASA and SSSA, Madison, USA, pp. 1123–1184.
40. TMECC 1997. *Test Methods for the Examination of Composting and Compost.* The US Composting Council. First Edition. The United States Composting Council, Amherst, OH.
41. Sparling, G. P. and West, A. W. A direct extraction method to estimate soil microbial C: calibration in situ using microbial respiration and "C labelled cells, *Soil Biol Biochem,* Vol. 20, No 3, 1988, pp. 337–343.
42. Zucconi, F., Forte, M., Monaco, A. and Beritodi, M. Biological evaluation of compost maturity, *Biocycle,* Vol. 22, 1981, pp. 27–29.
43. Francou, C., Poitrenaud, M. and Houot, S. (2005) Stabilization of organic matter during composting : influence of process and feedstocks, *Compost Sci Util,* Vol. 13, No. 1, 2005, pp. 72–83.
44. Wu, L. L., Ma, Q. and Martinez, G. A. Comparison of methods for evaluating stability and maturity of biosolids compost, *J Environ Qual,* Vol. 29, 200, pp. 424–429.
45. Tiquia, S. M. Microbiological parameters as indicator of compost maturity, *J Appl Microbiol,* Vol. 99, 2005, pp. 816–828.
46. Anderson, T. H. and Domsch, K. H. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils, *Soil Biol Biochem,* Vol. 25, 1993, pp. 393–395.
47. Fliessbach, A., Martens, R. and Reber, H. H. Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge, *Soil Biol Biochem,* Vol. 26, 1994, pp.1201–1205.
48. Perucci, P., Dumontet, S., Bufo, S. A., Mazzatura, A. and Casucci, C. Effects of organic amendment and herbicide treatment on soil microbial biomass, *Biol Fert Soils,* Vol. 32, 2000, pp. 17–23.
49. Salminen, E. A. and Rintala, J. U. Semi-continuous anaerobic digestion of solid poultry slaughterhouse waste: effect of hydraulic retention time and loading, *Waste Res,* Vol. 36, 2002, pp. 3175–3182.
50. Morel, J. L., Cohn, F., Germon, J. C., Godin, P. and Juste, C. 1985. Methods for the evaluation of the maturity of municipal refuse compost. In: Gasser, J. K. R. (ed) *Composting of Agricultural and Other Wastes.* Elsevier Applied Science, New York, USA, pp 56–72.
51. Wong, M. H., Cheung, Y. H. and Cheung, C. L., The effects of ammonia and ethylene oxide in animal manure and sewage sludge on seed germination and root elongation of Brassica parachinensis, *Environ Poll,* Vol. 30, 1983, pp. 109–123.
52. Tam, N. F. Y. and Tiquia, S. M., Assessing toxicity of 'spent sawdust pig-litter' using seed germination technique, *Resour Conserv Recycl,* Vol. 11, 1994, pp. 261–274.

Molecular Structures and Biofilm Characterization in Compost at Different Maturity Stages Using ^{13}C NMR Spectroscopy and Multiple Fluorescence Labeling Techniques

MINJIE WU, XINLAN MEI, QIRONG SHEN and GUANGHUI YU*

Jiangsu Key Lab for Organic Solid Waste Utilization, Key Laboratory of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, PR China

ABSTRACT: Understanding the development of the molecular structure of compost and biofilms is essential for improving the efficiency of composting processes. In the present study, ^{13}C NMR spectroscopy and multiple fluorescence labeling were used to characterize the development of the molecular structure and architecture of biofilms at different maturity stages in a full-scale compost facility. Results showed that the evolution of the molecular structure of bulk compost occurred predominantly at the thermophilic stage (from 2 to 36 d), while minor changes were observed in the cooling stage (from 37 to 69 d), as revealed by solid-state ^{13}C cross-polarization magic-angle- spinning (CPMAS) NMR spectra. Multiple fluorescence labeling combined with confocal laser scanning microscopy (CLSM) observation demonstrated that the biofilm was well-formed at the end of the thermophilic stage (i.e., 37 d) and presented a thickness of approximately 80 μm on the compost surface. ^{13}C CPMAS NMR spectra of dissolved organic matter in compost further indicated that the C in the biofilm consisted primarily of CHOH groups, carboxyl carbons, methyl carbons, and anomeric carbons. Increased knowledge about the molecular structure of compost and biofilms contributes to our understanding of composting and provides novel information for engineering applications as well as scientific research.

INTRODUCTION

COMPOSTING is a cheap, efficient, and sustainable method of transferring waste manure into commercial organic fertilizer [4,10,18]. Assessment of compost maturity is of utmost importance for achieving high quality compost, guaranteeing compost marketability and reducing the harmful effects of immature compost application [12]. However, common approaches for assessing compost maturity are based on empirical criteria, which are often not self-consistent [18]. The most appropriate and reliable criteria for evaluating compost maturity should be based on the molecular structure of organic matter (OM) in compost [13].

As a non-destructive spectroscopic technique, ^{13}C cross-polarization magic-angle- spinning nuclear magnetic resonance (^{13}C CPMAS NMR) is one of the most powerful tools for evaluating the structure and composition of OM during composting. However, the evolution of the molecular structure of compost is closely related to the in situ distribution pattern of biopolymers [19]. Although ^{13}C CPMAS NMR can provide details on the transformation of OM during composting, this technique cannot supply information concerning the in situ distribution of OM.

Recently, multiple fluorescence labeling combined with confocal laser scanning microscopy (CLSM) has been shown to be a powerful tool for identifying the distribution and role of macromolecules such as proteins, α-polysaccharides, cellulose, lipids, total cells and dead cells in biofilms, providing visual insight into their structure [2, 18, 3, 19]. Nevertheless, the identification and quantification of specific biofilm constituents is limited by the availability of fluorescently labeled probes [5]. CLSM and various methods based on chemical structural analyses, such as ^{13}C CPMAS NMR spectroscopy, can be combined to provide a comprehensive understanding of biofilm development [19].

*Author to whom correspondence should be addressed.
E-mail: yuguanghui@njau.edu.cn

1544-8053/12/02 065-08

The objectives were (1) to explore the development of the molecular structure of compost at different maturity stages in a full-scale compost facility by ^{13}C CPMAS NMR and (2) to evaluate the structure and function of biofilms in compost at different maturity stages in a full-scale compost facility by combining multiple fluorescence labeling and ^{13}C CPMAS NMR.

MATERIALS AND METHODS

Composting Process and Biofilm Sample Collection

A detailed description of the composting and sampling process could be seen in a previous report [15]. One windrow in a full-scale compost facility with a volume of 13 × 1 × 1.5 m (length × width × height) was filled with swine manure, rice husk, and straw. Composting was performed under aerobic conditions for 69 d. In particular, the primary fermentation was conducted for 44 d and the second fermentation was conducted for 25 d. Piles were turned at the 2, 4, 6, 8, 11, 15, 18, 22, 26, 29, 32, and 36 d.

During the composting process, four longitudinal sections were randomly dug from different parts of the piles to ensure that typical samples were collected. Sections were nearly 0.5 m wide from the pile surface and 1.5 m high. Samples were collected from the entire part of the section. The samples from the four sections were combined, mixed, and divided into four parts by quartile method to collect subsamples. About 2 kg of subsample was collected on days 6, 37, and 69 of compost and divided into two subsamples. Various chemical, biological, and spectral indices showed that the pile entered the thermophilic phase on day 2, reached maturity after the 37th day, and subsequently entered the cooling phase. The C/N ratio, moisture content, and germination index (GI) at the 37th day were 9.48, 31.8%, and 85%, respectively.

Multiple fluorescent labeling and CLSM observations were conducted on one subsample of compost. Biofilm was identified in the other subsample to determine their chemical structure and composition. Briefly, a biofilm was separated from the compost by shaking the sample in deionized water (a solid to water ratio of 1:10 w/v was applied) for 24 h on a horizontal shaker at room temperature [19]. Prior to NMR spectral analysis, microorganisms in biofilms from fresh compost were filtered through a 0.45 μm polytetrafluoroethylene filter under a vacuum of 30 cm of Hg in dead-end membrane filtration tests and were freeze-dried at –50°C for 48 h.

Solid-State CP and DD MAS ^{13}C NMR Spectroscopy

Solid-state ^{13}C CPMAS NMR spectroscopy was conducted on a Bruker AV-400 spectrometer equipped with a 4-mm wide-bore magic-angle- spinning (MAS) probe. NMR spectra were obtained by applying the following parameters: rotor spin rate = 13 kHz, recycle time = 1 s, contact time = 1 ms, acquisition time = 20 ms, number of scans = 4,000. The samples were packed in 4-mm zirconia rotors with Kel-F caps. The pulse sequence was applied using a ^{1}H ramp to account for the non-homogeneity of Hartmann-Hahn conditions at high spin rotor rates. Chemical shifts were calibrated with adamantine.

Dipolar dephasing (DD) identifies non-protonated carbons and highly mobile carbons by distinguishing between strong and weak dipolar interactions of carbons with neighboring protons [11]. DD spectra were generated by applying a decoupling delay of 68 μs between cross polarization and data acquisition. NMR signals of carbons that strongly interact with a neighboring ^{1}H rapidly lose intensity during the dephasing delay. In contrast, the signals of carbons without directly bound protons remain intense for longer dephasing times due to their weak C–H coupling [11].

NMR Spectrum Analysis

The following interpretation of the NMR data focuses on relative changes in C components due to decomposition rather than absolute amounts of C. Crystallinity indexes (CrI) were calculated from the areas of crystalline (86–92 ppm) and amorphous (79–86 ppm) C4 [8]:

$$\text{CrI}\ (\%) = \frac{A_{86\text{-}92\ \text{ppm}}}{A_{79\text{-}86\ \text{ppm}} + A_{86\text{-}92\ \text{ppm}}} \times 100 \qquad (1)$$

where A represents area of the NMR regions.

The signal in the 145–163 ppm region of the spectrum results from C_3 and C_4 phenolic carbons in lignin units, and the signal in the 47–60 ppm region is derived from lignin methoxy carbons. Therefore, the total lignin carbon content can be calculated using the following equation:

$$\text{Lignin carbon} = 4.5A_{145\text{-}163\ \text{ppm}} + A_{47\text{-}60\ \text{ppm}} \qquad (2)$$

where 4.5 represents the sum of the four aromatic (C_1, C_2, C_5, C_6) and two phenolic (C_3, C_4) carbons of lignin monomer units which can be calculated as

3 $A_{145\text{-}163\ ppm}$, and the three carbons of the lignin side chain (C_α, C_β, C_γ), as 1.5 $A_{145\text{-}163\ ppm}$ [Figure 1(c)].

The signals in the 60–95 ppm region arise from C_2, C_3, C_4, C_5, and C_6 of polysaccharide monomer units [Figure 1(b) and 1(c)] and the three side-chain carbons (C_α, C_β, and C_γ) of lignin units. Including the signal from the anomeric C1 of polysaccharide monomer units, the total contribution of polysaccharide carbons can be expressed as:

$$\text{Polysaccharide carbon} = 1.2(A_{60\text{-}95\ ppm} + 1.5A_{145\text{-}163\ ppm}) \quad (3)$$

where 1.2 represents the sum of C_2 to C_6 of cellulose and hemicellulose monomer units after removal of the three carbons of the lignin side chain (C_α, C_β, C_γ), as 1.5 $A_{145\text{-}163\ ppm}$ [Figure 1(c)].

Thus, the ratio of lignin to polysaccharide monomer units (γ) can be calculated according to the following expression:

$$\gamma = 0.6\frac{\text{Lignin carbon}}{\text{Polysaccharide carbon}} \quad (4)$$

Multiple Fluorescence Labeling and CLSM Observation

Proteins and other amine-containing compounds, cellulose, α-mannopyranosyl and α-glucopyranosyl sugar residues (i.e., α-polysaccharides), total cells, and dead cells were labeled with fluorescein isothiocyanate (FITC), calcofluor white (CW), Concanavalin A (Con A) conjugated with tetramethylrhodamine, SYTO 63,

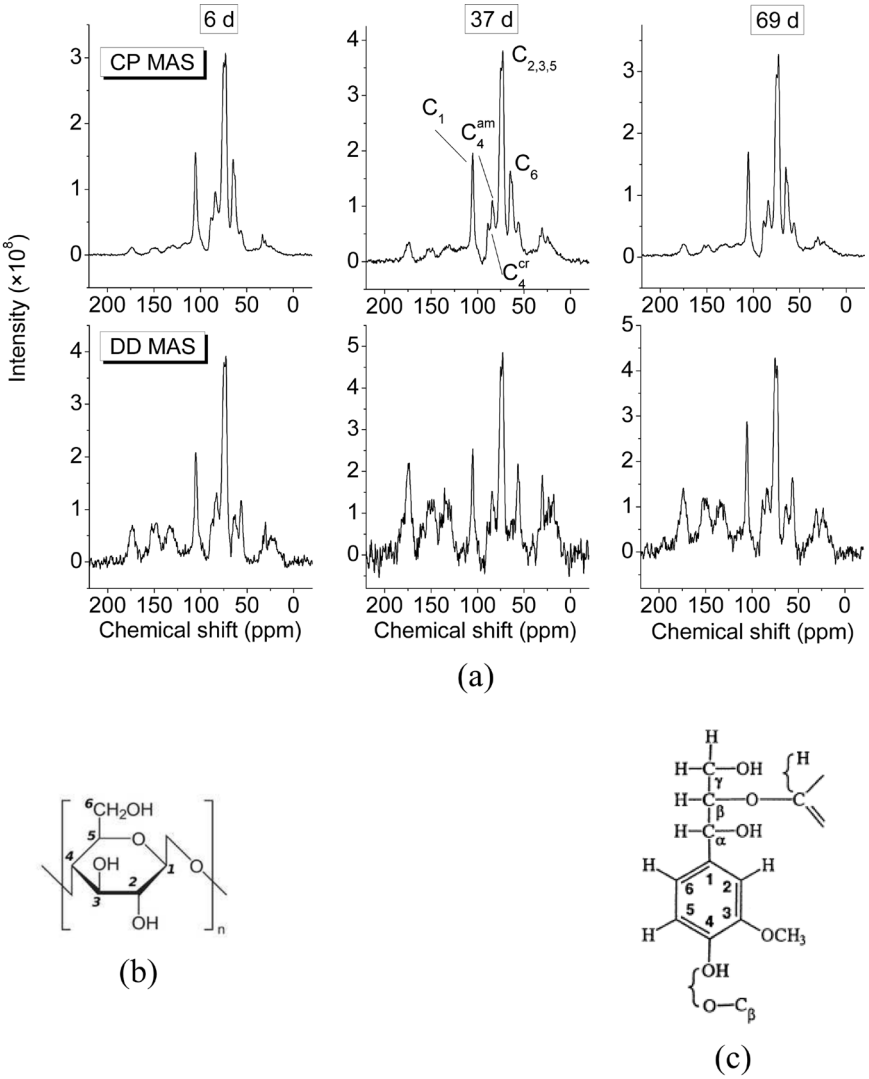


Figure 1. *Solid-state ^{13}C cross-polarization (CP) and dipolar dephasing (DD) magic-angle- spinning (MAS) nuclear magnetic resonance (NMR) spectra of bulk compost (a) at different maturity stages (i.e., 6 d, 37 d, and 69 d); and structural units of cellulose (b) and lignin (c). The numbers shown in (a) correspond to the chemical shifts (in ppm) of indicated resonances in the spectra.*

and SYTOX blue, respectively [2,17,19]. SYTO 63 (20 μM, 500 μL) was added to 0.5 g of a hydrated sample, and the resulting mixture was placed on a shaker table for 30 min. The sample was washed with phosphate-buffered saline, and 0.1 mol $NaHCO_3$ buffer (500 μL) was added to maintain a pH of 9. Subsequently, a solution of FITC (10 g L^{-1}, 200 μL) was added to the suspension. The resulting mixture was stirred for 1 h and washed again, and a solution of Con A (250 mg L^{-1}, 500 μL) was added. After 30 min, the sample was washed, CW (300 mg L^{-1}, 500 μL) was added, and the sample was incubated for 30 min. Finally, the unwashed sample was incubated with a SYTOX blue solution (2.5 μM, 500 μL). The stained samples were embedded in Cryo-STAT (McCormick Scientific, St. Louis MO) for cryosectioning and were frozen at –20°C. Next, 30-μm sections were cut on a cryomicrotome (Cryotome E, Thermo Shandon Limited, UK) and were mounted onto gelatin-coated (0.1% gelatin and 0.01% chromium potassium sulfate) microscopic slides for CLSM (Leica TCS SP2 confocal spectral microscope imaging system, Germany) observation.

RESULTS AND DISCUSSION

Molecular Structure of Organic Matter for Compost at Different Maturity Stages

To characterize structural changes in organic matter at different maturity phases, i.e., the beginning of the thermophilic stage (6 d), the end of the thermophilic stage (37 d), and the end of the cooling stage (69 d), solid-state ^{13}C CPMAS NMR spectra of bulk compost in different maturity stages were obtained by solid-state ^{13}C CPMAS NMR spectroscopy [Figure 1(a)]. Resonance peaks were assigned, and the results are displayed in Table 1. The strong signals of lignin (173, 148, 129 ppm), polysaccharides (cellulose and hemicellulose) (105, 87, 84, 75, 65 ppm), lipids, and waxes (33, 30 ppm) in the organic matter of compost at different maturity stages were detected in the ^{13}C CPMAS NMR spectra. The intensity of the resonance peaks of lignin (173, 148, 129 ppm), polysaccharides (65 ppm), lipids, and waxes (33, 30 ppm) decreased markedly on the 37th and 69th days compared to those observed on the 6th day. However, the intensity of resonance peaks on day 37 was similar to that observed on day 69 [Figure 1(a) and Table 2].

The content of polysaccharide carbons from the compost decreased from 72.3% on the 6th day to 56.6% on the 37th day and then increased slightly to 58.2% on the 69th day; however, the content of lignin carbon from the compost markedly increased from 14.0% on the 6th day to 21.3% on the 37th day and then increased slightly to 22.1% on the 69th day (Figure 2). The aforementioned results revealed that polysaccharide degradation occurred more often than lignin degradation. The CrI results further demonstrated that the degradation of polysaccharides was attributed to the amorphous region of polysaccharides. The lignin

Table 1. Assignment of Major ^{13}C NMR Absorption Bands of Compost.

Compound Class	Band Position (ppm)	Assignment	Reference
Carboxyl, ester and amide (160–190 ppm)	174	COOH in lignin and hemicellulose	[7]
Total aromatic pool (120–160 ppm)	148	C-5 in lignin without OMe	[9]
	128	Alkyl in lignin	[14]
	104.7	C-1 in cellulose	[9]
	107.1 and 102.4	C-1 of hemicellulose	[9]
	105	C-1 of polysaccharides	[16]
	87–90	C-4 in crystalline cellulose	[9]
Total carbohydrate pool (60–120 ppm)	80–86	C-4 in amorphous cellulose	[9]
	88,89	C-4 in polysaccharides	[16,11,7]
	83, 88	C-4 in amorphous and crystalline cellulose, respectively	
	72, 74–75,75	C-2, C-3, and C-5 in polysaccharides	[16,11,9,14,5]
	60–80	Extracellular polymeric substances (EPS)	
	62–66	C-6 in polysaccharides	[16,11,9,14]
Total aliphatic pool (0–60 ppm)	55, 56	CH_3 in lignin	[16]
	40–60	Proteins and sugars	[5,11,14]
	33, 30	CH_3 and CH_2 in saturated aliphatic chain (lipids and plant waxes), respectively	[7]
	21	CH_3 in acetyl groups in hemicellulose	[7]

Table 2. Relative Distribution of ^{13}C in Organic Matter from the Bulk and Dissolved Organic Matter (DOM) of Compost at Different Maturity Stages, as Determined by CPMAS NMR.

		Percentage Distribution of ^{13}C within Indicated ppm Region (%)							
		Total Aliphatic Pool		Total Carbohydrate Pool		Total Aromatic Pool		Carboxyl, Ester and Amide	Aldehyde and Ketone
Sample		Paraffin (CH_x, 0–45 ppm)	Methoxy group (CH_3O–, 45–60 ppm)	HCOH (60–90 ppm)	Anomeric carbon O–C–O (90–120 ppm)	C=C/Ar–C (120–140 ppm)	Ar–O (140–160 ppm)	COO/CON (160–190 ppm)	C=O (190–220 ppm)
Bulk	6 d	7	5	62	18	4	2	2	0
	37 d	13	7	51	14	6	4	4	0
	69 d	10	7	53	15	6	4	4	1
DOM	6 d	27	13	29	10	6	3	11	0
	37 d	21	12	34	12	6	4	11	0
	69 d	20	12	36	12	6	4	10	0

to polysaccharide monomer unit ratio (γ) was similar to the lignin to polysaccharide carbon ratio.

Solid-state ^{13}C DDMAS NMR spectra can be used to identify nonprotonated carbons as well as highly mobile carbons by distinguishing between strong and weak dipolar interactions of carbons with neighboring protons [11]. Figure 1(a) displays the ^{13}C DDMAS NMR spectra of the bulk compost in different maturity stages. The intensity of the NMR signal at 73 ppm decreased rapidly during the dephasing delay suggesting that the C-2, C-3, and C-5 groups on polysaccharides strongly interact with a neighboring 1H. In contrast, signals for the carbons at 174 ppm remained intense for a longer dephasing time, revealing that protons were not directly bound to the COOH groups of lignin and hemicellulose due to their weak C–H coupling. The DD spectra results obtained in the present study are consistent with those of Spaccini and Piccolo [14]. In conclusion, a combination of DD and CP spectra provided a more detailed molecular structure of OM than CP spectra at different maturity stages.

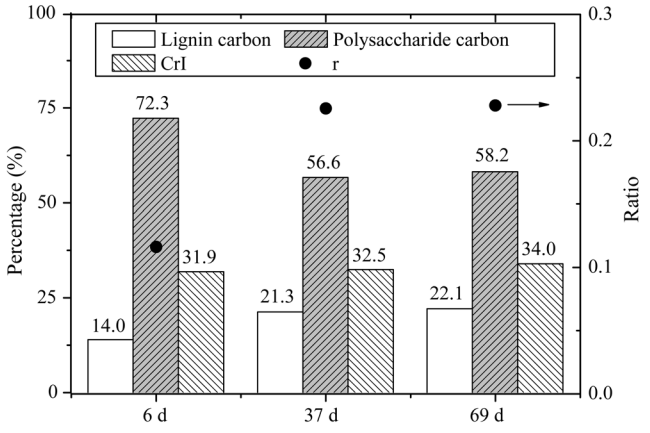
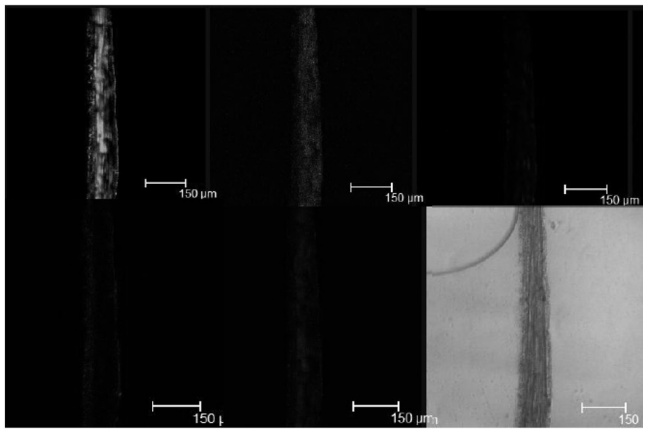


Figure 2. *Lignin C, polysaccharide C, and crystallinity index (CrI) of bulk compost at different maturity stages (i.e., 6 d, 37 d, and 69 d), as determined by ^{13}C CPMAS NMR. Note that γ = 0.6(Lignin carbon/Polysaccharide carbon).*

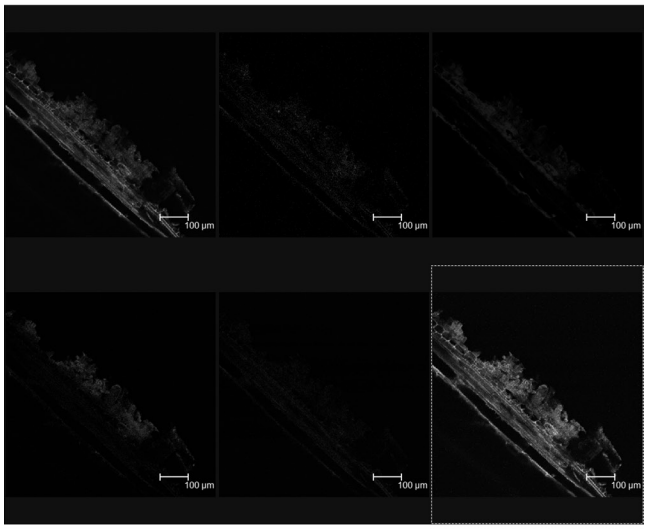
Biofilm Architecture at Different Maturity Stages Based on Multiple Fluorescence Labeling Combined with CLSM

Figure 3 displays CLSM images of a biofilm in the compost on days 6, 37, and 69 of composting. At the beginning of the thermophilic stage [Figure 3(a)], rice straw consisted of vascular plant material, and macromolecules were heterogeneously distributed. Specifically, proteins (FITC) formed the backbone of the straw and were distributed in the interior, whereas cellulose (CW) was distributed in clusters. In contrast, α-polysaccharides (Con A) presented a relatively homogeneous distribution pattern. Total cells (SYTO 63) were primarily detected on the outer surface, whereas dead cells (SYTOX blue) were mainly observed in the cores. Moreover, significant biofilm amounts did not form in this stage.

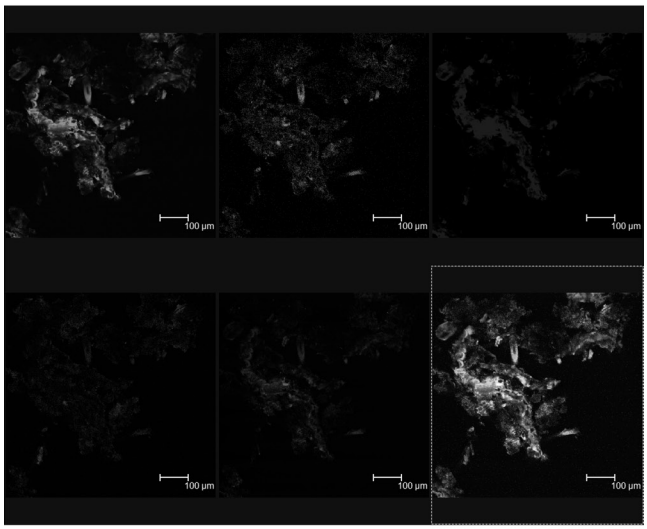
At the end of the thermophilic stage [Figure 3(b)], the structure of rice straw on the 37th day of composting was significantly looser than that observed on the 6th day due to the degradation of macromolecules during the thermophilic stage, as evidenced in the ^{13}C CP-MAS NMR data (Figures 1 and 2 and Table 2). Obviously, the CLSM images could be classified into two parts, i.e., a rice straw part and a biofilm part. In rice straw, protein (FITC) was distributed throughout the entire sample, and cellulose (CW) was only observed on the outer surface. Moreover, α-polysaccharides (Con A), total cells (SYTO 63), and dead cells (SYTOX blue) presented a distribution pattern that was



(a)



(b)



(c)

Figure 3. *Confocal laser scanning microscopy (CLSM) images of a biofilm in compost after 6 d (a), 37 d (b), and 69 d (c) of composting. The images were obtained with a ×20 objective lens: proteins (FITC) = green; α-polysaccharides (Con A) = light blue; cellulose (CW) = blue; total cells (SYTO 63) = red; dead cells (SYTO blue) = violet; right bottom panel, merged image of the above images.*

similar to that of protein (FITC); however, these materials displayed a weaker fluorescence intensity than that of protein (FITC). The thickness of the biofilm was approximately 80 µm, and a biofilm was only detected on one side of the surface of rice straw. In addition, total cells (SYTO 63) were observed on the outer surface of the straw and were closely connected with protein (FITC) and cellulose (CW). The presence of dead cells may be attributed to the poor adaptation of mesophilic bacteria to the thermophilic environment [19].

In the cooling stage [Figure 3(c)], the vascular structure of rice straw disappeared, and rice straw mainly consisted of protein (FITC) and cellulose (CW). Moreover, biofilms were observed throughout the entire compost. In the cooling stage, total cells (SYTO 63) and dead cells (SYTOX blue) were observed more frequently than in the thermophilic stage, which is consistent with the results of Yu *et al.* [19], who demonstrated that cell recolonization occurs during the cooling stage of composting.

Biofilm Structure at Different Maturity Stages Based on Solid-State ^{13}C CPMAS NMR Spectra

Solid-state ^{13}C CPMAS NMR spectra of dissolved organic matter in compost at different maturity stages (i.e., 6, 37, and 69 d) are shown in Figure 4. All of the spectra were dominated by the resonance lines of CHOH groups in the region between 60 and 80 ppm, suggesting that extracellular polymeric substances (EPS) were present in the biofilm [6,5]. In addition, the resonances of methyl carbon atoms at 23 ppm, anomeric carbons at 105 ppm, and carboxyl carbons at 178 ppm were observed in the spectra, revealing the presence of CH_3 groups in acetyl moieties, sugars, and carboxyl group carbons, respectively [5]. The spectrum of the biofilm on the 37th day of composting was similar to that obtained on the 69th day but was distinct from that observed on the 6th day, which was similar to the spectra of the bulk compost, indicating that the degradation of organic matter occurred in the thermophilic stage. Compared to the results obtained on days 37 and 69, the spectrum of the biofilm on the 6th day of composting contained more methyl group and carboxyl group carbon signals.

DISCUSSION

Solid-state ^{13}C CPMAS NMR spectra of the bulk compost demonstrated that the development of the molecular structure of bulk compost occurred pre-

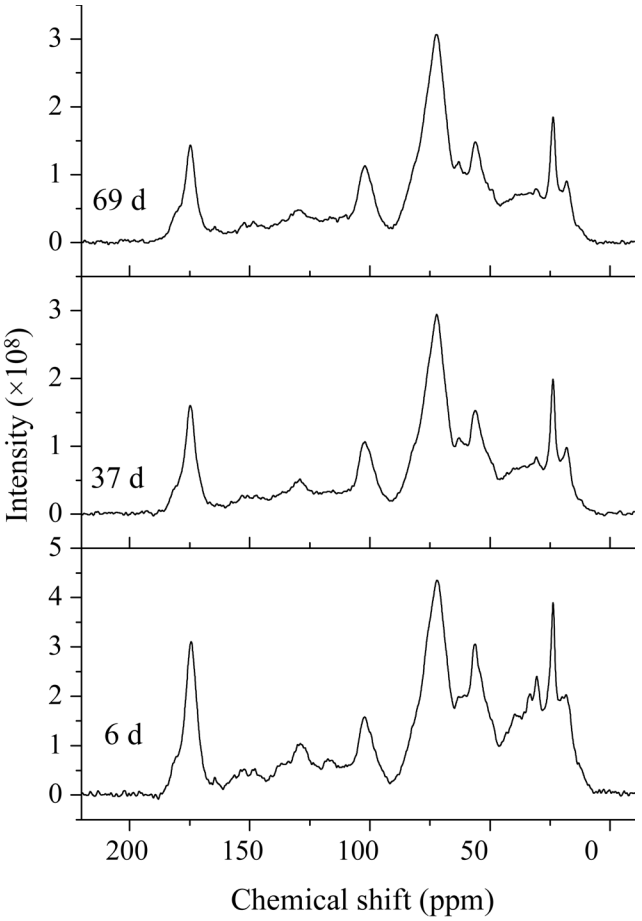


Figure 4. *Solid-state ^{13}C CPMAS NMR spectra of dissolved organic matter from compost at different maturity stages (i.e., 6 d, 37 d, and 69 d).*

dominantly in the thermophilic stage (from day 2 to 36). In addition, slight changes in the molecular structure of the bulk compost were observed in the cooling stage (from days 37 to 69), which was consistent with the results of fluorescence excitation–emission matrix (EEM) spectroscopy [15]. Specifically, in the thermophilic stage, a portion of the total carbohydrate pool (HCOH and anomeric carbon) was degraded and transformed into the total aliphatic pool (paraffin and methoxy group), total aromatic pool (C = C/Ar–C and Ar–O) and carboxyl, ester, and amide functional groups pool (Table 2). Solid-state ^{13}C CPMAS NMR spectra of dissolved organic matter during composting also suggested that development of molecular structure occurred in the thermophilic stage. Therefore, the cooling stage plays a trivial role in the evolution of the molecular structure of OM during composting. The evolution of the molecular structure of OM in the thermophilic stage was supported by the formation of biofilms, which was observed *in situ* CLSM (Figure 3). Thus, the molecular structure of compost becomes stable and mature in the thermophilic stage. As a result, the composting process can be optimized by shortening the duration of the cooling stage.

The molecular structure of compost is primarily altered by microorganisms embedded in the extracellular polymeric substances (EPS) matrix of biofilm. Multiple fluorescence labeling combined with CLSM observation provides information on the architecture and distribution of biofilms during composting. Specifically, biofilms did not form in the beginning of the thermophilic stage (i.e., 6 d) but were well-formed at the end of the thermophilic stage (i.e., 37 d) and presented a thickness of approximately 80 μm on one side of the surface of a piece of residue. The presence of well-formed biofilms supported the hypothesis that the molecular structure developed in the thermophilic stage. Furthermore, solid-state ^{13}C CPMAS NMR spectra of the dissolved organic matter of compost demonstrated that the composition of C in biofilms during composting consisted primarily of CHOH groups, carboxyl carbons, methyl carbons, and anomeric carbons.

Moreover, multiple fluorescence labeling combined with CLSM observation demonstrated that rice straw showed vascular plant structure, which was assumed to be highly resistant to decay [1]. Proteins and cellulose formed the backbone of rice straw. After mixing with a suitable amount of pig manure, rice straw was co-degraded by microorganisms during composting.

In summary, characterizing the development of molecular structure of organic matter and the architecture of biofilms will improve our understanding of the composting process and the mechanisms of maturity.

CONCLUSIONS

The development of molecular structure in bulk compost occurred predominantly during the thermophilic stage (from days 2 to 36). The molecular structure of bulk compost continued to change slightly during the cooling stage (from days 37 to 69). Thus, the molecular structure of compost mostly reaches stability or maturity at the end of the thermophilic stage. Multiple fluorescence labeling, combined with CLSM observation, demonstrated that the architecture of biofilms in different maturity stages during composting was distinct. Solid-state ^{13}C CPMAS NMR spectra further indicated that the C in the biofilm was mainly composed of CHOH groups, carboxyl carbons, methyl carbons, and anomeric carbons. In conclusion, solid-state ^{13}C CPMAS NMR spectroscopy combined with multiple fluorescence labeling provides novel infor-

mation for engineering applications and scientific research in the field of composting.

ACKNOWLEDGEMENTS

The authors thank the National Natural Science Foundation of China (No. 21007027), the National Basic Research Program of China (No. 2011CB100503), China Postdoctoral Science Foundation (No. 20100481156, 201104535, and 1002017B), the Agricultural Ministry of China (No. 2011-G27 and 201103004), Key Agricultural Project of Jiangsu Province (SX(2010)220), the Fundamental Research Funds for the Central Universities (No. KYZ201143), and the National Key Technology R&D Program (No. 2011BAD11B03).

REFERENCES

1. Bianchi, T.S., 2011. The role of terrestrially derived organic carbon in the coastal ocean: a changing paradigm and the priming effect. *Proc. Natl. Acad. Sci.* 108, 19473–19481.
2. Chen, M.Y., Lee, D.J., Tay, J.H., 2007. Distribution of extracellular polymeric substances in aerobic granules. *Appl. Microbiol. Biotechnol.* 73, 1463–1469.
3. Dominik, D.M., Nielsen, J.L., Nielsen, P.H., 2011. Extracellular DNA is abundant and important for microcolony strength in mixed microbial biofilms. *Environ. Microbiol.* 13, 710–721.
4. Gajalakshmi, S., Abbasi, S.A., 2008. Solid waste management by composting: state of the art. *Crit. Rev. Environ. Sci. Technol.* 38, 311–400.
5. Garny, K., Neu, T.R., Horn, H., Volke, F., Manz, B., 2010. Combined application of ^{13}C NMR spectroscopy and confocal laser scanning microscopy-Investigation on biofilm structure and physico-chemical properties. *Chem. Engineer. Sci.* 65, 4691–4700.
6. Lattner, D., Flemming, H.C., Mayer, C., 2003. ^{13}C NMR study of interaction of bacterial alginate with bivalent cations. *Int. J. Biol. Marcomol.* 33, 81–88.
7. Liang, Y.G., Zheng, Z., Luo, X.Z., Guo, F.H., Wang, L.M., Zhang, J.B., 2011. Effect of mesophilic and thermophilic conditions on changes of physicochemical characteristics of smooth cordgrass via dry digestion process. *Chem. Engineer. J.* 168, 544–552.
8. Liitiä, T., Maunu, S.L., Hortling, B., 2001. Solid state NMR studies on inhomogeneous structure of fibre wall in kraft pulp. *Holzforschung* 55, 503–510.
9. Matulova, M., Nouaille, R., Capek, P., Péan, M., Forano, E., Delort, A.M., 2005. Degradation of rice straw by Fibrobacter succinogenes S85: a liquid- and solid-state nuclear magnetic resonance study. *Appl. Environ. Microbiol.* 71, 1247–1253.
10. Moral, R., Paredes, C., Bustamante, M.A., Marhuenda-Egea, F., Bernal, M.P., 2009. Utilisation of manure composts by high–value crops: safety and environmental challenges. *Bioresour. Technol.* 100, 5454–5460.
11. Pichler, M., Knicker, H., KÖgel-Knabner, I., 2000. Changes in the chemical structure of municipal solid waste during composting as studied by solid-state dipolar dephasing and PSRE ^{13}C NMR and solid-state 15N NMR spectroscopy. *Environ. Sci. Technol.* 34, 4034–4038.
12. Said-Pullicino, D., Erriquens, F.G., Gigliotti, G., 2007. Changes in the chemical characteristics of water–extractable organic matter during composting and their influence on compost stability and maturity. *Bioresour. Technol.* 98, 1822–1831.
13. Senesi, N., Plaza, C., Brunetti, G., Polo, A., 2007. A comparative survey of recent results on humic-like fractions in organic amendments and effects on native soil humic substances. *Soil Biol. Biochem.* 39, 1244–1262.
14. Spaccini, R., Piccolo, A., 2009. Molecular characteristics of humic acids extracted from compost at increasing maturity stages. *Soil Biol. Biochem.* 41, 1164–1172.
15. Tang, Z., Yu, G.H., Liu, D.Y., Xu, D.B., Shen, Q.R., 2011. Different analysis techniques for fluorescence excitation-emission matrix spectroscopy to assess compost maturety. *Chemosphere* 82, 1202–1208.
16. Vinceslas-Akpa, M., Loquet, M., 1997. Organic matter transformations in lignocellulosic waste products composted or vermicomposted (*eisenia fetida andrei*): chemical analysis and ^{13}C CPMAS NMR spectroscopy. *Soil Biol. Biochem.* 29, 751–758.
17. Wang, L.P., Yu, G.H., Ran, W., Xu, Y.C., Shen, Q.R., 2012. Fate of biopolymers during rapeseed meal and wheat bran composting as studied by two-dimensional correlation spectroscopy in combination with multiple fluorescence labeling techniques. *Bioresour. Technol.* 105, 88–94.
18. Yu, G.H., Luo, Y.H., Wu, M.J., Tang, Z., Liu, D.Y., Yang X.M., Shen Q.R., 2010b. PARAFAC modeling of fluorescence excitation—emission spectra for rapid assessment of compost maturity. *Bioresour. Technol.* 101, 8244–8251.
19. Yu, G.H., Tang, Z., Xu, Y.C., Shen, Q.R., 2011. Multiple fluorescence labeling and two dimensional FTIR–^{13}C NMR heterospectral correlation spectroscopy to characterize extracellular polymeric substances in biofilms produced during composting. *Environ. Sci. Technol.* 45, 9224–9231.

Soil Texture Effects on Rhizodegradation of Crude Oil Contaminated Soil

FARHANA MAQBOOL[1,2], YING XU[3], DONGMEI GAO[1], ZULFIQAR AHMAD BHATTI[4] and ZHENYU WANG[1,*]

[1]*College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China*
[2]*Department of Microbiology, Hazara University Garden Campus, Mansehra, 21300, KPK, Pakistan*
[3]*College of Animal Science and Technology, Qingdao Agricultural University, Qingdao, 266109, China*
[4]*Department of Environmental Science, COMSATS Institute of Information Technology, Abbottabad, 22010, KPK, Pakistan*

ABSTRACT: The effect of soil texture on the rhizodegradation of crude oil by the *Sesbania cannabina* plant was evaluated. Soil texture amendment was difficult to carry out in the field of polluted soil so the soil was used in its original clay-like texture and bioaugmented with a microbial consortium. After 120 days of plant growth, crude concentration decreased non-significantly from 3,000 ppm to 2,200 ppm. Root morphological characteristics and microbial biomass was checked and did not show a pronounced difference. It was concluded that the *Sesbania cannabina* has a fibrous root structure not strong enough to support constricting and stretching forces of clayey textured soil. Therefore, texture amendment is indispensible for optimizing rhizodegradation of crude oil in soil.

INTRODUCTION

SOILS contamination from organic pollutants' accumulation is a widespread issue. Contaminated soils are found on all continents. Accidental spills occur mainly in areas near oil refineries, storage areas, and significant manufacturing activities. Specific techniques aimed at remediating these areas consume considerable industry and government resources [1].

A combination of two approaches using rhizoremediation and bioaugmentation both resulted in rhizodegradation. During rhizodegradation, exudates derived from the plant may help to stimulate survival and action of bacteria. The root system of a plant helps to spread bacteria through the soil and helps penetration into impermeable soil layers [2]. Soil texture, compactness, and drainage are the main soil characteristics related to plant growth. Compact soil layers limit root growth and effect properties related to water and air movement. According to Gerhardt [3] in spiked soils, chemicals tend to be bioavailable. Whereas, contaminants in naturally weathered soils are often not readily bioavailable due to hard soil structure. Hydrophobic characteristics of crude oil retard mass transfer of air, water, and contaminants from particles to microorganisms in soil, which limits rate of uptake and metabolism of contaminants by hydrocarbon-degrading of the bacteria [4, 5]. Moisture content and aeration were determined to be key factors associated with polyaromatic hydrocarbons (PAH) bioremediation [6] and these factors are related to soil texture. The specificity of the plant–bacteria interaction is dependent upon soil conditions that may alter contaminant bioavailability, root exudates composition, and nutrient levels [7].

Many studies have been carried out to check effects of nutrient addition and bioaugmentation on biodegradation of petroleum polluted soil [8, 9, 10] but very few literatures demonstrated the effect of soil physical texture in rhizodegradation experiments. Crude oil contamination is a very much serious problem in the Yellow River Delta in Dongying, Shandong due to its exploitation, manufacturing, and transportation. The size of a contaminated site is very long so the need for a cost effective technique able to remediate such large area is necessary. In the present study soil was bioaugmented without any other structural amendments to make the technique applicable for the large field area.

MATERIALS AND METHODS

Physicochemical Analysis of Soil

Soil texture was analyzed using the modified meth-

*Author to whom correspondence should be addressed.
E-mail: fairy_es@yahoo.com

1544-8053/12/02 073-07

od of Pansu and Gautheyrou [11]. First step, gravimetric soil water content was determined for this 10 gm air dried sample (AD) was oven dried for overnight at 105°C. The difference of these were divided by oven dried (OD) sample weight. In the next step 40 gm of AD soil sample was mixed with a dispersing solution of sodium hexametaphosphate (50g L^{-1}) and 100 ml deionized (DI) water and shaken overnight. Then, the aliquot was transferred to a graduated cylinder and filled up to 1,000 ml with DI water. One blank was also run. Mixing for 1 minute was done with a plunger and a hydrometer reading was taken after 40 seconds for percent sand and then after 2 hours for percent clay. A reading was corrected by subtracting from the blank. Percentage of sand, silt, and clay were calculated. Soil texture was estimated by using Table 1.

Soil pH was measured in 0.01 mol L^{-1} $CaCl_2$ solution with a soil-to-solution ratio of 1:2.5. Water holding capacity (WHC) was calculated by taking the difference of the weight of dry and wet soil after 4 to 8 hours. The soil pot was placed in the filled water tub or until the absorbent paper on the soil surface was completely wet and then percentage of WHC was calculated.

Moisture content (%) was calculated by dividing the loss in weight by drying for 24 hrs at 105ºC using initial sample weight and multiplying by 100.

Soil Preparation for Pot Experiment

Soil sample was initially air dried for one week, then pulverized and passed through a 2 mm sieve. A 1.5 Kg soil was used and initial pot weight was analyzed in order to keep the 40–50% of soil moisture content. Analysis was performed after 80 and 120 days of plant growth. Each pots used in triplicate had 8 plants each. Treatments were as follows: planted pots with free culture inoculation rhizosphere (FR) and non-rhizosphere (FN), planted pots with sterilized soil rhizosphere (SR) and non-rhizosphere (SN), and two controls was used. One control was planted pots without inoculation rhizosphere (CR) and non-rhizosphere (CN) and the other was soil without plants (C) which was used to compare phytoremediation effect of planted pots.

Table 1. Soil Textures.

Soil Classification	Clay Soil	Loam Soil	Sandy Soil
percent clay	40–100%	7–27%	1–10%
percent silt	0–40%	28–50%	1–15%
percent sand	0–45%	23–52%	85–100%

Table 2. Physicochemical Properties of the Soil Sample.

pH	8.03
WHC ($ml.gm^{-1}$)	0.4315
Moisture content	13.75%
*[a]Soil texture	clayey
sand	12.1%
silt	46.6%
clay	41.3%
Crude oil concentration (ppm)	3080
Total N (g kg^{-1})	0.816
Available N (mg kg^{-1})	1.68
Total P (g kg^{-1})	0.0288
Available P (mg kg^{-1})	13.44
Total C (g kg^{-1})	12.70017
CEC (c mol kg^{-1})	6.0996
*[a]Oil degraders (Num gm^{-1})	7.85×10^5
*[b]Oil degraders (CFU gm^{-1} dry soil)	1.093×10^3

Note. *[a]by DAPI method, *[b]by MPN method.

Regarding required amount of inoculums, initial soil load was checked using MPN. Total bacterial load in mixed consortium was checked using direct microscopic count with a Petroff-hausser counting chamber. Bacteria were introduced to a final concentration of 10^4 CFU kg^{-1} of dry soil. 37 ml of Pre-fresh culture in log phase was used and for a brief period 2 ml of old culture was added in 150 ml of MSM along with 100 µL of diesel oil after 2.5 days of incubation at 37ºC with 150 rpm to obtain the culture. First, it was centrifuged at 4,500 rpm for 10 min and then pellet washed 3 times with 0.9% saline and mixed thoroughly by hand in a 1.5 kg contaminated soil. Water content was adjusted to 60% of the WHC in the beginning and to 40–50% during plant growth. Sterilization of soil was done using steam-sterilization (i.e., three successive sterilizations 24 h apart in order to killed spores at 100°C for 1 h each) and soil humidity was adjusted with sterile distilled water.

Plant Cultivation and Analysis

A local dominant specie of plant *Sesbania cannabina* was selected for biodegradation in the lab experiment. First, seeds were selected then sterilized with 10% H_2O_2 for 30 minutes and kept in saturated $CaSO_4$ solution overnight. After 80 and 120 days of growth, plants were harvested and then analyzed. Plants were separated into shoots and roots with soil collected from adjacent areas to roots by hand-shaking. This was labeled as rhizosphere and area far from roots was re-

ferred to as non-rhizosphere soil [12]. Plant roots were scanned and analyzed using an Epson Scanning and WinRHIZO Pro 2005 to observe different root characteristics such as length, surface area, volume, and number of root tips [13]. Plant samples were oven dried at 105°C for 15 min then 60°C for 48 h and weighed before and after drying to get dry root and shoot biomass. See Figure 1.

Analytical Procedures

A modified Rahman method [14] was used for detecting crude oil. Briefly, oil extraction was done ultrasonically using hexane as solvent. An air-dried soil sample was mixed with 1 g anhydrous sodium sulphate and extracted 3 times in 10 mL of hexane using ultrasonication for 1 h each time. Analysis was conducted using a UV-2550 Spectrophotometer (Shimadzu) at 225 nm.

Cation exchange capacity (CEC) was determined using an ammonium chloride standard method with some modifications. Briefly, soil samples were treated with an ammonium chloride solution and boiled until NH_3 evolved which dissolved all calcium carbonate and formed calcium chloride. Soil was saturated with ammonium and excess ammonium was washed away using ethanol. In order to confirm Ca^{+2} ion conversion, a 5ml clear supernatant was mixed with 1 ml of buffer solution and a small amount of K-B powder. A blue color developed suggesting absence of the Ca^{+2} ion.

Seed germination under dark conditions

Soil and plant samples collection for analysis

Plant growth after 80 days

Plant growth after 120 days

Figure 1. *Soil and plant setup and plant growth.*

If a pink color would have appeared that would suggest the presence of Ca^{+2} and the step must then be repeated. Ammonium ion was removed by washing with 95% ethanol and centrifuging for 3 min and 5 min at speeds of 3,000 and 4,000 rpm. It was washed until the final ethanol solution was ammonium ion-free.

A sample was then transferred into a Kjeldahl's flask by dissolving with distill water. After sufficient distillate was collected and titrated with hydrochloric acid standard solution, calculated amount of ammonium in soil displayed cation exchange capacity [15, 16].

Total and available N and total P was detected using Kjeldahl's method [17]. Available P was detected using the Olsen P method [18]. Briefly, 20 ml of extracting solution of bicarbonate (0.5M $NaHCO_3$, pH 8.5) for 1 gm of soil was used. The flask was shaken for 30 min at 200 rpm at room temperature and filtered. 20–30 ml of distill water was added along with 2 drops of dinitrophenol indicator to 10 ml of filtered sample. Then, 50% NaOH was used to gain a yellowish color and 5 ml of Molybdenum-antimony anti-reagent was added and distilled water was used to make up a volume of 50 ml and left for 30 minutes. A UV-probe spectroscope set at 700 nm was used to check absorbance. Using a standard curve, concentration of sample P (mg/L) was calculated.

Microbial Analysis

Petroleum degraders were enumerated using the most-probable-number (MPN) technique [19]. A 96-well microtiter plate containing sample, mineral salt medium (MSM), and crude oil was used to calculate the MPN of hydrocarbon degraders in colony forming units (CFU) g^{-1} dry soil. Total microbial activity in soil was measured using fluoresce in diacetate (3', 6'-diacetylfluorescein (FDA) hydrolysis rate) [20]. The amount of FDA hydrolyzed was measured by absorbance at 490 nm (A_{490}). The value of A_{490} per gram soil was referred to as microbial activity in the soil.

RESULTS AND DISCUSSION

Detection of Crude Oil Degradation

Crude oil degradation was analyzed to check effectiveness of bioaugmentation along with *S. cannabina* in a clayey textured soil. Results showed that crude oil concentration was decreased more in the rhizosphere of an uninoculated control from 3,080 ppm in the beginning to 2,077 and 1,632 ppm after 80 and 120 days of plant growth, respectively (See Table 2). Bioaugmented planted soil FR showed insignificant higher degradation at 2,280 and 2,057 ppm. Then, it was sterilized. SR showed insignificant higher degradation at 2,712 and 2,641 ppm. An unvegetated control soil C showed insignificant higher degradation at 3,040 and 2,900 ppm after 80 and 120 days, respectively.

The difference of crude oil degradation among different samples was low during 80 days of plant growth (See Table 3) but this difference was higher after 120 days of plant growth. Rhizospheric differences were found in all samples as compared to their respective non-rhizosphere soil sample. Rhizosphere is vitally important regarding degradation of xenobiotics, Both the microbial biomass and number of decomposers were greater in the rhizosphere than in the bulk soil so contribution of plants in pollutants removal and degradation is strongly dependent on rhizosphere processes [21].

Plant Growth Characteristics

Plant growth was under stress condition due to clayey textured soil. Dry shoot biomass was found more in the case of FP and SP than CP after 120 days while

Table 3. Crude Oil Concentration and Oil Degraders Load After 80 and 120 Days of Plant Growth.

Samples	Crude oil concentration (ppm) 80 days	Crude oil concentration (ppm) 120 days	Oil degraders load[1] 80 days	Oil degraders load[1] 120 days
FR	2280	2057	4.09×10^3	4.23×10^3
FN	2760	2291	3.55×10^3	3.90×10^3
SR	2712	2641	9.712×10	1.56×10^2
SN	3008	2746	8.65×10	7.82×10
CR	2077	1632	1.61×10^2	4.2×10^3
CN	2218	1722	2.09×10^2	1.14×10^3
C	3040	2900	5.22×10	3.55×10

Notes. [1]CFU gm^{-1} dry soil. Average of triplicate samples value mentioned here. Treatments include FR & FN = bioaugmented soil plant rhizosphere & non-rhizosphere, SR & SN = sterilized soil plant rhizosphere & non-rhizosphere, CR & CN = uninoculated soil plant rhizosphere & non-rhizosphere, respectively, and C = control soil without plant.

no difference was observed in plant length during 80 and 120 days (See Figure 1). The more structured soils have a higher proportion of effective stress on total stress. Therefore, all root parameters displayed an irregular trend among different treatments (See Figure 2). Most values were found more in the case of sterilized pots. The reason may be that the sterilization technique aerated soil very well due to penetration of steam under pressure. However, due to an absence of oil degraders in sterilized pots crude oil degradation was not observed. Dynamic forces may affect the pore system and composition of soil by rearrangement of single aggregates resulted in an increased bulk density and a less aerated and less rootable soil volume. Thus, the smaller texture dependent soil strength coincides with more intensive soil compaction due to loading [22].

Insufficient plant and root growth was a key factor responsible for failure of crude oil degradation. Due to the clayey textured soil, moisture content was not maintained properly as water leaked out due to fracturing within the soil. Sometimes welting of a stem near the soil surface was observed after watering due to low absorption of water in the pots. The reason was that hydraulic conductivity is more in a sandy soil and less in a clay soil. Since the hydraulic conductivity is also affected by structure and texture it will be higher if the soil is highly porous, fractured, or aggregated and will be lower if it is tightly compacted and dense [22].

Soil-plant interaction depends on hydraulic gradients [23]. Differences in hydraulic gradients should be greater in aggregated dense soils resulting in reduced plant water uptake efficiency and less root length density may be detected which again can be correlated with hydraulic properties [24].

Root surfaces which have evolved specifically to adsorb elemental nutrients from soil and pore water have extraordinarily large surface areas [25] and high-affinity chemical receptors [26, 27]. In the process of adsorption root surfaces bind many elemental pollutants as well as nutrients. [28]. In this study and due to constricting and stretching forces of clayey texture soil *S. cannabina* did not grow well. According to Horn [22], in such soil ion transport by mass flow as well as by diffusion are delayed whereby the length of flow path in such tortuous finer pores further retards chemical exchange processes. *S. cannabina* has fibrous roots

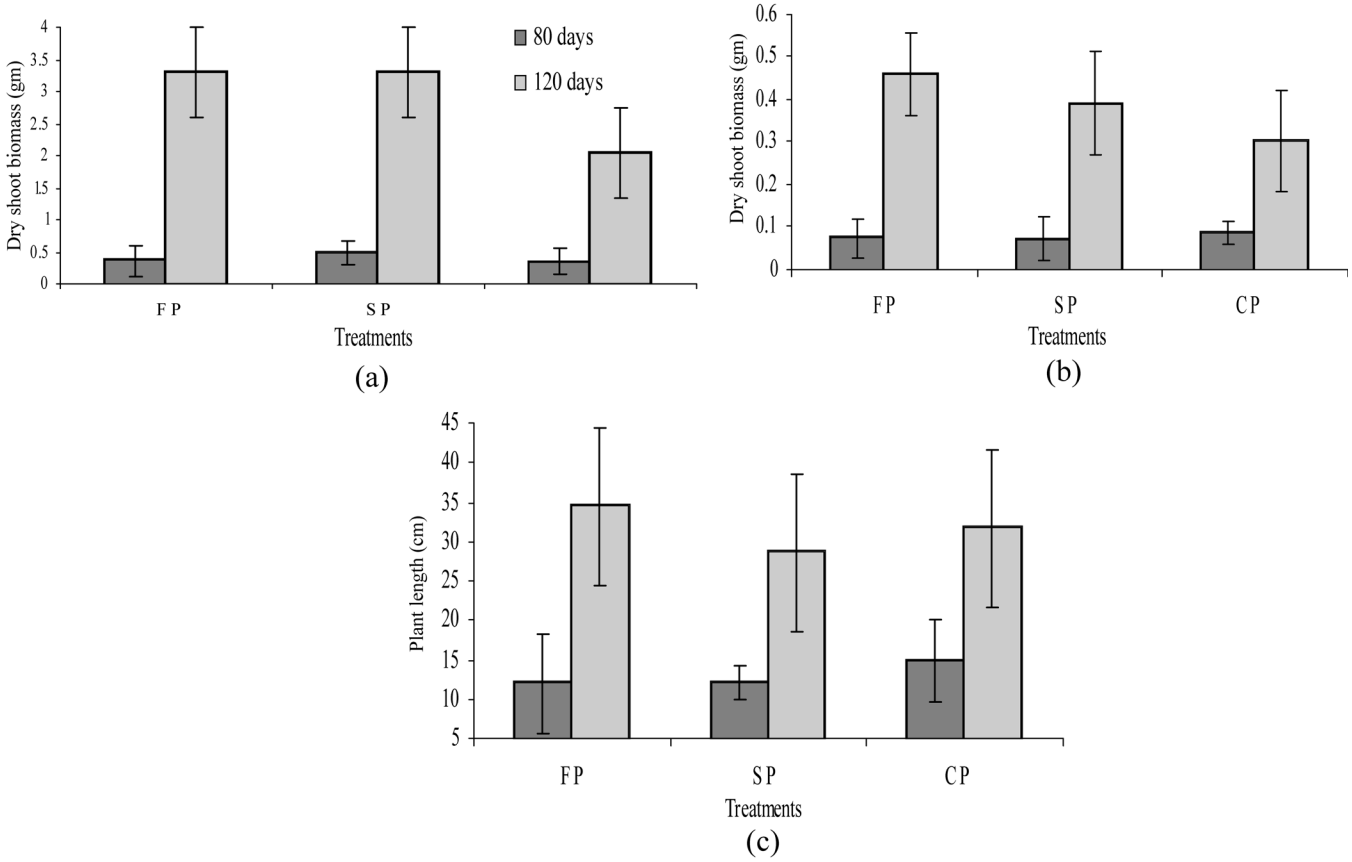


Figure 2. *(a) dry shoot biomass (b) dry root biomass (c) plant length, among different treatments during 80 and 120 days of plant growth. Sample FP = bacterial inoculated soil plant, SP = sterilized plant and CP = uninoculated plant.*

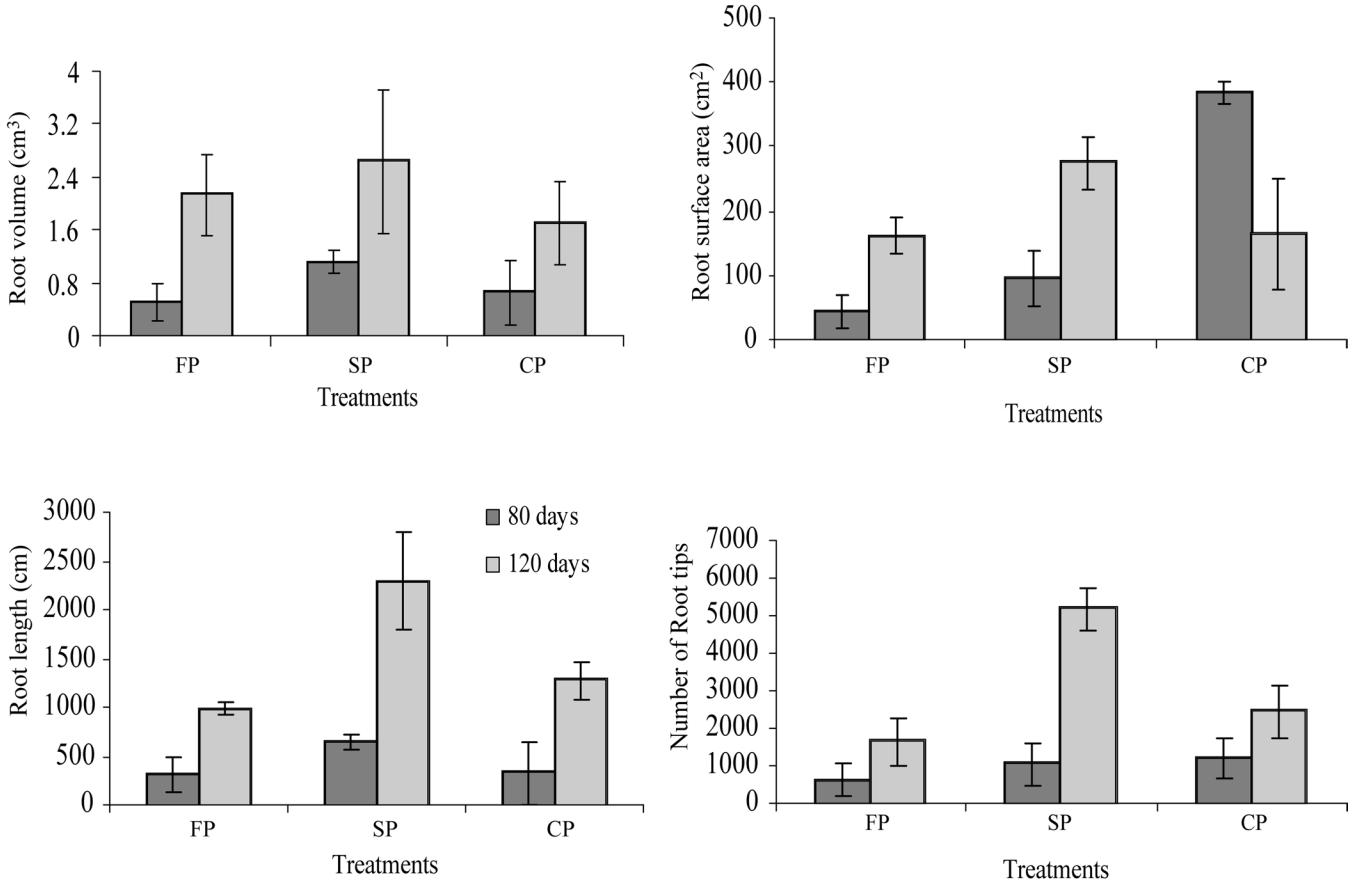


Figure 3. *Showing different root morphological characteristics among different treatments during 80 and 120 days of plant growth. Sample FP = inoculated soil plant, SP = sterilized plant and CP = uninoculated soil plant.*

which did not proliferate in the soils deeper layers so contaminant interaction with plant and oil degraders did not achieve sufficient rhizodegradation. See Figures 2 and 3.

MPN Analysis

MPN was estimated after 80 and 120 days in order to check the effect of oil degraders bioaugmentation and indigenous microbial population in clayey textured planted soil (See Table 3). Higher CFU gm^{-1} of dry soil was found in FR and FN after 80 days due to addition of oil degraders consortium but after 120 days no significant difference was observed in FR and in the planted control soil CR. Addition of bacteria in the beginning increases the heterotrophic number. After competition with indigenous bacteria a stable community structure was observed. In CR after 120 days CFU gm^{-1} dry soil was gradually increased due to plant growth which contributed root exudates and production of enzymes [29]. In the control soil without plant oil degrader's the number was very low. Hence, no degradation was observed during the course of study. A previous study demonstrated that rates of natural degradation typically have been found to be low and limited by environmental factors such as contaminant or nutrient (e.g., N and P) bioavailability, physical conditions (e.g. temperature, salinity, and pH), or microbial competition [30].

CONCLUSION

Higher degradation of TPH was observed in vegetated soil with a 47% TPH net removal rate versus bioaugmented vegetated soil at 33%, and a unvegetated sample at 6% net TPH removal rate. Therefore, it is concluded that bioaugmentation showed ineffectiveness. Due to compactness, the present soil sample retained more water and caused depletion of oxygen which results in insufficient root growth and is unfavorable for hydrocarbon degraders growth. Overall, root morphological parameters suggest that *S. cannabina* could be a suitable candidate for rhizoremediation application based on potential for root depth penetration and this can only be possible with suitable textured soil.

It is concluded that *Sesbania cannabina* has a fibrous root structure which are not strong enough to support constricting and stretching forces of clayey textured soil. Texture amendment is indispensible along with other amendments for optimizing rhizodegradation of crude oil in soil.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (No. 41073067) and the Foundation for Key Program of the Education Ministry, China (No. 308016).

REFERENCES

1. Cunningham, S. D., Anderson, T. A., Schwab, A. P., Hsu, F. C. Phytoremediation of soils contaminated with organic pollutants, *Advances in Agronomy,* Vol. 56, 1996, pp. 55–114.
2. Kuiper, I., Lagendijk, E. L., Bloemberg, G. V. and Lugtenberg, B. J. J. 2004. Rhizoremediation: A Beneficial Plant-Microbe Interaction, MPMI, 17, pp. 6–15. The American Phytopathological Society.
3. Gerhardt, K. E., Huang, X. D., Glick, B. R. and Greenberg, B. M. Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges, *Plant Science,* Vol.176, 2009, pp. 20–30.
4. Bosma, T.N.P., Middeldorp, P.J.M., Schraa, G., Zehnder, A.J.B. Mass transfer limitation of biotransformation: quantifying bioavailability. *Environ. Sci. Technol.* Vol. 31, 1997, pp. 248–252.
5. Semple, K.T., Morriss, A.W.J., Paton, G.I., Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. *European Journal of Soil Science,* Vol. 54, 2003, pp. 809–818.
6. Vinas M., Sabate J., Espuny, M. J. and Solanas, A. M. Bacterial Community Dynamics and Polycyclic Aromatic Hydrocarbon Degradation during Bioremediation of Heavily Creosote-Contaminated Soil. *Applied and Environ. Microbiology,* Vol. 71. No. 11, 2005, pp. 7008–7018.
7. Siciliano S.D. and Germida, J.J. Mechanisms of phytoremediation: biochemical and ecological interactions between plants and bacteria, *Environmental Reviews,* Vol. 6, 1998, pp. 65–79.
8. Sherman, D.F. and Stroo, H.F. Biological Treatment of PAHs in Soil Residues in Simulated Land Treatment Systems. *Proceedings: Environmental Engineering* 89, 1989, pp. 726–733.
9. Frick, C., R. Farrell and J. Germida. Assessment of phytoremediation as an in-situ technique for cleaning oil-contaminated sites. *Calgary, Petroleum Technology Alliance of Canada* (PTAC). 1999.
10. Thangarajan, R., Adetutu, E.M., Moore, R.B., Ogunbanwo, S.T. and Ball, A.S. Comparison between different bio-treatments of a hydrocarbon contaminated soil from a landfill site. *African J. of Biotech.* 10, 2011, 15151–15162.
11. Pansu, M. and Gautheyrou, J. Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods. In: Mineralogical analysis; Particle size analysis, Springer-Verlag Berlin Heidelberg. 2006. Pg. 31–56.
12. Phillips, L.A., Greer, C.W. Farrell, R.E., Germida, J.J. Field-scale assessment of weathered hydrocarbon degradation by mixed and single plant treatments, *Appl. Soil. Ecol.* Vol. 42, 2009, pp. 9–17.
13. Wang, Z. Y, Xu, Y., Zhao, J., Li, F., Gao, D. and Xing, B. Remediation of petroleum contaminated soils through composting and rhizosphere degradation. *J of Hazardous Materials* Vol. 190, 2011, pp. 677–685.
14. Rahman, K. S. M., Banat, I. M., Thahira, J., Thayumanavan, T., Lakshmanaperumalsamy, P. Bioremediation of gasoline contaminated soil by a bacterial consortium amended with poultry litter, coir pith and rhamnolipid biosurfactant. *Biores. Technol.,* Vol. 81, 2002, pp. 25–32.
15. LY/T1243, 1999. Forest soil cation exchange content. Available at http://www.cn-standard.net/ebzdetail/EEF/D9D771ED.shtml, access on July 14, 2009.
16. Lu, R. K. 1999. Soil and Agricultural Chemistry Analysis. Beijing: China Agricultural Science and Technology Press., China Analysis Network.
17. Bremner, J. M. and Mulvaney, C. S. 1982. Nitrogen-total. In Page, A. L., Miller, R. H. and Keeney, D. K. (eds.) Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Madison, WI, USA. pp. 595–624.
18. Pansu, M. and Gautheyrou, J. 2006. Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods. In chapter 29 Phosphorous. Springer-Verlag Berlin Heidelberg. Pg. 793–814.
19. Kirk, J. L., Klironomos, J. N. Lee, H. Trevors, J. T.. The effects of perennial ryegrass and alfalfa on microbial abundance and diversity in petroleum contaminated soil, *Environ. Pollut.* Vol. 133, 2005, pp. 455–465.
20. Green, V.S., Stott, D.E. and Diack, M. Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples, *Soil Biol. Biochem.* Vol. 38, 2006, pp. 693–701.
21. Ma, B., He, Y., Chen, H. H., Xu, J. M., Rengel, Z. Dissipation of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere: Synthesis through meta-analysis. *Environmental Pollution.* Vol. 158, 2010, pp. 855–861.
22. Horn, R., Taubner, H. Wuttke, M., Baumgartl, T. Soil physical properties related to soil structure. *Soil & Tillage Research* Vol. 30, 1994, pp. 187–216.
23. Bohne, H. 1988. Utilization of water by rye-seedlings under conditions of restricted rooting. Proc. 11 th Conf. ISTRO, Edingburgh, pp. 13–19.
24. Vetterlein D, Marschner H. Use of a microtensiometer technique to study hydraulic lift in a sandy soil planted with pearl millet (Pennisetum americanum). *Plant and Soil,* Vol. 149, 1993, pp. 275–282.
25. Schnoor, J. L. Licht, L. A. McCutcheon, S. C., Wolfe, N. L., Carreira, L. H. Phytoremediation of organic and nutrient contaminants, *Environ. Sci. Technology,* Vol. 29, 1995, pp. 318A–323A.
26. Salt, D. E, Prince, R. C, Pickering, I. J., Raskin, I. Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiol,* Vol. 109, 1995, pp. 1427–1433.
27. Dushenkov V, Nanda-Kumar PBA, Motto H, Raskin I. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environ Sci Tech.* Vol. 29, 1995, pp. 1239–1245.
28. Meagher, R.B. Phytoremediation of toxic elemental and organic pollutants, *Curr. Opin. Plant Biol.,* Vol. 3, 2000, pp. 153–162.
29. Anderson, T. A., Guthrie, E. A., Walton, B. T. Bioremediation in the rhizosphere: plant roots and associated microbes clean contaminated soil. *Environ Sci Tech,* Vol. 27, 1993, pp. 2630–2636.
30. Atlas, R.M. 1991. Bioremediation of fossil fuel contaminated soils. In: In situ Bioreclamation. Applications and Investigations for Hydrocarbon and Contaminated Site Remediation. Hinchee, R.E. and Olfenbuttel, R.F., Eds. Butterworth-Heinemann, Stoneham, M A. pp. 14–33.

Inherent Error and Dangers of "Once-in-a-Lifetime" Exposure Limits

ROD O'CONNOR[1,*] and P. "BRENT" DUNCAN[2]

[1]*Chemical Consulting Services, College Station, TX 77840*
[2]*Department of Biology, Forensic Sciences, University of North Texas, Denton, TX 76203*

ABSTRACT: Various organizations and agencies have proposed inhalation levels of toxic chemicals that may be experienced with negligible health risks on a once-in-a-lifetime exposure basis. Many of the chemicals for which such exposure levels have been defined are present in any given area on a regular basis, which means there are chronic as well as acute exposures to consider. Others represent repeated exposures for emergency response personnel because of multiple release incidents from the same source, or multiple sources in the area. Additionally, special risks of high level acute exposures such as cancer initiators vs. cancer promoters may have been ignored. The inherent error and dangers are illustrated by specific cases of arsenic, benzene, mercury, and vinyl chloride. It is recommended that no "minimal risk" or "safe" exposure levels for emergency response personnel or the general public should ever be promulgated that are less protective than the most conservative short-term exposure limits for the workplace.

INTRODUCTION

FOR many years the National Institute for Occupational Safety and Health (NIOSH) has published exposure limits called "Immediately Dangerous to Life or Health" (IDLH) as minimal warnings to workers and emergency response personnel for inhalation risks in industrial environments [1]. These limits express exposures above which approved respiratory protection is essential for an atmospheric concentration of a substance that poses an immediate threat to life or that would cause irreversible or delayed adverse health effects, or that would interfere with an individual's ability to escape from a dangerous atmosphere. IDLH values are reviewed periodically and many have been lowered on the basis of studies demonstrating health risks more serious than those previously recognized. No IDLH is now listed in some cases because of the recognition of special hazards such as potential occupational carcinogens [2]. Because these limits are designed for the workplace they recognize that additional continuous or periodic exposures are likely.

Other organizations and agencies have assumed that only a "peak" exposure is likely, and have established once-in-a-lifetime "safe" exposure levels even for carcinogens. These include:

- AEGLs (Acute Exposure Guideline Levels) that claim to apply to "the general population, including susceptible individuals". These levels are provided by the U.S. EPA's AEGL Program [3] and supposedly apply to once-in-a-lifetime, or rare, exposures. There are three levels of AEGLs: AEGL-1 is the airborne concentration above which it is predicted that the general population including susceptible individuals could experience unpleasant effects that are not disabling and are reversible upon cessation of exposure; AEGL-2 is the concentration above which irreversible or other serious long-term effects are predicted; and AEGL-3 is the concentration predicted to cause life-threatening health effects or death. Exposure times of 10 minutes, 30 minutes, 1 hour, 4 hours, and 8 hours have been calculated for each of these levels. It follows that a concentration lower than AEGL-2 would be predicted as "safe" with respect to any long term adverse health effects.
- ERPGs (Emergency Response Planning Guidelines) have been developed by the American Industrial Hygiene Association (AIHA) [4]. They are also calculated at three different levels: ERPG-1 is the maximum airborne concentration below which it is

*Author to whom correspondence should be addressed.
E-mail: docroc34@hotmail.com

1544-8053/12/02 081-06

believed that nearly all individuals could be exposed for up to one hour with only transient adverse health effects; ERPG-2 is the maximum airborne concentration below which it is believed that nearly all individuals could be exposed for up to one hour without developing irreversible serious health effects or symptoms that could impair an individual's ability to take protective action; ERPG-3 is the maximum concentration below which it is believed nearly all individuals could be exposed for up to one hour without developing life-threatening effects. It follows that a concentration lower than ERPG-2 would be predicted as "safe" with respect to any long term adverse health effects.

- TEELs (Temporary Emergency Exposure Limits) were developed by the Chemical Exposures Working Group of the U.S. Department of Energy Office of Emergency Management [5] and defined for a fifteen minute period. These values are always subject to change, being replaced by AEGLs or ERPGs when new values are published. There are four levels for these values: TEEL-0 is the threshold concentration below which most people will experience no adverse health effects; TEEL-1 is the concentration above which most people are predicted to experience discomfort which is transient and reversible upon cessation of exposure; TEEL-2 is the concentration above which the general population is predicted to experience irreversible or other serious adverse health effects or an impaired ability to escape; TEEL-3 is the concentration above which the general population could experience life-threatening health effects or death. It follows that a concentration lower than TEEL-2 would be predicted as "safe" with respect to any long term adverse health effects.
- PACs (Protective Action Criteria) were developed by the Subcommittee on Consequence Assessment and Protective Actions (SCAPA) for the U.S. Department of Energy. These criteria use AEGLs, ERPGs, and TEELs selecting the one deemed most appropriate for a PAC level. Exposure time for PACs varies depending on which of the aforementioned values were selected. Guidelines for PAC levels were set by a policy established in 2008 [6] and in the absence of ERPG or AEGL data may use OSHA short term (i.e., typically 15 minute) values. A problem with this approach is that OSHA values are compromises that include financial considerations of factors such as respiratory protection or ventilation costs as well as health effect data.
- IDLHs, WEELs (Workplace Environmental Exposure Levels), STELs (Short Term Exposure Levels), RELs (Recommended Exposure Levels), PELs (Permissible Exposure Levels), and TLVs (Threshold Level Values) are used by various organizations and governmental agencies for workplace safety and health. This paper will deal primarily with types of once-in-a-lifetime exposure levels designed to apply to emergency response personnel and the general public. However, it is revealing to compare these with workplace values which recognize health risks considered unacceptably high for the general public (Table 1).

ONCE-IN-A-LIFETIME EXPOSURES ARE RARE FOR MANY HAZARDOUS CHEMICALS

While there are certainly areas and populations for which a large exposure to certain hazardous chemicals would be a rare or once-in-a-lifetime event, it must be recognized that many toxic chemicals are ubiquitous in all but the most remote of areas. The only U.S. government agency that seems to be fully aware of serious limitations regarding once-in-a-lifetime exposure values is the National Oceanograph-

Table 1. Comparison of "Safe" Once-in-a-Lifetime Exposure Levels.

Chemical	AEGL-2 (1 hr.)	ERPG-2	PAC-2	NIOSH	OSHA
arsenic	[a]3.0 mg/m^3	NA	0.58 mg/m^3	Ceiling 0.002 mg/m^3	PEL (TWA) 0.010 mg/m^3
benzene	2,540 mg/m^3	480 mg/m^3	2,540 mg/m^3	15 min. STEL 3.18 mg/m^3	15 min. STEL 16.4 mg/m^3
mercury	1.7 mg/m^3	2.0 mg/m^3	1.7 mg/m^3	Ceiling 0.1 mg/m^3	PEL (TWA) 0.1 mg/m^3
vinyl chloride	3,120 mg/m^3	13,000 mg/m^3	3,120 mg/m^3	respirator required at any detectable concentration	Ceiling 13 mg/m^3
Levels below which no long-term adverse health effects are predicted.					

[a]As arsenic trioxide

ic and Atmospheric Administration (NOAA) which warns that once-in-a-lifetime exposure levels such as ERPGs *should not be used "as guidelines for workers who are routinely exposed to chemicals for longer durations" or "as guidelines for members of the public who are exposed to background chemical releases for longer durations"*[7].

Arsenic levels in air are typically low ranging from < 0.001 μg/m^3 in most remote areas to 0.03 μg/m^3 in urban areas [8]. However, there is enough arsenic in cigarette smoke alone to double arsenic intake each day [9]. In addition, arsenic is emitted as an air pollutant from external combustion boilers, waste incinerators, copper, lead and zinc smelters, glass manufacturing, and coal-fired power plants [10]. Persons living near arsenic acid plants were routinely exposed to airborne arsenic at levels up to 0.1 μg/m^3 [11]. Even in rural areas there were often significant sources of airborne arsenic from applications of arsenical-contaminated poultry litter to agricultural fields [12, 13]. Of equal or greater importance is the fact that most arsenic intake is from food and water [8]. Air, water, and soil contain significant levels of arsenic in most areas worldwide [14]. Accordingly, for most persons there is no such thing as a once-in-a-lifetime exposure to arsenic. Dangers of acute exposures as an example and at levels below the PAC-2 together with lower level chronic exposures including those from food and water create general health effects that include cardiovascular and peripheral vascular disease, developmental abnormalities, neurologic and neurobehavioral disorders, and significantly higher standardized mortality rates and cumulative mortality rates for cancers of the skin, lung, liver, urinary bladder, kidney, and colon [15]. Studies of respiratory cancer at mean exposures well below PAC-2 levels with both periodic acute and chronic exposures demonstrated that "the excess relative risk for a fixed cumulative exposure was greater when delivered at a higher concentration and shorter duration than when delivered at a lower concentration and longer duration" [16].

Most people are exposed to benzene in small amounts on a daily basis. Air levels recorded in remote regions range from 0.047 to 0.27 μg/m^3 [17], but people living or working near petroleum refineries or gasoline stations may be exposed to levels up to 52 mg/m^3 [18]. Major sources of benzene are tobacco smoke, gasoline stations, motor vehicle exhausts, and industrial emissions and these are extensive [19, 20]. Clearly, for most persons there is no such thing as a once-in-a-lifetime exposure to benzene. Exposures at levels far lower than PAC-2 values have resulted in acute myeloid leukemia and aplastic anemia [19, 21].

A principle source of mercury for most people is contaminated seafood, and outdoor air levels are typically < 2 ng/m^3 [22]. Much higher exposures occurring in the home are most commonly from mercury spilled from a broken thermometer, thermostat, or mercury switch and many such incidents are reported annually [23]. Additional indoor exposures occur in medical or dental offices, often from mercury vapor released by blood pressure devices. Coal-fired power plants are a major source of mercury emissions and, surprisingly, recent research shows that vegetation growing on mercury-contaminated soil emits large amounts of mercury into the air [24]. When both indoor and outdoor sources of mercury are considered [25, 26] along with the significant contributions of mercury in seafood, it is apparent that for most persons there is no such thing as a once-in-a-lifetime exposure to mercury. Accidental inhalation of mercury vapor at moderate concentrations has resulted in severe lung damage [27], neuropsychological damage [28], and even death [29, 30].

Until they were banned in 1974, refrigerants, hairsprays, and other aerosols with vinyl chloride as the propellant were in common use [31]. Air levels of vinyl chloride in most areas are quite low, but persons living downwind from one or more of the more than fifty U.S. vinyl chloride-emitting industries are typically exposed to significant levels [32]. Major sources of vinyl chloride releases are the vinyl chloride and PVC manufacturing industries, but there are significant levels in tobacco smoke and waste incinerator emissions [33]. There is no such thing as a once-in-a-lifetime exposure to vinyl chloride for many people. Exposures at levels far below PAC-2 have resulted in hepatotoxicity, vascular disorders, and cancers of the liver, lung, brain, and digestive tract [34].

AREAS MOST LIKELY TO USE ONCE-IN-A-LIFETIME EXPOSURE GUIDELINES ARE THE VERY ARES IN WHICH ONCE-IN-A-LIFETIME EXPOSURES *ARE LEAST LIKELY*

In Bryan, Texas there was a plant operation from the 1940s that produced arsenic acid. During its latter years, the plant was purchasing crude arsenic trioxide from outside the U.S. Many batches contained enough iron to cause a "runaway" reaction when peroxide was added to convert raw material to arsenic pentoxide. Therefore, the plant site and nearby residential areas normally experiencing air arsenic levels as high as 0.1

$\mu g/m^3$ were periodically bombarded by clouds of arsenic oxides that fell in the area like snow [35]. What might otherwise have been "once-in-a-lifetimes" massive exposures were, instead, frequent repeated occurrences over a period of years.

A refinery in the Houston, Texas area had a massive release of over 17,000 pounds of benzene over a period of less than 960 hours in April of 2010. Between 2000 and 2007 that refinery had been cited for at least thirty-nine Emission Events [36]. Plant personnel, emergency responders, and other persons in the vicinity who might otherwise have been exposed on a once-in-a-lifetime basis to high levels of benzene were, instead, exposed repeatedly at levels well above workplace STELs.

Mercury spills are not uncommon and resulting vapors often pose serious health hazards. Some students at an Arizona high school for example recently poured out mercury in various classrooms and in the boys' locker room. The school had to be closed for an expensive cleanup. Although no information was released as to the level of mercury vapors in the school, some nearby homes were found to have indoor air levels of up to 50,000 ng/m^3 [37]. Students, teachers, emergency responders, and nearby residents who might otherwise have been impacted for a brief time as a "once-in-a-lifetime" exposure were, instead, repeatedly exposed to high levels over several days before cleanup was completed.

Within the Calcasieu Industrial Area near Lake Charles, Louisiana there are three vinyl chloride monomer and polymer facilities. Just one of these alone had 16 unauthorized vinyl chloride release events during the 2000–2001 period. There was a major pipeline rupture that released huge amounts of vinyl chloride along with ethylene dichloride and a plant fire that released large enough quantities to require downwind residents to shelter in place [38]. Workers, emergency personnel, and others in the area were exposed to high levels of vinyl chloride on much more than a "once-in-a-lifetime" basis.

ARE HIGH ACUTE EXPOSURES NO MORE DANGEROUS THAN LONG-TERM LOW LEVEL CUMULATIVE CHRONIC EXPOSURES?

Although most governmental and private groups attempting to develop once-in-a-lifetime exposure limits seem to minimize cancer risks from large acute exposure, one agency has recognized the danger of underestimating carcinogenicity. NIOSH has subscribed to a carcinogen policy published in 1976 by Edward J. Fairchild, II, Associate Director of Cincinnati Operations, which called for "no detectable exposure levels for proven carcinogens" [39]. Consequently, NIOSH recommended exposure levels for known or probable carcinogens that are now being based primarily on analytical limits of detection or technological feasibility.

As new information is developed and reviewed an increasing number of extremely dangerous chemicals, both carcinogens and non-carcinogens, will have no IDLH values but will—as has already been done with vinyl chloride—receive a warning that "respiratory protection is required at any detectable concentration" [2].

Part of the problem in attempting to set once-in-a-lifetime exposure levels is that there are very few studies of acute exposures to carcinogens, and especially to those for which the latency period is long. Typical animal studies focus on cancer production after repeated exposures occurring over fairly long time periods. Most information from workplace studies also involves cumulative chronic exposures. Yet, there is increasing interest in cancer risks from short-term exposures and increasing recognition that use of cancer potency estimates from long-term studies may seriously underestimate risk from acute exposure [40].

Recognition that cancer initiators may be of special concern in short-term exposures has generated increasing interest regarding attempts to assess risk for acute exposures. Evaluation of limited human data and available experimental data using laboratory animals has led to the conclusion that short-term or single exposure to genotoxic chemicals can cause and/or promote cancer development [41].

The very fact that cancer risk from acute exposure has yet to be fully defined may be one of the strongest arguments against use of once-in-a-lifetime exposure levels that have failed to recognize such risk and are based primarily on studies of non-cancer health effects such as damage to the respiratory system or neurological system.

A REASONABLE ALTERNATIVE

In recognition of the problems discussed above, and with the premise that emergency response personnel and the general public should be no less protected than persons in the workplace, it is reasonable to rely on the careful research of the National Institute for Occupational Safety and Health (NIOSH). Guidelines for respiratory protection for emergency response personnel or for shelter in place or evacuation decisions for

the general public should be based on NIOSH STEL recommendations when such information is available. In the absence of STEL values, REL values should be used or, if they are not available, the most protective of ACGIH or TEEL values should be used.

CONCLUSIONS

Attempts to produce once-in-a-lifetime exposure guidelines by mathematical manipulations of limited data, or with simple "black and white" metrics, without thorough review of the literature, including case studies, are irresponsible and should be stopped. Use of non-cancer or generalized cancer endpoints for such calculations is especially egregious for chemicals known or suspected to be cancer initiators or promoters. This places both emergency responders and the general public at unacceptable risk. Continuing such a practice, even with the best of intentions, cannot be condoned. Since most toxicological studies of chemical health effects in humans are based on workplace exposures, it is reasonable to rely upon the most protective workplace information available.

REFERENCES

1. NIOSH. 1994. Documentation for Immediately Dangerous to Life or Health Concentrations (IDLHs). Centers for Disease Control and Prevention, Atlanta, GA [available at www.cdc.gov/niosh/idlh/idlhintr.html]
2. NIOSH. 2010. NIOSH Pocket Guide to Chemical Hazards (NPG) Appendix A. Centers for Disease Control and Prevention, Atlanta, GA. [available at www.cdc.gov/niosh/npg/ nengapdxa.html]
3. USEPA. 2011, Acute Exposure Guideline Levels (AEGLs), United States Environmental Protection Agency, Washington, DC [available at www.epa.gov/oppt/aegl/]
4. AIHA. 2011. Emergency Response Planning Guidelines, American Industrial Hygiene Association, Fairfax, VA [available at www.aiha.org/insideaiha/.../ERPG/ 2011erpgweelhandbook_table]
5. SCAPA. 2010. Protective Action Criteria (PAC) with AEGLs, ERPGs, & TEELs: Rev. 26 for Chemicals of Concerns (09/2010). Subcommittee on Consequence Assessment and Corrective Actions (SCAPA), U.S. Department of Energy (DOE), Washington, Dc [available at www.orise.orau.gov/emi/scapa/teels.htm]
6. DOE Handbook. 2008. Temporary Emergency Exposure Limits for Chemicals: Methods and Practice. DOE-HDBK-1046-2008, U.S. Department of Energy, Washington, DC.
7. NOAA. 2012. Emergency Response Planning Guidelines, NOAA Office of Response and Restoration. [available at http://response.restoration.noaa.gov/erpgs]
8. Exponent. 2009. Arsenic Fact Sheet, Exponent, Bellevue, WA [available at www.tva.com/kingston/exponent/Arsenic%20fact%20sheet.pdf]
9. HPA.2011. HPA Compendium of Chemical Hazards: Inorganic Arsenic, Health Protection Agency, London, England. [available at www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/ 1202487025752]
10. USEPA. 1998. Locating and Estimating Air Emissions from Sources of Arsenic and Arsenic Compounds. EPA-454/R-98-013. U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards. Research Triangle Park, NC [availably at www.epa.gov/Exe/ZyPURL.cgi?Dockey=2000D52M.txt]
11. Kilburn, K.H. 1997. Neurobehavioral Impairment from Long-Term, Residential Arsenic Exposure. Chapter 14 in Arsenic Exposure and Health Effects, Chapman & Hall, New York, NY.
12. O'Connor, R., M. O'Connor, K. Irgolic, et al. 2005. Transformation, Air Transport, and Human Impact of Arsenic from Poultry Litter. *Environmental Forensics,* 6: 83–89.
13. O'Connor, R. and P.B. Duncan. 2008. Estimation of Chronic and Acute Air Arsenic Levels from House Dust Composition in Poultry Waste Disposal Areas. *Journal of Residuals Science & Technology,* 5: 127–136.
14. 14. ATSDR. 2007A. Toxicological Profile for Arsenic. Agency for Toxic Substances and Disease Registry, Atlanta, GA.
15. 15. Tchounwou, P.B., A.K. Patlolla, and J.A. Centeno. 2003. Carcinogenic and Systemic Health Effects Associated with Arsenic Exposure—A Critical Review. *Toxicologic Pathology.* 31:575–588.
16. Lubin, J.H., L.E. Moore, J.F. Fraument, Jr., and K.P, Cantor. 2008. Respiratory Cancer and Inhaled Arsenic in Copper Smelters Workers: A Linear Relationship with Cumulative Exposures that Increases with Concentration. *Environmental Health Perspectives.* 116: 1661–1665.
17. McCarthy, M.C., H.R. Hafner, and S.A. Montzka. 2006. Background Concentrations of 18 Air Toxics for North America. *Journal of the Air & Waste Management Association,* 56: 3–11.
18. EBI. 2011. Benzene. Environmental Bureau of Investigation. Toronto, Ontario, Canada. [available at www.e-b-i.net/ebi/contaminants/benzene.html]
19. ATSDR. 2007B. Toxicological Profile for Benzene. Agency for Toxic Substances and Disease Registry, Atlanta, GA.
20. Wallace, L. 1996. Environmental Exposure to Benzene: An Update. *Environmental Health Perspectives.* 104(Suppl. 6); 1129–1136.
21. WHO. 2010, Exposure to Benzene: A Major Public Health Concern. World Health Organization, Geneva Switzerland. 5 pages
22. USEPA 1997, Mercury Study Report to Congress, Volume IV: An Assessment of Exposure to Mercury in the United States. U.S. Environmental Protections Agency, Washington DC. [available at www.eps.gov/ttn/oarpg/t3/reports/volumr4.pdf]
23. NEWMOA 2003. Indoor Air Mercury. Northwest Waste Management Officials' Association, Boston, MA [available at www.newmoa.org/prevention/mercury/MercuryIndoor.pdf
24. TCEQ. 2006. Mercury in Texas: Background, Federal Rules, Control Technologies, and Fiscal Implications. Texas Commission on Environmental Quality, Austin. TX [available at www.tceq.state.tx.us/publications]
25. ATSDR. 1999. Toxicological Profile for Mercury. Agency for Toxic Substances and Disease Registry, Atlanta, GA
26. Lutter, R. and E. Irwin. 2002. Mercury in the Environment: A Volatile Problem. *Environment* 44: 24–40.
27. Lien, D.C., D.N. Todoruk, H.R. Rajani, D.A. Cook, and F.A. Herbert. 1983. Accidental Inhalation of Mercury Vapour: Respiratory and Toxicologic Consequences. *Canadian Medical Association Journal.* 129: 591–595.
28. Yeates, K.O. and M.E. Mortensen. 1994. Acute and Chronic Neuropsychological Consequences of Mercury Vapor Poisoning in Two Early Adolescents. *Journal of Clinical and Experimental Neuropsychology.* 16: 209–222.
29. Rowens, B., D, Guerrero-Betancourt, C.A. Gottlieb, R.J. Boyes, and M.S. Eichenhorn. 1991. *Chest.* 99: 185–190.
30. Asano, S., K. Eto, E. Kurlsaki, *et al.* 2000. *Pathology International.* 50: 169–174.
31. NTP. 2011. Report on Carcinogens, Twelfth Edition, National Toxicology Program, U.S. Department of Health and Human Services, Washington, DC, pages 437–442.
32. EWG. 1998. The Inside Story: Vinyl Chloride. Chemical Industry Archives, a Project of the Environmental Working Group. [available at www.chemicalindustryarchives.org/the inside story/ vinyl chloride]
33. ATSDR. 2006. Toxicological Profile for Vinyl Chloride. Agency for Toxic Substances and Disease Registry, Atlanta, GA.
34. Faust, R.A. 1993. Formal Toxicity Summary for Vinyl Chloride. The Risk Assessment Information System (RAIS), [available at http://rais.ornl.gov/tox/profiles/vinyl/html]. 15 pages.

35. O'Connor, R. and M. O'Connor. 1990-91. Personal interviews with plant personnel and persons living and working within a mile of the Bryan, Texas arsenic acid plant.
36. Cause No. D-1-GV-10-001237 in the District Court of Travis County, Texas, State of Texas vs. BP Products B North America, Inc., Plaintiff's Original Petition. Office of the Attorney General of Texas, Environmental Protection and Administrative Law Division, Austin, TX, filed August 9, 2010.
37. Gordon, M. 2009. How School's Huge Mercury Cleanup Unfolded. The Arizona Republic, November 29, 2009. [available at www.azcentral.com/.../news/.../11/.../20091129 mercuryspill1129.htm]
38. Subra, W. 2001. Environmental Impacts in Communities Adjacent to PVC Production Facilities, Subra Company, New Iberia, LA [available at www.pvcinformation.org/.../Wilma_Subra_report_on_PVC_fencelin...]
39. Fairchild, E.J. 1976. Guidelines for a NIOSH Policy on Occupational Carcinogenesis. Annals of the New York Academy of Sciences. 271: 200–207.
40. Halmes, N.C., S.M. Roberts, J.K. Tolson, and C.J. Portier. 2000. Revaluating Cancer Risk Estimates for Short-Term Exposure Scenarios. *Toxicological Sciences.* 58:32–42.
41. Bos, P.M.J., B. Baars, M.T.M. van Raaij. 2004. Risk Assessment of Peak Exposures to Genotoxic Carcinogens: A Pragmatic Approach. Toxicology Letters. 151: 43–50.

Comparative Analysis of Structural and Socio-cultural Properties of Agricultural Farm Enterprises in Terms of Sludge Application[1]

K. YAMAN[1,*] and E. OLHAN[2]

[1]*Assistant Professor, Karabük University, Faculty of Economics and Administrative Sciences, Division of Urbanization and Environmental Issues, Karabuk, Turkey*
[2]*Professor, Ankara University, Faculty of Agriculture, Department of Agricultural Economy, Dışkapı, Ankara, Turkey, 06100*

ABSTRACT: Socio-cultural characteristics of 39 farm enterprises which applied sewage sludge produced in a central waste water treatment plant established in Ankara, capital city of Turkey, and 42 farm enterprises which did not apply sewage sludge are comparatively examined. According to t-test statistical results, the difference between two groups in terms of educational levels and crop production is significant at the 5% level, whereas difference of average amount of owned land is significant at the 1% level. Relationship between educational levels of farmers and sludge using tendencies is opposite and is found to be significant at the 1% level.

INTRODUCTION

FIRST agricultural usage of sewage sludge reportedly started on different dates ranging from 1845 to 1959 to the 1990s [1, 2, 3]. The first time when sewage sludge was applied as fertilizer in the United States was in 1927 through the foundation of a large wastewater treatment plant (WTP). The U.S. Environmental Protection Agency (USEPA) and local authorities have promoted recycling of sewage sludge since 1970. However, in the European Union (EU) and in Turkey usage of sewage sludge on agricultural land is rather restricted. Conversely, results of scientific researches revealed that sewage sludge related issues have been recently threatening the environment, animals, and humankind.

It is noteworthy that there are very few scientific researches which examine structural and socio-cultural characteristics of farm enterprises that have applied sewage sludge and those which did not. Farmers who used and who did not use sewage sludge produced in the Ankara Wastewater Treatment Plant (AWTP) were interviewed and differences were revealed.

*Author to whom correspondence should be addressed.
E-mail: kyaman@karabuk.edu.tr
[1]Published as proceedings of IWA Specialist Conference Sustainable Management of Water and Wastewater Sludges in Harbin, China, 2009.

MATERIAL AND METHOD

Basic materials of this research are primarily composed of survey data obtained from farm enterprises which applied and did not applied the sewage sludge produced in AWTP, located in Tatlar village, which is 45 km away from the west of Ankara province (Figure 1). The second type of material is the data gained from various scientific researchers conducted at national and international level.

AWTP is one of the largest treatment plants in Europe established in 2000 in order to provide service to a population of approximately 4 million at Ankara. The AWTP has a treatment capacity of 765,000 m^3 waste water and has a daily production of sewage sludge of 704 m^3 [4].

Farmers who live in Gökler (Ayaş), Tekkeköy and Anayurt (Sincan), and Türkobası (Polatlı) villages within Ankara that applied sewage sludge from the AWTP during the production period of 2004–2005 were selected as a target group. Two different questionnaires were prepared for farmers having applied sewage sludge and for those who did not. 50 questions in total were posed to the group which applied sewage sludge, whereas 26 questions were posed to the group that did not apply sewage sludge. Both groups were asked identification questions as well as questions about the size of the enterprise, tenure forms,

1544-8053/12/02 087-07

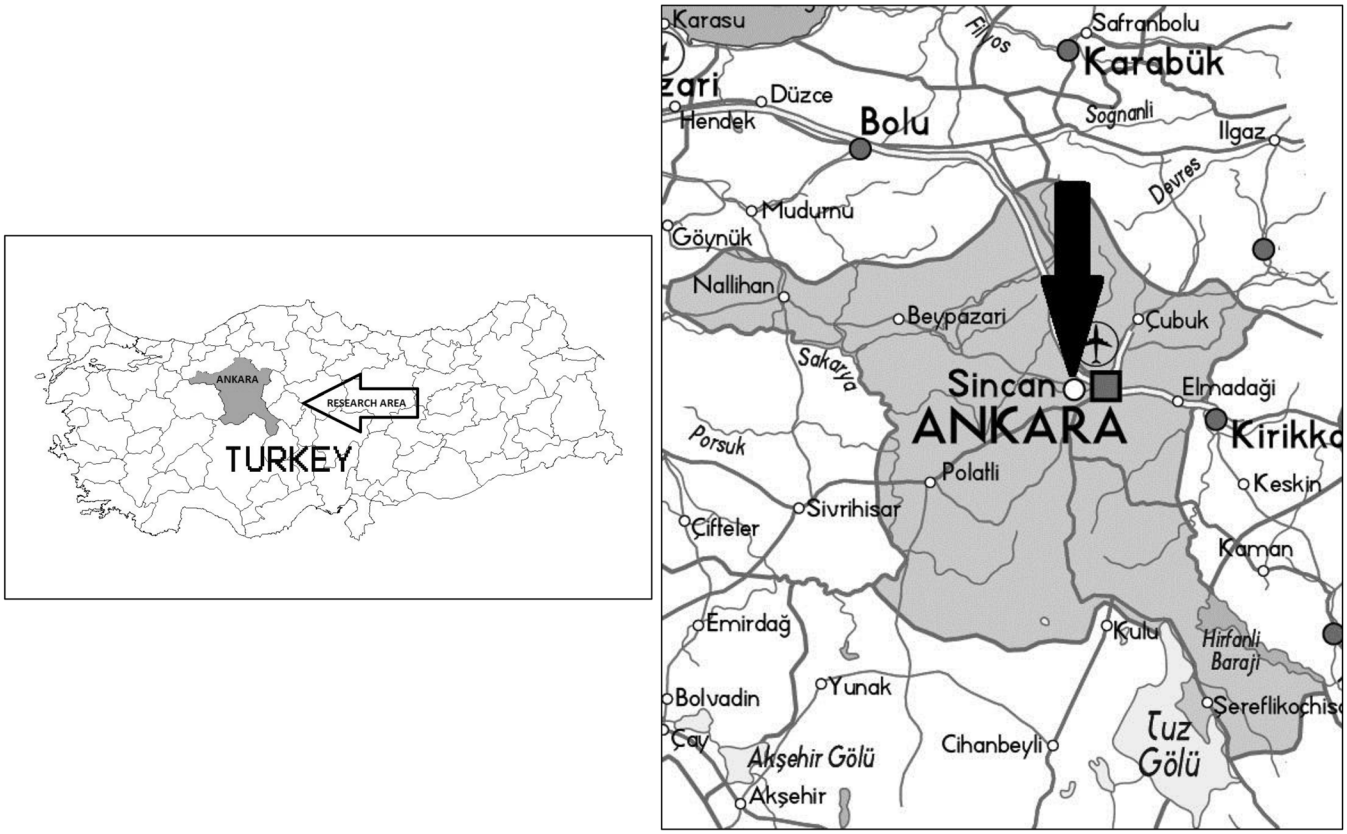


Figure 1. *Location of the research area (www.maps.google.com; www.1resimler.com).*

crop rotation system, population, work force capacity of the enterprise, educational levels of farmers, membership behaviors towards agricultural organizations, and socio-cultural status of the enterprises. Surveys were carried out through face-to-face interviews with the farmers.

While selecting enterprises which applied sewage sludge, a full counting method was used from data collection from the AWTP in 2005 and 39 enterprises were interviewed in the survey. Regarding selection of enterprises which did not apply sewage sludge, data for the aforementioned villages was obtained from the Farmer Recording System (FRS) of 2005 under the District Directorate of Agriculture Ministry. Among them, the enterprises that used sewage sludge in 2005 were left out and enterprises which did not use sewage sludge were chosen as the main target group. In the target group, 42 enterprises which would be examined in the surveys were chosen on the basis of land size and through a method of random sampling. [5]. Total number of enterprises was determined in accordance with population ratio in the four villages selected namely, Gökler, Anayurt, Türkobası, and Tekke.

Depending on family members' age groups and gender, an available man labor force unit (MLU) for the enterprises was calculated with a technique applied by Erkuş [6]. In conclusion, survey data obtained from both groups were comparatively analyzed. An independent-samples t-test was applied in order to compare estimated mean at the significance level of 1% and 5%, and the correlation analysis was applied in order to find out direction and magnitude of a relationship between educational level of farmers and sewage sludge application.

RESULTS AND DISCUSSION

Water Reuse

Water is an essential and vital natural resource for humans, animals, and plants and can't be easily generated here. Therefore, reuse of treated waste in different ways such as urban, industrial, agricultural, recreational purposes, and for habitat restoration has been encouraged [7]. Treated waste water from the AWTP has not been reused and discharged into the Ankara stream [8]. Although it is treated, disposal of reclaimed water to the natural environment causes serious environmental pollution. To illustrate, it has been found that high volumes of treated waste water disposal from the San

Jose/Santa Clara Water Pollution Control Plant at the San Francisco Bay has threatened the area's natural salt water marsh [9].

The first known regulation of water reuse which belongs to the State of California in the United States was published in 1918 [10]. However, safe reuse of waste water has been regulated starting from 1989 by the World Health Organization (WHO). Subsequently, the USEPA developed a guideline for water reuse in 1993. The WHO eventually published a guideline for safe use of water in 2006. In the latter guideline, reuse of waste water in agriculture is restricted for certain conditions and health-related parameters are defined [11]

Comparison of Structural Characteristics of Enterprises

Total land size of a farm enterprise consists of owned, rented, and tillable lands of the enterprise. Among enterprises examined for the group that applied sludge land size was 40.1 ha and 34.6 ha of which (86.50%) was owned, 3.6 ha (9%) was rented, and 1.8 ha (4.5%) was tillable land. Enterprises which did not apply sewage sludge had the values of 17.1 (75%) for owned, 3.2 (14.04%) for rented, and 2.5 ha (10.96%) for tillable lands (See Figure 2). Average of total land size was 31.16 ha and it was 5.1 times more than the average for all of Turkey which was 6.10 ha in 2001 [12].

When qualities of agricultural land in enterprises were examined it was observed that 81.47% of total land size of enterprises which applied sludge and 88.14% of total land size of enterprises that did not apply were dry land fields. Percentages of irrigated land in the enterprises which applied sewage sludge and enterprises that did not apply sewage sludge were 10.28% and 17.38%, respectively. Other types of land use were vegetable and fruits gardens. The ratio of dry land enterprises were found higher in comparison to enterprises examined in Central Anatolian Region (See Figure 3).

Comparison of Family Labor Force Structures in the Enterprises

Family labor force structures in the enterprises were calculated in terms of Family Labor force Unit (FLU) in accordance with the technique explained in the materials and method section. The ratio of male population was 2.33 FLU for the group which applied sewage sludge, whereas it was 1.95 FLU in the group that did not apply sewage sludge. Rate of female population was calculated at 1.92 and 1.95 FLU for groups in previously mentioned order. Rate of female population was found higher in the sludge applying group versus the non-sludge applying group whereas the rate of female population was found higher in the non-sludge applying group. It was found that the rate of family labor force was 4.25 FLU in the sludge applying enter-

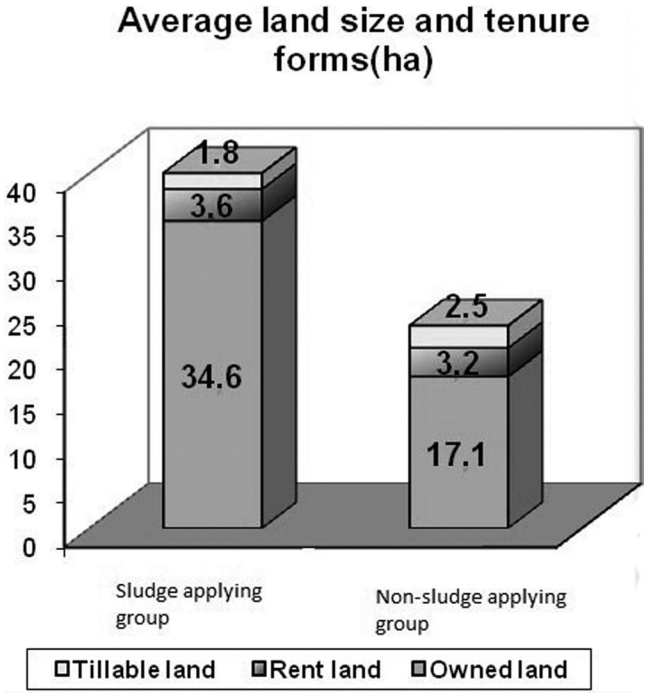


Figure 2. Land size and land tenure forms by enterprises.

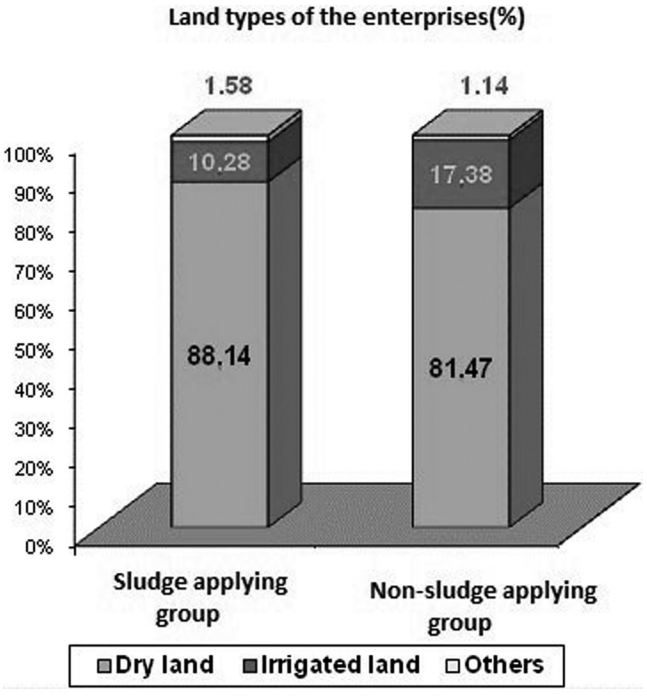


Figure 3. Land types by enterprises.

Table 1. Educational Level of Farmers.

	Primary		Secondary		Total	
Enterprise Groups	**Number**	**%**	**Number**	**%**	**Number**	**%**
Sludge applying group	22	56	17	44	39	100
Non sludge applying group	35	83	7	17	42	100
Total	**57**	**70**	**24**	**30**	**81**	**100**

prises while it was only 3.80 in the non-sludge applying group.

Educational Level of Farmers

When a relationship between educational level and sewage sludge application was compared it was discovered that 56% of farmers were graduates of primary school[2] and 44% were graduates of middle school[3] in the sludge applying group, whereas ratios for graduates of primary school and secondary schools were 83% and 17%, respectively (See Table 1). Regarding total, 70% and 30% of farmers were graduates of primary and middle school, respectively. In another study conducted in the Region of Central Anatolia, percentages for graduates of primary, middle, and high schools were 66%, 16%, and 12%, successively [13]. A higher rate for usage of sewage sludge by primary school educated farmers indicates sewage sludge usage tendency decreases as educational level of farmers increases. According to results from the correlation analysis, a relationship between educational level and sludge usage tendency has a reverse correlation and in terms of statistics is significant at the level of 1% (Pearson estimation: –0,295).

In Table 2 below it is noteworthy to mention that educational level of the household within the enterprise was examined on the bases of gender. The rate of primary schooled females and middle schooled males as well as the rate of college educated males and females and rate of pre-schooled females are higher in the sludge applying group in comparison to the non-sludge applying group. It was found that the rate of a female gaining primary education, the rate of a male gaining secondary education, the rate of a male and a female gaining university education and the rate of a male and a female at the age of pre-school education were higher in the group that applied sewage sludge when compared to those in the group that did not apply sludge.

[2]Training period of five years
[3]Training period of three years

Table 2. Percentage of Household Members in the Enterprises on the Basis of Educational Level and Gender.

	Sludge Applying Group		Non Sludge Applying Group	
	Male	**Female**	**Male**	**Female**
Preschool	18.68	16.00	8.54	9.75
Primary	43.96	54.67	63.41	51.22
Middle	34.07	16.00	26.83	24.39
University-College	2.20	2.67	1.22	0
Illiterate	1.10	10.67	0.00	14.63

Factors that Affect Sludge Application

When farmers were asked about their preference for sewage sludge application it was found that the reason for the highest rate of sewage sludge application which was 33.33% resulted from the fact that it was free of charge. Subsequently, while 30.77% of farmers presented the ability of sewage sludge application as a reason for their preference, 20.51% of them indicated both its being free of charge and its ability to increase productivity as a reason for their choice. Consequently, it was observed that farmers preferred to use sewage sludge largely for economic reasons and in fact adverse effects of sewage sludge application on the soil in the

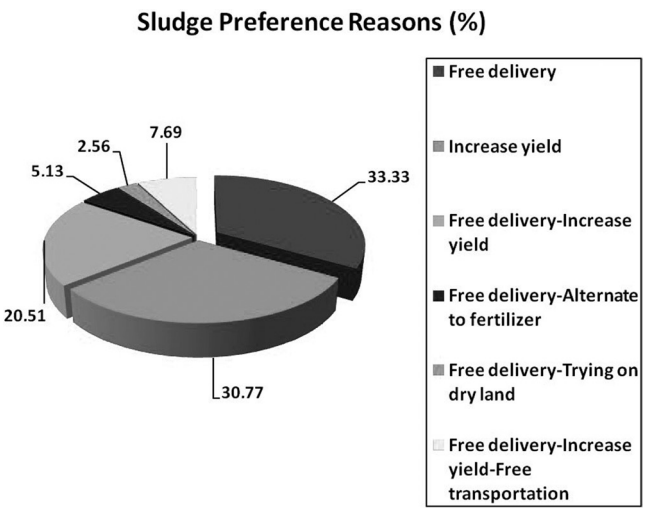


Figure 4. Sludge preference reasons by enterprises.

immediate term and loss of productivity in long term are of no importance to those farmers (See Figure 4).

Status of Membership–Partnership for Agricultural Organizations

An affinity towards agricultural organization membership in the sewage sludge applying group and non-sewage sludge applying group were compared using agricultural holdings (See Table 3). It was found that 39 holdings which applied sewage sludge had 77 memberships in total whereas 42 holdings which did not apply sewage sludge had 88 memberships in total. Therefore, it was calculated that a coefficient for membership was 1.97 for the sewage sludge applying farmers and 2.09 for the non-sewage sludge applying farmers.

In accordance with results from another study conducted for 400 farmers in the Southeastern Anatolian Region in Turkey, the coefficient for membership was 0.15 [14]. The coefficient for organizational membership was found to be higher when compared to those of the previous research.

The difference of coefficient for membership in both studies may be explained by educational levels of the farmers, product patterns, and current legislations. Furthermore, it is a well-established fact that farmers in Turkey generally become members of agricultural organizations in order to receive Direct Income Support (DIS) to purchase fertilizer with credit and to provide themselves warranty sales for their products.

Visiting Frequency for Agricultural Organizations

Visiting frequencies to the District Directorate of Agricultural Affairs and for other agricultural organizations all located in the center of districts where agricultural enterprises were operating are displayed in Table 4. It was discovered that 45.24% of farmers who used sewage sludge and 65.24% of those who did not occasionally visited agricultural organizations. Visiting frequency of farmers to agricultural organizations in both groups occurred each time. They went to town ranges from 23.08% to 21.43%. 10.26% of farmers who applied sewage sludge and 9.52% of farmers who did not apply sewage sludge stated they never paid a visit to those organizations.

Table 3. Membership Behavior of Farmers for Agricultural Organizations.

Groups	Number of Farmers	Number of Total Membership	Membership Ratio
Sludge Applying Group	39	77	1.97
Non Sludge Applying Group	42	88	2.09

Table 4. Visiting Frequency of Enterprises to Agricultural Organizations.

	Sludge Applying Group		Non Sludge Applying Group	
	Number	Percentage	Number	Percentage
For every visit to town	9	23.08	9	21.43
Occasionally	16	41.03	19	45.24
Never	4	10.26	4	9.52
For business	8	20.51	7	16.67
N/A	2	5.13	3	7.14
Total	**39**	**100**	**42**	**100**

Socio-Cultural Structures of the Enterprises

Reasons for a high percentage of having private cars in these areas were high income rate of farmers and distance to Ankara from these areas. Second, percentages for daily newspaper purchases were 5.13 and 4.76 in the examined group, respectively. Third, successive percentages for computer usage and internet connection were 5.13, 2.56, 7.14, and 0.0 in those groups. It is noteworthy mentioning that average internet subscription of urban areas in Turkey was 12.81% in 2005 and 22.7% in 2011 [15]. Next, percentages for mobile phone usage in both groups were 85.05 and 76.19 respectively, while it was 82.9 in urban areas in Turkey in 2009 [16]. Finally, it was observed that all holdings had at least one television in their home (Table 5).

Table 5. The socio-cultural Structures of Enterprises (%).

Parameters	Sludge Applying Group	Non Sludge Applying Group	Total
Private car	48.72	57.14	**53.09**
Daily newspaper	5.13	4.76	**4.94**
Personal computer	5.13	7.14	**6.17**
Internet connection	2.56	0	**1.28**
TV	100	100	**100**
Fixed line telephone phone	100	97.62	**98.77**
Mobile phone	82.05	76.19	**79.01**

Statistical Assessments

Basic indicates were compared to analyze structural and socio-cultural characteristics of both sewage sludge and non -sewage sludge applying enterprises. Whether these differences are significant or not are displayed in Table 6. It was further discovered that the difference between the two groups in terms of land owning is significant at a level of 1% and that the difference between the groups in terms of gross production value and educational level is significant at a level of 5%. Usage of sewage sludge in those villages under inquiry is found to be novel and sewage sludge application is detrimental to human health because of contaminants.

The significant difference in terms of land size of holdings may be explained by a measure of those enterprises to try sewage sludge application. It is estimated that differences in other socio-cultural parameters were not statistically significant at the level of 1% and 5%.

CONCLUSIONS

According to results, farmers who applied sewage sludge in comparison to those who did not are younger, more educated, more wealthy, owners of larger enterprises and land, and one of a greater family labor force.

Furthermore, number of individuals in the family, male population, and use of mobile phone are higher in the first group. On the contrary, rate of owning private cars, membership to an agricultural organization, and rate of female population per home are higher in the non-sewage sludge applying group.

Application of sewage sludge increases productivity especially in places where dry land farming is carried out within a short period of time. According to various other study results application of sewage sludge leads to various damages to soil structure, product quality, and animals as well as human health [17, 18, 19]. Far more prefer sewage sludge generally for its free distribution and low production costs.

In sum, it is well understood that farmers avoiding use of sewage sludge are ones of higher education. It is essential that relevant institutes and cooperatives explain negative effects of sewage sludge application and train farmers to use alternative and less potentially harmful methods.

Table 6. Statistical Assessments of Enterprises.

Parameters	Group Applying Sludge	Group Not Applying Sludge	P-value
Family population	4.25	3.90	NS
Age of the farmers	49	53	NS
Gross crop production value ($)	21.337	13.817	*
Education level***	2.43	2.6	*
Land owned (ha)	34.2	17	**
Male population	2.33	1.95	NS
Female population	1.92	1.95	NS
Number of membership to agricultural organizations	1.97	2.09	NS
Private car	0.38	0.54	NS
Cell phone	0.58	0.50	NS

NS: Not Significant, *P < 0.05 **P < 0.01, ***(2 = Primary, 3 = Secondary).

REFERENCES

1. Anonymous. 1996. *Use of Reclaimed Water and Sludge in Food Crop Production.* National Academy Pres. Washington. USA.
2. Chang, A.C., Pan, G., Page, A. L. and Asano, T. 2002. Developing human health-related chemical guidelines for reclaimed water and sewage sludge application in agriculture. WHO publication.
3. Anderson M.S. 1959. "Fertilizing characteristics of sewage sludge, Sludge Ind. Wates". 31: pp. 678–682.
4. Anonymous 2009. Ankara Water and Sewage Directorate (ASKI), Offical web site. Ankara.
5. Güneş, T. and Arıkan, R., 1998. *Agricultural Economics Statistics.* AÜZF Pub. No: 1049, Ankara (Turkish).
6. Erkuş, A., Bülbül, M., Kıral, T., Açıl, A.F. and Demirci, R. 1995. *Agricultural Economics,* Ankara University Faculty of Agriculture, Education, Research and Development Foundation Publications, No: 5, Ankara. (Turkish).
7. US EPA. 1992. Guidelines for Water Reuse. US EPA Office of Technology Transfer and Regulatory Support. EPA/625/R-92/004. p. 262. September 1992.
8. Demirel, G. 2009. Ankara Wastewater Treatment Plant and Disposal of Its Sewage Sludge. 2th National Symposium of Sewage Sludges.ACS-2009. Proceedings, p 145–154 Izmir (Turkish).
9. US EPA. 1998. Water Recycling And Reuse: The Environmental Benefits, p: 9, USA.
10. UNEP. 2004. Water and Wastewater Reuse: An Environmentally Sound Approach for Sustainable Urban Water Management. p. 48.
11. WHO. 2006. Guidelines for the safe use of wastewater, excreta and grey water. Volume 2: Wastewater use in agriculture.
12. Anonymous 2007. Turkish Statistical Institute. Turkish Statistical Yearbook. Pub. No: 3063, Ankara (Turkish).
13. Dernek, Z. 2000. "Determining Irrigation Possibilities and Agricultural Problems in the Wheat Growing Areas of Ankara, Konya and Eskisehir Provinces". Turk J Agric For 25 (2001) 451–458 TÜBİTAK.
14. Eraktan, G., Aksoy, S., Kuhnen, F., Olhan, E. and Winkler, W. 2002. *Effects Possible of the change in agriculture Technologies in southeastetn Anatolia on producer bahaivor and that on economy.* ÇESAV Foundation Pub. No. 3, pp. 204. Ankara (Turkish).
15. TUIK. 2011. Turkish Statistical Institute, Information And Communication Technology (ICT)Usage Survey In Households And Ind?v?duals, Press Release, No. 170, Date:18.08.2011.
16. Uğuz, H.E. 2011. *Digital Divide in Turkey and Bridging the Digital Divide,* Journal of US-China Public Administration, Vol. 8, No. 6, pp. 629–639.
17. Witter, E. 1999. *Limit values for heavy metal concentrations in sewage sludge and soil that protect soil microorganisms.* Proceedings of the workshop on "Problems around sludge", 18–19 November 1999, EC Joint Research Center. 93 pp., Stresa-Italy.

18. Eriksson, J. 2001. "Concentrations of 61 trace elements in sewage sludge, farmyard manure, mineral fertilizer, precipitation and in oil and crops". Swedish Environmental Protection Agency, 44 pp., Sweden.

19. Lewis. D.L., Gattie, D.K., Novak, M.E., Sanchez, S. and Pumphrey, C. 2002. "Interactions of pathogens and irritant chemicals in land-applied sewage sludges (biosolids)". BMC Public Health 2002, 2:1.1