**FÁBIO SATOSHI HIGASHIKAWA**

# RESÍDUOS ORGÂNICOS E SOLOS:
## FORMULAÇÃO, ÍNDICES DE MATURAÇÃO DE SUBSTRATOS E COMPOSTOS ORGÂNICOS VOLÁTEIS ALVOS

**LAVRAS – MG**

**2013**

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciência do Solo, área de concentração em Fertilidade do Solo e Nutrição de Plantas, para a obtenção do título de Doutor.

Orientador
Dr. Carlos Alberto Silva

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**Ficha Catalográfica Elaborada pela Divisão de Processos Técnicos da Biblioteca da UFLA**

Higashikawa, Fábio Satoshi.
Resíduos orgânicos e solos : formulação, índices de maturação de substratos e compostos orgânicos voláteis alvos / Fábio Satoshi Higashikawa. – Lavras : UFLA, 2013.
146 p. : il.

Tese (doutorado) – Universidade Federal de Lavras, 2013.
Orientador: Carlos Alberto Silva.
Bibliografia.

1. Subprodutos. 2. Compostagem. 3. Calibração multivariada. 4. Meios de crescimento alternativos. 5. Gases. I. Universidade Federal de Lavras. II. Título.

CDD – 631.875

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APROVADA em 16 de abril de 2013.

| | |
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| Dr. Miguel Angel Sánchez-Monedero | CEBAS/CSIC |
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Dr. Carlos Alberto Silva
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**LAVRAS - MG**
**2013**

A minha mãe, Lucia, pela torcida, incentivo, amor, carinho e dedicação;
Ao meu pai, Tsukasa, pelo apoio, ensinamentos e dedicação;
A minha namorada, Roberta, pelo apoio, paciência, companhia em todos os momentos, carinho e amor;
A minha avó, Tomoe, pelo carinho, preocupação e pelo simpático acolhimento de sempre;
A minha família materna e paterna, pelo apoio e consideração em todos os momentos da minha vida;
A todos os meus amigos, que são muito importantes na minha vida.

OFEREÇO.

Ao meu avô paterno, Kihei Higashikawa (*in memoriam*) e a minha avó paterna Toshiyo Higashikawa (*in memoriam*), que certamente estariam felizes por mim;

Ao meu avô materno, Carlos Anraku (*in memoriam*), pelo exemplo de luta, simpatia e pelos inesquecíveis momentos;

DEDICO

# AGRADECIMENTOS

Agradeço, primeiramente, a Deus, à Virgem Maria e a Nossa Senhora Aparecida, por me iluminar e guiar por todos os caminhos e momentos;

À Universidade Federal de Lavras, em especial ao Departamento de Ciência do Solo, por fornecer infraestrutura necessária para o desenvolvimento da pesquisa e pelas oportunidades em todas as etapas de minha formação durante a Pós-Graduação.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, pela concessão da bolsa de estudos e à Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG (Processo: CAG 20008-09), pelo financiamento da minha estadia na Espanha e pelo custeio da pesquisa. Ao CNPq, pelo financiamento de parte do projeto de pesquisa;

Ao Centro de Edafologia y Biologia Aplicada del Segura (CEBAS/CSIC), em Murcia, Espanha, e ao Ministério de Ciência e Inovação da Espanha, pelo apoio dado para a realização dos projetos de pesquisa no CEBAS (CTM2009-14073-C02-02 e AGL2012-40143-C02-01);

Ao Professor Carlos Alberto Silva, pela orientação, ensinamentos, incentivo, amizade e confiança ao longo desses anos;

Ao cooreintador, Miguel Angel Sánchez-Monedero, pela ótima recepção, amizade, ensinamentos e apoio durante minha estadia na Espanha;

Aos amigos e colegas do Departamento de Ciência do Solo, pela boa convivência e aos amigos e companheiros do futebol semanal;

Ao amigo, Bruno Dias, por ser sempre solícito e pelas valiosas dicas e ajuda na minha estadia na Espanha durante o doutorado sanduíche;

Aos amigos da república Edson, Rafael e Ricardo, pelo acolhimento e pela harmoniosa convivência;

Aos amigos, Gabriela Pinheiro e Davi, pela amizade e por serem sempre solícitos quando precisei de ajuda;

Aos amigos Daniel, Leandro Heiji e Renato, pela longa amizade e companheirismo em todos os momentos;

Aos colegas do CEBAS/CSIC Asuncion, Roig, Mariluz, Pepa, Rafael, Maite e Keiji, pela boa convivência e auxílios durante a minha estadia em Murcia;

Aos amigos, Douglas Amaral, Douglas Marques, Clério Hickmann, Raphael Siqueira, Hugo Catão, Alexandre Galvão, Romulo Duarte, Carlinha, Fábio Ono, Guilherme Amaral, Fabrício Ávila, Silvio, Geila, Glauco, Gustavo (Maizena) e Henrique Ferro, pela boa convivência dentro e fora da UFLA;

Aos funcionários do Departamento de Ciência do Solo, em especial -à Dirce, José Roberto (Pezão), Maria Alice, Humberto, Cristina, Roberto Lelis e João Gualberto, pelo auxílio imprescindível nas atividades do cotidiano;

A minha família, por toda dedicação, apoio e carinho;

A família da Roberta, em especial ao João Bosco, Alexandra e dona Zilda, pelo apoio e simpático acolhimento de sempre;

A minha namorada, Roberta, por toda ajuda paciência, carinho, amor e companheirismo para superar as dificuldades da vida;

Agradeço a todos que colaboraram de forma direta ou indireta na execução deste trabalho.

Muito Obrigado!

“Não tentes ser bem sucedido, tenta antes ser um homem de valor.”

Albert Einstein

# RESUMO GERAL

Os compostos orgânicos, quando maturos, podem ser utilizados na agricultura como componentes de substratos para crescimento de plantas. Entretanto, durante a compostagem de resíduos orgânicos, ocorre liberação de diversos gases que englobam os compostos orgânicos voláteis (COVs). Diante do exposto, três experimentos foram conduzidos para estudar diferentes usos e aplicações de resíduos orgânicos. Objetivou-se nesses experimentos avaliar: a) o crescimento de mudas de eucalipto em substratos alternativos obtidos por meio da compostagem de resíduos orgânicos de origens diversificadas, em comparação com substratos comerciais; b) o desenvolvimento de modelos de predição para índices de maturação de amostras de substratos, combinando-se técnicas de espectroscopia de infravermelho (FTIR) e regressão por mínimos quadrados parciais (PLS); c) o desenvolvimento e otimização da metodologia de microextração por fase sólida (SPME) em *headspace* para análises de COVs alvos de grupos comuns (hidrocarbonetos, alcoóis, aromáticos, hidrocarbonetos clorados, cetonas e terpenos) em uma série de matrizes que inclui solos com diferentes texturas, matrizes orgânicas de estercos e compostos de diferentes origens e biocarvões. No primeiro experimento, concluiu-se que os substratos anternativos estavam desbalanceados quanto aos nutrientes. Somente o teor de boro disponível se correlacionou positivamente com a produção de matéria seca de eucalipto. A condutividade eletrolítica não afetou significativamente a produção de biomassa total, no entanto, pode ser utilizada como preditora de teores disponíveis de $NH_4^+$, P, $K^+$, $Mg^{2+}$, $Na^+$, $Cu^{2+}$ e $Fe^{2+}$. A utilização de análise de componentes principais auxiliou na identificação dos atributos que diferenciaram os substratos quanto à produção de biomassa de eucalipto. Em relação ao segundo experimento, todos os modelos ajustados para os índices de maturação foram preditivos, robustos e não apresentaram correlação aleatória. Os modelos ajustados permitiram predizer os índices de maturação em substratos formulados com diferentes misturas de resíduos orgânicos e com larga faixa de variação do grau de maturação. No terceiro experimento, as características das matrizes, especialmente o pH e a matéria orgânica, tiveram acentuado impacto na SPME, devido a competição de analitos por sítios ativos na fibra. A metodologia apresentou boa performance para solos de texturas variadas e para matrizes orgânicas, exceto para *biochars*, que foram caracterizados por uma forte capacidade de adsorção, que resultou na competição por sítios ativos da fibra SPME, limitando, desse modo, a extração dos analitos das matrizes.

Palavras-chave: Subprodutos. Compostagem. Meios de crescimento alternativos. Calibração multivariada. Gases.

# GENERAL ABSTRACT

Organic compounds, when mature, may be used in agriculture as substrate components for the growing plants. However, many gases, including volatile organic compounds (VOCs), are released during the composting of organic residues. Three experiments were conducted in order to study different uses and applications for organic residue. The objective of these experiments was to evaluate: a) the growth of eucalyptus seedlings in alternative substrates obtained through the composting of organic residues of different origins, in comparison to commercial substrates; b) the development of prediction models for substrate samples maturity indexes, combining Fourier transform infrared (FTIR) spectroscopy and partial least squares (PLS) regression techniques; c) the development and optimization of the solid phase microextraction (SPME) methodology in headspace for the analyses of common groups (hydrocarbons, alcohols, aromatics, chloride hydrocarbons, ketones and terpenes) targeted VOCs in a series of matrices which include soils with different textures, manure organic matrices and compounds of different origins and biochars. In the first experiment, we concluded that the alternative substrates were unbalanced in regard to the nutrients. Only the content of available boron was positively correlated to the production of eucalyptus dry matter. The electrical conductivity did not significantly affect the production of total biomass, however, it may be used as predictor of available content of $NH_4^+$, P, $K^+$, $Mg^{2+}$, $Na^+$, $Cu^{2+}$ and $Fe^{2+}$. The use of principal component analysis aided in identifying the attributes which differentiated the substrates in regard to the production of eucalyptus biomass. In regard to the second experiment, all the models adjusted to the maturation indexes were predictive, robust and did not present a random correlation. The adjusted models allowed us to predict the maturation indexes in substrates formulated with different organic residue mixtures and with a wide range of maturation degree variation. In the third experiment, the matrices characteristics, especially pH and organic matter, had an accentuated impact over the SPME, due to the competition of analytes for the fiber's active sites. The methodology presented a good performance for soils of varied textures and for organic matrices, except for biochar*s*, which were characterized by a strong absorption capacity, which resulted in the competition for active sites of the SPME fiber, thus, limiting the extraction of analytes from the matrices.

Keywords: By-products. Composting. Alternative growing media. Multivariate calibration. Gases.

# LISTA DE ABREVIATURAS

| | |
|---|---|
| Ac | Peak area of compound |
| Ais | Peak area of internal standard |
| Ammonium-N | Ammonium nitrogen |
| AvAm | Available ammonium content |
| AvB | Available boron content |
| AvCa | Availablecalcium content |
| AvCu | Available copper content |
| AvFe | Available iron content |
| AvK | Available potassium content |
| AvMg | Available magnesium content |
| AvMn | Available manganese content |
| AvNa | Available sodium content |
| AvNit | Available nitrate content |
| AvP | Available phosphorus content |
| AvS | Available sulfur content |
| AvZn | Available zinc content |
| BD | Bulk density |
| BMA | Brazilian Minitry of Agriculture |
| $B_{OAK}$ | Oak biochar |
| $B_{POULTRY}$ | Poultry biochar |
| BTEX | Benzene, toluene, ethylbenzene and xylene |
| CAR/PDMS | carboxen/polydimethylsiloxane |
| $CB_{POULTRY}$ | compost of poultry manure with biochar |
| Cc | Concentration of compound |
| CE | Condutividade eletrolítica |
| CEC | Cation exchange capacity |

| | |
|---|---|
| Cis | Concentration of internal standard |
| $C_{MSW}$ | Compost of municipal solid waste |
| COV | Composto orgânico volatile |
| COVs | Compostos orgânicos voláteis |
| $C_{POULTRY}$ | Compost of poultry manure |
| CTC | Capacidade de troca de cátions |
| DOC | Dissolved organic carbon |
| DP | Degree of polymerization |
| DVB/CAR/PDMS | divinilbenzene/carboxen/polydimethylsiloxane |
| EC | Electrical conductivity |
| EXC | Alkaline extractable carbon |
| FAC | Fulvic acid carbon |
| FTIR | Fourier transform infrared |
| GC | Gas chromatograph |
| GC-FID | Gas chromatograph-flame ionization detector |
| HAC | Humic acid carbon |
| HI | Humification index |
| HR | Humification ratio |
| ID | Internal diameter |
| IV | Infravermelho |
| LD | Limit of detection |
| LOO-CV | Leave-one-out cross-validation |
| LQ | Limit of quantification |
| LV | Latent variable |
| LVs | Limit values |
| $M_{SHEEP}$ | Sheep manure |
| MSW | Municipal solid waste |
| Nitrate-N | Nitrate nitrogen |

| | |
|---|---|
| NM | No matrix |
| OM | Organicx matter |
| OR | Optimal range |
| OXC | Oxidizable organic carbon |
| PAC | Polyacrylate |
| PC1 | Principal component 1 |
| PC2 | Principal component 2 |
| PCA | Principal component analysis |
| PDMS | Polydimethylsiloxane |
| PHA | Percentage of humic acid |
| PLS | Partial least square |
| PTFE | Polytetrafluoroethlylene |
| $R^2$ | Squared correlation coefficient |
| $R^2_0$ | Squared correlation coefficient values between observed and predicted values for the test set without intercept |
| $R^2$cal | Squared correlation coefficient of calibration |
| $R^2$cv | Squared correlation coefficient of cross-validation |
| $r^2$m | $r^2$m metric |
| $r^2$p | $r^2$p metric |
| $R^2$pred | Squared correlation coefficient of prediction |
| $R^2$y-rand | Squared correlation coefficient of y-randomization |
| RMSE | Root mean square error |
| RMSEc | Root mean square error of calibration |
| RMSEcv | Root mean square error of cross-validation |
| RMSEp | Root mean square error of prediction |
| RMSEy-rand | Root mean square error of y-randomization |
| rpm | revolutions per minute |
| $S_{CLAY}$ | Clay soil |

| | |
|---|---|
| $S_{LOAM}$ | Sandy loam soil |
| SPME | Solid phase microextraction |
| $S_{SANDY}$ | Sandy soil |
| TC | Total content of carbon |
| TDW | Total biomass dry weight |
| USDA | United States Department of Agriculture |
| VOC | Volatile organic compound |
| VOCs | Volatile organic compounds |
| W | Deionized water |
| $W_{NaCl}$ | NaCl saturated solution |

# SUMÁRIO

PRIMEIRA PARTE

# PRIMEIRA PARTE

## 1 INTRODUÇÃO

Os resíduos orgânicos, quando atendem a legislação vigente, podem ser utilizados na agricultura para finalidades diversas. Uma delas preconiza a produção de compostos orgânicos, por meio do processo de compostagem, para serem utilizados de forma segura na agricultura (BERNAL et al., 1998b). A compostagem é a decomposição de materiais orgânicos por meio de processos biológicos sob condições controladas, onde ocorre a transformação do resíduo orgânico sólido em um composto maduro (SHARMA et al., 1997).

A utilização de compostos orgânicos na fabricação de substratos é uma alternativa viável do ponto de vista econômico e ambiental, em relação ao uso de substratos comerciais. Isso ocorre em razão do emprego de resíduos orgânicos na formulação de substratos proporcionarem redução de custos na produção de mudas, reciclagem de nutrientes e disposição adequada de resíduos no ambiente. No entanto, a obtenção de substratos alternativos que atendam às exigências das plantas é uma tarefa complexa, devido à heterogeneidade em composição química dos resíduos e dos compostos orgânicos. Por isso, são necessárias pesquisas no sentido de que as combinações de materiais propiciem a obtenção de substratos cujas características físicas, químicas e biológicas atendam ao requerimento das espécies vegetais cultivadas.

O composto, quando estabilizado quimicamente, é o componente ideal para compor substratos para crescimento de plantas, pois, nesse estádio, apresenta matéria orgânica humificada e ausência de patógenos de plantas (BERNAL et al., 1998b), e as frações fenólicas com propriedades fitotóxicas (ORTEGA et al., 1996) estão menos presentes (SÁNCHEZ-MONEDERO et al., 1999). Os métodos convencionais para mensurar o grau de maturação dos compostos orgânicos são morosos, exigem grande esforço e geram resíduos químicos no laboratório (JANIK et al., 2007; MORGANO et al., 2005; TAVARES et al., 2012). Além disso, para determinar o estádio de maturação de matriz orgânica quase sempre, vários atributos devem ser analisados simultaneamente. Assim, a técnica que combina a espectroscopia de infravermelho (FTIR) e a regressão por mínimos quadrados parciais (PLS) é uma alternativa rápida, de baixo custo e com menor produção de resíduos químicos, em relação aos métodos convencionais de avaliação do grau de maturação. Além disso, os modelos de predição resultantes da combinação das técnicas de FTIR e PLS permitem a avaliação de amostras de compostos orgânicos de forma confiável e segura (MEISSL; SMIDT; SCHWANNINGER, 2007).

Os resíduos orgânicos liberam, durante o processo de compostagem, diversos gases que são influenciados pelo manejo e pela origem dos resíduos (BROWN; KRUGER; SUBLER, 2002). Entre esses gases, destacam-se os compostos orgânicos voláteis (COVs), que são um extenso grupo de compostos de origem antropogênicas ou biogênicas (TURAN; AKDEMIR; ERGUN, 2009); podem, ainda, apresentar potencial de poluição, mau odor, periculosidade, contribuir para o aquecimento global, reduzir o ozônio estratosférico e promover a formação de ozônio troposférico (KOMILIS; HAM; PARK, 2004).

No Brasil, no âmbito da responsabilidade compartilhada pelo ciclo de vida dos produtos, a lei nº 12305 (BRASIL, 2010), que institui a Política Nacional de Resíduos Sólidos, determina a implantação do sistema de compostagem para resíduos orgânicos sólidos, quando há um plano municipal de gestão integrada desses materiais. Segundo essa mesma lei, na recuperação energética dos resíduos sólidos urbanos, deve ser implantado um programa de monitoramento de gases tóxicos. Existem, assim, no país, demandas recorrentes quanto às pesquisas pertinentes aos tipos e quantidades de gases e substâncias voláteis produzidas durante o processo de compostagem de resíduos orgânicos.

Diante do exposto, os objetivos com o presente trabalho foram avaliar a influência de combinações de resíduos orgânicos e de suas composições químicas sobre o crescimento de mudas de eucalipto; predizer o grau de maturação de substratos por meio da combinação das técnicas FTIR e PLS e avaliar a emissão de compostos orgânicos voláteis (COVs) após incubação em diversas matrizes de importância ambiental.

# 2 REFERENCIAL TEÓRICO

## 2.1 Compostagem de resíduos orgânicos

Os resíduos orgânicos são originados da produção vegetal e animal, do manuseio de subprodutos, grau de processamento, à espécie animal e vegetal e à idade dos animais, seus manejos e esquemas de arraçoamento. As misturas e usos de camas em estercos, a espécie vegetal e o sistema de manejo da biomassa produzida, o grau de compostagem dos materiais utilizados etc., são todos fatores ligados à origem ou aos processos de produção e exercem influência sobre a sua composição química (ABAD et al., 2002; ABREU-JÚNIOR et al., 2005; SILVA, 2008). A aplicação direta de resíduos orgânicos ao solo pode implicar em perdas de nutrientes, danos fisiológicos às plantas, volatilização de nitrogênio, entre outros efeitos negativos associados ao descarte inadequado, ou ao uso desses materiais sem obedecer a critérios técnicos (ABREU-JÚNIOR et al., 2005). A reciclagem, por meio da agricultura, de resíduos orgânicos e outros resíduos originados de atividades antrópicas, como o lodo de esgoto, resíduos sólidos urbanos, resíduos da indústria agro - alimentar, resíduos de processamento de alimentos e resíduos de culturas agrícolas, é reconhecida como uma alternativa promissora para o manejo de resíduos sólidos orgânicos (PROVENZANO et al., 2001). Nesse sentido, a compostagem, um processo natural disponível para a estabilização desses materiais, é necessária para a disposição adequada de resíduos sólidos no ambiente (ZHANG; HE, 2006). A compostagem é o tratamento de resíduos orgânicos mais utilizados e bem desenvolvidos para o manejo de resíduos orgânicos urbanos (BERNAL et al., 1998a). Essa técnica consiste na decomposição, sob condições aeróbicas, da matéria orgânica por meio de uma rápida sucessão de microrganismos que transformam o resíduo em um produto estabilizado (DIAS et al., 2009; SENESI, 1989; SHARMA et al., 1997). Durante a primeira fase do processo de compostagem, os compostos simples de carbono orgânico são facilmente mineralizados e metabolizados por microrganismos, produzindo $CO_2$, $NH_3$, $H_2O$, ácidos orgânicos e calor; à medida que o processo evolui, há acúmulo de calor, que aumenta a temperatura na pilha do composto, de modo que, ao final do processo, há redução do volume do resíduo orgânico e diminuição da germinação de sementes de ervas daninhas e eliminação de microrganismos patogênicos (BERNAL; ALBURQUERQUE; MORAL, 2009).

O principal requerimento de um composto para ser usado seguramente na agricultura é o seu grau de estabilidade ou maturidade, o que implica em presença de matéria orgânica humificada e ausência de compostos fitotóxicos e de patógenos de plantas e animais (BERNAL et al., 1998b). Os compostos não podem, também, conter metais e compostos orgânicos de elevada persistência no ambiente (SHARMA et al., 1997). Em relação à compostagem, o termo estabilidade refere-se ao composto cuja temperatura é igual a do ambiente e o termo maturidade é relacionado ao produto final e de melhor qualidade (ZMORA-NAHUM et al., 2005).

## 2.2 Formulação de substratos com resíduos orgânicos

Os resíduos orgânicos são originados das diversas atividades humanas e podem ser de origem animal, vegetal, urbana, agroindustrial ou industrial (SHARMA et al., 1997; SILVA, 2008). Entre as opções de disposição desses resíduos, a utilização para fins agrícolas tem sido apontada como opção interessante econômica, ambiental e socialmente viável, pelo fato da reciclagem de resíduos minimizarem os problemas resultantes do descarte inadequado desses materiais (PIRES; MATTIAZZO, 2008). A própria agricultura gera diferentes resíduos orgânicos de composições químicas e grau de humificação variados. Quando esses materiais são propriamente combinados e incubados, eles podem ser usados para produzir substratos para crescimento de planta (BARDHAN; WATSON; DICK, 2008; ESTÉVEZ-SCHWARZ et al., 2009; SÁNCHEZ-MONEDERO et al., 2004).

Entre as características que variam nos resíduos estão a concentração de nutrientes, condutividade eletrolítica, relação C/N, sais, capacidade de troca catiônica, pH, densidade aparente, capacidade de retenção de água, etc. (BARDHAN; WATSON; DICK, 2008; HIGASHIKAWA; SILVA; BETTIOL, 2010; JAYASINGHE; ARACHCHI; TOKASHIKI, 2010; SÁNCHEZ-MONEDERO et al., 2004). O desbalanço e a variabilidade de nutrientes em relação às exigências das plantas (WESTERMAN; BICUDO, 2005), bem como de outros atributos, sejam de ordem química ou física, podem ser limitantes para o uso agrícola de resíduos orgânicos e de seus compostos para a produção de substratos. Essas variações na composição devem ser consideradas para produzir meios de crescimento de qualidade. Substratos são utilizados para o desenvolvimento das plantas, sustentando-as com fornecimento de nutrientes, podendo ser formados por matérias-primas de origem mineral, orgânica ou sintética, de um único material ou por diversos materiais em misturas (ABREU; ABREU; BATAGLIA, 2002; ANDRADE NETO, 1998). Os meios de crescimento devem possibilitar a produção de plantas de alta qualidade, com baixo custo e em menor tempo (ABREU; ABREU; BATAGLIA, 2002).

A compostagem é um processo empregado para estabilizar resíduos orgânicos, mineralizar nutrientes de plantas, principalmente N e P, e para aumentar a capacidade de troca catiônica dos meios de crescimento (BARDHAN; WATSON; DICK, 2008; SHIRALIPOUR; MCCONNEL; SMITH, 1992). Alguns substratos como aqueles produzidos com resíduos de plantas podem ser utilizados sem diluição, uma vez que eles apresentam baixa condutividade eletrolítica, reduzida concentração de sais e nutrientes e menor densidade aparente, em relação àqueles produzidos com resíduos de origem urbana e animal (BENITO et al., 2006; ESTÉVEZ-SCHWARZ et al., 2009; RIBEIRO et al., 2007). Por essa razão, dependendo do resíduo, o composto obtido pode requerer a mistura dos materiais vegetais com outros resíduos mais ricos em nutrientes (BENITO et al., 2005), para atender as exigências nutricionais de uma determinada cultura.

Resíduos vegetais como cascas e serragem, devem passar por compostagem mais intensa, uma vez que podem conter compostos fenólicos fitotóxicos que, dependendo da concentração, prejudicam o desenvolvimento de plantas (ORTEGA et al., 1996). Comparado com resíduos de plantas, os compostos produzidos com lodo de esgoto, estercos e resíduos industriais, por exemplo, apresentam maior teor de substâncias húmicas, maior densidade de cargas e maior capacidade de reter água e, por essa razão, podem substituir com êxito a turfa (JAYASINGHE; ARACHCHI; TOKASHIKI, 2010; SÁNCHEZ-MONEDERO et al., 2004), que é um recurso não renovável utilizado globalmente na fabricação de substratos.

A concentração de sais deve ser considerada com cautela, pela sensibilidade das culturas a meios de cultivo com condutividade eletrolítica superior a 2 dS $m^{-1}$, especialmente na fase inicial de crescimento das plantas (BARDHAN; WATSON; DICK, 2008; BRAGG; WALKER; STENTIFORD, 1993; RIBEIRO; VASCONCELOS; SANTOS, 2000; SÁNCHEZ-MONEDERO et al., 2004). Por isso, o uso exclusivo de materiais de origem animal ou urbana como componentes únicos de substratos não é recomendável.

O uso de compostos sem as devidas modificações em sua composição é desaconselhável, sendo um dos impedimentos à produção de substratos o pH fora da faixa considerada ideal (pH em água, de 5,3 a 6,5) para cultivo de plantas (ABAD; NOGUERA; BURÉS, 2001). O esterco bovino em razão de sua elevada salinidade (BARDHAN; WATSON; DICK, 2008), pode influenciar a condutividade eletrolítica (CE) e limitar a sua quantidade nos substratos, enquanto o lodo de esgoto, dependendo da composição química, pode aumentar os teores de metais pesados e elevar a condutividade eletrolítica (BARDHAN; WATSON; DICK, 2008; INGELMO et al., 1998; OSTOS et al., 2008).

Para a redução de custos e de uso de fertilizantes (BARDHAN; WATSON; DICK, 2008; GIL; CARBALLO; CALVO, 2008; INGELMO et al., 1998), e para reciclar nutrientes e resíduos orgânicos (MELO; SILVA; DIAS, 2008; PIRES; MATTIAZO, 2008), é interessante produzir substratos em que os componentes substituam parcialmente materiais não renováveis utilizados na fabricação de substratos comerciais (FARRELL; JONES, 2010; GARCIA-GOMEZ; BERNAL; ROIG, 2002; JAYASINGHE et al., 2010; SÁNCHEZ-MONEDERO et al., 2004) ou totalmente (BARDHAN; WATSON; DICK, 2008; BENITO et al., 2006; ESTÉVEZ-SCHWARZ et al., 2009; JAYASINGHE; ARACHCHI; TOKASHIKI, 2010) especialmente para os substratos em que a turfa é o componente principal.

O que justifica a produção de substratos alternativos a partir de resíduos e compostos orgânicos é que a performance desses meios pode ser melhor do que a obtida por substratos comerciais, dependendo da espécie da planta cultivada, como foi observado por Bardhan, Watson e Dick (2008), Estévez-Schwars et al. (2009) e Ribeiro et al. (2007). No Brasil, resíduos como lodo de esgoto, estercos, composto de lixo, fibra de coco, casca de café e resíduos de madeira podem ser utilizados na formulação de substratos. Alguns desses materiais são ricos em nutrientes e outros não, o que indica a necessidade de combiná-los em proporções capazes de garantir propriedades físicas, químicas e biológicas que atendam as exigências das plantas.

## 2.3 Predição do grau de maturação de substratos

Resíduos orgânicos sólidos de diferentes origens podem ser utilizados como meio de crescimento, quando apresentam adequadas propriedades para o desenvolvimento das plantas (ABAD; NOGUERA; BURÉS, 2001). Compostos preparados a partir de diferentes tipos e combinações de materiais orgânicos podem ser usados puros ou como componente predominante de substratos (ESTÉVEZ-SCHWARZ et al., 2009). Em relação ao composto, o termo grau de maturação é usado para se referir à qualidade do produto final (ZMORA-NAHUM et al., 2005), portanto, materiais maturados são preferíveis para cultivar plantas, dado que, nesse estádio, frações fenólicas, que apresentam propriedades fitotóxicas (ORTEGA et al., 1996), são menos presentes nos compostos (SÁNCHEZ-MONEDERO et al., 1999). Além disso, os materiais mais humificados apresentam maior capacidade de troca de cátions (CTC), são capazes de reter mais nutrientes e água e não apresentam o risco de causar danos fisiológicos em plantas, em razão da decomposição de matrizes não estabilizadas e ricas em nutrientes. Assim, atingir grau de maturação é a condição ideal do produto final antes do cultivo de mudas.

Para a determinação de índices de maturação em compostos, os parâmetros propostos por Iglesias-Jiménez e Pérez-García (1992) e Senesi (1989) são: relação C/N, capacidade de troca de cátions, razão de humificação, índice de humificação, percentagem de carbono na forma de ácido húmico e grau de polimerização. A relação $NH_4^+/NO_3^-$ foi um dos índices de maturidade utilizado por Bernal et al. (1998b) para avaliar sete diferentes compostos. Esses parâmetros citados anteriormente são determinados em amostras de compostos e são considerados em conjunto para avaliar o grau de maturação. Entretanto, são determinações analíticas que demandam tempo, possuem custo elevado e, ainda, produzem resíduos químicos no laboratório (JANIK et al., 2007; MORGANO et al., 2005; TAVARES et al., 2012). A medição de carbono orgânico dissolvido em água é, também, um atributo simples e alternativo que permite a avaliação do grau de maturidade de compostos de diferentes origens e processos (ZMORA-NAHUM et al., 2005). Diferentes autores (BERNAL et al., 1998b; IGLESIAS-JIMÉNEZ; PÉREZ-GARCÍA, 1992; ZMORA-NAHUM et al., 2005) sugerem uma variedade de métodos, ou seus usos em conjunto, de modo que não há consenso na literatura quanto aos atributos que devem ser utilizados para determinar o grau de maturação de compostos.

A determinação de diferentes índices de maturação em diversos compostos orgânicos gera uma grande quantidade de variáveis que podem requerer a utilização de métodos de tratamento de dados multivariados. Um desses métodos é a análise de componentes principais (PCA), que fundamenta outros métodos da estatística multivariada. A PCA representa as variações dos dados por meio de um sistema de eixos construídas a partir de variáveis denominadas componentes principais, no qual os dados multivariados são representados em dimensões reduzidas (FERREIRA et al., 1999); o que facilita a compreensão e assimilação das informações presentes no conjunto de dados.

O método de espectroscopia de infravermelho com transformada de Fourier (FTIR) é uma valiosa ferramenta para estudos de composição química da matéria orgânica de resíduos, compostos orgânicos e turfeiras (ARTZ et al., 2008; CASTILHOS et al., 2008; HSU; LO, 1999; JOURAIPHY et al., 2005). Além disso, esse método permite a análise e o monitoramento das transformações da matéria orgânica durante o processo de compostagem (HSU; LO, 1999; JOURAIPHY et al., 2005) e, portanto, é uma técnica que possibilita a avaliação da maturidade em compostos (PROVENZANO et al., 2001). Na espectroscopia de infravermelho, os diversos tipos de ligações químicas e de estruturas moleculares existentes numa molécula absorvem radiação eletromagnética na região do infravermelho, em comprimentos de onda característicos, que sensibilizam átomos em seus ambientes químicos característicos de vibração (CERETTA et al., 2008).

A técnica de espectroscopia de infravermelho gera um enorme conjunto de dados e a avaliação desses por meio da análise multivariada permite a extração de informações adicionais, com redução do tempo e do custo no manejo de resíduos (FORINA; LANTERI; CASALE, 2007; MEISSL; SMIDT; SCHWANNINGER, 2007). Os espectros gerados após a análise de infravermelho das amostras podem ser utilizados para calibração multivariada por meio da regressão por mínimos quadrados parciais (PLS), que utiliza um determinado número de fatores (variáveis latentes) para construção de modelos preditivos (FERREIRA et al., 1999).

A combinação de espectroscopia de infravermelho e regressão por mínimos quadrados parciais (PLS) permite uma simples e rápida predição das propriedades das amostras (JANIK et al., 2007). Ludwig, Schmilewski e Terhoeven-Urselmans (2006) utilizaram a espectroscopia de infravermelho próximo combinado com PLS para predizer características químicas e a fitotoxidade de turfas em meios de crescimento. Os modelos de predição resultantes da combinação das técnicas de FTIR e PLS mostraram-se apropriados para aferir a estabilidade de compostos, permitindo também a avaliação de amostras de compostos orgânicos de composição desconhecida, de forma confiável e segura (MEISSL; SMIDT; SCHWANNINGER, 2007). As principais vantagens da técnica que combina FTIR e PLS são a redução do tempo de análises químicas e dos resíduos no laboratório, menor manipulação de amostras e, ainda, evita o uso de reagentes perigosos, quando comparado com métodos convencionais (JANIK et al., 2007; MORGANO et al., 2005; TAVARES et al., 2012). Desse modo, é desejável desenvolver um método simples e de baixo custo, pouco invasivo e que permita a verificação da maturidade de substratos obtidos a partir de compostos preparados com diferentes tipos de resíduos, após a compostagem.

## 2.4 Compostagem e a emissão de COVs

Muitos dos gases emitidos por compostos e resíduos orgânicos são conhecidos como compostos orgânicos voláteis (COVs). Os COVs são geralmente definidos como compostos orgânicos (exceto $CH_4$) com ponto de ebulição menor que 80 ºC, enquanto os compostos semi-voláteis apresentam ponto de ebulição entre 80º e 180 ºC (KOMILIS; HAM; PARK, 2004). Segundo estes autores, os COVs podem apresentar potencial de poluição do ar devido ao odor desagradável ou à periculosidade, além de ainda poderem contribuir para o aquecimento global, diminuir o ozônio estratosférico e formar o ozônio troposférico. Os COVs formam um extenso grupo de compostos xenobióticos ou biogênicos com pressão de vapor relativamente alta e com baixa solubilidade em água, sendo que cada composto possui um valor limite em que as pessoas podem ficar expostas sem que ocorram efeitos adversos (TURAN; AKDEMIR; ERGUN, 2009). A liberação desses gases poluentes para a atmosfera pode ser uma fonte de mau odor e/ou de efeitos nocivos para trabalhadores e residentes locais (DOMINGO; NADAL, 2009).

A volatilização de COVs não ocorre somente durante o processo de compostagem; também ocorrem emissões nas áreas de recepção e armazenamento de resíduos nas estações de tratamento desses materiais (FONT; ARTOLA; SÁNCHEZ, 2011). Assim, a acumulação de resíduos sólidos combinada ao clima quente, pode contribuir para a geração de odores indesejáveis e, até mesmo, impactar a saúde pública (STATHEROPOULOS; AGAPION; PALLIS, 2005). Dentre os resíduos sólidos, os mais estudados em relação aos COVs são aqueles de origem urbana, que possuem diferentes origens. Statheropoulos, Agapion e Pallis (2005), ao analisarem a emissão de compostos emitidos de lixeiras expostas em via públicas, identificaram mais de 150 COVs, entre os quais, os principais poluentes foram tolueno, etilbenzeno, naftaleno e o benzeno, que possuem propriedades carcinogênicas. Em estudo realizado por Pierucci et al. (2005) sobre a produção de COVs durante decomposição aeróbica de resíduos sólidos municipais, foi verificado a presença, além do benzeno, de compostos halogenados, como o dicloroetileno, tricloroetileno e o tetracloroetileno, que são também tóxicos. Segundo Komilis, Ham e Park (2004), o composto de lixo urbano pode ser fonte de COVs perigosos, sendo estes gases são liberados no início do processo de compostagem, quando há umidade e calor. Os autores sugerem a utilização de biofiltros nas unidades de tratamento de gás das instalações onde se faz a compostagem de resíduo urbano com finalidade de reduzir o odor e a emissão de COVs. Mao et al. (2006) verificaram que, na compostagem de resíduos de alimentos, o biofiltro humidificado apresentou maior eficiência na remoção de odor, $NH_3$, aminas, compostos de enxofre e COVs, em relação ao purificador químico e ao biofiltro. Para remover COVs durante a compostagem de cama de frango, Turan, Akdemir e Ergun (2009) utilizaram pedra-pomes, zeólita natural, vermiculita expandida e perlita expandida, e encontraram eficiência de remoção de 79,7%, 54,6%, 88,2% e 61,5%, respectivamente. Em função das propriedades físico-quimicas desses materiais naturais e da biodegradabilidade dos COVs, houve a remoção de quatro famílias de poluentes que foram os oxigenados, alifáticos, aromáticos e os clorados. Wilkins e Larsen (1996) identificaram cerca de 170 COVs emitidos da compostagem de podas de jardim e constataram a presença de dimetil dissulfeto, que tem toxicidade semelhante ao do metanotiol.

Algumas matrizes de importância ambiental, como solos, lodo e sedimentos podem apresentar limitações metodológicas quanto à análise de COVs, devido à baixa recuperação dos analitos, como consequência das suas interações químicas com a matriz sólida (ALENXANDROU; LAWRENCE; PAWLISZYN, 1992; ZHANG; PAWLISZYN, 1993). Além disso, de acordo com Kissel, Henry e Harrison (1992), as interações entre grupos orgânicos de COVs e a superfície do composto orgânico resultam em uma forte adsorção desses compostos, que, posteriormente, podem ser oxidados sob condições aeróbicas.

# 3 CONSIDERAÇÕES FINAIS

Foram formulados substratos cuja performance agronômica ficou aquém dos substratos disponíveis no mercado. Mesmo assim, avanços foram alcançados, pois foram feitas associações entre as características dos meios de cultivo com a biomassa de mudas de eucalipto. Os custos de produção e de comercialização de substratos comerciais foram superficialmente abordados e basearam-se nas informações encontradas na literatura. Para afirmar a real redução de custos proporcionados pela utilização de resíduos orgânicos na fabricação de substratos, como mencionado no artigo 1, um estudo de relação custo/benefício deveria ser conduzido, de modo que é uma sugestão para futuras pesquisas, dado que esse não foi o foco do presente estudo.

Com base no estudo conduzido no artigo 1, a formulação e fabricação de novos substratos à base de compostos orgânicos deve priorizar, além do estudo da composição química, a investigação também das propriedades físicas dos meios de crescimento. As características físicas devem se manter estáveis durante todo o período de desenvolvimento das plantas e a composição pode ser facilmente modificada por uso de insumos ao longo do ciclo de crescimento das plantas, é natural que a aeração, densidade, retenção de água etc devam ser priorizadas ao se formular meios de crescimento de eleva eficiência agronômica. Devido à alta concentração de nutrientes nos estercos, os mesmos devem ser utilizados com cautela e em baixas proporções na composição de substratos alternativos.

Em futuros estudos, todo esforço deve ser feito para produzir substratos menos concentrados em nutrientes, com baixa salinidade, e com balanço adequado de macro e micronutrientes e com adequadas propriedade físicas. Por isso, nós sugerimos o uso de menores quantidades de estercos e lodo de esgoto e maiores proporções de resíduos de plantas, como casca de pinus, fibra de coco, carvão e outro materiais vegetais na formulação de substratos a base de compostos, para garantir a melhor performance como o que foi apresentado pelo substrato 6.

Em relação ao melhor custo/benefício que seria proporcionado pela utilização do método combinado de FTIR e PLS mencionado no artigo 2, há algumas ressalvas: o método proposto é vantajoso em relação aos métodos convencionais, em caso de já haver aparelho de infravermelho e pessoal capacitado para a condução das análises. O uso de infravermelho (IV) pode não ser vantajoso economicamente se for necessário construir e instalar toda a estrutura necessária para realizar as análises de FTIR.

O substrato 16 mencionado no artigo 2 foi considerado um *outlier*, não pela qualidade desse meio de crescimento, mas sim pelo método analítico utilizado, que foi o IV. O método de IV não possibilitou a obtenção de espectros com informações necessárias para caracterizaão do substrato 16, pela alta quantidade de carvão presente nessa amostra. Para analisar em profundidade o substrato 16, o método de espectrometria por fluorescência de raios X seria o mais adequado, por não sofrer interferência devido à alta quantidade de carvão.

O grau de maturação de compostos orgânicos é um parâmetro imprescindível a ser considerado na utilização ou não desses materiais para cultivo de plantas, pois o composto maduro apresenta características físicas, químicas e biológicas estáveis e apropriadas para utilização na agricultura.

O estudo do efeito de diferentes matrizes quanto à sorção de compostos orgânicos voláteis (COVs), cujos dados foram discutidos no artigo 3, poderia futuramente ser feito em matrizes ambientais do Brasil. A volatilização de COVs de resíduos orgânicos e de plantas de compostagem industriais são temas que poderiam ser estudados no Brasil, dado o volume de resíduos orgânicos gerados no país, que demandam pesquisas quanto aos impactos ambientais.

No artigo 3, o *biochar* de carvalho apresentou forte adsorção dos COVs alvo, o que o caracteriza como de alto potencial para ser utilizado como componente de biofiltros visando a purificação de ar em plantas de compostagem, por exemplo.

O tema *biochar* (biocarvão) é muito pouco estudado no Brasil, diferentemente do que ocorre em outros países. Teoricamente, todo tipo de biomassa pode ser transformada em *biochar*; o que condiciona o país como grande potencial produtor de *biochar*. O *biochar*, além de condicionador de solo, apresenta vários benefícios ambientais, como sequestro de carbono, mitigação de gases de efeito estufa e redução de lixiviação de nutrientes do solo.

Diante dos tópicos abordados no presente trabalho, diversas outras pesquisas podem ser conduzidas com temas relacionados à utilização de resíduos orgânicos, compostagem e emissão de gases, notadamente os que abrangem os estudos de biocarvões nas condições de uso dos solos/culturas brasileiras.

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# SEGUNDA PARTE - ARTIGOS

## ARTIGO 1 Growing eucalyptus seedlings in compost-based substrates

**Normas da revista *Compost Science & Utilization***

# Growing Eucalyptus Seedlings in Compost-Based Substrates

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Mixes of organic wastes in suitable proportions allows the production of substrates that can support plant nutrition; recycling of organic wastes can also help to preserve non-renewable resources largely used in the production of substrates in Brazil. Growth of eucalyptus cultivated on five compost-based substrates, formulated with different proportions of sewage sludge, coffee husk, peat, chicken manure, cattle manure, pine bark, sawdust, and coconut fiber, were evaluated. Compost-based and two commercial substrates were characterized by the available contents of macro and micronutrients, $Na^+$, pH, electrical conductivity (EC), and bulk density. Substrate attributes and biomass production were submitted to principal component analysis (PCA) to detect patterns of similarities. In addition, Pearson correlation analysis was performed to assess the degree of correlation between the analyzed attributes. Compost-based substrates had adequate pH, above threshold EC limit level and notable high contents of available P, K, $NH_4^+$-N and $Na^+$. The highest biomass was produced in the commercial substrate rich in available B and Ca, less concentrated in most of the nutrients and with low EC and $Na^+$ content. The substrate 4, for having more chicken manure and sewage sludge, resulted in the highest biomass production among the compost-based substrates. Boron availability was the only attribute that was correlated to the eucalyptus biomass variation. Electrical conductivity did not exert influence on the eucalyptus biomass, probably due to salts and ions leaching during the experiment; EC was an adequate index to predict the ammonium-N, P, K, Mg, Na, Cu and Fe availability of the investigated substrates.

## *Introduction*

In Brazil, agriculture activities generate several organic residues with different chemical compositions and degrees of humification. When these materials are properly combined, they can be used to produce substrates that can support good plant growth (Sánchez-Monedero *et al.* 2004; Bardhan *et al.* 2008; Estévez-Schwarz *et al.* 2009). Organic wastes, however, present chemical [nutrient concentrations, electrical conductivity (EC), C:N ratio, salts, cation exchange capacity (CEC), pH, etc.] and physical (bulk density, water holding capacity, etc) composition which vary greatly (Sánchez-Monedero *et al.* 2004; Bardhan *et al.* 2008; Higashikawa *et al.* 2010; Jayasinghe *et al.* 2010a). Considering the proportions of residues in organic formulated substrates, these variations in chemical composition should be taken into account in order to produce high-quality growth media. According to Garcia-Gomez *et al.* (2002) and Gouin (1998), mixing of organic residues should produce substrates with high degree of humification and adequate physical and chemical properties.

Composting is an adequate process to stabilizing organic residues, mineralizing plant nutrients, mainly N and P, and increasing the CEC of the growth media (Shiralipour *et al.* 1992; Bardhan *et al.* 2008). By adequately combining organic wastes, final substrate EC must also be considered, since crops are sensitive to growth media which are rich in ions and salts, especially in the initial plant growth stage (Bragg *et al.* 1993; Ribeiro *et al.* 2000; Sánchez-Monedero *et al.* 2004; Bardhan *et al.* 2008). Substrates produced with composted plant residues can be used without dilution, since they have lower EC, salts concentration, nutrients, and bulk density, when related to substrates that were made using urban or animal wastes (Benito *et al.* 2006; Ribeiro *et al.* 2007; Estévez-Schwarz *et al.* 2009). However, depending on the plant residue, the obtained compost may require mixing the plant materials with some other nutrient-rich residues (Benito *et al.* 2005), in order to provide the crop nutritional requirements. Comparing to composts that were formulated with plant residues, those composts that were produced with sewage sludge, manures and industrial wastes have higher content of humic substances, higher charge density, and higher water holding capacity; for this reason, they successfully can replace peat (Sánchez-Monedero *et al.* 2004; Jayasinghe *et al.* 2010a). Depending on their constituents and proportions in composts, the presence of heavy metals and other contaminants in organic wastes may represent a potential constraint to plants and environment (Soumarè *et al.* 2003; Barzegar *et al.* 2002).

Some wastes such as sewage sludge, garbage composts, coconut fiber, pine bark, coffee bark, and wood wastes, have frequently been used to compose growth media in Brazil. Such use can reduce fertilizer addition and costs (Ingelmo *et al.* 1998; Bardhan *et al.* 2008; Gil *et al.* 2008), and provide recycling of nutrients and organic residues (Melo *et al.* 2008; Pires and Mattiazo, 2008). When properly formulated, organic substrates can partially (Garcia-Gomez *et al.* 2002, Sánchez-Monedero *et al.* 2004; Farrell and Jones, 2010; Jayasinghe *et al.* 2010b) or fully replace (Benito *et al.* 2006; Bardhan *et al.* 2008; Estévez-Schwarz *et al.* 2009; Jayasinghe *et al.* 2010a) some non-renewable materials that are used to produce commercial substrates, especially those in which peat is the main component. The use of manure as a component of forest seedling substrates may reduce the growth media production costs (Trazzi *et al.* 2012), by decreasing the use of fertilizers (Trigueiro and Guerrini, 2003) and high price of commercial substrate. Differences in composition of organic residues indicate the necessity of combining these materials in adequate proportions for guaranteeing chemical, physical and biological properties of substrates to meet the eucalyptus nutritional requirements.

This work investigated the effect of varying proportions of organic residues on chemical and physicochemical characteristics of substrates to be used in the seedling production of eucalyptus. Principal component analysis was used to group substrates in terms of similarities in chemical and physicochemical characteristics, as related to eucalyptus biomass production.

## ***Materials and Methods***

### *Organic Residues*

Composted sewage sludge, chicken and cattle manures, peat, eucalyptus sawdust, charcoal obtained from pyrolysis of eucalyptus, coffee husk, coconut fiber, pine bark and vermiculite were used in the formulation of compost-based substrates. The main chemical and physical characteristics of composted and mixed organic residues in this study are presented in Higashikawa *et al.* (2010).

### *Substrate Formulation*

Organic residues were air-dried and sieved in 8 mm mesh. Then, they were mixed in different proportions in order to obtain substrates (Table 1). The compost-substrates were numbered from 1 to 5. Coffee husk, pine bark, sawdust, peat and sewage sludge were used in these substrates to ensure appropriate substrate physicochemical characteristics; manures were used as the main source of nutrients. Higher amounts of chicken manure were used in the substrate formulation, compared to cattle manure, due to its largest concentration of N and other nutrients. In the tested substrates, the amount of chicken manure, cattle manure and sewage sludge increased from 8.3 to 41.7 %, 8.3 to 25% and 8.3 to 33.3%, respectively (Table 1), in order to check possible influence of the proportion of these materials on eucalyptus plant growth. Sewage sludge was composted before being mixed with the other residues. Thus, sewage sludge was used in different proportions in these substrates to assure stable physicochemical conditions to plants, which, in most commercial substrates, are assured by the use of peat. The basic difference between substrates 1 and 2 is the proportion of manures and sewage sludge. The substrate 3 was formulated in order to test plant residues as the main materials in growth media and, thus, to obtain substrates less concentrated in nutrients than the other mixtures. Substrate 4 was prepared in order to prioritize the use of chicken manure and sewage sludge as sources of nutrients, respectively, in relation to plant residues. Only the substrate 5 did not have chicken manure in its composition and, in this growth media, the main sources of nutrients were cattle manure and sewage sludge. Agronomic performance of all the formulated substrates was compared with two commercial substrates, 6 and 7, that are currently used in the Brazilian market as growth media for eucalyptus seedlings. Eucalyptus seedlings were cultivated in these growth media without any additional fertilizers. Substrate 6 was prepared with pine bark, vermiculite, corrective of acidity, urea, ammonium sulfate, superphosphate, and potassium nitrate. Substrate 7 was formulated with organic components, mainly peat, pine bark and vermiculite, and slow release fertilizers.

All compost-based substrates were incubated during 30 days before planting. During this period, they were daily aerated and weekly supplied with distilled water. Water was added to keep humidity close to water holding capacity of each substrate. To avoid leaching of nutrients, the mixed organic residues were incubated in a closed 27-liter plastic container under greenhouse conditions. After 30 days of incubation, the temperature of substrates did not differ from the surrounding environment, indicating the stability of the composted mixtures. Charcoal and vermiculite were added in compost-based substrates at 8.3% each, wich was calculated based on the dry weight determined after a 30-day incubation period.

TABLE 1.
Relative proportions (dry basis, %) of organic wastes used in the production of the five compost-based substrates.

| Organic waste | Substrate* | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Chicken manure | 16.7 | 41.7 | 8.3 | 33.4 | 0 |
| Cattle manure | 16.7 | 8.3 | 8.3 | 8.3 | 25.0 |
| Coffee husk | 12.5 | 8.3 | 21.0 | 16.7 | 8.3 |
| Pine bark | 8.3 | 4.2 | 16.7 | 0 | 4.2 |
| Sawdust | 4.2 | 4.2 | 12.5 | 0 | 4.2 |
| Sewage sludge | 16.7 | 8.3 | 8.3 | 25.0 | 33.3 |
| Peat | 8.3 | 8.4 | 8.3 | 0 | 0 |
| Coconut fiber | 0 | 0 | 0 | 0 | 8.4 |

*Charcoal and vermiculite were added in the proportion of 8.3 % to all compost-based substrates.

### *Chemical analysis*

Available nutrient contents were measured following analytical protocols described in Higashikawa *et al.* (2010). The extraction of the available concentration of nutrients in the substrate solution was performed using water as extractant (substrate:water ratio of 1:2 v/v), according to Abreu *et al.* (2006), with some modifications. The extract was obtained by adding substrate to 100 mL of water up to a final volume of 150 ml. The suspension was shaken for 20 minutes at 220 rpm and filtered through cellulose membrane. In these extracts, S and P were determined by colorimetry, $Na^+$ and $K^+$ by flame photometry; and the other chemical elements, with the exception of N and B, were quantified by atomic absorption. In the extracts, by analyzing an aliquot of 30 mL, the levels of N as ammonium and nitrate were determined by steam distillation. The procedures used for determining $NO_3^-$-N and $NH_4^+$-N, followed the methodology described in Embrapa (1999). To determine the B content in extracts, the methodology proposed by Malavolta *et al.* (1997) for determination of total B in plant tissue was used. Electrical conductivity and pH in the aqueous substrate extracts (2:1 v/v) were also determined. To determine the substrate bulk density (BD), the same method of self-compacting used by Higashikawa *et al.* (2010) was used.

### *Eucalyptus Growth*

The initial phase of clone rooting was carried out in a commercial forestry nursery. Each plastic tube was filled with 180 $cm^3$ of substrate, 100 ml of deionized water was added one day before mini-cuttings insertion in the growth media, in order to leach out the excess of salts and to moisten the substrates. Mini-cuttings were collected in clonal garden at July 07, 2009, and, in a maximum interval of 30 minutes, transferred to the seven substrates contained in the tubes, using three replicates for each substrate. The mini-cuttings were commercial clones of *Eucalyptus grandis* x *Eucalyptus urophylla*. The surface of each substrate was covered with a layer of fine-grained vermiculite before the planting phase. After planting, the substrates were taken to an acclimatized greenhouse facility for rooting (with relative humidity above 80% and temperature of 27 ± 2 °C), remaining there for 25 days. Subsequently, the mini-cuttings were taken to a shaded environment for greenhouse acclimatization during 10 days. After this period, the mini-cuttings were transported to a greenhouse facility, and all plants were protected by a 50% shade cover that allowed enough passage of sunlight for plants. The experiment was carried out during 77 days. Fertilizers were not added to the eucalyptus plants during the experiment. At the end of the experiment, the total (root and aerial part) biomass dry weight of each plant was evaluated.

### *Statistical Analysis*

The mean of each attribute was submitted to principal component analysis (PCA) using the Chemoface software version 1.4 (Nunes *et al.* 2012). Before processing PCA, data were submitted to autoscaling in order to assign an equal weight for each attribute. All chemical, physicochemical and biomass data were subjected to analysis of variance and treatment means were compared using the Skott-Knott test at $p < 0.05$, using the statistical program Sisvar version 5.3 (Ferreira, 2010). Simple correlation analysis of Pearson was performed in order to assess the degree of correlation between the analyzed attributes.

## ***Results and Discussion***

### *Substrate characteristics*

Chemical and physicochemical characteristics of the different substrates investigated are shown in Table 2. Most of the substrates tested attended the optimal range of pH values for plant growth (Abad *et al.* 2001). Substrates 1, 5 and 7 presented pH values slightly lower than the minimum ideal value, but a constraint on plant growth is only observed when pH < 4 or > 7.5 (Bardhan *et al.*

2008; Ingram *et al.* 2003). The EC values are above the maximum level (3.5 dS $m^{-1}$) of the ideal range for plant growth, in all substrates tested. EC values ranged from 4.1 to 21.6 dS $m^{-1}$ and were affected by the proportions of organic residues in the substrates. The highest EC values of substrates 1, 2 and 4 were influenced by the amount of chicken manure in the mixes. Using wastes as components of substrates, Sánchez-Monedero *et al.* (2004) and Bardhan *et al.* (2008) verified that the EC of the organic formulated substrates reached values far above the upper threshold for containerized growing media. The components which most contribute to the salinity are $Na^+$, $K^+$, $Cl^-$, ammonia, nitrate and sulfate (Jayasinghe *et al.* 2010a) which, with the exception of $Cl^-$, were found in greater amounts in the compost-based substrates (Table 2). The bulk density (BD) of all substrates were significantly different ($p < 0.05$), but within or slightly above the established ideal substrate range (Abad *et al.* 2001). High values of BD decrease the flux of oxygen through the substrate and increase its transportation cost (Jayasinghe *et al.* 2010a).

Most of the substrates did not achieve the ideal range for $NO_3^-$-N, mainly the commercial ones which are very low in nitrate-N. Compost-based substrates presented the highest available contents of $NH_4^+$-N and $NO_3^-$-N (Table 2) which probably are associated to the higher proportions of chicken and cattle manure, coffee husk and sewage sludge in the mixes. According to Guerrero *et al.* (2002), the addition of composted sewage sludge increased the nitrate-N in leachates, although, in this study, instead of sewage, the main source of available nitrate-N is probably chicken manure.

Presence of chicken manure in the mixes increases the availability of P (Table 2). Commercial substrates were characterized by the low levels of available P. Among the compost-based substrates, increase proportions of plant residues in the mixes decrease the availability of P. All substrates attended the ideal value for available content of S (below 960 mg $L^{-1}$), but S varied quietly intensive among the substrates, from 56 to 244 mg $L^{-1}$. In the other hand, available K contents (Table 2) in the compost-based substrates are very high, far above the maximum threshold level for substrates in general.

Levels of Ca were high in all substrates, including the commercial ones. These values are much higher than the range of 7.6 to 97.6 mg $L^{-1}$ of ideal available Ca presented by Abad *et al.* (2002) in coir dusts from different sources. The high Ca contents in substrates could be explained by the presence of manure and sewage sludge in compost-base substrate and mineral fertilizer and lime in the commercial growing media.

Differences in chemical and physicochemical properties (Ao *et al.* 2008) of the organic residues and in their proportions in the mixes influenced markedly the available contents of nutrients in the substrates. With exception of substrate 6, the available K levels were higher than Mg for all substrates. Higher total content of K compared to Mg was verified in substrates studied by Grigatti *et al.* (2007); this imbalance among cation nutrients induced Mg deficiency in *Salvia splendens* but not in other plant species. Only commercial substrates presented adequate values for available Na (below 115 mg $L^{-1}$).

Compost-based substrates (1 to 5), with exception of BD and available contents of Ca and B, presented the highest values for all other attributes, in comparison to commercial substrates (6 and 7). The data suggests that, in general, compost-based substrates presented excess of Na and nutrients in relation to commercial substrates (Table 2). Substrates 1, 2 and 4, which were prepared with higher proportions of chicken and cattle manures than plant residues (Table 1) are more enriched in nutrients than other growing media. Substrate 3 presents higher proportion of plant residues (58.5%) and this was reflected in lower availability of nutrients among the compost-based substrates. Substrate 4 and 5 presented the high levels of available Zn, which could be explained by the elevated proportions of sewage sludge used in their formulation. Sewage sludge used in this experiment contains high levels of zinc (Higashikawa *et al.* 2010). High level of Mn in substrate 5 can also be due to high proportion of sewage sludge in this media.

In the substrates studied by Perez-Murcia *et al.* (2006), the increased proportions of composted sewage sludge in the mixes promoted the elevation of total content of Zn. Guerrero *et al.* (2002) and Mupondi *et al.* (2006) also verified that substrates composed with sewage sludge presented higher total content of Zn, in comparison with other organic materials tested. Lopes *et al.* (2008) also used water as extractant (substrate:water ratio of 1:2 v/v) to evaluate two substrates and only available content of Cu is similar with the results of this study. The reason of this difference is that these authors did not study substrates that had manures or sewage sludge in the formulation. Available content of Fe, as was verified for other micronutrients, with the exception of B, was higher for compost-based substrates in comparison to commercial growing media.

TABLE 2.

Physicochemical properties and available contents of macro and micronutrients and sodium in substrates used for eucalyptus cultivation.

| | | | | Available content | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sb | pH | EC | BD | $NH_4^+$-N | $NO_3^-$-N | P | $SO_4^{2-}$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $Na^+$ | $Mn^{2+}$ | $Zn^{2+}$ | $Cu^{2+}$ | $B^{3+}$ | $Fe^{2+}$ |
| | | dS $m^{-1}$ | kg $m^{-3}$ | mg $L^{-1}$ | | | | | | | | | | | | |
| 1 | 5.0d | 13.3c | 407a | 191c | 11.6b | 2575b | 79c | 8440b | 920a | 1073a | 740c | 3.7b | 2.1c | 0.5c | 0.8c | 0.9c |
| 2 | 5.6a | 15.3b | 359b | 462b | 7.2b | 2951a | 70c | 8600b | 449b | 716c | 830b | 1.4c | 1.5c | 1.0b | 1.7b | 2.4b |
| 3 | 5.6a | 7.9e | 336b | 63d | 7.3b | 1295c | 56c | 3720d | 368b | 366d | 327e | 1.4c | 1.2c | 0.2 d | 0.03e | 1.6b |
| 4 | 5.5b | 21.6a | 393a | 840a | 212a | 2594b | 244a | 12040a | 894a | 1073a | 1240a | 3.6b | 4.7b | 1.9a | 0.2e | 12.5a |
| 5 | 5.1c | 9.8d | 328b | 100d | 12.6b | 282d | 105b | 5712c | 868a | 890b | 487d | 9.6a | 5.1a | 0.2d | 1.0c | 1.3b |
| 6 | 5.5b | 4.1g | 417a | 18e | 22b | 35d | 114b | 250e | 799a | 481d | 42f | 3.2b | 0.3d | 0.03e | 5.6a | 0.9c |
| 7 | 4.7e | 6.6f | 350b | 3e | 14.3b | 8d | 87c | 920e | 966a | 338d | 109f | 1.4c | 2.0c | 0.01e | 1.5b | 0.3c |
| OR and LVs* | 5.3-6.5 | 0.75-3.49 | < 400 | — | 100-199 | — | < 960 | 150-249 | — | — | < 115 | — | — | — | — | — |

Sb: substrate; EC: electrical conductivity; BD: bulk density. Means followed by same letter in the column not differ significantly by Scott-Knott test ($p<0.05$). * OR – optimal range and LVs – limit values according to Abad *et al.* (2001).

### *Eucalyptus growth response*

Eucalyptus seedlings biomass production varied depending on the tested growing media (Figure 1). Commercial substrates 6 and 7 were tested as reference to evaluate the performance of plants in compost-based substrates. Substrate 6 presented the best performance and produced a biomass of 1.37 g while the best compost-based substrate (4) produced 0.78 g (Figure 1). Total biomass dry weight (TDW) of substrate 7 was 0.81 g, which is higher than the biomass produced by number 4. However, the biomass produced by both substrates are very close. Biomass production (Figure 1) were lower than those obtained by Silva *et al.* (2012) in a study with the same eucalyptus clone. Nevertheless, these authors provided additional nutrients in the substrates during the seedling development, which resulted in proper balance of nutrients for plant nutritional requirements. In this study, nutrient from conventional fertilizers were not provided to the eucalyptus plants.

One goal of this study was to use only organic wastes as sources of nutrients, in order to promote reduction of fertilization use and costs (Trigueiro and Guerrini, 2003; Bardhan *et al.* 2008). Possibly, the reason for the better performance of plants cultivated in substrate 4 (Figure 1), among the compost-based substrates, is its higher availability of $NH_4^+$-N (Table 2). The clone cultivated in this study (*Eucalyptus grandis* and *Eucalyptus urophylla)* absorbs more $NH_4^+$-N than $NO_3^-$-N (Grespan *et al.* 1998). However, as it was found by Atiyeh *et al.* (2000), the high $NH_4^+$-N content in chicken manure could affect plant roots, which may cause detrimental effect on the plant growth. Substrate 4, even with the highest EC value, was the compost-based substrate that allowed the highest biomass production.

Salt leaching during irrigation could have mitigated the possible negative effect of high EC. High EC in substrates are an important factor to be considered when young plants are cultivated. However, levels of salts are manageable for adequate levels (Hernández-Apaolaza *et al.* 2005) when irrigation is adequately used (Hicklenton *et al.* 2001). According to Atiyeh *et al.* (2001), in the end of growth phase, no significant difference in EC was observed among commercial substrate (control) and other pig manure vermicompost substrates.

Considering the cultivation of eucalyptus on substrates composed with 100% of compost without dilution with other materials or commercial substrate, probably, imbalance of nutrients on the compost-based substrates (Table 2) may have affected the growth of eucalyptus seedlings. However, substrates consisting of 100% of compost can provide better agronomic performance than commercial substrates, depending on the plant species (Ribeiro *et al.* 2007; Estévez-Schwars *et al.* 2009).

Imbalanced nutrients in substrates may affect exportation of photosynthates and allocation of nutrients by plants (Grigatti *et al.* 2007). Furthermore, other physical properties (porosity, water holding capacity etc.) not analyzed in this study could have affected the performance of plants cultivated in the substrates. In addition to high soluble salt concentration, Atiyeh *et al.* (2001) verified that tomato seedlings cultivated in substrates containing 100% of pig manure vermicompost were affected by the poor porosity and aeration, which probably influenced negatively the root growth. Similarly, Trigueiro and Guerrini (2003) and Guerrini and Trigueiro (2004) found that high proportion of sewage sludge in substrates resulted in an increment of bulk density and microporosity and, consequently, reduction of macroporosity, which, caused poor aeration, and affected the eucalyptus seedlings development. This hypothesis was supported by the poor performance of substrate 5, which is a mix with high proportion of sewage sludge in its formulation.

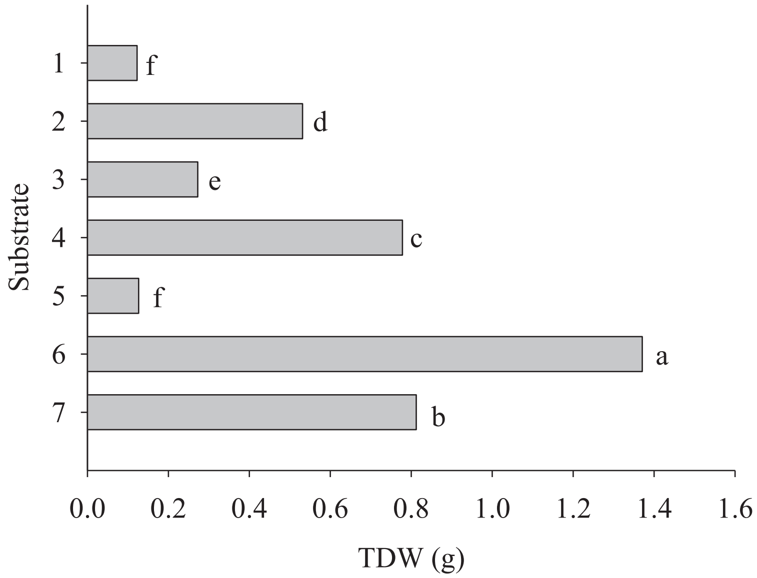

FIGURE 1. Total biomass dry weight (TDW) of eucalyptus grown on the substrates studied. Means followed by same letter did not differ significantly by Scott-Knott test ($p<0.05$).

### *Principal component analysis of substrate characteristics and biomass*

The PCA separated the individual contributions of the descriptors (physicochemical properties and biomass production) and discriminate graphically the overall characteristics and performance of substrates. Most of vectors representing variables (AvZn, AvMg, AvK, AvNa, EC, AvP, AvCa, AvCu, AvAm, AvFe, AvNi, AvS, pH and BD) characterized substrates 1, 2 and 4 that are located in the left side of plot (Figure 2). In addition, these vectors clearly show the high values for available nutrients of these substrates that suffered influence of chicken manure in their composition; chicken manure proportions in these substrate could explain theses residues since this manure is rich in nutrients (Ao e t al., 2008; Higashikawa *et al.* 2010).

The compost-based substrates, compared to the commercial ones, showed higher levels of available K (Table 2) which could be one of the main factors contributing to the substrate high EC, as also shown by Spiers and Fietje (2000). Substrate 1 and 5 are situated closer to available Mn, which could be explained by the fact that these substrates presented the highest contents of this nutrient, specially the substrate 5 (Table 2), which was enriched with sewage sludge. On the other hand, substrates 1 and 5 are in the opposite side of TDW because of low biomass production (Figure 1), which could mean that high Mn availability affected the eucalyptus growth.

The relatively short distance between substrates 3 and 7 show the similarity characteristics between them. These two substrates are in opposite side of the most parameters that characterized substrates 1, 2 and 4. Substrate 3 presented the lowest values for most of available nutrients, among the compost-based substrates. This could be due to the lower proportion of chicken and cattle manure and sewage sludge used in its formulation (Table 2). This substrate did not show low levels of nutrients such as those presented by Benito *et al.* (2006). Nevertheless, the substrate 3 is very poor in AvB and had lower TDW in relation to substrate 7.

Substrate 6 presented the highest availability of B (Table 2) and the highest biomass production (Figure 1) and both parameters differentiated this growing media from the others, according to PCA (Figure 2). The available content of B is the unique variable in positive correlation with TDW which could mean that AvB contributed in higher extent to better growing media in comparison to others properties analyzed. The positions of variables indicated by PCA (Figure 2) show that the origin of materials used to make substrates influenced their physicochemical characteristics which conditioned different substrates (Zoes *et al.* 2001) with distinct agronomic performances.

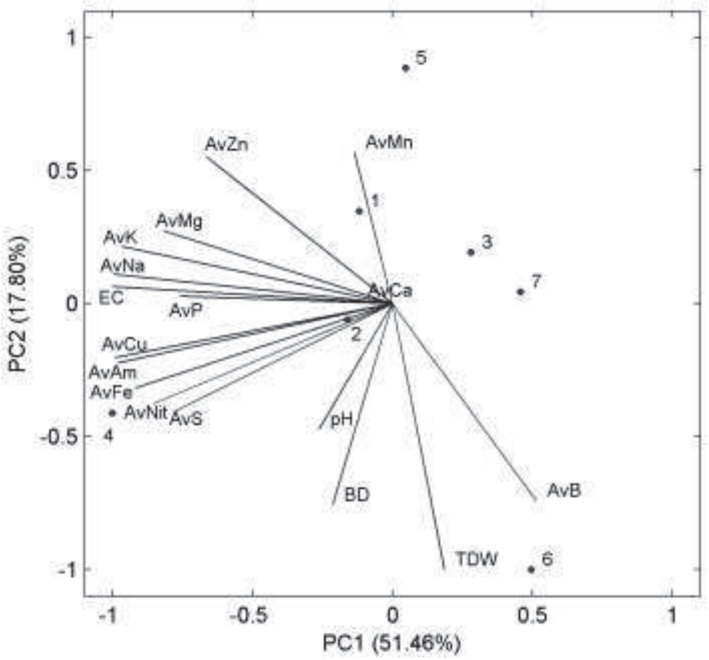

FIGURE 2. Principal component analysis (PCA) of the physical and chemical characteristics of substrates and eucalyptus biomass production in relation to the first two principal components (PC1xPC2). Legend: AvAm, AvNit, AvP, AvS, AvK, AvCa, AvMg, AvNa, AvMn, AvZn, AvCu, AvB and AvFe are, respectively, available contents of $NH_4^+$-N, $NO_3^-$-N, P, $SO_4^{2-}$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Na^+$, $Mn^{2+}$, $Zn^{2+}$, $Cu^{2+}$, $B^{3+}$ and $Fe^{2+}$; BD: bulk density; EC: electrical conductivity; TDW: total dry weight.

### *Biomass and substrate characteristics correlation*

Bulk density, pH, available content of Ca, Mn and Zn did not correlate with none of attributes and negative correlation was not found between any parameter of Table 3. Electrical conductivity presented significant and high coefficient correlation with available content of $NH_4^+$-N, P, K, Mg, Na, Cu and Fe. Available content of K and Na were the parameters that most contributed to EC, according to the Pearson test (Table 3). However, EC did not show any significant correlation with TDW which means that high values of EC above the range considered ideal by Abad *et al.* (2001) did not affect the biomass production. Therefore as discussed before, salt leaching during daily irrigation could have mitigated a possible negative effect of high EC.

Total dry weight correlated only with available content of B. Thus, AvB could have affected the development of eucalyptus plant, reflecting the importance of availability of this nutrient in substrates for eucalyptus production. This observation supports the hypothesis discussed in the previous section about the high contribution of AvB in the biomass production. Daily irrigation might have reduced the availability of B during the experiment period below the critical level which was from 0.09 to 0.24 mg $L^{-1}$ for clones of *E. grandis* x *E. urophylla* (Barretto *et al.* 2007). In the case of substrate 3 (Table 2), the available content of B was below the critical level and could have been an important factor that affected the development of plant and biomass production.

## TABLE 3.

Pearson analysis correlation coefficient for physicochemical properties, biomass production and available contents of macro and micronutrients and sodium in substrates used for eucalyptus cultivation

| | pH | EC | BD | $NH_4^+$-N | $NO_3^-$-N | P | $SO_4^{2-}$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $Na^+$ | $Mn^{2+}$ | $Zn^{2+}$ | $Cu^{2+}$ | $B^{3+}$ | $Fe^{2+}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $dS\ m^{-1}$ | $kg\ m^{-3}$ | | $mg\ L^{-1}$ | | | | | | | | | | | |
| EC | 0.21 | | | | | | | | | | | | | | | |
| BD | 0.12 | 0.15 | | | | | | | | | | | | | | |
| $NH_4^+$-N | 0.38 | 0.95* | 0.23 | | | | | | | | | | | | | |
| $NO_3^-$-N | 0.23 | 0.73 | 0.31 | 0.83* | | | | | | | | | | | | |
| P | 0.36 | 0.83* | 0.24 | 0.75 | 0.36 | | | | | | | | | | | |
| $SO_4^{2-}$ | 0.16 | 0.63 | 0.37 | 0.75 | 0.96* | 0.20 | | | | | | | | | | |
| $K^+$ | 0.23 | 0.97* | 0.13 | 0.87* | 0.60 | 0.87* | 0.51 | | | | | | | | | |
| $Ca^{2+}$ | -0.72 | 0.04 | 0.36 | < -0.01 | 0.29 | -0.29 | 0.45 | < -0.01 | | | | | | | | |
| $Mg^{2+}$ | -0.01 | 0.77* | 0.33 | 0.64 | 0.49 | 0.61 | 0.51 | 0.85* | 0.37 | | | | | | | |
| $Na^+$ | 0.26 | 0.99* | 0.16 | 0.92* | 0.68 | 0.85* | 0.59 | 0.99* | < 0.01 | 0.81* | | | | | | |
| $Mn^{2+}$ | -0.20 | 0.02 | -0.20 | -0.06 | 0.02 | -0.27 | 0.19 | 0.14 | 0.41 | 0.49 | 0.08 | | | | | |
| $Zn^{2+}$ | -0.21 | 0.57 | -0.29 | 0.48 | 0.54 | 0.11 | 0.59 | 0.58 | 0.43 | 0.65 | 0.57 | 0.71 | | | | |
| $Cu^{2+}$ | 0.37 | 0.95* | 0.23 | 0.99* | 0.86* | 0.74 | 0.77* | 0.88* | 0.01 | 0.65 | 0.93* | -0.05 | 0.50 | | | |
| $B^{3+}$ | 0.14 | -0.55 | 0.51 | -0.36 | -0.25 | -0.46 | -0.07 | -0.58 | 0.12 | -0.34 | -0.55 | -0.05 | -0.54 | -0.39 | | |
| $Fe^{2+}$ | 0.35 | 0.81* | 0.24 | 0.91* | 0.98* | 0.49 | 0.91* | 0.71 | 0.13 | 0.52 | 0.78* | < 0.01 | 0.55 | 0.93* | -0.33 | |
| TDW | 0.18 | -0.25 | 0.52 | 0.02 | 0.25 | -0.34 | 0.34 | -0.42 | 0.18 | -0.39 | -0.31 | -0.35 | -0.40 | < -0.01 | 0.77* | 0.15 |

EC: electrical conductivity; BD: bulk density; TDW: total dry weight. * Significant at $p < 0.05$.

## *Conclusions*

The compost-based substrates had adequate pH, EC above the threshold limit level and high contents of available P, $K^+$, $NH_4^+$-N and $Na^+$. Commercial substrates are enriched in B and Ca and presented lower concentrations of $NH_4^+$-N, P, $K^+$ and micronutrients.

EC values and $Na^+$ contents of commercial substrates are also lower and adequate to grow plants.

The compost-based substrate 4, for having more chicken manure and sewage sludge, resulted in the highest biomass production, among the compost-based substrates. Boron availability was the only attribute that explained most of the eucalyptus biomass variation.

Electrical conductivity (EC) did not affect the eucalyptus biomass, probably due to salts and ions leaching during the experiment. However, EC is an adequate index to predict the $NH_4^+$-N, P, $K^+$, $Mg^{2+}$, $Na^+$, $Cu^{2+}$ and $Fe^{2+}$ availability of the investigated substrates.

## *Acknowledgements*

The authors would like to thank the Bela Vista Forestry Nursery for providing the eucalyptus seedlings and the initial support for the conduction of the experiment. Authors also acknowledge the financial support from CNPq (Project 574921/2008-7) and FAPEMIG.

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# ARTIGO 2 Fourier transform infrared spectroscopy and partial least square regression for the prediction of substrate maturity indexes

**Normas da revista *Science of the Total Environment***

# Fourier transform infrared spectroscopy and partial least square regression for the prediction of substrate maturity indexes

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## Abstract

Traditional methods to evaluate the stability and maturity of organic wastes and composting matrices are laborious, time-consuming and generate laboratory chemical wastes. This study focused on the development of partial least square (PLS) regression models for the prediction of substrates maturity indexes based on Fourier transform infrared (FTIR) spectroscopy. The following parameters, selected as conventional maturity indexes, were modeled: dissolved organic carbon (DOC), C/N and $NH_4^+/NO_3^-$ ratios, cation exchange capacity (CEC), degree of polymerization (DP), percentage of humic acid (PHA), humification index (HI) and humification ratio (HR). Models were obtained by using data from a wide range of growing media of different origin and composition, including 4 commercially available substrates and 11 compost-based substrates prepared with varying proportions of different organic wastes. The PLS models presented correlation coefficient of calibration ($R^2_{cal}$) close to 0.90 and correlation coefficient of cross validation ($R^2_{cv}$) presented acceptable values (> 0.6), ranging from 0.67 (HR) to 0.92 (C/N). Y-randomization tests confirmed a low probability of chance correlation and indicate the good performance of the models. $R^2$ for test samples ($R^2_{pred}$) ranged from 0.66 (C/N) to 0.97 (HI) confirming the good correlation between measured and PLS predicted maturity indexes. FTIR spectroscopy combined with PLS regression represents a fast and alternative method to assess

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substrate maturity with reduction of time, lower generation of laboratory chemical residues and lower cost per sample compared to conventional analytical methods. All models adjusted for maturity indexes are predictive, robust and did not present chance correlation.

Keywords: FTIR; Multivariate calibration; Compost; Growing media.

## 1. Introduction

Solid organic wastes from different sources may be used as growing media when these materials present adequate properties for plant development (Abad et al., 2001). Composts prepared using different types and combinations of materials could be used pure or as a predominant component of substrates (Estévez-Schwarz et al., 2009). One of the main characteristics of these compost-based substrates should be their stability and maturity, which are used to refer to the quality of final product (Zmora-Nahum et al., 2005). For this reason, the measurement of compost stability and maturity is a key issue in assessing the suitability of different growing media components. Growing media components with poor stability should be avoided to prevent further degradation of the materials during cultivation. Mature materials are preferable to avoid the negative effects of phenol fractions and other potentially phytotoxic substances (Ortega et al., 1996) which can be present at early stages of the composting process (Sánchez–Monedero et al., 1999). Thus, maturity reflects the ideal condition of the final product before planting seedlings.

Different parameters have been proposed as compost maturity indexes. Senesi (1989) and Jiménez and García (1992) proposed the following parameters: C/N ratio, cation exchange capacity (CEC), humification ratio (HR), humification index (HI), percent of C as humic acid (PHA) and degree of polymerization (DP). The $NH_4^+/NO_3^-$ ratio was also proposed as a maturity parameter by Bernal et al. (1998), who evaluated the suitability of seven different composting mixtures. These previous parameters are determined in composting samples and are also considered to evaluate the maturaty degree, however, the evaluation of all these parameters are time consuming, expensive and produce large amount of laboratory chemical residues. The measurement of dissolved organic carbon (DOC) or water soluble carbon concentration is a simple parameter that allows evaluating the maturity of composts from different sources and composting processes (Zmora-Nahum et al., 2005). Therefore, there is no consensus on which parameters can be used to measure compost maturity, as reflected by the large variety of parameters and indexes suggested by different authors in the literature.

Fourier transform infrared (FTIR) spectroscopy method is a valuable tool to study the chemical composition of the organic matter of residues, organic composts and peatlands (Hsu and Lo, 1999; Jouraiphy et al., 2005; Artz et al., 2008; Castilhos et al., 2008). FTIR spectroscopy analysis allows monitoring the transformation of organic matter during composting (Hsu and Lo, 1999; Jouraiphy et al., 2005) and thereby it is a technique that enables to access compost maturity (Provenzano et al., 2001). Infrared spectroscopy analysis generates a huge dataset and the multivariate analysis support the extraction of additional information with reduction of time required for the data analysis (Meissl et al., 2007). The combination of infrared spectroscopy and partial-least square (PLS) regression allows a simple and quick prediction of sample properties (Janik et al., 2007). Ludwig et al. (2006) used near infrared spectroscopy combined with PLS to predict chemical characteristics and phytotoxicity of peats and growing media. The combination of FTIR-PLS prediction models was proven to be an appropriate technique to assess compost stability, also allowing the evaluation of unknown compost samples with accuracy (Meissl et al., 2007). The main advantages of technique that combine FTIR and PLS are the reduction of the analysis time and chemical residues in the laboratory, less manipulation of samples and avoid using hazardous chemical reagents, when compared with chemical conventional methods (Morgano et al., 2005; Janik et al., 2007; Tavares et al., 2012). Accordingly, it is desirable to develop a simple and inexpensive method that allows to verify the maturity of substrates produced by composts prepared using different types of wastes and composting processes.

This study developed PLS regression models for predicting substrate maturity indexes based on Fourier transform infrared (FTIR) spectroscopy wich was calibrated and tested against conventional stability and maturity attributes used for the evaluation of composting mixtures. Prediction of these parameters would allow evaluating compost-based and commercial growing media as a support decision tool to select substrates before plant cultivation.

## 2. Materials and methods

### *2.1. Preparation of substrates*

Composted sewage sludge, chicken and cattle manures, peat, eucalyptus sawdust, charcoal obtained from pyrolysis of eucalyptus, coffee husk, coconut fiber, pine bark, and vermiculite were used in the formulation of the compost-based substrates. The composted sewage sludge is a mix of domestic sewage sludge and pruning waste that was composted during 100 days by a private company located in São Paulo state. The main chemical and physical characteristics of the organic residues used in the formulation of the compost-based substrates of this study are presented in Higashikawa et al. (2010). Organic residues were air-dried and passed through an 8 mm sieve. Then, they were mixed in different proportions to obtain the substrates (Table 1). All compost-based substrates were incubated during 30 days; during incubation, these composts were daily aerated and weekly supplied with distilled water. Water was added to keep moisture close to water holding capacity of each substrate. To avoid leaching of nutrients, the mixed organic residues were incubated in a closed 27 L polyethylene container under greenhouse conditions. After 30 days of incubation, the temperature of the substrates was stable and close to the temperature of the environment, indicating the stability of the composted mixtures. Charcoal and vermiculite were added only on the compost-based substrates in fixed amounts of 8.3% each after the incubation period. This amount was based on the dry weight, determined after a 30-day incubation period. These compost-based (number 1 to 11) and commercial substrates (number 12 to 16) were analyzed to obtain chemical parameters and maturity indexes. The general compositions of commercial substrates include the presence of green waste composts, mineral fertilizer, peat, pine bark, vermiculite and milled charcoal, in the case of substrate 16.

**Table 1**

Relative proportions (dry basis, %) of organic residues used for the production of the 11 compost-based substrates.

| Materials | compost-based substrates[a] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| | (%) | | | | | | | | | | |
| Chicken manure | 16.7 | 25.0 | 33.4 | 41.7 | 25.1 | 16.7 | 8.3 | 33.3 | 16.7 | 33.4 | — |
| Cattle manure | 16.7 | 8.3 | 8.3 | 8.3 | 8.3 | 8.3 | 8.3 | 16.7 | 8.3 | 8.3 | 25.0 |
| Coffee husk | 12.5 | 12.5 | 8.3 | 8.3 | 8.3 | 16.7 | 21.0 | 4.2 | 16.7 | 16.7 | 8.3 |
| Pine bark | 8.3 | 8.3 | 4.2 | 4.2 | 8.3 | 16.7 | 16.7 | 4.2 | 16.7 | — | 4.2 |
| Sawdust | 4.2 | 4.2 | 4.2 | 4.2 | 4.2 | 8.3 | 12.5 | 4.2 | — | — | 4.2 |
| Sewage sludge | 16.7 | 16.7 | 16.7 | 8.3 | 25.0 | 8.3 | 8.3 | 16.7 | 8.3 | 25.0 | 33.3 |
| Peat | 8.3 | 8.4 | 8.3 | 8.4 | 4.2 | 8.4 | 8.3 | 4.1 | 8.4 | — | — |
| Coconut fiber | — | — | — | — | — | — | — | — | 8.3 | — | 8.4 |

[a] Charcoal and vermiculite were added in the proportion of 8.3 % to all compost-based substrates.

*2.2. Determination of the maturity indexes in the substrates*

Total content of C (TC) and dissolved organic carbon (DOC) were determined by an elemental analyzer (Elementar, Vario TOC Cube model). Water extracts preparations before determination of DOC were done according to Zmora-Nahum et al. (2005). Oxidizable organic carbon (OXC) of samples was determined following the method proposed by Yeomans and Bremner (1988). The extraction and quantification of fulvic acid carbon (FAC) and humic acid carbon (HAC) were done according to Benites et al. (2003). The sum of FAC and HAC was considered the alkaline extractable carbon (EXC). Total nitrogen was determined by the *Kjeldahl* method and to measure $NH_4^+$ and $NO_3^-$ it was used the method proposed by Bremner and Keeney (1965). The cation exchange capacity (CEC) was measured following the Brazilian Ministry of Agriculture (BMA) official analytical methods for substrate and soil conditioners (BMA, 2007). Previous attributes already mentioned were used to calculate humification degree indexes (Senesi, 1989; Iglesias Jiménez and Pérez García, 1992), that are: humification ratio (HR = EXC/OXC x 100), humification index (HI = HAC/OXC x 100), percent of humic acid carbon (PHA = HAC/EXC x 100), degree of polymerization (DP = HAC/FAC) and C/N ratio. The follow parameters are also considered to evaluate the stability and maturity of the substrates: DOC (Zmora-Nahum et al., 2005), $NH_4^+/NO_3^-$ ratio (Bernal et al., 1998; Jouraiphy et al., 2005) and CEC (Senesi, 1989). Maturity indexes data were compared using the Skott-Knott test at $p < 0.05$, using the statistical program Sisvar version 5.3 (Ferreira, 2010).

A principal component analysis (PCA) was done in order to explore the substrates in relation to the conventional maturity indexes. The dataset (Table 2) was autoscaled previously to the PCA. Calculations were carried out in the Chemoface version 1.4 (Nunes et al., 2012).

### *2.3. Fourier transform infrared (FTIR) spectroscopy analysis*

FTIR spectroscopy analysis was performed using samples of each substrate prepared in triplicate. Previously, the samples were dried at 60° C in an oven with forced air flow and, after this step, they were ground and sieved (< 0.149 mm). Then, KBr pellets were obtained by applying a pressure of 4 Mg cm$^{-2}$, using a hydraulic press for 30 seconds to a mixture of 1 mg of substrate sample and 100 mg of KBr (previously dried). Subsequently, these pellets were analyzed on a spectrophotometer Excalibur FT 3000 Series DTGS detector, operating in a 4000-600 cm$^{-1}$ wavelength range with a resolution of 8 cm$^{-1}$. FTIR spectra were submitted to multiplicative signal correction.

### *2.4. PLS modeling procedures*

The substrate 16 was an outlier by FTIR method, probably due to the presence of milled charcoal in the composition, and therefore has no PLS regression model. Thus, 45 samples (three replicates of 15 substrates) were used for PLS regression analysis.

The FTIR spectra (all absorbance from 4000 to 600 cm$^{-1}$) of the substrates were calibrated against the respective maturity indexes (previously determined by classical methods) using PLS regression.

The calibration performance was evaluated using the root mean square error of calibration ($RMSE_c$) and the squared correlation coefficient of calibration ($R^2_{cal}$) (Kiralj and Ferreira, 2009).

The models were validated by leave-one-out cross-validation (LOO-CV). This is one of the simplest procedures and a cornerstone for model validation. It consists of excluding each sample once, constructing a new model without this sample, and predicting the value of its dependent variable. Then the residuals (differences between experimental and estimated values from the model) are used to calculate the root mean square error of cross-validation ($RMSE_{cv}$) and the squared correlation coefficient of cross-validation ($R^2_{cv}$) (Kiralj and Ferreira, 2009).

A y-randomization test was also used to validate the PLS models. This test consists of several runs for which the original descriptors matrix X is kept fixed, and only the vector **y** is randomized. As for cross-validation, the performance parameters root mean square error of y-randomization ($RMSE_{y\text{-}rand}$) and squared correlation of y-randomization ($R^2_{y\text{-}rand}$) were also calculated. The models obtained under such conditions should be of poor quality and without any real meaning (randomization). A performance on the y-randomization test equivalent to the performance of the calibration indicates a model adjusted due to chance correlation (Kiralj and Ferreira, 2009).

Finally, the models were tested through the external group of test samples (samples not included in the calibration, *i.e.* independent from all model building processes) in order to test the predictive capacity of the models. The root mean square error of prediction ($RMSE_p$) and squared correlation coefficient of prediction ($R^2_{pred}$) were used as statistical parameters to judge the model performance on prediction. Among the 45 observations (15 substrates in triplicate), 10 (about 20% of the observations) were methodologically selected by Kennard Stone algorithm (Kennard and Stone, 1969) as test samples.

The root mean square errors (RMSE) and the squared correlation coefficients ($R^2$) were calculated by:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}} \quad (1)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{n}(y_i - \bar{y})^2} \quad (2)$$

where $y_i$ is the reference value of the dependent variable, $\hat{y}_i$ is the predicted value, $\bar{y}$ is the mean value, and *n* is the number of samples.

Statistical parameters, such as squared correlation between the experimental and predicted values for the test set ($r^2_m$) and $R^2$ of y-randomization prediction ($r^2_p$) (Eqs. 3 and 4), were also used for validation purpose (Roy et al., 2009).

$$r^2_m = R^2[1-(R^2-R^2_0)^{1/2}] \quad (3)$$

where $R^2$ and $R^2_0$ correspond to the squared correlation coefficient values between observed and predicted values for the test set with and without intercept, respectively.

$$r^2_p = R^2_{cal}(R^2_{cal}-R^2_{y\text{-}rand})^{1/2} \quad (4)$$

When $r^2m > 0.5$ indicates a good correlation between the experimental and predicted values for the test set, and that those values are congruent. The $r^2_P > 0.5$ guarantees a valid difference between $R^2_{cal}$ and $R^2_{y\text{-}rand}$ (Roy et al., 2009).

All calculations were carried out in the Chemoface version 1.4 (Nunes et al., 2012).

## 3. Results and discussion

The evaluation of the different substrates according to the conventional maturity indexes (Table 2) showed that the selected substrates cover a wide range of chemicalcompositions and characteristics. The five commercial substrates showed an acceptable degree of maturity, whereas the maturity indexes obtained for the compost-based substrates were generally above the typical threshold values characteristic of mature composts. Means presented by substrate 15 and 16 are statistically higher for CEC, DP, PHA, HI and HR in comparison with the others growing media, according to Scott-Knott test. Reference values for maturity indexes are listed in Table 2 and none of substrates attend to the required values for C/N and $NH_4^+/NO_3^-$ ratio, according to Jiménez and García (1992) and Jouraiphy et al. (2005), respectively. Based on the C/N ratio, commercials substrates present a general tendency to have, proportionally, higher levels of C than N, in comparison to the compost-based substrates; the opposite tendency was verified when $NH_4^+/NO_3^-$ ratio was evaluated. Substrates 6, 7, 9, 11 and all the commercial growth media are considered to be mature if the reference value for DOC proposed by Zmora-Nahum et al. (2005) is considered. Substrates 14, 15 and 16 are also typified as being mature, when DP, PHA and HI are used as indexes; substrates 15 and 16 also attend CEC parameter, according to the values suggested by Jiménez and García (1992). For the HI parameter, only the alternative substrate number 10 is considered to be mature. In relation to HR, substrates 10, 14, 15 and 16 attend the value proposed by Jouraiphy et al. (2005) wich is used to separate between mature and immature composts.

**Table 2**

Conventional maturity indexes of the studied substrates.

| Substrate | DOC | C/N | CEC | DP | PHA | HI | HR | $NH_4^+/NO_3^-$ |
|---|---|---|---|---|---|---|---|---|
| | g kg$^{-1}$ | | cmol kg$^{-1}$ | | | % | | |
| 1 | 5.21b | 17.7f | 40.2e | 0.86e | 46.1e | 8.02e | 17.3f | 1.95c |
| 2 | 5.29b | 16.5f | 41.1e | 0.72e | 41.4f | 6.87e | 16.8f | 11.5c |
| 3 | 5.55b | 15.9f | 34.6f | 0.89e | 46.9e | 8.36e | 17.8f | 39.3a |
| 4 | 4.50c | 14.0f | 32.1f | 0.79e | 43.7f | 7.33e | 16.7f | 36.8a |
| 5 | 4.35c | 18.0f | 46.9d | 0.79e | 44.2f | 9.15e | 20.7e | 8.54c |
| 6 | 3.55d | 22.0e | 46.9d | 0.96e | 48.7e | 7.67e | 15.7f | 4.17c |
| 7 | 3.07e | 24.2e | 46.8d | 1.00e | 49.9e | 7.51e | 15.0f | 7.28c |
| 8 | 5.36b | 17.0f | 38.9e | 0.91e | 49.9e | 11.0d | 22.0e | 24.8b |
| 9 | 3.83d | 22.6e | 43.8d | 1.39d | 58.0d | 9.42e | 16.2f | 4.40c |
| 10 | 8.35a | 15.1f | 40.8e | 0.70e | 41.0f | 13.2d | 32.2d | 39.9a |
| 11 | 3.21e | 20.6e | 38.1e | 0.57f | 36.2f | 8.65e | 23.9e | 1.00c |
| 12 | 1.73f | 58.7a | 41.0e | 0.26g | 20.1g | 3.43f | 16.9f | 3.56c |
| 13 | 1.34g | 49.0b | 61.8c | 0.30g | 23.1g | 3.00f | 13.0f | 1.28c |
| 14 | 1.26g | 23.5e | 58.1c | 2.54c | 71.4c | 27.1c | 37.8c | 0.60c |
| 15 | 1.17g | 29.6d | 65.8b | 3.66b | 78.5b | 49.8a | 63.4a | 0.58c |
| 16 | 0.25h | 45.8c | 82.3a | 9.08a | 90.8a | 37.7b | 41.6b | 0.43c |
| Critical maturity indexes | ≤ 4[a] | < 12[b] | > 67.0[b] | > 1.6[b] | > 62[b] | > 13[b] | 28[c] | 0.2[c] |

Means (n=3) followed by the same letter do not differ significantly by the Scott-Knott test ($p < 0.05$). [a] Zmora Nahum et al. (2005). [b] Iglesias Jiménez and Pérez García (1992). [c] Jouraiphy et al. (2005). Legend: DOC: dissolved organic carbon; CEC: cation exchange capacity; DP: degree of polymerization; PHA: percent of C as humic acid; HI: humification index; HR: humification ratio.

Data presented in Fig. 1 show a PCA characterization of the substrates based on the maturity indexes. Substrates 12 and 13 presented similar composition with higher C/N ratios. Substrates 3, 4, 8 and 10 presented high values for DOC and $NH_4^+/NO_3^-$ indexes. Based on the PCA, HI, HR and PHA were maturity indexes that distinguished substrates 14, 15 and 16 from others. Substrate 16 also presented high DP and CEC. Substrates 1, 2, 5, 6, 7, 9 and 11 (in the center of the graph) presented intermediary values for the maturity indexes in comparison with the others growing media. Regarding maturity, generally, low values are desirable, when considering DOC, C/N and $NH_4^+/NO_3^-$. However, lower values for HI, HR, PHA, CEC and DP indexes indicate immature substrates. Most of these parameters (HI, HR, PHA, CEC and DP) are considered high for the substrates 14, 15 and 16, as opposing to the low DOC; therefore, these growing media can be considered of higher maturity compared to the other growing media.

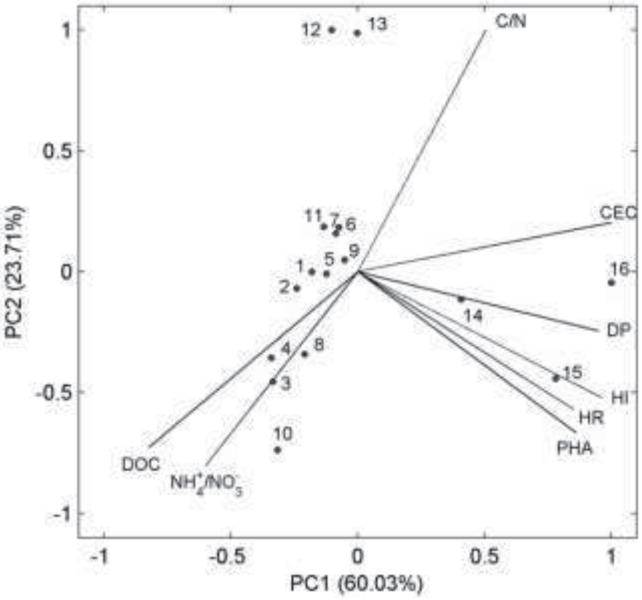

**Fig. 1.** Principal component analysis for the maturity indexes of substrates showing the scores (samples) and loadings (variables) in relation to the first two principal components (PC1 x PC2). Legend: DOC: dissolved organic carbon; CEC: cation exchange capacity; DP: degree of polymerization; PHA: percent of C as humic acid; HI: humification index; HR: humification ratio.

The main signals in the FTIR spectra (Fig. 2) of the substrates are O-H axial deformation around 3300 cm$^{-1}$, CH axial deformation at 2900 cm$^{-1}$, C=C axial deformation close to 1650 cm$^{-1}$, and C-O axial deformation at 1050 cm$^{-1}$. These results are similar to those found elsewhere in composted organic wastes (Hsu and Lo, 1999; Sánchez-Monedero et al., 2002; Jouraiphy et al., 2005; Smidt and Meissl, 2007).

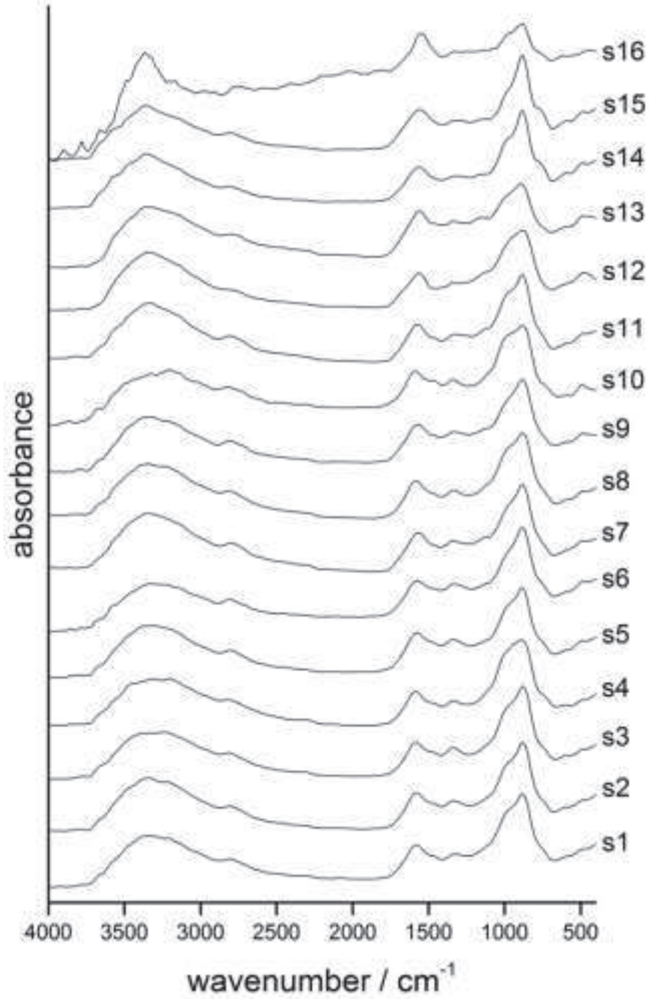

**Fig. 2.** Fourier transform infrared spectra of the substrates (s) after multiplicative scatter correction. Each spectra refers to corresponding substrate that is numbered from s1 to s16.

FTIR spectra of the substrates were calibrated against the respective values of the maturity indexes. The parameters of PLS regression for the models of each maturity index are presented in Table 3. Substrate 16 was detected as an outlier, probably because of its composition, which appears to be predominantly charcoal, and resulting in an infrared spectrum of poor quality (with few spectral information).

Multivariate calibration by PLS was applied to substrate 1 to 15 in order to obtain models that are able to predict different types of maturity index. The number of latent variable (LV) used for each model was established according to small value of root mean square error of cross validation ($RMSE_{cv}$).

The PLS models showed good correlation coefficient of calibration ($R^2_{cal}$), with values slightly lower than 0.90 only for DOC, CEC and $NH_4^+/NO_3^-$, and mean square error for calibration ($RMSE_c$) with low values in relation to the predicted range for each index. Squared correlation of cross validation ($R^2_{cv}$) were also acceptable (> 0.6), ranged from 0.64 (DOC) to 0.93 (C/N) (Kiralj and Ferreira, 2009). Galvez-Sola et al. (2010) on agroindustrial compost, using the PLS regression method, obtained a $R^2_{cv}$ value of 0.81 and a $RMSE_{cv}$ value of 1.53, that are significantly lower than the presented in this study.

To attest the robustness of the models, the y variables were randomized for the calibration set. The low $R^2$ of y-randomization ($R^2_{y\text{-rand}}$) and the elevated $RMSE_{y\text{-rand}}$ values of models suggest that the correlation obtained was not aleatory. The $r^2_p$ values > 0.5 confirm the valid differences between $R^2_{cal}$ and $R^2_{y\text{-rand}}$ (Roy et al., 2009).

The test of the models with external samples (test set) also suggests PLS models as good predictors ability. Squared correlation for test samples ($R^2_{pred}$) ranged from 0.70 (PHA) to 0.97 (HI). The $r^2_m$ values > 0.5 confirm the good correlation between measured and PLS predicted indexes (Roy et al., 2009). Fig. 3 shows the predicted against measured values of maturity indexes of calibration, cross-validation and test set validation. The distribution of calibration points around the line for all models reflects good coefficient correlation (Table 3).

**Table 3**

Parameters of PLS regression for models of substrate maturity indexes.

| | DOC | C/N | CEC | DP | PHA | HI | HR | $NH_4^+/NO_3^-$ |
|---|---|---|---|---|---|---|---|---|
| | g kg$^{-1}$ | | mmol kg$^{-1}$ | | | % | | |
| LV | 4 | 14 | 7 | 15 | 15 | 15 | 15 | 7 |
| $RMSE_c$ | 0.96 | 1.95 | 3.74 | 0.15 | 2.88 | 2.22 | 2.84 | 6.25 |
| $R^2_{cal}$ | 0.75 | 0.98 | 0.84 | 0.97 | 0.97 | 0.96 | 0.95 | 0.81 |
| $RMSE_{y\text{-}rand}$ | 1.80 | 8.70 | 7.93 | 0.60 | 11.1 | 8.14 | 9.13 | 11.3 |
| $R^2_{y\text{-}rand}$ | 0.11 | 0.55 | 0.30 | 0.50 | 0.49 | 0.48 | 0.47 | 0.38 |
| $r^2_p$ | 0.60 | 0.64 | 0.62 | 0.66 | 0.67 | 0.67 | 0.66 | 0.53 |
| $RMSE_{cv}$ | 1.16 | 3.52 | 5.26 | 0.27 | 4.95 | 5.32 | 7.36 | 7.79 |
| $R^2_{cv}$ | 0.64 | 0.93 | 0.70 | 0.90 | 0.90 | 0.79 | 0.67 | 0.71 |
| $RMSE_p$ | 0.96 | 3.74 | 3.58 | 0.23 | 6.65 | 2.78 | 3.66 | 8.40 |
| $R^2_{pred}$ | 0.76 | 0.94 | 0.86 | 0.93 | 0.70 | 0.97 | 0.95 | 0.72 |
| $r^2_m$ | 0.62 | 0.77 | 0.70 | 0.92 | 0.59 | 0.87 | 0.84 | 0.55 |

Legend: DOC: dissolved organic carbon; CEC: cation exchange capacity; DP: degree of polymerization; PHA: percent of humic acid carbon; HI: humification index; HR: humification ratio; LV: latent variable; RMSE: root mean square error; $R^2$: squared correlation; $RMSE_c$: RMSE of calibration; $R^2$cal: $R^2$ of calibration; $RMSE_{y\text{-}rand}$: RMSE of y-randomization; $R^2_{y\text{-}rand}$: $R^2$ of y-randomization; $r^2_p$: $r^2_p$ metric; $RMSE_{cv}$: RMSE of cross-validation; $R^2$cv: $R^2$ of cross-validation; $RMSE_p$: RMSE of prediction; $R^2_{pred}$: $R^2$ of prediction; $r^2_m$: $r^2_m$ metric

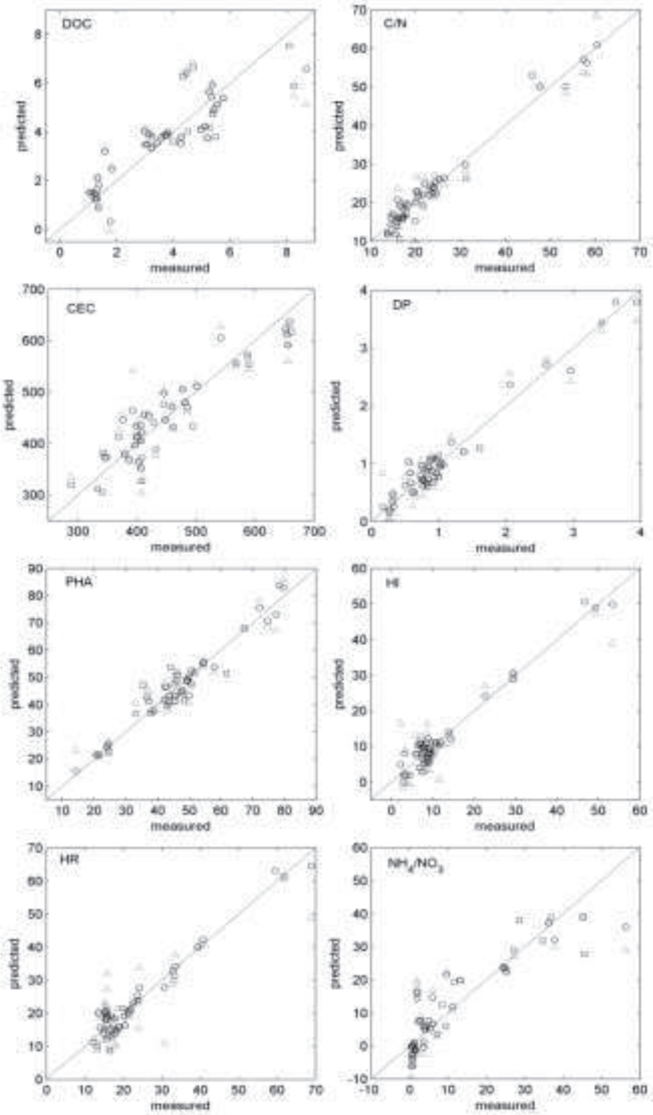

**Fig. 3.** Predicted vs. measured values for DOC, C/N, CEC, DP, PHA, HI, HR and $NH_4^+/NO_3^-$. Legend: DOC: dissolved organic carbon; CEC: cation exchange capacity; DP: degree of polymerization; PHA: percent of humic acid carbon; HI: humification index; HR: humification ratio; ○ calibration; Δ cross-validation; □ test.

## 4. Conclusions

Among the substrates modeled by PLS regression, substrates 14 and 15 showed characteristics of typical mature composts according to the conventional indexes because attended critical maturity values for DOC, DP, PHA, HI and HR.

FTIR spectroscopy combined with PLS regression allowed to predict maturity in substrate composed by mixture of organic residues and with different maturation degree.

This combined method was less time consuming to assess substrate maturity, genetate much less chemical residues and have low cost per sample in relation to conventional methods.

All the adjusted models for maturity indexes are predictive and robust; in addition, they did not present chance correlation.

The models were adjusted for a selection of growing media that covered a wide range of properties with characteristics typical of mature composts (substrates 14 and 15) and others outside these characteristics.

The prediction of maturity using FTIR spectra and PLS regression model allowed the simplification of different methods to assess the degree of maturity starting from a single sample analysis.

## Acknowledments

The first author wishes to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-CAPES for the award of a doctoral training fellowship. This research was supported by funds from the Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq (Process: 574921/2008-7) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais-FAPEMIG.

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# ARTIGO 3 Matrix effect on the performance of headspace solid phase microextraction method for the analysis of target volatile organic compounds (VOCs) in environmental samples

**Normas da revista *Chemosphere***

# Matrix effect on the performance of headspace solid phase microextraction method for the analysis of target volatile organic compounds (VOCs) in environmental samples

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## Abstract

Solid phase microextraction (SPME) is a fast, cheap and solvent free methodology that has been widely used for environmental analysis. A SPME methodology has been optimized for the analysis of VOCs in a range of matrices covering different soils of varying textures, organic matrices from manures and composts from different sources, and biochars. The performance of the technique was compared for the different matrices spiked with a multicomponent VOC mixture tha was selected to cover several VOC families of environmental relevance (ketone, terpene, alcohol, aliphatic hydrocarbons and alkylbenzenes). VOC recovery was dependent of the nature itself of the VOC and the matrix characteristics. The SPME analysis of non-polar compounds, such as alkylbenzenes, terpenes and aliphatic hydrocarbons, were markedly affected by the type of matrix as a consequence of the competition for the adsorption sites in the SPME fiber. These polar compounds were strongly sorbed on the biochar surfaces, limiting the use of SPME for these matrices. However, this adsorption capacity was not evident when the biochar had undergone a weathering/aging process through composting.

Polar compounds (alcohol and ketone) showed a similar behavior in all matrices, as a consequence of the hydrophilic characteristics, affected by the water content in the matrix. SPME showed a good performance for soils and organic matrices especially for non-polar compounds, achieving a limit of detection (LD) and limit of quantification (LQ) of 0.005 and 0.01 ng $g^{-1}$ for non-polar compounds and poor extraction for more hydrophilic and polar compounds (LD and LQ higher 310 and 490 ng $g^{-1}$). The characteristics of the matrix, especially pH and organic matter, had a marked impact on SPME, due to the competition of the analytes for active sites in the fiber, but VOC biodegradation should also be considered when dealing with matrices with active microbial biomass.

**Keywords** Biochar, soil, organic wastes, limit of detection, limit of quantification, SPME, GC-FID

## 1. Introduction

Solid phase microextraction (SPME) is a sampling and sample preparation technique, initially developed by Arthur and Pawliszyn

(1990), that has been optimized for a wide range of analytical applications (Risticevic et al, 2010). This technique combines in one unique process the extraction and pre-concentration of analytes which can be directly analyzed chromatographically without further treatment. The extraction is performed with a SPME fiber with appropriated coatings which enables the extraction of analytes, either by immersing the fiber directly in aqueous solution or by exposing the fiber to the headspace above the sample (Zhang and Pawliszyn, 1993; Valente and Augusto, 2000). The SPME is based on the simultaneous partition equilibrium of analytes between the sample and the fiber coating where a non-exhaustive extraction removes only a small quantity of the analyte from the sample matrix (Ouyang and Pawliszyn, 2008). Therefore, there is an increasing acceptance of the SPME as a fast, simple and inexpensive sample preparation technique (Demeestere et al., 2007) since it does not require the use of expensive equipment, such as automatic thermal desorber, sorbent tubes or cryogenic trapping techniques (Tuduri et al., 2001).

The headspace SPME is a solvent-free analytical method that is being widely used for the monitoring and quantitative analysis of different environmental samples (Zhang and Pawliszyn, 1993; Parreira and Cardeal, 2005). From the wide range of the potential analytical applications, the SPME fibers have been successfully used for the analysis of volatile organic compounds (VOCs) in landfills (Davoli et al., 2003; Banar et al., 2007), landfill leachates (Banar et al., 2006), manures (Miller and Woodbury, 2006; Woodbury et al., 2006), groundwater (Cho et al., 2003), vegetable oil (Jeleń et al., 2000; Vichi et al., 2003) and municipal solid wastes (MSW) (Pierucci et al., 2005). The VOCs are xenobiotic or biogenic organic compounds with relative high vapor pressures, including compounds with boiling points in the range from 0 to 50-100° C, wich are considered to be very volatile organic compounds, and up to 240-400°, which are known as semivolatile organic compounds (WHO, 1989). The release of these air pollutants to the atmosphere can be a source of malodor and/or harmful effects to site workers and local residents (Domingo and Nadal, 2009).

Risticevic et el. (2010) recommended the development of a separate SPME method for different target analytes and sample matrices by selecting the most suitable fiber coating and optimizing operational parameters such as the ionic strength of the sample solution, the water and solvent contents, sampling and extraction time and temperatures, GC conditions, etc. However, the optimization of the analysis of VOC is a complex objective due to the large variety of compounds at different concentrations that can be found in a single sample. Font et al. (2011) reported that typical groups of VOCs emitted from MSW treatment facilities include hydrocarbons, ketones, alcohols, aldehydes, ethers, esters, terpenes, nitrogen compounds, sulphur compounds, halogenated compounds and carboxylic acids. Several types of fibers and operational conditions have been tested and compared for the analysis of VOC in different environmental samples, showing a good performance for vegetable oils (Jeleń et al., 2000), landfills (Davoli et al., 2003) and MSW (Pierucci et al., 2005). However, other matrices of environmental relevance such as fly ash, soils, sludge and sediments may present methodological limitations due to the poor recovery of analytes as a consequence of their chemical interaction with the solid matrix (Alexandrou et al, 1992; Zhang and Pawliszyn, 1993).

The aim of this work was to develop and optimize a headspace SPME methodology for the analysis of target VOCs in environmental samples, representing the most common families of volatile organic compounds (hydrocarbons, alcohols, aromatics, chlorinated hydrocarbons, ketones and terpenes). The performance for this methodology was assessed for a number of soils, water and a selection of rich organic matrices such as manures, MSW, composts, biochars and biochar blended compost by comparing the limit of detection and quantification for each target analyte.

## 2. Materials and Methods

### *2.1. Sample description and preparation*

A selection of environmental samples was chosen to cover a wide range of matrices. Three agricultural soils with contrasting textures (according to the USDA textural classification): $S_{CLAY}$ (clay soil; pH 8.16; 15.9 g $kg^{-1}$ organic matter (OM); 20.8 % sand; 40.0 % clay; 39.2 % silt); $S_{SAND}$ (sandy soil; pH 9.09; 10.0 g $kg^{-1}$ OM; 87.8 % sand; 6.5 % clay; 5.7 % silt) and $S_{LOAM}$ (sandy loam soil; pH 8.68; 17.0 g $kg^{-1}$ OM; 75.5 % sand; 14.4 % clay; 10.1 % silt). Two biochars prepared by slow pyrolysis of organic wastes and characterized by a large organic matter contents and high sorption capacities: $B_{POULTRY}$ (poultry manure biochar; pH 7.73; 458 g $kg^{-1}$ C) and a $B_{OAK}$ (oak biochar; pH 9.4; 701 g $kg^{-1}$ C).

Four treated organic wastes: $M_{SHEEP}$ (sheep manure; pH 9.0; 456 g $kg^{-1}$ OM); $C_{MSW}$ (compost obtained from municipal solid wastes; pH 7.55; 543 g $kg^{-1}$ OM), $C_{POULTRY}$ (compost obtained from a mixture of poultry manure and rice straw (pH 8.2; 552 g $kg^{-1}$ OM) and $CB_{POULTRY}$ (biochar blended compost obtained by mixing 90% $C_{POULTRY}$ and 10% $B_{OAK}$, in dry weight, at the beginning of the composting process; pH 7.8; 730 g $kg^{-1}$ OM). These relevant environmental matrices were compared to deionized water (W). A NaCl saturated solution ($W_{NaCl}$) was used as reference matrix to standardize the method performance. Solid samples were oven dried (24 h at 80 ºC) prior to analysis, to avoid the interference of any native VOC. Dry samples were brought to a 20% of moisture content (or water holding capacity in the case of soils) prior to analysis, to enhance the release of the pollutants from the solid matrix to the headspace (Llompart et al., 1999).

### *2.2. Target VOC selection and description*

A series of target volatile compounds was selected in order to evaluating the performance of the SPME method for the most common VOCs groups emitted from relevant environmental sources and posing a risk for site workers and the environment: ketone, terpene, alcohol, aliphatic and aromatic groups (Font et al., 2011; Scaglia et al., 2011; Ni et al., 2012). The selected compounds were (boiling point within brackets): 4-methyl-2-pentanone (117-118 °C), β-pinene (165-167 °C), n-butanol (117-118 °C), dodecane (215-217 °C) and 2-ethyltoluene (164-165 °C). An external standard stock solution (ES) was prepared by dissolving the appropriate amount of each target VOC in methanol to the following concentrations in mg $L^{-1}$: 4-methyl-2-pentanone (801); β-pinene (173); n-butanol (810); dodecane (75) and 2-ethyltoluene (89). Moreover, 1-bromo-3-chloropropane with a boiling point range of 142-145 °C was used to prepare an internal standard stock solution (IS) in the concentration of 477 mg $L^{-1}$. Both the ES and IS were used to do the calibration curves. A multicomponent stock solution was prepared by diluting the ES (1:100) and IS (3:100) with methanol. This multicomponent solution was used to perform the SPME tests. All analytes used were analytic reagent grade (Sigma-Aldrich Co., Germany).

### *2.4. Development and optimization of the SPME methodology*

A SPME fiber holder for manual sampling (Supelco$^{TM}$, Sigma Aldrich Co., Germany) was used. The holder protects the coated fiber and controls the exposure of the fiber to the analytes by an adjustable depth guide. Glass vials (20 mL) were used to expose the fiber to the sample headspace for all the tests in duplicate. The vial caps had a flexible silicon/PTFE septum that allowed introducing the SPME fiber holder for sampling. The following operational parameters were optimized with the following tests:

#### *2.4.1. Fiber test*

Three types of SPME fibers were evaluated in order to select the most suitable fiber for the target VOCs (largest peak areas): PAC (polyacrylate, coating 85 µm), PDMS (polydimethylsiloxane, coating 100 µm) and DVB/CAR/PDMS (divinilbenzene/carboxen/polydimethylsiloxane, coating 50/30 µm). These fibers were selected according to the molecular weight and polarity of the target VOCs, as recommended by the manufacturer. Before the first use, the fibers were preconditioned in the GC injection port, according to the manufacturer instructions. The preconditioned fibers were placed into the headspace of the vials containing 5 μL of the multicomponent stock solution during 20 minutes at 20 °C. The final headspace concentrations for the different compounds in μg $L^{-1}$ were: 4-methyl-2-pentanone (2.00); ß-pinene (0.43); n-butanol (2.02); dodecane (0.18); 2-ethyltoluene (0.22) and 1-bromo-3-clhoropropane (3.58). The fibers were then inserted in the injection port in the gas chromatograph (GC) at 250 °C (See section 2.6.).

### *2.4.2. Desorption temperature test*

Once the most suitable fiber (DVB/CAR/PDMS) was selected, three different desorption temperatures (injector temperature in the GC) were tested: 230, 250 and 270 °C following the operational range recommended by the fiber manufacturer. The DVB/CAR/PDMS fiber was exposed during 20 minutes in the headspace of the vial containing 5 μL of the multicomponent stock solution at 20 °C (as in the previous test).

### *2.4.3. Fiber exposure time test*

Five exposure times were tested: 5, 10, 20, 40 and 60 minutes. The DVB/CAR/PDMS fiber was exposed in the headspace of the vial containing 5 µL of the multicomponent stock solution at 20 °C (as in the previous test) for the five selected exposure periods. The injector temperature in the GC was kept at 230 °C.

### *2.4.4. Fiber exposure temperature test*

Four exposure temperatures were tested: 20, 40, 60 and 80 °C. The DVB/CAR/PDMS fiber was exposed during 20 minutes at each temperature in the headspace of the vial containing 5 µL of the multicomponent stock solution (as in the previous test). The injector temperature was kept at 230 °C.

## *2.5. Calibration of the developed SPME methodology for different types of matrices.*

After the SPME methodology was optimized, a calibration was performed for the target VOCs in the presence of different types of matrices. The following environmental matrices were tested: W, $W_{NaCl}$,

$S_{CLAY}$, $S_{SANDY}$, $S_{LOAM}$, $M_{SHEEP}$, $C_{MSW}$, $C_{POULTRY}$, $CB_{POULTRY}$, $B_{POULTRY}$ and $B_{OAK}$. To evaluate the effect of the matrix in the transfer of the analytes to the headspace, a five-level calibration study was performed by adding 1, 3, 5, 7 and 9 µL of the ES solution to a 20 mL vial containing the appropriated amount of matrix (0.5 mL for liquid matrices, 1.0 g for soils and 0.1 g for organic materials). For each target analyte, the concentration (µg $L^{-1}$) ranged from: 4-methyl-2-pentanone (40.05 to 360.45); ß-pinene (8.66 to 77.84); n-butanol (40.50 to 364.50); dodecane (3.75 to 33.75) and 2-ethyltoluene (4.44 to 39.92). A volume of 5µL of internal standard (1-bromo-3-chlropropane) was injected separately to each single vial to reach a concentration of 119.4 µg $L^{-1}$. The vials containing the analytes and the matrices were incubated for 1 hour at 20 °C and then exposed to the fiber during 20 minutes before injecting in the GC. For each calibration point, the area (Ac) and concentration (Cc) of each compound was divided by the area (Ais) and concentration (Cis) of internal standard. Then, these Ac/Ais and Cc/Cis ratio values were used to calculate the linear regression. In order to compare the performance of the SPME technique in the different matrices, the peak area for each compound was normalized by setting the peak area obtained in NaCl saturated solution to 100%.

The limit of detection (LD) and limit of quantification (LQ) were calculated for each environmental matrix, according to Mocak et al. (1997). Five blank injections were performed, consisting of 5 μL of IS in each matrix. The peak areas of each compound in these blanks were considered to calculate the LD and LQ.

### *2.6. Gas chromatography conditions*

The analysis of the VOCs were performed in a gas chromatograph with flame ionization detector (Varian 450 GC-FID). The carrier gas used was nitrogen at a flow rate of 1 mL min$^{-1}$. A 0.75 mm ID liner was fitted in the split/splitless injection port. After each manual injection, the SPME fiber remained 5 minutes in the injector in splitless mode to ensure the complete desorption of analytes. The compounds were separated on a Varian CP-WAX58 capillary column (30m x 0.25 mm ID, 0.20 μm film thickness). The oven temperature was kept at 35 °C for 5 min and ramped to 85 °C at 6.0 °C min$^{-1}$, and then ramped to 240 °C at 60 °C min$^{-1}$, and held for 2 min. The total run time was 17.92 minutes. The detector temperature was set at 300 °C.

## 3. Results and discussion

### *3.1. Optimization of the SPME methodology for the target VOCs*

The fibers tested showed a different performance for each specific compound (Fig. 1). The three-phase fiber (DVB/CAR/PDMS) had the best performance in terms of peak areas and reproducibility, in particular for dodecane and 2-ethyltoluene. The PAC fiber showed a poor response for dodecane, with a standard variation of 59.29%, however this was the most sensitive fiber for n-butanol and 4-methyl-2-pentanone. The PDMS fiber presented the best performance only for β-pinene and low sensibility for n-butanol and 4-methyl-2-pentanone. In the study of Tuduri et al. (2001), the three-phase fiber presented better performance than PDMS which showed poorer sensitivity to extract 11 target VOCs from air. Superior performance of three-phase fiber in relation to PDMS was also found by Jeleń et al. (2000), for the characterization of VOCs in vegetable oils and by Davoli et al. (2003), when they analysed VOCs from MSW.

When the three-phase fiber was compared to PAC and PDMS for the analysis of VOCs in MSW, the three-phase fiber adsorbed and extracted the largest number of compounds (Pierucci et al., 2005). PAC and PDMS had reduced extraction capacities when they were used in a waste gas from a fat refinery (Kleeberg et al., 2005). PDMS applications include the analysis of non-polar volatiles and semivolatiles, while PAC is indicated for polar semivolatiles, and DVB/CAR/PDMS fiber to large range ($C_2$ – $C_{20}$) of analytes (Risticevic et al., 2010). Thus, the coatings characteristics of the fibers influence their performance. The SPME fibers also present different sorption mechanisms, either for absorption or adsorption sample extraction (Tuduri et al., 2001), that can influence their performance for different analytes. PDMS and PAC are absorption-type fibers and three-phase is a mixed polarity adsorbent-based fiber (Parreira and Cardeal, 2005).

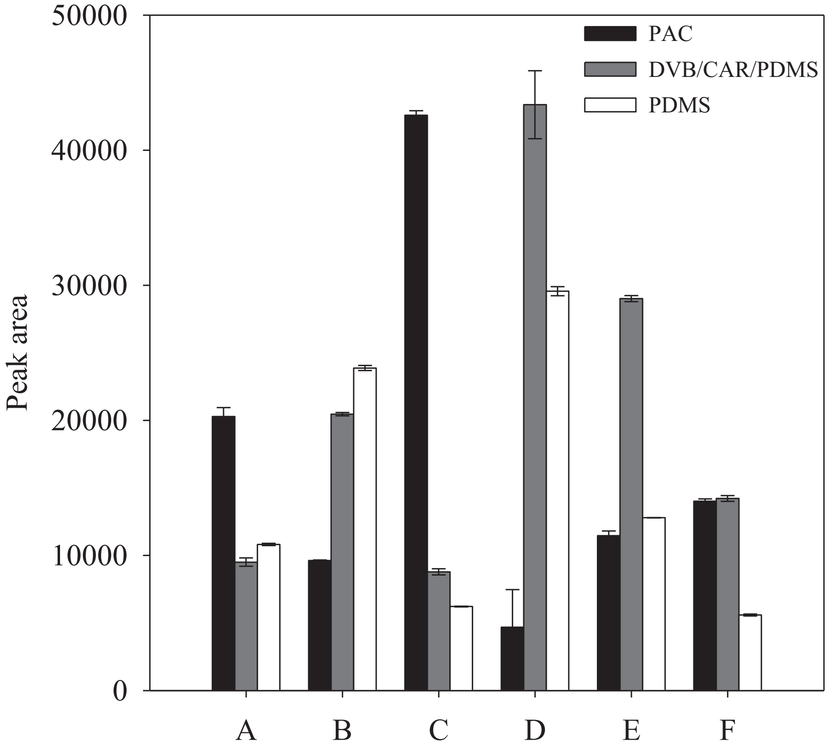

Fig. 1. Comparison between three different types of SPME fibers: PAC; DVB/CAR/PDMS; PDMS. Peak area of A (4-methyl-2-pentanone), B (β-pinene), C (n-butanol), D (dodecane), E (2-ethyltoluene) and F (IS: 1-bromo-3-chloropropane). Error bars represent the standard error (n = 2).

Once the three-phase fiber was chosen as the most suitable fiber for this study, different injector temperatures were tested to choose the best desorption temperature (Fig. 2). For all the tested compounds, the best results were obtained for an injector temperature of 230°C, since the peak areas of the analytes decreased inversely with the desorption temperature. This fact, probably was due the degradation of compounds with the increase of temperature. However, the opposite performance was observed for PDMS, when analyzed volatile compounds of vegetable oils (Steenson et al., 2002) and for CAR/PDMS fiber when increase desorption temperature for hexanal and (E,E)-2,4-decadienal (Kleeberg et al., 2005). Better response of VOCs when increase desorption temperature occur probably because of better desorption of these compounds at higher temperature (Steenson et al., 2002).

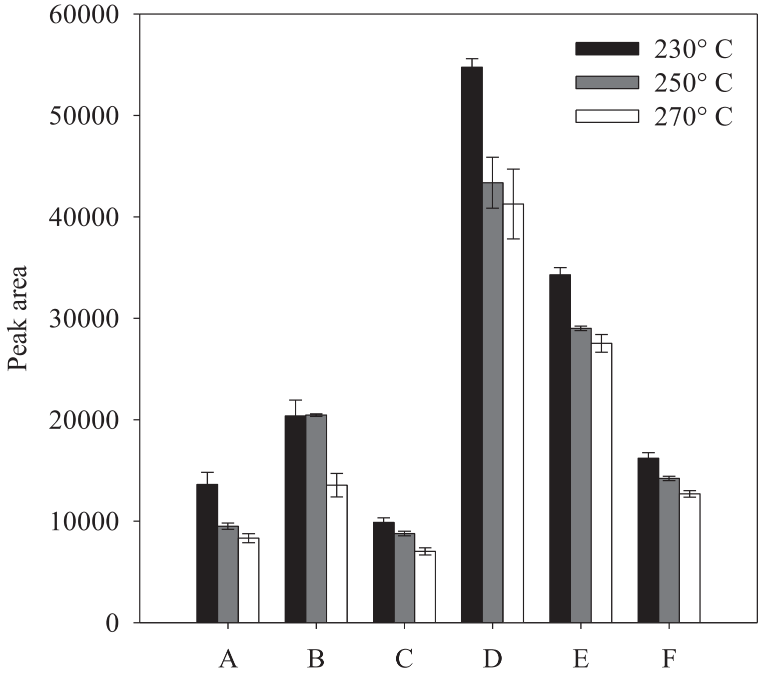

Fig. 2. Peak areas at different desorption temperature after SPME with DVB/CAR/PDMS fiber for the following compounds: A (4-methyl-2-pentanone), B (β-pinene), C (n-butanol), D (dodecane), E (2-ethyltoluene) and F (IS: 1-bromo-3-chloropropane). Error bars represent the standard error (n = 2).

Different exposure times were tested at 20° C (Fig. 3). Larger peak areas were found for 4-methyl-2-pentanone, β-pinene, n-butanol and 2-ethyltoluene when the fiber was exposed for 20 minutes. For IS (1-

bromo-3-chloropropane), a time exposure of 10 minutes gave the largest peak area. Cho et al. (2003) verified a competitive extraction under multi-component conditions in water with BTEX (benzene, toluene, ethylbenzene and xylene) for a CAR/PDMS fiber. According to these authors, different affinities of substances with the fiber result in differences on the shapes of the curves. On the other hand, Tuduri et al. (2001) did not observe displacement effects using the DVB/CAR/PDMS fiber in a gaseous mixture of 11 VOCs. In previous tests of this study, poor affinity of n-butanol and 4-methyl-2-pentantone with the three-phase fiber was proven and to compensate that these compounds were added at higher concentrations in the ES.

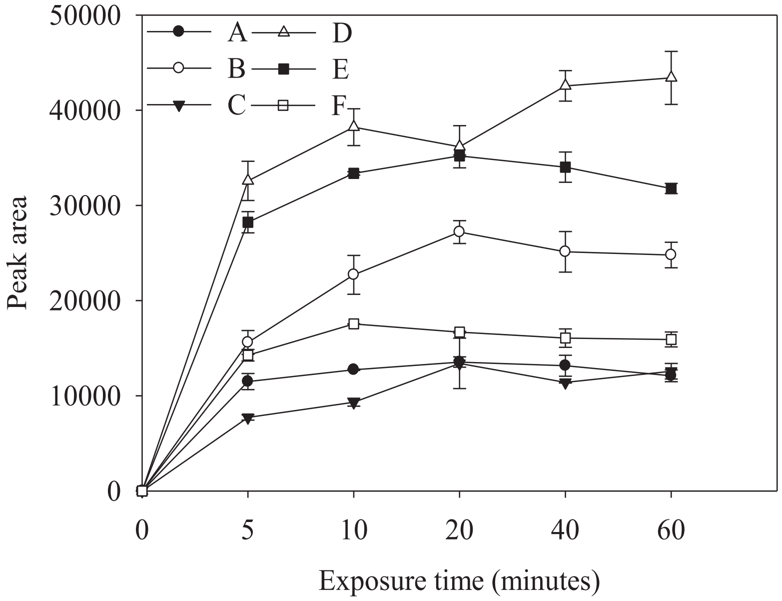

Fig. 3. Peak area of A (4-methyl-2-pentanone), B (β-pinene), C (n-butanol), D (dodecane), E (2-ethyltoluene) and F (IS: 1-bromo-3-chloropropane) after different exposure times. Error bars represent the standard error (n = 2).

When the compounds were exposed in the vials headspace during 20 minutes at different temperatures (Fig. 4), they all, with the exception of dodecane, reduced their peak area when the temperature was increased. The higher performance of dodecane at 40° C could be due to the fact that dodecane has the highest boiling point compared to the other tested compounds. These results are in agreement with Jeleń et al. (2000), who demonstrated that headspace SMPE with a three-phase fiber from vegetable oil was more effective at 50° C than at 20° C for the extraction of compounds with higher boiling points. According to the same authors, the extraction at 20° C showed larger peak areas compared to the extraction at 50° C for the most volatile compounds.

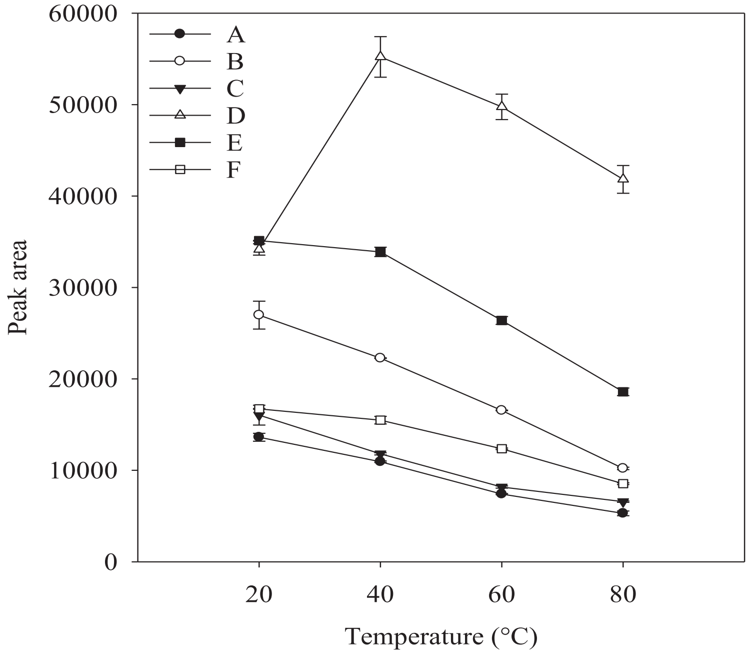

Fig. 4. Peak area of A (4-methyl-2-pentanone), B (β-pinene), C (n-butanol), D (dodecane), E (2-ethyltoluene) and F (IS: 1-bromo-3-chloropropane) after exposing the fiber during 20 minutes in the headspace at different temperatures. Error bars represent the standard error (n = 2).

### *3.2. Validation of the methodology for different environmental matrices. Limit of detection and limit of quantification*

Fig. 5 shows the normalized areas for the selected VOC at the highest concentration used for the preparation of the calibration curves (see supplementary material) in different matrices. The VOC areas were normalized to the peak areas registered in $W_{NaCl}$, which was set as a reference since this matrix presented the highest area for most compounds, with the exception of β-pinene and IS.

The addition of salt to water facilitates the transfer of the VOCs molecules from the sample to headspace, but the extracted amount of each target analyte would depend on the nature of the compound (Risticevic et al., 2010). The normalized peak areas for the different VOCs decreased in the order: water > soils > organic materials > biochars, except for n-butanol and 4-methyl-2-pentanone, probably because of their higher hydrophilicity.

In the case of the calibration curve prepared with no matrix, the increasing amounts of methanol added with the increasing concentration of ES may have influenced the extraction of the fibers, reducing the peak areas of the compounds, especially in the case of 4-methyl-2-pentanone and β-pinene. Other factor that influenced the area was the competition between compounds and IS for the fiber adsorption sites. For matrices with high content of organic matter (biochars, composts and organic wastes), beyond the competition for adsorption sites between the different VOCs, the adsorption effect of the matrix itself was the other factor that influenced the peak areas.

According to Kissel et al. (1992) the interactions between organic groups from VOC and the compost surface leads to a strong adsorption of these compounds which could be further oxidized under aerobic conditions. Thus, VOC biodegradation should not be discarded during sample preparation and analysis, especially in biologically active organic matrices, such as manures and composts, wich can be colonized by a large microbial biomass. However, Poulsen et al., (1998) found that VOC biodegradation had a negligible effect in soils. In the case of soils, the adsorption of VOCs and IS by the matrix itself seems less pronounced than in organic matrices with the exception of n-butanol.

An active role of clays was expected to affect adsorption of VOCs in soils through dispersion, π-electron interactions, and H-bond acidity interaction (Ruiz et al., 1998; Serrano and Gallego, 2006; Insam and

Seewald, 2010). However, $S_{CLAY}$ matrix had a reduced interaction of VOCs with clay minerals because of the hydrophilic character of clays (preferably occupied by water molecules) and the alkaline pH of the soils. Strong interaction between VOCs and clays has been reported in acidic soils but only limited sorption was observed in alkaline soils, where clays and organic matter are negatively charged and can form ion bridges with Ca and Mg, giving clay-humic complexes (Serrano and Gallego, 2006). Furthermore, the strong dipole interaction of molecule of water in the adsorption sites displaces non-polar organic molecules as aliphatic and aromatic compounds (Ruiz et al., 1998). Therefore VOCs sorption to soil minerals is dominant only at low soil water content and low pH (Poulsen et al., 1998).

In the case of biochar matrices, there was a strong interaction of the biochar and VOCs, reducing markedly the peak areas of all the studied compounds. Biochar and related products present a high affinity and sorption capacity for different organic compounds (Smernik, 2009). For this reason biochar is considered a high efficient sorbent to remove different kinds of pollutants in the environment (Chen et al., 2011). The high sorption capacity of biochar compete with the fiber sorption sites, reducing the ability of the fiber to adsorb the analytes. $B_{OAK}$ led to the smallest areas for all the VOCs with the only exception of β-pinene, which was the lowest in $B_{POULTRY}$. Probably the different behaviour between $B_{OAK}$ and $B_{POULTRY}$ are the influence of distinct biomass source and the production conditions such as pyrolysis temperature (Lehmann, 2007; Singh et al., 2010). In the case of non-polar compounds, such as dodecane and 2-ethyltoluene, the strong sorption capacity of biochar, probably through π-interactions, reduced the headspace concentration to levels below the GC detection limit.

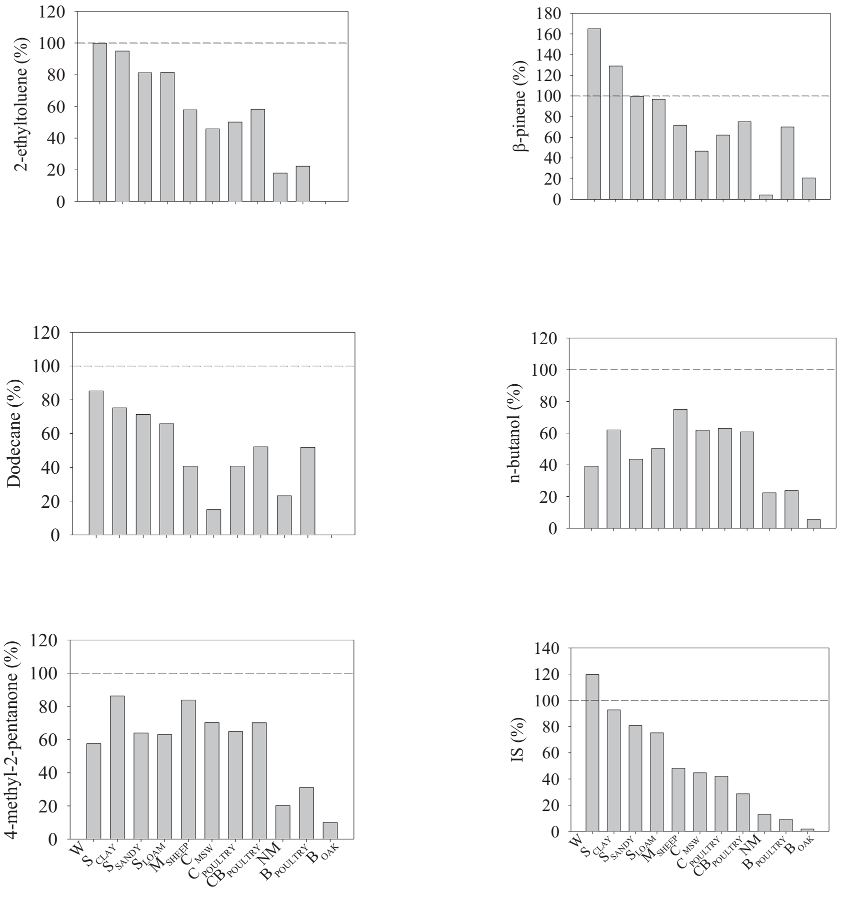

Fig. 5. Normalized peak areas of selected VOCs at the highest concentration of the calibration curves in different matrices. The peak areas of the VOCs obtained from salt water are set to 100% and are represented by a dash line. The matrices are: deionized water (W); clay soil ($S_{CLAY}$); sandy soil ($S_{SANDY}$); sandy loam soil ($S_{LOAM}$); sheep manure ($M_{SHEEP}$); municipal solid waste compost ($C_{MSW}$); poultry manure and rice straw compost ($C_{POULTRY}$); poultry manure and rice straw compost blended with oak biochar ($CB_{POULTRY}$); no matrix (NM); poultry manure biochar ($B_{POULTRY}$) and oak biochar ($B_{OAK}$).

The LD and LQ for the selected VOCs in the studied matrices are shown in Table 1. Both biochar matrices ($B_{POULTRY}$ and $B_{OAK}$) led to the highest LD and LQ values, which show their foremost capacity to adsorb VOCs in comparison with other matrices that limited the suitability of SPME for this type of matrix, as already observed for the analysis of polyaromatic hydrocarbons (PAH) which require initial extraction at high temperatures and preconcentration of analytes prior to analysis (Llompart et al., 1999).

Fabbri et al. (2012) developed a Soxhlet method for the determination of PAHs in biochar and found the following ranges for LD and LQ: 0.01 – 0.4 ng $g^{-1}$ and 0.03 – 1 ng $g^{-1}$ respectively. These ranges are much lower than the range presented by biochars in this study, but it is necessary to consider the different nature of compounds and the method used. SPME showed the best performance in the air matrix (NM) where LD and LQ presented the lowest values (0.005 and 0.01) in ppb scale.

The LD of NM (Table 1) ranged from 0.005 to 2.60 ng $mL^{-1}$ that is lower than the range found by Martos and Pawliszyn (1997) that presented values from 0.02 to 5.5 ng $mL^{-1}$ for SPME on a PDMS fiber in air samples. $W_{NaCl}$ and W (liquid matrices) showed similar values for non-polar compounds as dodecane, β-pinene and 2-ethyltoluene,

Table 1

Limit of detection (LD) and limit of quantification (LQ) of compounds for each matrix.

| Compound | $(ng\ g^{-1})$ | Matrix | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $W_{NaCl}^{*}$ | $W^{*}$ | $S_{CLAY}$ | $S_{SANDY}$ | $S_{LOAM}$ | $M_{SHEEP}$ | $C_{MSW}$ | $C_{POULTRY}$ | $CB_{POULTRY}$ | $NM^{*}$ | $B_{POULTRY}$ | $B_{OAK}$ |
| dodecane | LD | 0.04 | 0.04 | 0.02 | 0.03 | 0.02 | 0.09 | 1.61 | 0.28 | 0.76 | 0.005 | 87.08 | n.d. |
| β-pinene | | 0.13 | 0.11 | 0.08 | 0.10 | 0.07 | 1.34 | 3.11 | 0.89 | 0.71 | 0.02 | 6.31 | 13.20 |
| 2-ethyltoluene | | 0.12 | 0.12 | 0.14 | 0.12 | 0.09 | 3.63 | 10.19 | 2.37 | 2.63 | 0.11 | 23.91 | n.d. |
| n-butanol | | 1.97 | 16.86 | 6.90 | 2.29 | 0.67 | 29.56 | 21.68 | 28.34 | 22.80 | 0.15 | 135.17 | $1x10^{3}$ |
| 4-methyl-2-pentanone | | 22.04 | 132.23 | 29.50 | 44.54 | 28.90 | 269.89 | 309.96 | 171 | 111.72 | 2.60 | $1x10^{3}$ | $23x10^{3}$ |
| dodecane | LQ | 0.07 | 0.07 | 0.05 | 0.06 | 0.03 | 0.16 | 2.43 | 0.50 | 0.84 | 0.01 | 147.41 | n.d. |
| β-pinene | | 0.26 | 0.17 | 0.12 | 0.12 | 0.08 | 2.66 | 4.20 | 1.23 | 1.22 | 0.04 | 10.27 | 21.23 |
| 2-ethyltoluene | | 0.16 | 0.18 | 0.30 | 0.16 | 0.16 | 5.53 | 15.61 | 2.69 | 3.25 | 0.19 | 54.37 | n.d. |
| n-butanol | | 3.76 | 29.03 | 16.74 | 4.26 | 1.28 | 33.08 | 39.64 | 40.17 | 41.30 | 0.34 | 267.90 | $2.2x10^{3}$ |
| 4-methyl-2-pentanone | | 26.27 | 196.63 | 52.56 | 53.37 | 48.43 | 490.37 | 400.89 | 258.64 | 169.53 | 2.69 | $1.9x10^{3}$ | $51x10^{3}$ |

The matrices are: salt water ($W_{NaCl}$); deionized water (W); clay soil ($S_{CLAY}$); sandy soil ($S_{SANDY}$); sandy loam soil ($S_{LOAM}$); sheep manure ($M_{SHEEP}$); municipal solid waste ($C_{MSW}$); compost of poultry manure ($C_{POULTRY}$); compost of poultry manure with biochar ($CB_{POULTRY}$); no matrix (NM); biochar manure ($B_{POULTRY}$) and oak biochar ($B_{OAK}$). n.d.: not detected. *The values of $W_{NaCl}$, W and NM are expressed in $ng\ mL^{-1}$.

however for n-butanol and 4-methyl-2-pentanone the presence of salt in $W_{NaCl}$ reduced the LD and LQ. As previously reported, the addition of salt improved the sensitivity of HS-SPME extractions (Risticevic et al., 2010). Conversely, in the study of Llompart et al. (1998) the saturation of water samples with KCl did not produce changes in the response of volatile and semi-volatile pollutants. The LD and LQ observed in $W_{NaCl}$ (Table 1) are higher than in a previous study by Llompart et al., (1998) who used the headspace SPME for the determination of volatile and semivolatile pollutants in water. SPME showed a good performance in soils since LD and LQ were in general slightly lower than those of water. The three soil matrices tested ($S_{CLAY}$, $S_{SANDY}$ and $S_{LOAM}$) led to similar LD and LQ for dodecane, β-pinene and 2-ethyltoluene. However, the differences in texture notably influenced the LD and LQ for 4-methyl-2-pentanone and n-butanol. Sandy soil reduced the performance of SPME by increasing the LD of methyl-2-pentanone from 1 to 2 (doubled the LD compared to the other soil textures). The lowest values for LD and LQ presented by soils (Table 1) are lower than those showed in soil by Llompart et al. (1999). However the highest values of LD and LQ observed in the soils of this study were higher than the highest values presented by this previous study. Organic matrices ($M_{SHEEP}$, $C_{MSW}$, $C_{POULTRY}$ and $CB_{POULTRY}$) did not present a clear pattern for target compounds despite the different content of OM. Probably the different maturity degree of these organic matrices influenced the adsorption of target compounds. Despite the strong sorption capacity of biochars, the presence of $B_{OAK}$ in the poultry manure compost ($CB_{POULTRY}$) did not have a significant effect on the LD and LQ for most of compounds, what may be related to the aging of biochar during the composting process (Spokas, 2012).

## Conclusions

A SPME methodology has been optimized for the analysis of key families of VOC in different matrices of environmental relevance. The methodology showed a good performance for soils of varying textures and organic matrices, except for biochars, which are characterized by a strong sorption capacity that compete with the active sorption sites of the SPME fiber, limiting the extraction of analytes from these matrices.

Wood biochar showed the highest capacity of adsorbing the tested VOCs.

The LD and LQ for each compound was markedly affected by the matrix, whose interaction with the specific VOC is an important factor to be considered for the quantification of VOCs.

The LD for non-polar compounds ranged from 0.005 to 3.63 ng $g^{-1}$ and LQ ranged from 0.01 to 5.53 ng $g^{-1}$, whereas for polar compounds LD and LQ ranged from 0.15 to 309.96 ng $g^{-1}$ and 0.34 to 490.37 ng $g^{-1}$ in the matrices of this study.

In addition to the properties of VOCs, the surface character of sorbents as humic acids and clay mineral and also the soil pH can affect the sorption of VOCs by soils, which could furthermore suffer microbiological degradation before the analysis.

## Acknowledgements

The authors thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG (Process: CAG 20008-09) for financing the predoctoral stay of Mr. Fábio Satoshi Higashikawa at CEBAS-CSIC (Spain). This research was financed by a grant from the Spanish Ministry of Science and Innovation, research projects CTM2009-14073-C02-02 and AGL2012-40143-C02-01.

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## Supplementary material

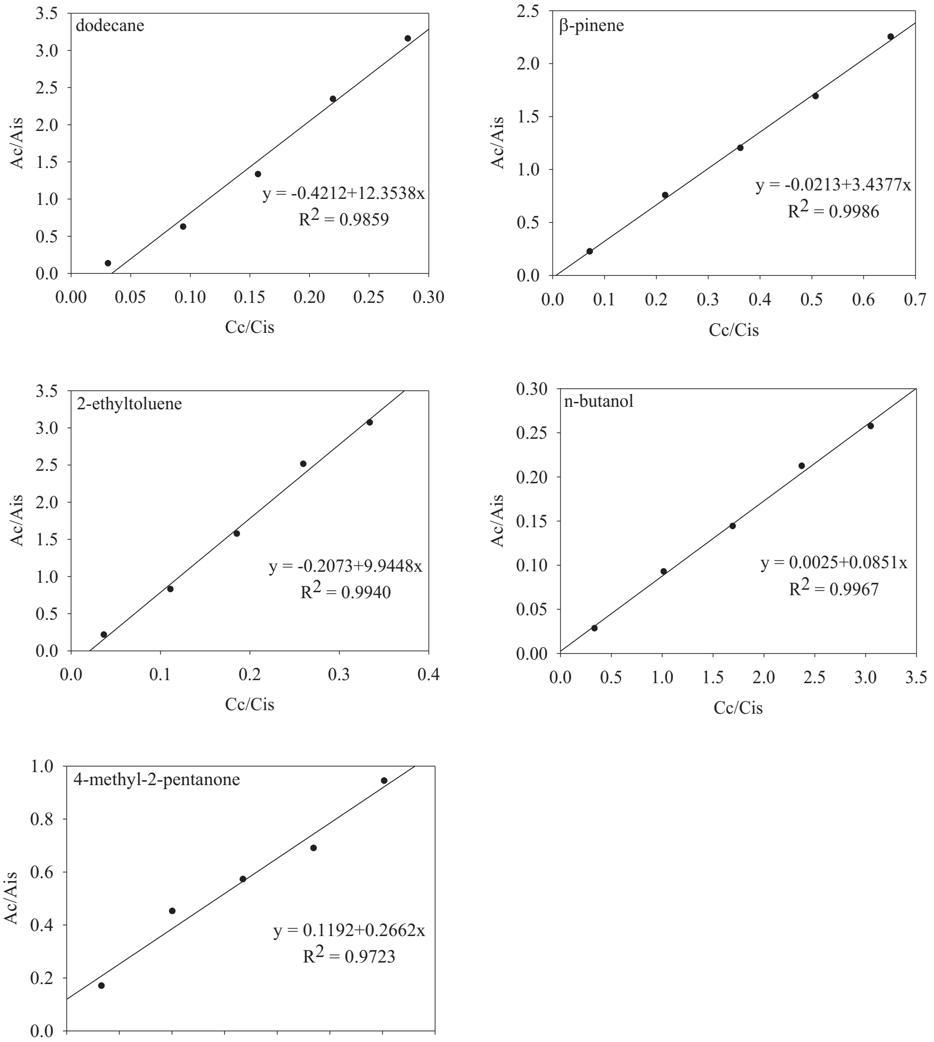

Fig. S1. Calibration curves of selected volatile organic compounds in deionized water saturated with NaCl ($W_{NaCl}$)

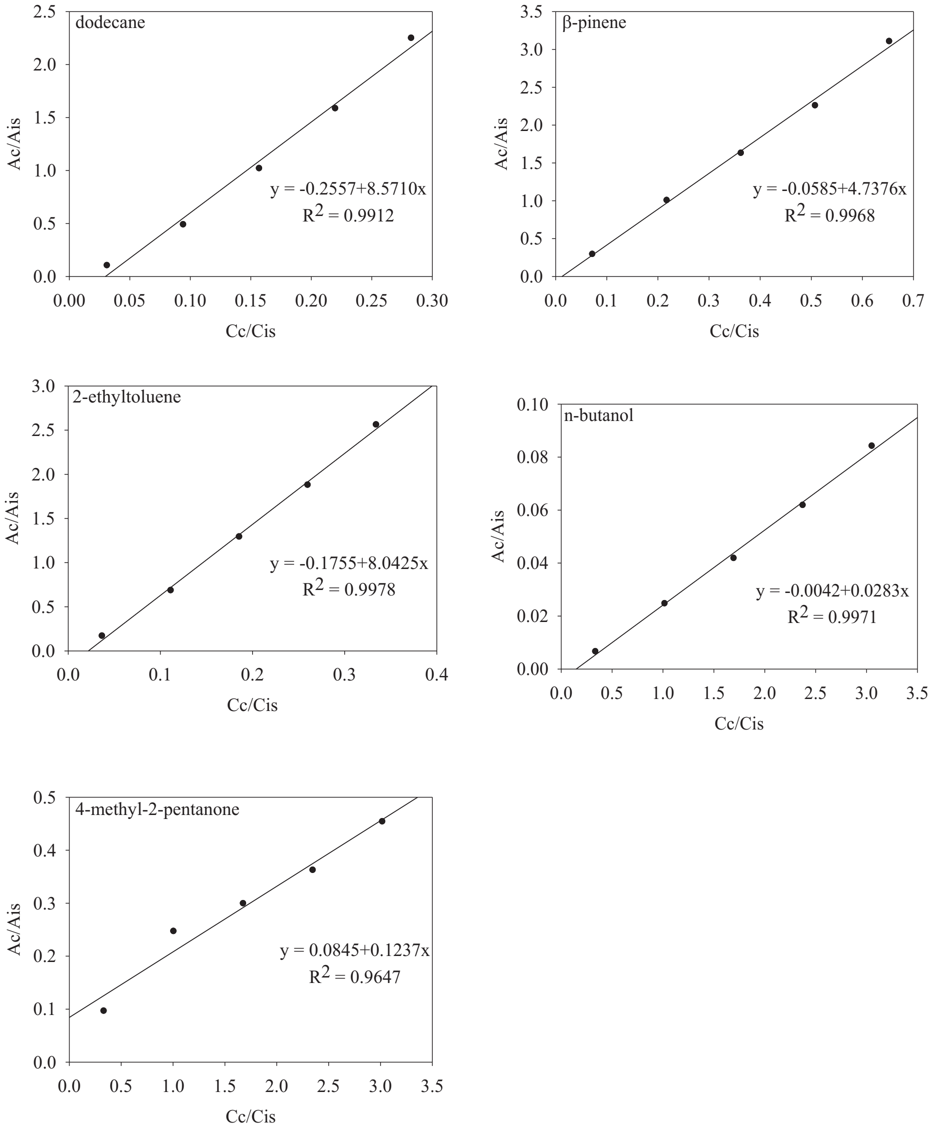

Fig. S2. Calibration curves of selected volatile organic compounds in deionized water (W).

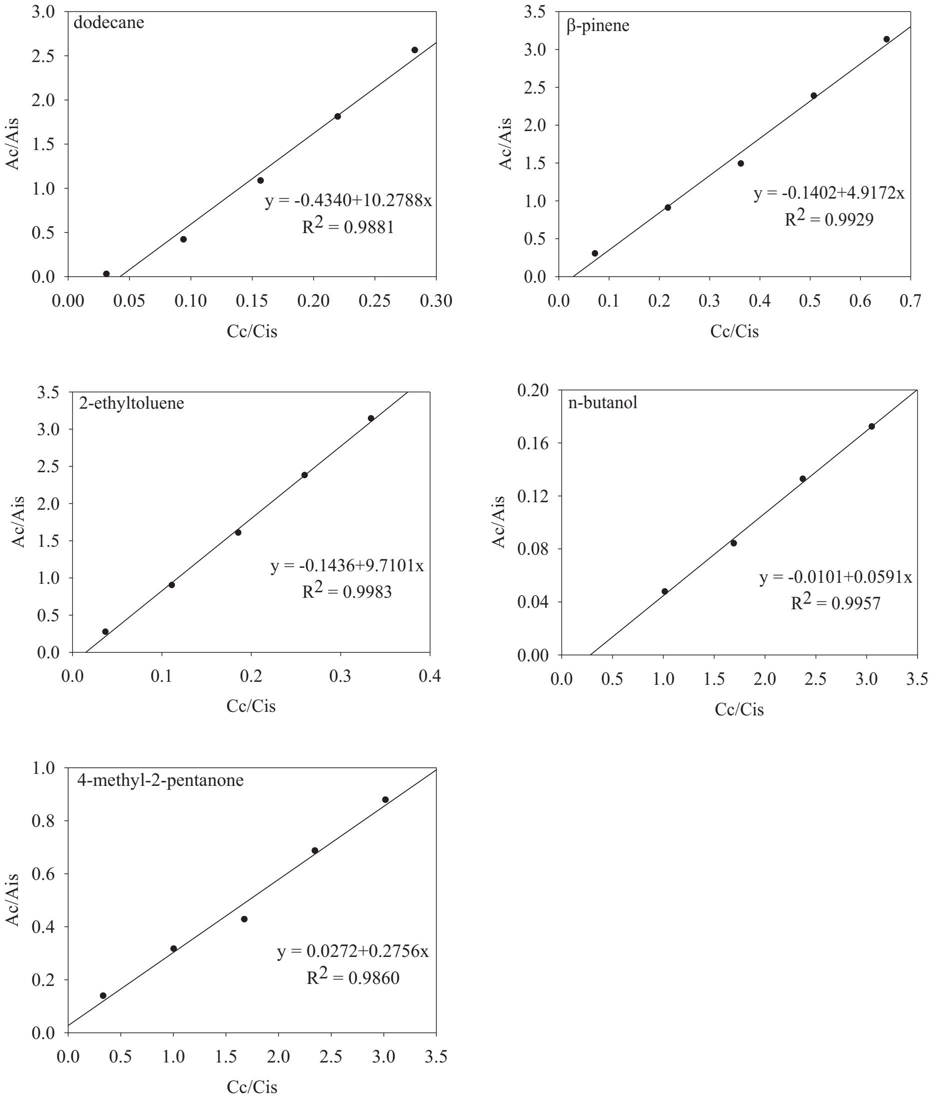

Figure S3: Calibration curves of selected volatile organic compounds in clay soil ($S_{CLAY}$)

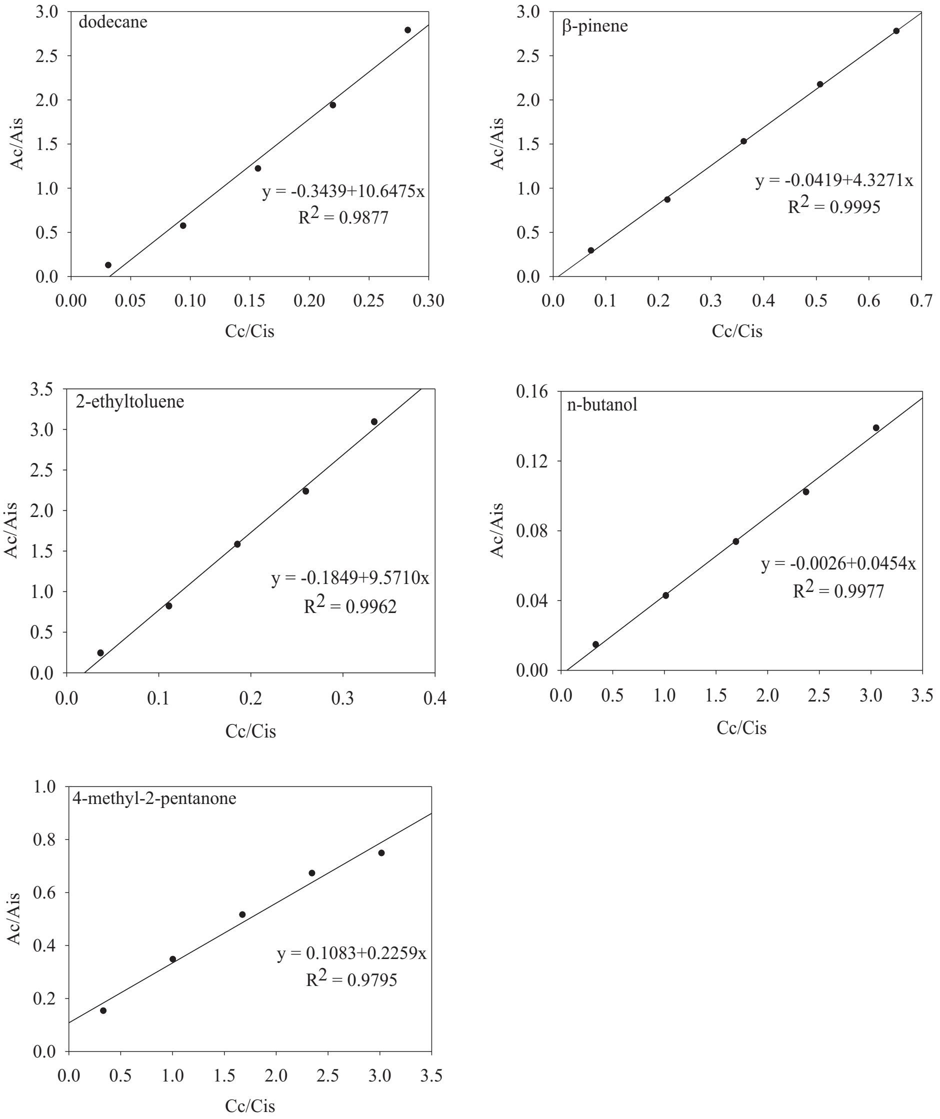

Fig. S4. Calibration curves of selected volatile organic compounds in sandy soil ($S_{SANDY}$).

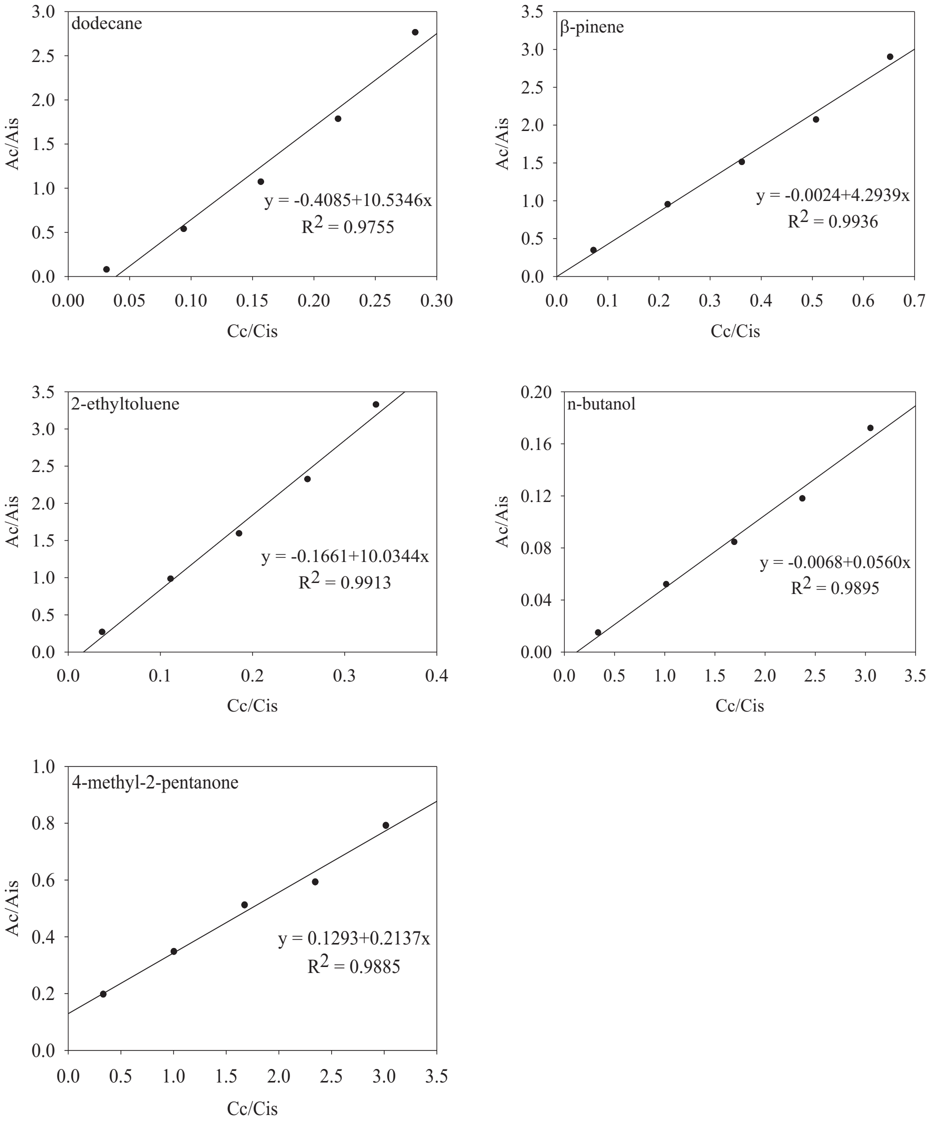

Fig. S5. Calibration curves of selected volatile organic compounds in sandy loam soil ($S_{LOAM}$).

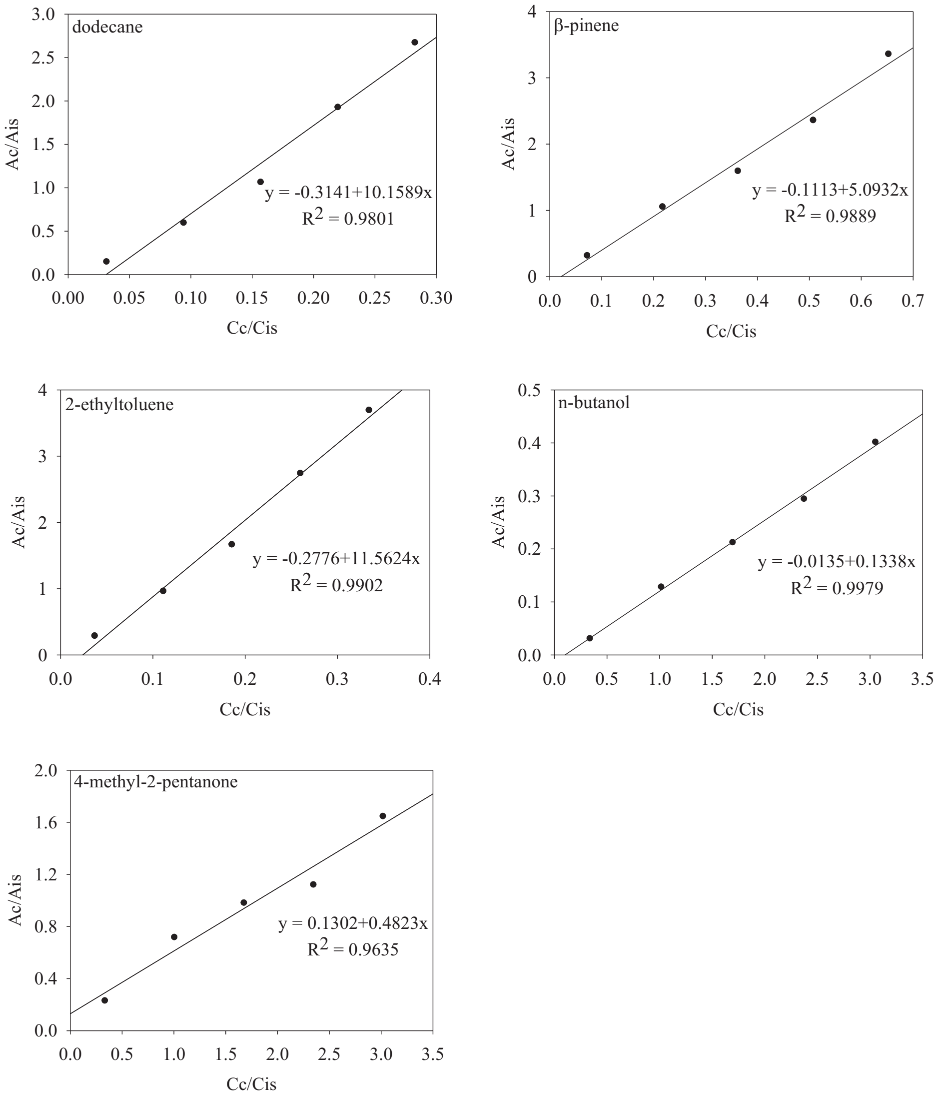

Fig. S6. Calibration curves of selected volatile organic compounds in sheep manure ($M_{SHEEP}$).

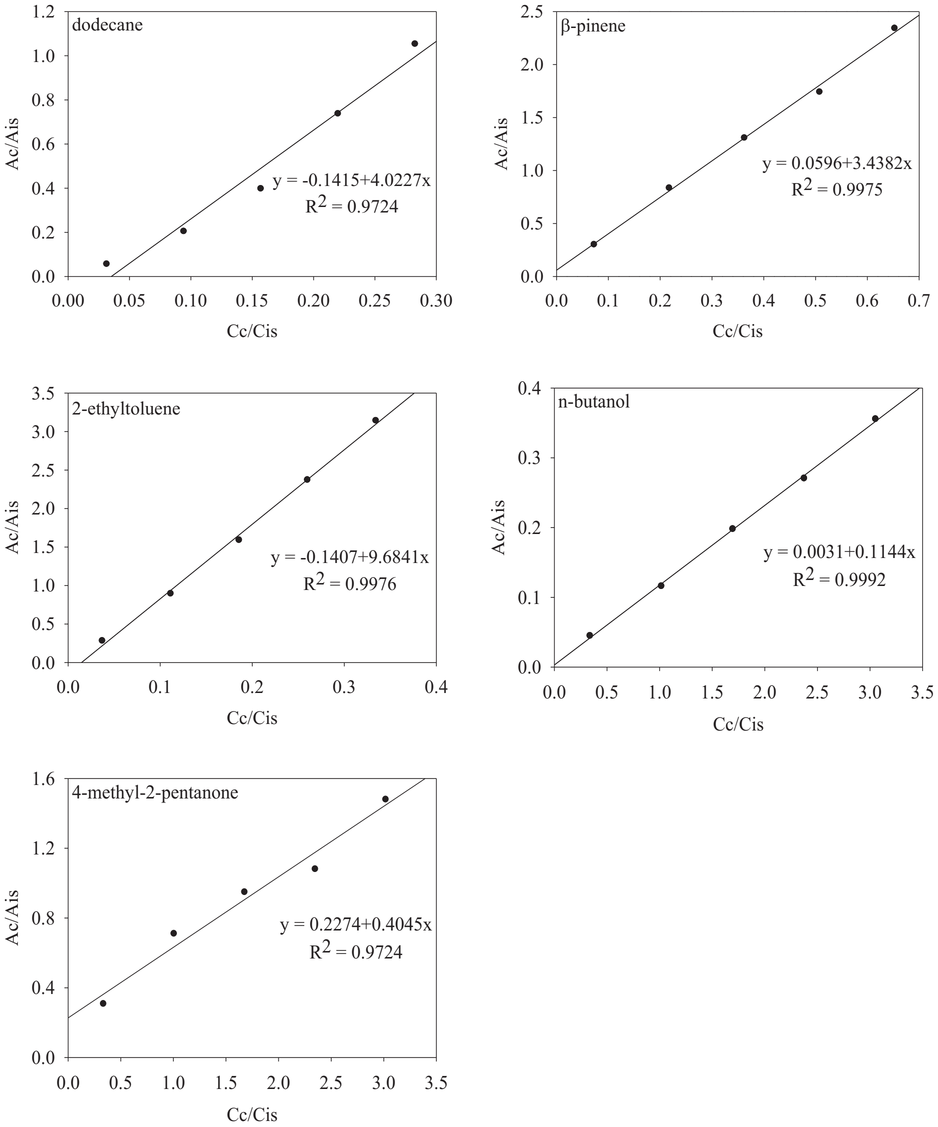

Fig. S7. Calibration curves of selected volatile organic compounds in municipal solid waste compost ($C_{MSW}$).

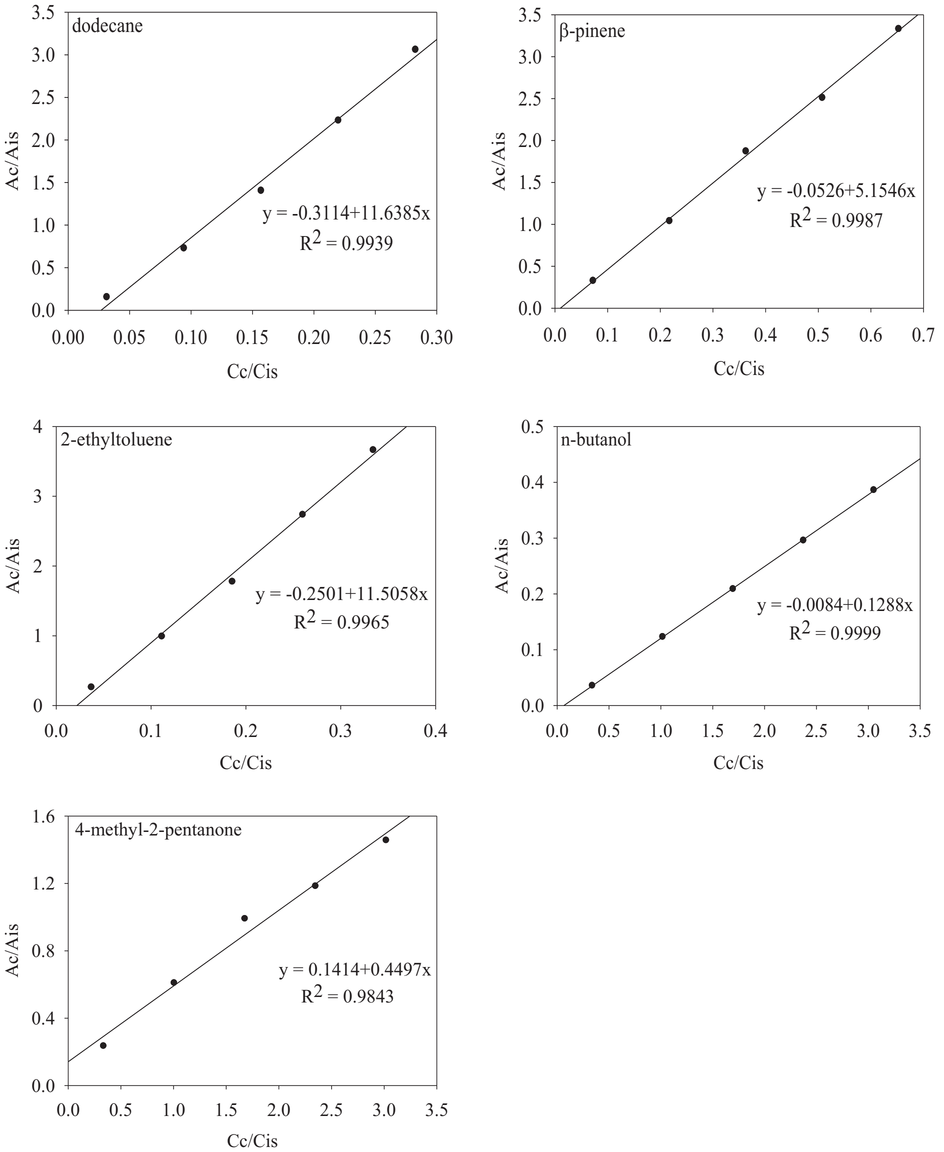

Fig. S8. Calibration curves of selected volatile organic compounds in poultry manure and rice straw composts ($C_{POULTRY}$).

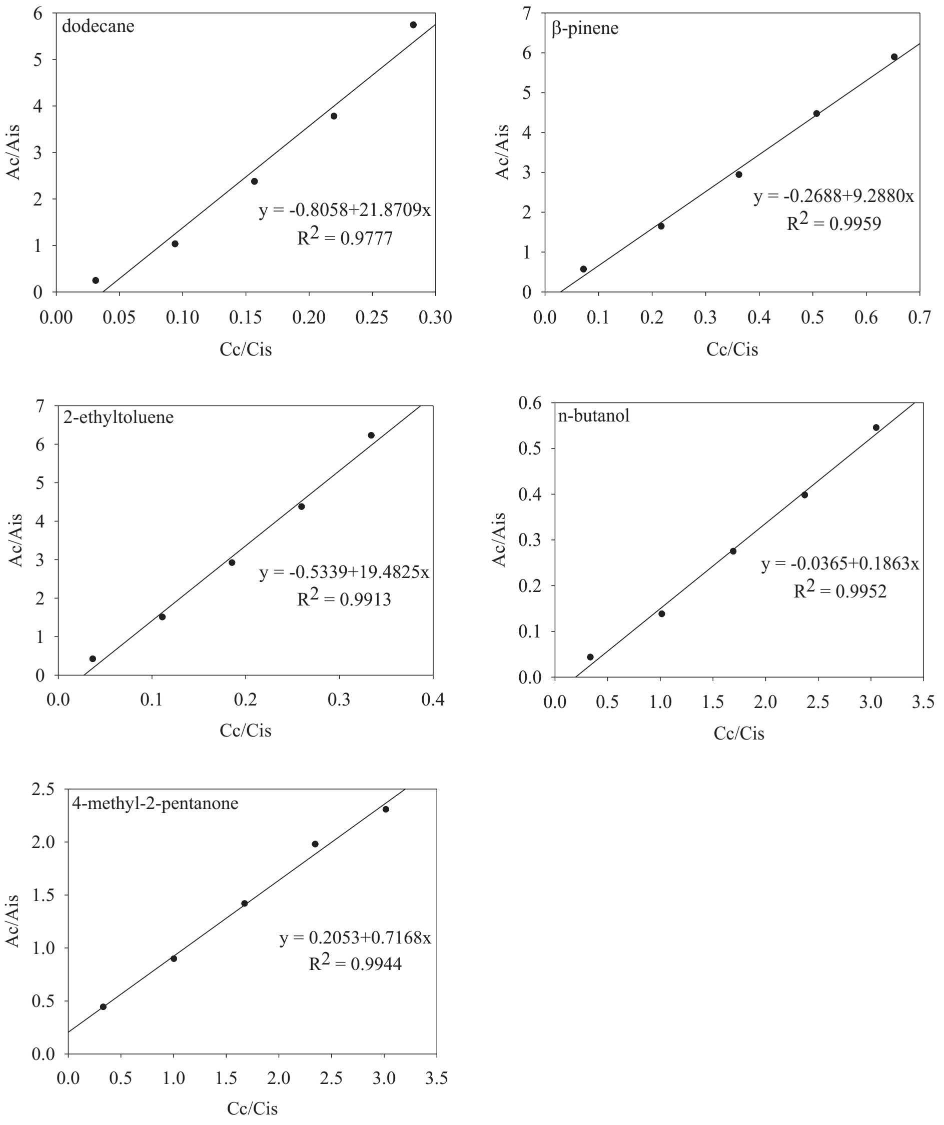

Fig. S9. Calibration curves of selected volatile organic compounds in poultry manure and rice straw compost blended with oak biochar ($CB_{POULTRY}$).

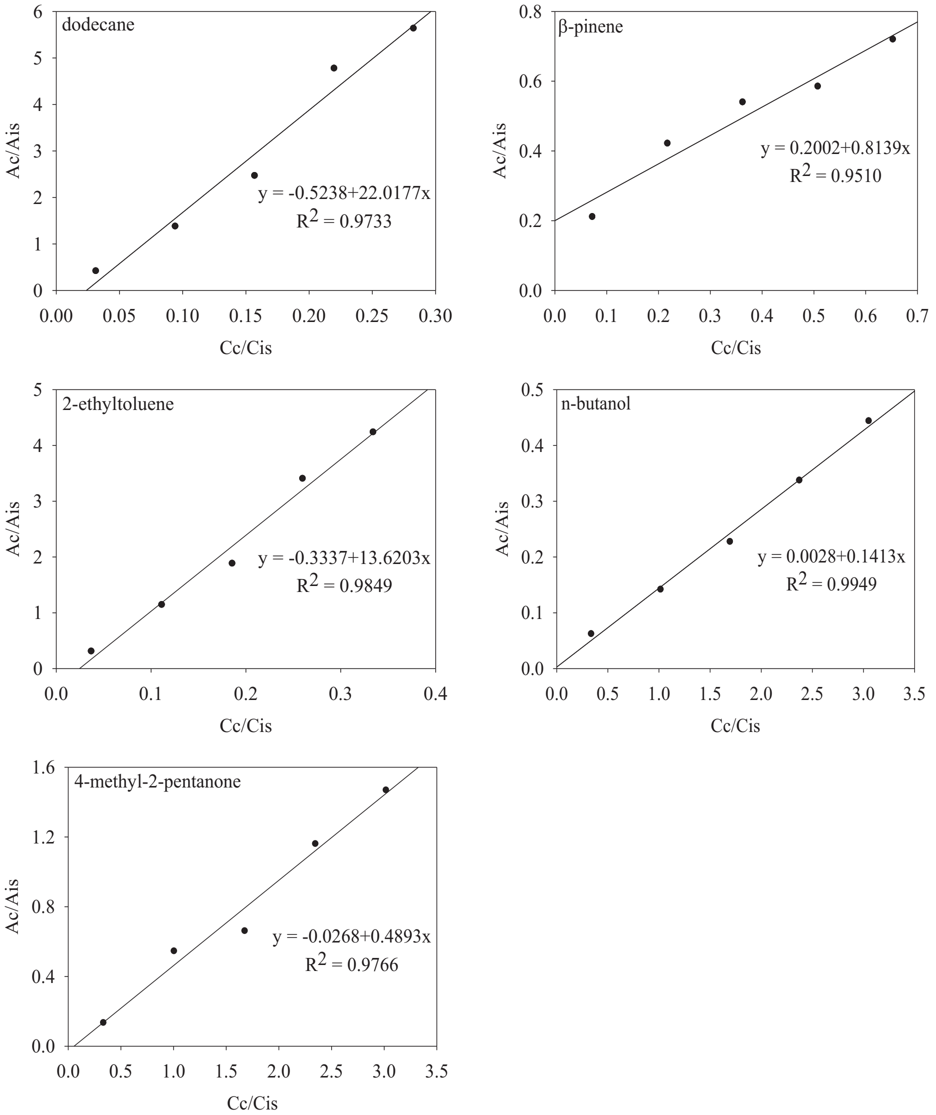

Fig. S10. Calibration curves of selected volatile organic compounds in no matrix (NM).

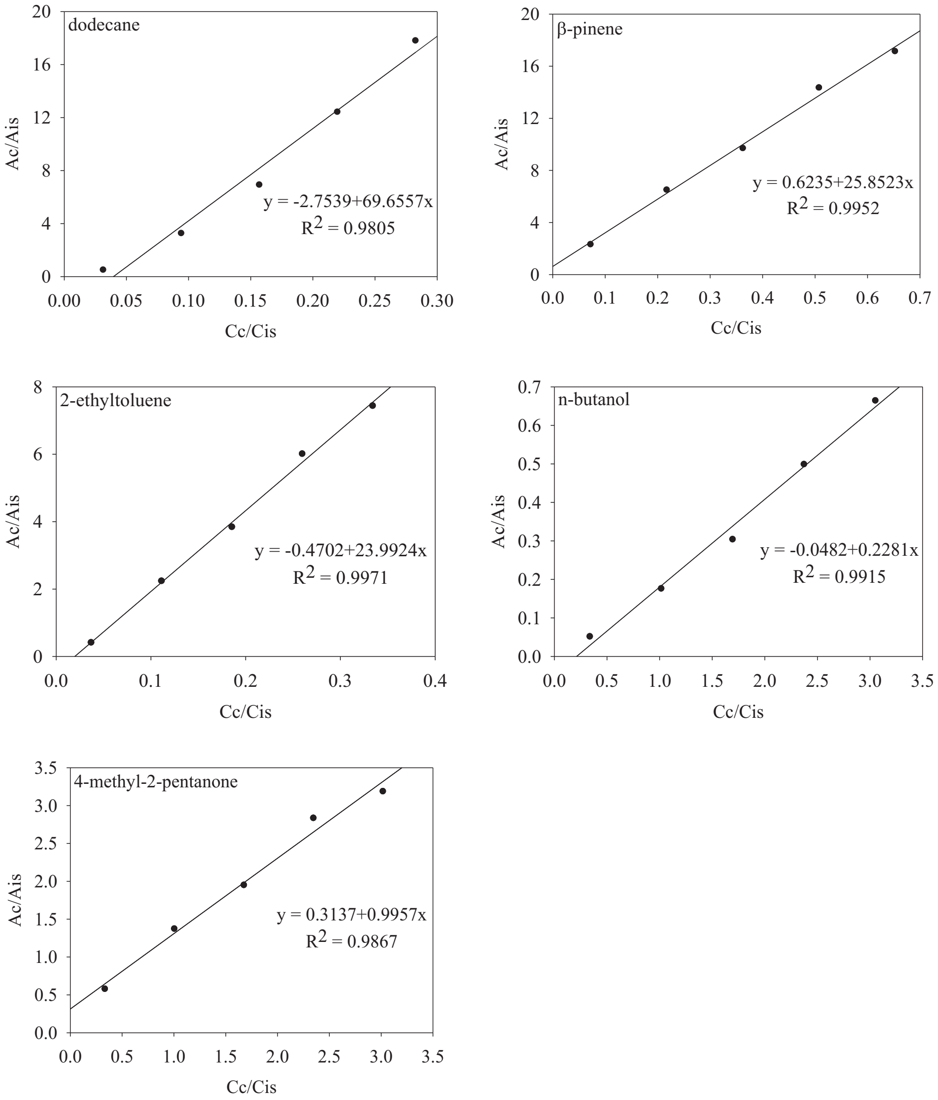

Fig. S11. Calibration curves of selected volatile organic compounds in poultry manure biochar ($B_{POULTRY}$).

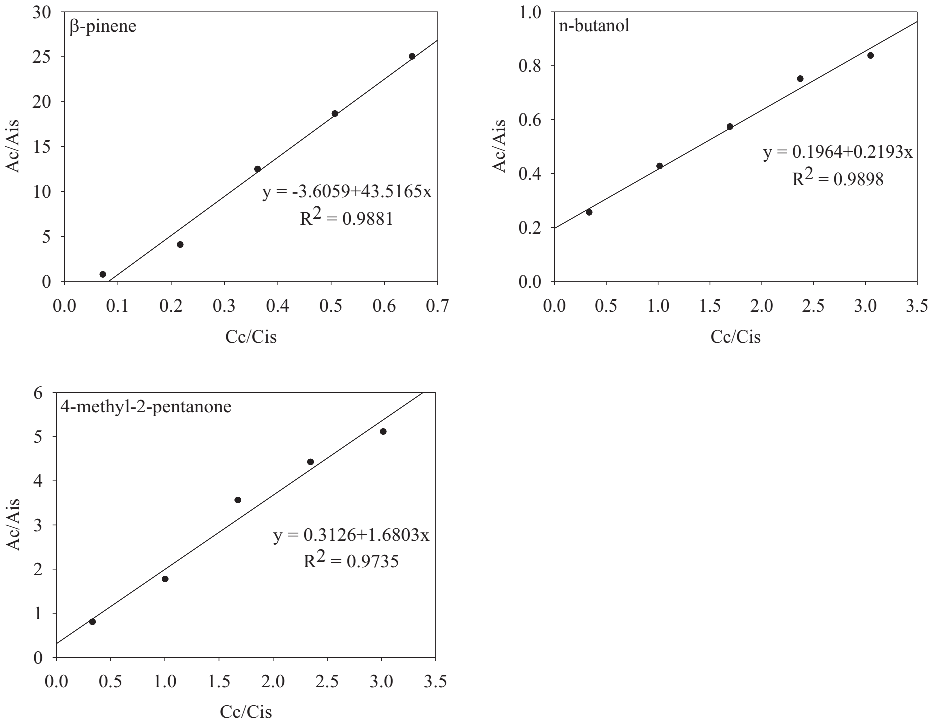

Figure S12: Calibration curves of selected volatile organic compounds in oak biochar ($B_{OAK}$).