Nativa, Sinop, v. 10, n. 3, p. 400-409, 2022.
Pesquisas Agrárias e Ambientais
DOI: https://doi.org/10.31413/nativa.v10i3.14123
ISSN: 2318-7670

Wood vinegar: chemical characteristics, phytotoxic effects, and impacts on greenhouse gas emissions

Marina Moura MORALES[1]*, Wyllian Winckler SARTORI[2], Bruno Rafael SILVA[2], Silvio Tulio SPERA[3], Andréa Beatriz Divério MENDES[4], Eliane Papa AMBROSIO-ALBUQUERQUE[4]

[1]Brazilian Agricultural Research Corporation (Embrapa Florestas), Colombo, PR, Brazil.
[2]Federal Institute of Education, Science and Technology of Mato Grosso, Sinop, Brazil.
[3]Brazilian Agricultural Research Corporation (Embrapa Agrosilvopastoral), Sinop, MT, Brazil.
[4]State University of Maringá, Maringá, PR, Brazil.
E-mail: marina.morales@embrapa.br
ORCID: (0000-0001-9125-7239; 0000-0001-8561-7808; 0000-0001-5458-7941; 0000-0001-5344-7509; 0000-0002-7955-1168; 0000-0002-8874-6047)

Submitted on 2022/07/15; Accepted on 2022/09/05; Published on 2022/09/19.

ABSTRACT: Wood vinegar has been used for over a century as a fertilizer and antimicrobial agent, but its impacts on ecosystems are poorly understood; further research is necessary to understand its chemical characteristics and avoid negative impacts. This study assessed the chemical characteristics, phytotoxicity, potential cytotoxicity, and greenhouse gas emissions of wood vinegar made from slow pyrolysis in a hot-tail kiln using cambara wood (*Qualea sp.*). Incubation experiments with varying concentrations of wood vinegar were established in samples of clayey, loamy, and sandy tropical soils, measuring CO_2, N_2O, and CH_4 over a 120-day period. Toxic effects on the germination, root tips, and meristematic cells of *Lactuca sativa* were also assessed. The findings confirmed that wood vinegar can function as a chemical fertilizer and pesticide, as well as a co-solvent for chemicals, particularly in agricultural and pharmaceutical applications, while the phytotoxicity indicated that this substance must be diluted for agricultural uses. Wood vinegar was seen to inhibit CO_2 and N_2O emissions from loamy and clayey soils, but this effect was not observed in sandy soil. Wood vinegar also blocked cell division in some dilutions, but at concentrations of less than 0.5% it did not present a potential risk to the environment or plants in general.
Keywords: pyroligneous acid; biomass; pyrolysis; residues; cytotoxicity.

Vinagre de madeira: características químicas, efeitos fitotóxicos e impactos nas emissões de gases

RESUMO: O vinagre de madeira é usado há mais de um século como fertilizante e agente antimicrobiano, mas seus impactos nos ecossistemas são pouco conhecidos; pesquisas são necessárias para entender suas características e evitar impactos negativos. Este estudo avaliou as características químicas, fitotoxicidade, potencial citotoxicidade e emissões de gases do vinagre de madeira obtido a partir de pirólise lenta em forno de cauda quente utilizando madeira de cambará (*Qualea sp.*). Experimentos de incubação com concentrações variadas do vinagre foram estabelecidos em amostras de solos tropicais argilosos, textura média e arenosos, medindo CO_2, N_2O e CH_4 durante 120 dias. Efeitos tóxicos no modelo *Lactuca sativa* também foram avaliados. Os resultados confirmaram que o vinagre de madeira pode funcionar como fertilizante químico e pesticida, bem como um co-solvente para produtos químicos, principalmente em aplicações agrícolas e farmacêuticas, enquanto a fitotoxicidade indicou que essa substância deve ser diluída para uso agrícola. O vinagre de madeira parece inibir as emissões de CO_2 e N_2O de solos argilosos e textura média, mas esse efeito não foi observado em solo arenoso. O vinagre de madeira também bloqueou a divisão celular em algumas diluições, mas em concentrações inferiores a 0,5% não apresentou risco potencial ao meio ambiente ou às plantas em geral.
Palavras-chave: ácido pirolenhoso; biomassa; pirólise; resíduos; citotoxicidade.

1. INTRODUCTION

Charcoal has been used as an energy source for thousands of years. Although for most of this time it was the main product (biofuel and biochar) of conventional pyrolysis, recently by-products of this process such as wood vinegar have become increasingly important, specifically as a pesticide and a solvent for chemical pesticides, but also for fertilizer, once it has plant nutrients bioavailable (TIILIKKALA et al., 2010).

Wood vinegar (also known as pyroligneous acid) starts as smoke from the charcoal kiln that is usually channeled into a long pipe to permit condensation. This liquid is then left to stand for several weeks, forming three layers: light oil on top, translucent brown wood vinegar in the middle, and thick wood tar at the bottom. Only translucent brown wood vinegar is used for agricultural purposes (MUNGKUNKAMCHAO et al., 2013).

Wood vinegar can contain more than 200 compounds including phenols, polyphenols, acetic acid (Velmurugan et al., 2009), ketones, esters, aldehydes, and alcohols (ZHAI et al., 2015). Because of their low pH and high organic load, these substances cannot be disposed in the environment without treatment (Fagernas et al., 2012), and must be diluted or neutralized.

Depending on its concentration, wood vinegar exhibits a high degree of antimicrobial activity against various microorganisms (Ma et al., 2011; Yang et al., 2016) and can stimulate microbial activity in soils (STEINER et al., 2008).

Additionally, because it is manufactured under various conditions, wood vinegar can differ in its chemical composition and toxicity, and risks to human health have been reported (MUKHTAR et al., 1982; SCHOKET et al., 1990; SCHMID; KORTING, 1996). Similar risks must be considered for plant and soil uses; for example, it is important to determine whether polyaromatic hydrocarbons (PAHs) are present, since they are stable in the environment and rapidly transported to humans through the food chain (BASAVAIAH et al., 2017; PETROVA et al., 2017). The US Environmental Protection Agency (1993) has listed 16 PAHs as priority pollutants, including naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(α)anthracene, chrysene, benzo(β)fluoranthene, benzo(ϰ)fluoranthene, benzo(α)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a, a)anthracene, and benzo(ghi)perylene.

Applying wood vinegar to the soil can also impact production of greenhouse gases; because a main constituent, acetic acid, is substrate for methanogens, it may increase CH4 emissions (KYUMA, 2004). It can also decrease N_2O emissions by preventing the dissociation of NH4+ into liquid NH_3 concentration by reducing pH (SIGUNGA et al., 2002). Furthermore, CO_2 emission is stimulated by microbial activity in the soil after application of wood vinegar (Steiner et al., 2008). It is important to note that greenhouse gases such as CO_2, N_2O, and CH_4 account for 60% of total atmospheric emissions (CHANGE, 2007).

However, the impact of wood vinegar on plant and soil toxicity, amendments to reduce greenhouse gas emissions, as well the different concentrations of this substance, are not well understood in soils. Further, research is necessary to understand the chemical characteristics that justify agricultural applications of wood vinegar and avoid negative environmental consequences. This study investigated wood vinegar's effects on greenhouse gases emission (CO_2, CH_4, and N_2O) from soil, soil toxicity via PAHs and organic compounds, bioavailable nutrient content, pH buffer capacity, and phytotoxicity.

2. MATERIAL AND METHODS

2.1. Wood vinegar production

The wood vinegar was made from cambara wood (*Qualea* sp.) sawdust by local small-scale producers in a hot-tail kiln in Vilhena, Rondonia state-Brazil. The wood vinegar production details are described in Morales et al., 2019.

2.2. Chemical characterization of wood vinegar

The total carbon (TC), total inorganic carbon (TIC), and total organic carbon (TOC) were analyzed in WVcam by Elementar Analysensysteme GmbH. The pH titration curve was analysed by continued addingadition of 0.5 ml of 0.1 N NaOH to 50 ml of WEBcam, until pH stabilization. Additional chemical analysis of plant nutrients (Ca, Mg, Cu, Mn, K and Zn), organic compounds and Poly-aromatic Hydrocarbon (PAH) Analysis The total PAH analysis are described in detail in Morales et al. (2019).

The majority and main chemical and/or pharmaceutical usefulness of the organic compounds was investigated using the flow databases: SciFinder (scifinder-cas.ez103.periodicos.capes.gov.br) and ChemSpider (http://www.chemspider.com) databases.

2.3. Soil preparation and incubation

Soils chemical analysis (Table 1) was performed according to Brazil, 2007 to determine: total N by the oxidation method with perchloric acid and extraction by sulphuric acid determined by semi-micro Kjeldahl distillation; pH in water (1:2.5); organic C by the volumetric oxidation method with $K_2Cr_2O_7$ and titration with ammonium ferrous sulfate; total Ca, Mg, Cu, Fe, Mn, and Zn via extraction with nitric-perchloric acid solution and spectrometry of atomic absorption; total K by extraction with nitric-perchloric acid solution and determination by flame photometry; total S by extraction with nitric-perchloric acid solution and determination by photocolorimetry; total P was analyzed by digestion with H_2SO_4 and H_2O_2.

The incubation experiment was set up at 25 °C at field capacity, which offers optimum conditions for many microbial processes and was used to maximize microbial activity. The WVcam was added at field capacity, at rates of 0, 1.25, 2, 50, and 100%, to 5 g samples of tropical soil classes, namely clayey (Ferralsol Haplic Dystric Clayic), loamy (Ferralsol Haplic Dystric Loamic), and sandy (Ferralsol Plinthic Dystric Arenic) soil (FAO, 2014).

The CO_2, N_2O, and CH4 were measured at 1, 3, 7, 11, 14, 21, 28, 49, 60, 81, and 120 days in a gas chromatograph equipped with a dual electron capture detector (ECD) for N_2O, flame ionization detector (FID) for CO_2 and CH_4, and column and injector. Ultrapure nitrogen was used as the carrier gas at an inlet pressure of 300 kPa (40 psi) and as the detector make-up gas at a flow rate of 25 ml min^{-1}.

To CO_2, N_2O, and CH_4 emissions were calculated by the equation below, exemplified for CO_2:

$$([CO_2\ (mg\ \ell\text{-}1) = \{\ [CO_2]\ (g\zeta^{-1}).\ \text{Pot volume}\ (\zeta)]/\ \text{soil mass}\ (g))\ .\text{Pot pressure}\ (atm)\ /\ R\ (atm\ \zeta\ mol^{-1}\ K^{-1}.\text{Pot temperature}\ (K)\ .\ CO_2 gmol^{-1}$$

2.4. Cytotoxicity analysis

2.4.1. Germination and root growth assay

This study was previously established to determine the concentrations used in phytotoxicity and cytotoxicity testing. Seeds of *L. sativa* L. (2n = 2x = 18) var. "Grand rapids TBR" (Feltrin Seeds Brazil, Farroupilha, RS, Brazil) were purchased at local garden supply stores, separated into plates containing 100 seeds, then placed on germination paper with 5 mℓ of WVcam solution at 0.5, 1.25, 5, 25, 50, and 100%. Distilled water was used as a negative control solution. The tests were performed with two replicates and incubated for 4 days at 25 °C in a BOD incubator. The percentage of germinated seeds (total germinated seeds/total seeds per treatment × 100) was determined and root growth was measured after 48, 72, and 96 h of exposure time.

Based on pre-tests, a cell cycle analysis was conducted with 0.5% WVcam to evaluate the wood vinegar's phytotoxic potential. The roots were collected per Petri dish per treatment and fixed in a fresh cold solution of ethanol and acetic acid (3:1 v/v). To prepare the slides, the meristematic region was boiled in 2% acetic orcein, transferred to a slide, covered with a coverslip, and carefully pressed into a drop of 2% acetic orcein solution. These were analyzed under a light microscope and approximately 3,000 cells per treatment were counted. The parameters analyzed included mitotic index (calculated as the number of dividing cells as a fraction of the total observed cells) and chromosomal aberrations (expressed as percentage of aberrations found in the total number).

2.5. Statistical analysis

Statistical analysis of WVcam biodegradation (CO_2) and N_2O emissions were performed via three-way ANOVA to test the effects of soil texture, concentration of WVcam, and time; the differences in mean values were tested using the Tukey test ($p<0.05$). Because CH_4 emission was unaffected, this data will not be presented.

The data were also tested by comparing curves for cumulative CO_2 and N_2O; the linear model was best suited and utilized for the treatments in soil textures separately, and the model coefficients were compared for equality. To do so, the likelihood ratio test was used, with accuracy determined by the chi-square (χ 2) statistic (REGAZZI; SILVA, 2010). This method involves adding two independent variables, D1 and D2, to calculate the maximum likelihood estimates of the parameters under no restrictions in the parametric space representing the complete model, and under restriction in the reduced model. The complete model was adjusted under no restrictions and the reduced model was adjusted to restrictions defined in H0.

Seed germination and root growth data were subjected to repeated measure ANOVA, and Tukey's test ($p<0.05$) was applied to the differences in mean values.

Table 1. Chemical analysis of the clayey, loamy, and sandy soil samples.
Tabela 1. Análises químicas das amostras de solo argiloso, textura média e arenoso.

Class	pH	N	P	K	Ca	Mg	Al	H+Al	SOM	OC	C/N	S
Soil	-	g kg^{-1}	mg kg^{-1}	------------------------cmol$_c$ dm^{-3}----------					--------g kg^{-1}--------		-	mg kg^{-1}
Clayey	5.6	1.3	25.9	0.08	1.36	0.25	0.33	5.00	34.2	19.9	15	11.0
Loamy	6.0	0.9	78.9	0.13	2.66	1.28	0	3.00	37.3	21.7	24	3.0
Sandy	5.4	0.7	19.4	0.12	1.29	0.66	0.20	6.10	24.8	14.4	21	1.0
Class	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**B_{Sum}**	**CEC**	**V**	**m**	**Clay**	**Silt**	**Sand**
Soil	-------------------------- mg kg^{-1}-------------------------					------cmol$_c$ dm^{-3}----		-----------%----------		----------------g kg^{-1}------------		
Clayey	0.13	0.4	44	4.6	1.4	1.7	6.7	25	16	632	34	334
Loamy	0.28	1.1	43	14.5	4.0	4.1	7.1	57	0	247	20	753
Sandy	0.20	1.0	71	4.7	3.1	2.1	8.2	25	9	182	43	815

CEC = cation exchange capacity; SOM= soil organic matter; H+Al = potential acidity; BSum = sum of soil bases (Ca, Mg and K); V (%) = soil base percent saturation; m (%) = soil aluminum percent saturation.

3. RESULTS

3.1. Chemical characteristics of wood vinegar

WVcam exhibited the capability to partially replace chemical fertilizer (Table 2), most notably due to its Mn content (Brasil e Abastecimento., 2016). Use of this substance as a fertilizer is becoming more important for many crops; it cannot entirely replace soil fertilization but can supplement a sound soil fertilization program (POLTHANEE et al., 2015). However, its use is generally based on local knowledge rather than scientific research (Tiilikkala et al., 2010), which poses certain environmental risks for soil and water contamination, toxicity, and impacts on soil microbiota.

Table 2. Wood vinegar (WVcam) chemical characteristics.
Tabela 2. Características químicas do vinagre de madeira.

Parameters		WVcam
pH	___	2.68
P	(mg l^{-1})	0.46
K		3.33
Ca		4.87
Mg		0.76
Mn		5.63
Cu		
Zn		0.13
TIC		1.64
TOC		275.59

Source: Morales et al., 2019.

WVcam contains 38 kinds of organic compounds belonging to four main groups (Table 3). The phenol group is the primary group: the main chemicals compounds are cresol (11.70%), guaiacol (6.6%), and syringol (3.17%). Chemical compounds of wood vinegar determined via CG-MS analysis. The main chemical compound in the carboxyl group is acetic acid (10.28%); this weak acid is most likely the main reason for the low pH values and buffer capacity (Table 3, Figure 1).

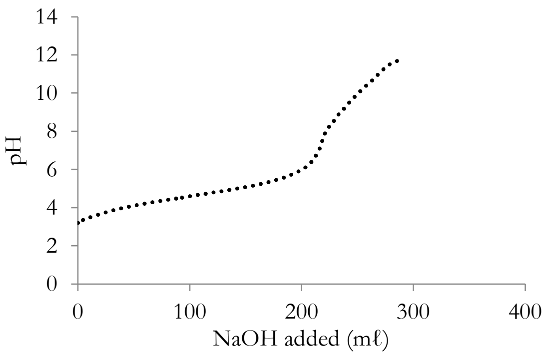

Figure 1. Titration curve for wood vinegar (WVcam) made with Cambara (*Qualea sp.*). Source: Adapted from Morales et al., 2019.
Figura 1. Curva de titulação do vinagre de madeira (WVcam) feito com Cambará (*Qualea sp.*). Fonte: Adaptado de Morales et al, 2019.

The Figures 3 and 4 and Table 5 illustrates the results of cytotoxicity analysis. In the cell cycle analysis, germination and root growth was impeded in *L. sativa* seeds treated with WVcam in concentrations above 1.25% (Figure 3a,b). At 0.5%, WVcam inhibited seed germination and root growth, which can be explained by the inhibitors such as phenolic compounds contained in wood vinegar (Table 3).

Table 3. Wood vinegar (WVcam) chemical compounds determined by CG-MS analysis.
Tabela 3. Componentes químicos do vinagre de madeira determinado por CG-MS.

Compounds	Usefulness WV_{cam}	References	
5-methyl-2-Furancarboxaldehyde or 5-methylfurfural	3.84	Chemical for synthesis/manufacture of fine chemicals,[1,2] potential candidate for treating sickle cell disease[3]	[1](Merck, 2017b); [2](Sigma-Aldrich, 2017b); [3](Abdulmalik et al., 2005).
1-hydroxy-2-propanone or acetol	0.43	Important intermediate used to produce polyols and acrolein;[1] widely used as a reduced dye in the textile industry[2] and as a skin tanning agent in the cosmetic industry, also adds aroma and flavor to foods[3]	[1](Zhu et al., 2013); [2](Soucaille et al.); [3](Mohamad et al., 2011).
2-Cyclopenten-1-one	0.44		
2-methyl-cyclopenten-1-one	0.32		
2-acetylfuran	0.50		
3-methyl-2-cyclopenten-1-one	0.39		
Methyl 4-Hydroxy-3-methoxybenzoate	0.46		
Carbonyl	6.38		
Acetic acid	10.28	Mainly used in industrial chemicals, to produce polymers derived from vinyl acetate production of purified terephthalic acid, which is used to produce polyethylene terephthalate (PET). Raw material for acetic anhydride and acetate esters, which like acetic acid itself, are widely used as solvents.[1,2] In the food industry, used as an acidity regulator[3]	[1](Icis, 2017); [2](Le Berre et al., 2013); [3](Scienceofcooking).
Propionic acid	0.82		
Ethenyl ester	0.72		
Butanoic acid	0.36		
Octanoic acid	0.28		
Carboxyl	12.46		
2-methoxy-Phenol or Guaiacol	6.6	Antimicrobial,[1,2] reduces gastric erosions induced by classic anti-inflammatory drugs (ibuprofen)[3], antidiarrheal agent,[4] and antioxidant[5]	[1](Sigma-Aldrich, 2017d); [2](Cooper, 2013); [3](Fossati et al., 1991); [4](Greenwood-Van Meerveld et al., 1999); [5](Yang et al., 2016).
4-methoxy-3-methyl-Phenol	0.93		
2,6-dimethyl-Phenol	0.52		
2-methoxy-5-methyl-Phenol	0.95		
2-methoxy-4-methyl-Phenol or creosol	11.70	Flavor Standards, Food and Cosmetic Component Standards[1,2], antidiarrheal agent[3]	[1](Sigma-Aldrich, 2017a); [2](Sigma-Aldrich, 2017c); [3](Greenwood-Van Meerveld et al., 1999).
2-methyl-Phenol or o-cresol	2.42		
Phenol	1.75		
4-ethyl-2-methoxy-Phenol or 4-Ethylguaiacol	8.92		
4-ethyl-3-methyl-Phenol	0.77		
3-ethyl-5-methyl-Phenol	0.38		
2-ethyl-Phenol	0.30		
2,5-dimethyl-Phenol	0.91		
2,4-dimethyl-Phenol	0.49		
2-methoxy-4-propyl-Phenol	3.46		
2,6-dimethoxy-4-(2-propyl) Phenol	0,48		
2-methoxy-4-(1-propyl-Phenol)	0.48		
2-methoxy-4-(2-propenyl) Phenol	1.16		
3,4-dimethyl-Phenol	0.77		
3,4,5-trimethyl-Phenol	0.81		
4-ethyl-Phenol	1.44		
2,6-dimethoxy-Phenol or syringol	3.17	antioxidant[1,2]	[1](Yang et al., 2016); [2] (Loo et al., 2008)
4-propyl-syringol	3.10	Component of wood adhesives[1]	[1](Pimenta, 2007)
Phenol	58.26		
7,8-dimethyl benzo cyclooctene	4.55		
Silicates	4.55		
3,4-dimethoxy-toluene	0.82		
Benzenethanol or Phenylethyl alcohol	4.33	Chemical for synthesis,[1] anti-infective agent and desinfectant[2]	[1][(Merck, 2017a)], [2][(Chemspider, 2017)].
Others	5.62		

Source: Morales et al., 2019.

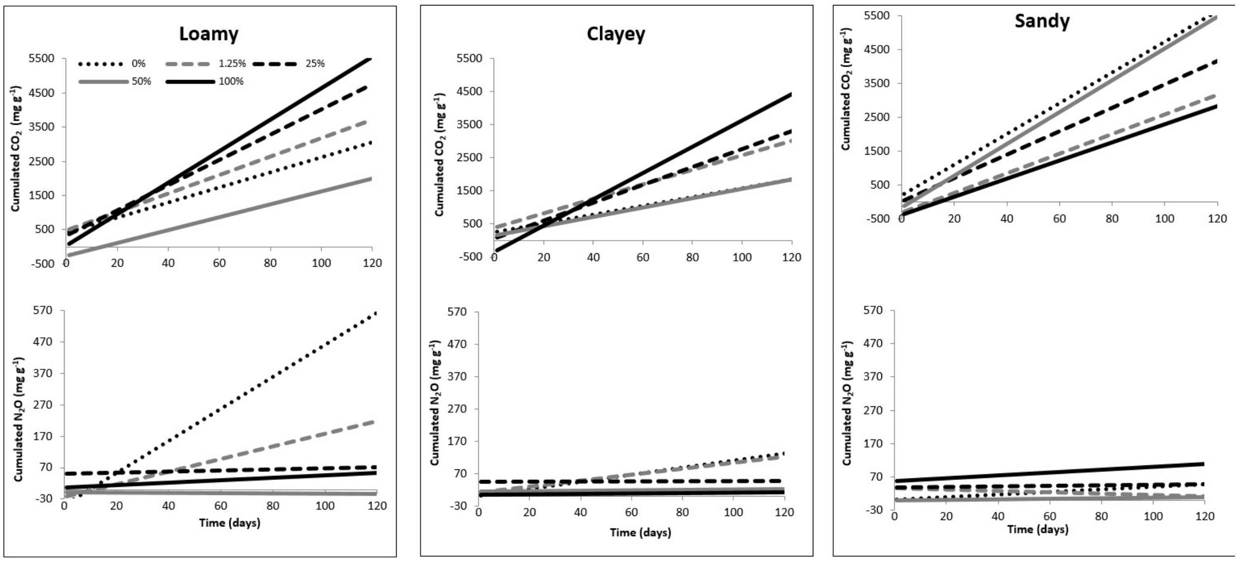

Figure 2. Cumulative C-CO_2 and N-N_2O emissions in incubations of loamy, clayey and sandy soils without WVcam (0%), and treated with 1.25, 25, 50, and 100%, until 120 days.
Figura 2. Emissões acumuladas de C-CO_2 e N-N_2O em solo argiloso, arenoso e de textura média sem WVcam (0%) e tratados nas concentrações de 1.25, 25, 50 e 100%, por 120 dias.

Table 4. Concentration of individual and total polyaromatic hydrocarbons (PAHs) in wood vinegar.
Tabela 4. Concentrações individuais e totais dos hidrocarbonetos poliaromáticos (HPAs) no vinagre de madeira.

Polyaromatic hydrocarbons	WV_{cam} (ng g^{-1})
Naphthalene	18.73
2-Methylnaphthalene	5.25
1-methylnaphthalene	151.72
Acenaphthylene	134.00
Acenaphthene	31.49
Fluorene	10.50
Phenanthrene	4.81
Anthracene	0.58
Fluoranthene	-
Pyrene	-
Benzo(a)anthracene	-
Chrysene	-
Benzo(b)fluoranthene	-
Benzo(k)fluoranthene	-
Benzo(a)pyrene	-
Indeno(1.2.3-cd)anthracene	-
Dibenz(a,h)anthracene	-
Benzo(g,h,i)perylene	-
Total	**357.12**

Source: Morales et al., 2019.

Table 5. Mean percentage of chromosome aberrations in meristematic lettuce cells, total cells evaluated, and mean mitotic index from lettuce root cells
Tabela 5. Porcentagem média de aberrações cromossômicas em células meristemáticas de alface, total de células avaliadas e índice mitótico de células de raiz de alface

$WV_{cam\ concentration}$		0% (Control)	0.5%
Cell cycle phase		%	%
Interphase	Normal	91.51	92.39
Prophase	Normal	3.49	1.93
	Abnormal	0.00	0.1
Metaphase	Normal	2.44	1.70
	Abnormal	0.00	0.46
Anaphase	Normal	1.27	0.98
	Abnormal	0.14	0.85
Telophase	Normal	1.15	1.32
	Abnormal	0.00	0.27
N Total		100	100
Total of abnormal cells		0.13	1.63
Mitotic Index		8	17

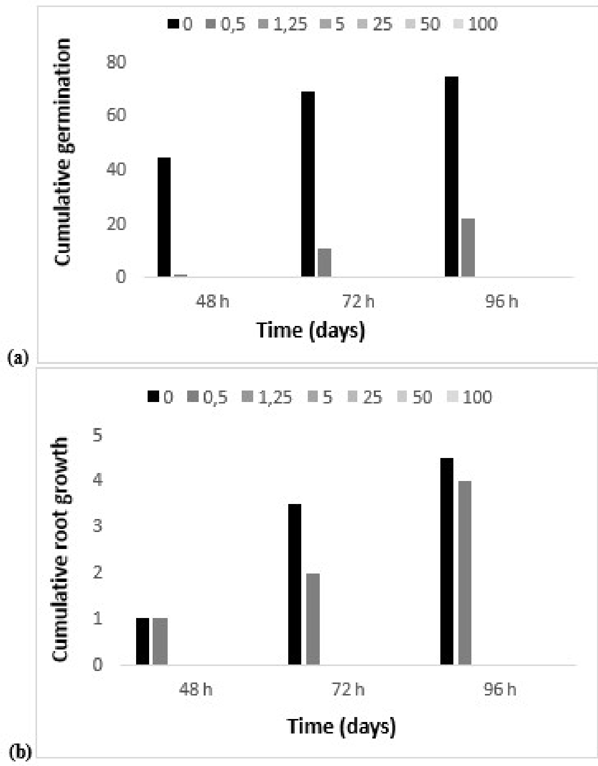

Figure 3. Cumulative germination (a) and root growth (b) of *L. sativa* over time when treated with different concentrations of WC_{cam}.
Figura 3. Germinação cumulativa (a) e crescimento da raiz (b) de *L.sativa* em diferentes tempos e concentrações de WC_{cam}.

4. DISCUSSION

A good example of how wood vinegar can be used as a fertilizer can be seen in dry weather, because of the water it contains. It is well known that a minimum level of soil moisture is necessary for plants to absorb nutrients via roots. During prolonged periods of dry weather, even fertilization with wood vinegar will not be able to positively impact yields, but during short dry spells this substance may offer an alternative to maintain productivity.

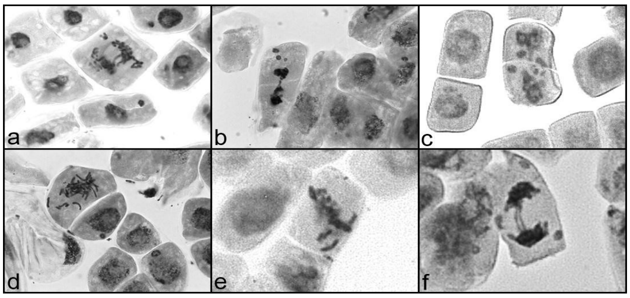

Figure 4. Chromosomal alterations observed: a, f) bridges at anaphase; b) bridge at telophase; c,d) C-metaphases; e) unoriented chromosomes at metaphase.
Figura 4. Alterações cromossômicas observadas: a, f) pontes na anáfase; b) ponte na telófase; c,d) C-metáfases; e) cromossomos não orientados na metáfase.

The total organic carbon (TOC) that wood vinegar adds to the soil (Table 2) may also be an easily accessible source of energy for microorganisms, and may stimulate or inhibit plant growth and development due to its components, especially low-molecular organic compounds (GONET; DEBSKA, 2006).

It is important to remember that wood vinegar cannot be indiscriminately disposed of without treatment in the environment because of its low pH and high TOC values, or applied as a pesticide or chemical fertilizer in agriculture. The beneficial effects of wood vinegar for soils or plants are directly related to the dosage used (MU et al., 2004; MUNGKUNKAMCHAO et al., 2013; ZHAI et al., 2015; MAHMUD et al., 2016). The pH of WVcam can be inversely correlated with titratable acidity, as seen in Figure 1 (MONTAZERI et al., 2013). Unlike strong acids that are fully dissociated, the acids in wood vinegar are only partially ionized, which can be positively correlated with its carbonyl and carboxyl group content.

Wood vinegar may also be used as a co-solvent for agrochemicals, such as pesticides and growth regulators. For many of these substances pH is a critical factor, and some pesticides (particularly carbamate and organophosphate insecticides) are broken down when combined with high pH water. The rate and severity of the reaction are determined by the pesticide's susceptibility to hydrolysis (BAILEY; BILDERBACK, 1998). A pH of 5.5 to 6.5 is ideal for mixing most pesticides, which is why the directions for most commercial pesticides recommend adding a buffering or acidifying agent to the spray tank (FISHEL; FERREL, 2016). Wood vinegar can act as a pH buffer and acidifying agent (Figure 1) for mixing pesticides that require low pH, and can also add readily available plant nutrients (Table 2). Mixing wood vinegar and pesticides can improve costs and even boost the effectiveness of the pesticide (ALEXANDER, 1986; KIM et al., 2008).

A wide range of total parent PAH concentrations can be found in WVcam, from 0.58 to 151.72 ng g^{-1} (Table 4). While wood vinegar is mainly used in agriculture as a partial substitute for fertilizers and pesticides, contamination rates must be considered since these substances may spread throughout the surface soil and can potentially cause cancer in humans. Some PAHs are lipophilic and are easily dissolved and transported by human cell membranes (ROCHE et al., 2002).

According to Canadian soil quality guidelines to protect the environment and human health, the soil quality criteria level for PAHs is 1000 ng g^{-1}, which is higher than the concentrations observed in WVcam. We also determined that the WVcam had no potential carcinogenic risk, since the seven carcinogenic compounds (benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1.2.3-cd)anthracene, and dibenzo(a,h)anthracene) (SCHOENY; POIRIER, 1993) were not found.

Various factors in the production of wood vinegar such as chemical composition of the biomass and temperature can affect the yield and chemical composition, including the PAHs formed. Thermal PAH formation can occur over a wide range of temperatures; when they are low, the compound distribution is governed by thermal stability and more stable isomers are formed, while PAHs of higher formation enthalpy can be generated at higher temperatures (BAUMARD et al., 1999).

4.1. Effect of wood vinegar amendments on carbon dioxide and methane emissions from soil

CO_2 emissions initially increased, reaching maximum concentration at 28 days. At 80 days of incubation CO_2 emissions leveled off, indicating the stability of WVcam biodegradation (Figure 2). WVcam did not negatively affect the soil microbes responsible for soil mineralization, even though it contains a large number of phenolic compounds (Table 3) with antibacterial properties (LUCCHINI et al., 1990; EVANS et al., 1999).

Cumulative concentrations of CO_2 did not vary according to soil type; at 120 days the samples treated with WVcam emitted average values of 3281.5, 2653.3, and 3341.2 mg g^{-1} of CO_2 in clayey, loamy and sandy soils, respectively, while the control soil samples emitted 2,618.0, 1,465.7 and 4,816.0 mg g^{-1}, respectively. The sandy soil treated with the WVcam mixture had lower CO_2 emissions than the control, possibly because of the type of soil microbiota that was inhibited by the wood vinegar. Soil microbes in the loamy and clayey soils treated with the WVcam emitted more CO_2, which was also seen by another group of researchers investigating charcoal and wood vinegar (STEINER et al., 2008). Previous studies have reported that microorganisms and microbial activity change in agricultural soils as a result of the organic content in wood vinegar that is added, since these organic compounds provide a source of carbon for microorganisms in the soil (HANGER, 2013; LU et al., 2015).

The soils that were not treated with WVcam had no detectable CH_4 emissions. Similar results were observed in moist soil cores incubated in the laboratory at 25 ºC in Germany (KOSCHORRECK; CONRAD, 1993) and in Sri Lanka incubated at 30ºC (SENEVIRATNE; VAN HOLM, 1998). The soils treated with the WVcam mixtures also did not emit CH_4, even though wood vinegar contains a significant amount of acetic acid, which is a substrate for methanogens (KYUMA, 2004). It is important to remember that methane is 23 times more potent than carbon dioxide in trapping heat in the atmosphere (Foster et al., 2007), absorbing 15 to 40 times more radiation than CO_2 (LENZI; FAVERO, 2009).

4.2. Effect of amendments on nitrous oxide emissions

Soil texture, WVcam ratios, and incubation time affected N_2O emissions (Figure 2). In the sandy and loamy soil samples, emissions fell as WVcam concentrations increased, while they did not decrease in the clayey soil; the decrease was most significant in the loamy soil, peaking at 49 days of incubation. The clayey soil stabilized at 80 days, while the loamy soil continued to emit nitrous oxide. These differences can be related to physical attributes of the soil such as porosity and pore size distribution, and chemical and biological attributes such as organic matter content and soil microbiota activity (GAILLARD et al., 2016; SIGNOR; CERRI, 2013).

Clayey soils have greater protection provided by aggregates, which may explain the lesser effect of WVcam application in different concentrations. Microporosity is usually not significant in sandy soils, causing less of an effect from the WVcam, while loamy soils tend to have a balanced distribution of pores, which can significantly reduce N_2O emissions (GAILLARD et al., 2016; SKIBA; BALL, 2006).

WVcam inhibited N_2O emissions in the clayey and loamy soil samples at 1.25%, which could have resulted from the organic C input and soil pH change; amendment with wood vinegar could potentially encourage the activity of N_2O reductase from denitrifying microorganisms while inhibiting the activity of reductases involved in the conversion of NO_3^- to N_2O (YANAI et al., 2007; RANATUNGA et al., 2018).

Indeed, changes in soil microbial community structure and enzyme activity after the addition of wood vinegar have been reported (Lu et al., 2015; Yang et al., 2016). Factors such as soil microbe communities that contribute to reductions in N_2O in soils where wood vinegar is utilized, particularly alongside nitrogen fertilization, require additional study.

The chemical properties of WVcam that alter the soil environment (such as C source, pH, and microbial activity) are directly related to greenhouse gas emissions from agricultural soils (NIU et al., 2017). Understanding these changes and the effects of soil texture on gas emissions can help guide the proper use of wood vinegar in agriculture.

4.3. Phytotoxicity and cytotoxicity

The phenolic compounds are some of the most important and common plant allelochemicals in the terrestrial ecosystem (Li et al., 2010), the inhibition of seed germination and root growth occurred due this exposition by WV. In assessing cytotoxicity, mitotic index and chromosomal alterations were used to verify changes in the cell cycle. Table 5 presents the data for 0.5% concentrations of WVcam and the control (water), since no germination occurred at other concentrations. The mitotic index was higher than the control, suggesting cellular proliferation occurred. Meristematic tissues are susceptible to many biotic and abiotic stressors, which makes it possible to test the toxicity of some substances (MOLINA et al., 2006).

According to Souza et al. (2010), the mitotic index may be lower when exposed to cytotoxic substances because these may inhibit the cell cycle. However, cytotoxic potential can vary according to concentration used. Our findings indicate that 0.5% WVcam has no phytotoxic potential. Some chromosomal abnormalities were observed; the predominant abnormalities were bridges at anaphase and telophase, C-metaphases, and unoriented chromosomes at metaphase (Figure 4).

Table 6 shows the percentage of abnormal cells per phase of mitosis. Aneugenic events are characterized by loss of chromosomes resulting from the formation of c-metaphases and loss or non-orientation of chromosomes, indicating that the cell contains components that prevent microtubule polymerization which impedes the formation of the mitotic spindle. On the other hand, clastogenic events cause chromosome breakage, forming bridges and chromosomal adhesion. Breakage of chromosome segments may cause inter- or intrachromatin fusion, generating irreversible forms of adhesion or leading to cell death (CHIAVEGATTO et al., 2017).

Chromosomal bridges can break in random regions, generating telomere-free chromosomes that can be transferred to the next generation and start the bridge-fusion-break cycle. Increased condensation and nuclear fragmentation are the first signs of apoptosis.

According to Lemme; Marin-Morales (2008) a complex mixture of hydrocarbons may demonstrate clastogenic and aneugenic activities or even induce cell death in *Allium cepa* genetic material, due to the presence of PAHs. Wood vinegar contains a variety of PAHs (Table 4), and may have a similar effect in *L. sativa*. Despite the effects of PAHs on genetic material, Nobrega et al. (2021) investigated the impact of these substances on lettuce physiology and demonstrated that the changes in physiological behavior as well as morphology induced by these compounds are only significant in high concentrations.

5. CONCLUSIONS

Wood vinegar has the potential to partially replace chemical fertilizers and pesticides and serve as a co-solvent for agrochemicals. The findings of chemical analysis indicate that alongside its established uses in the chemical and pharmaceutical industries. Also, WVcam can be used in soils and alongside nitrogen fertilization, since it can prevent or reduce N_2O and CO_2 emissions, particularly in clayey and loamy soils.

WVcam presents no risk for environmental and vegetal behavior in concentrations less than 0.5%, which was confirmed by the fact that seeds germinated in this concentration and the mitotic index did not decrease compared to the control. Nevertheless, the chromosome alterations detected suggest that WVcam must be diluted for agricultural applications.

6. ACKNOWLEDGEMENTS

This work was supported by the Mato Grosso Foundation for Research Support (FAPEMAT), project 148211/2014. The soil samples were kindly provided by Marcos da Silveira. Thanks also to Embrapa Agrossilvipastoral librarian Aisten Baldan.

7. REFERENCES

ABDULMALIK, O.; SAFO, M. K.; CHEN, Q.; YANG, J.; BRUGNARA, C.; OHENE-FREMPONG, K.; ABRAHAN, D. J.; ASAKURA, T. 5-hydroxymethyl-2-furfural modifies intracellular sickle hemoglobin and inhibits sickling of red blood cells. **British Journal of Haematology**, v. 128, n. 4, p. 552-561, 2005. DOI: 10.1111/j.1365-2141.2004.05332.x

ALEXANDER, A. Optimum Timing of Foliar Nutrient Sprays. In: ALEXANDER, A. (Ed.). Foliar Fertilization: International Symposium on Foliar Fertilization, I, **Proceedings…** Organized by Schering Agrochemical Division, Special Fertilizer Group, Berlin (FRG) March 14–16, 1985. Dordrecht: Springer Netherlands, 1986. p. 44-60.

BAILEY, D.; BILDERBACK, T. Alkalinity control for irrigation water used in nurseries and greenhouses. North

Carolina: North Carolina State University, 1998. Available: < http://www.hort.vt.edu/ghvegetables/documents/Irrigation%20Fertility%20Media/NCSUAlkalinityContrl.pdf >.

BASAVAIAH, N.; MOHITE, R. D.; SINGARE, P. U.; REDDY, A. V. R.; SINGHAL, R. K.; BLAHA, U. Vertical distribution, composition profiles, sources and toxicity assessment of PAH residues in the reclaimed mudflat sediments from the adjacent Thane Creek of Mumbai. **Marine Pollution Bulletin**, v. 118, n. 1-2, p. 112-124, 2017. DOI: https://doi.org/10.1016/j.marpolbul.2017.02.049

BAUMARD, P.; BUDZINSKI, H.; GARRIGUES, P. Polycyclic aromatic hydrocarbons in recent sediments and mussels (Mytilus edulis) from the Western Baltic Sea: occurrence, bioavailability and seasonal variations. **Marine Environmental Research**, v. 47, n. 1, p. 17-47, 1999. DOI: https://doi.org/10.1002/etc.5620170501

BRASIL_Ministério da Agricultura, Pecuária e Abastecimento (MAPA). **Instrução Normativa nº 46 de 22 de novembro de 2016**. Brasília: Diário Oficial da União, n. 234, p. 4- 13, 07 dez. 2016. Available on: < http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=07/12/2016&jornal=1&pagina=4&totalArquivos=148 >. Acesso em: 20 ago. 2018.

BRAZIL_ Ministério da Agricultura, Pecuária e Abastecimento (MAPA). **Normative Instruction n. 28**. Manual de Métodos Analíticos Oficiais para Fertilizantes Minerais, Orgânicos, Organominerais e Corretivos. Brasília: Diário Oficial da União, n. 28, p. 11, 31 jul. 2007. Available on: https://www.gov.br/agricultura/pt-br/assuntos/insumos-agropecuarios/insumos-agricolas/fertilizantes/legislacao/manual-de-metodos_2017_isbn-978-85-7991-109-5.pdf. Acesso em: 15 set. 2022.

CANADIAN_**Canadian Soil Quality Guidelines for the Protection of Environmental and Human Health: Polycyclic aromatic hydrocarbons**. Yukon: Council of Ministers of the Environment, 2010. Available: < http://ceqg-rcqe.ccme.ca/download/en/320 >.

CHANGE., I.-I. P. O. C. **Climate change 2007:** fourth assessment report on climate change impacts, adaptation and vulnerability of the intergovernmental panel on climate change. Cambridge: Cambridge University, 2007. Available: < https://www.ipcc.ch/pdf/assessment-report/ar4/wg2/ar4_wg2_full_report.pdf >.

CHEMSPIDER. Phenylethyl alcohol. CHEMSPIDER 2017. Available on: http://www.chemspider.com/Chemical-Structure.553122.html

COOPER, R. A. Inhibition of biofilms by glucose oxidase, lactoperoxidase and guaiacol: the active antibacterial component in an enzyme alginogel. **International Wound Journal**, v. 10, n. 6, p. 630-637, 2013. DOI: https://doi.org:10.1111/iwj.12083

DILUSTRO, J. J.; COLLINS, B.; DUNCAN, L.; CRAWFORD, C. Moisture and soil texture effects on Soil CO2 efflux components in southeastern mixed pine forests. **Forest Ecology and Management**, v. 204, n. 1, p. 85-95, 2005. DOI: https://doi.org/10.1016/j.foreco.2004.09.001

EVANS, G. B.; FURNEAUX, R. H.; GRAVESTCK, M. B.; LYNCH, G. P.; SCOTT, G. K. The synthesis and antibacterial activity of totarol derivatives. Part 1: Modifications of ring-c and pro-drugs. **Bioorganic & Medicinal Chemistry**, v. 7, n. 9, p. 1953-1964, 1999. DOI: https://doi.org/10.1016/S0968-0896(99)00162-5

FAGERNAS, L.; KUOPPALA, E.; TIILIKKALA, K.; OASMAA, A. Chemical Composition of Birch Wood Slow Pyrolysis Products. **Energy & Fuels**, v. 26, n. 2, p. 1275-1283, 2012. https://doi.org/10.1021/ef2018836

FISHEL, F. M.; FERREL, J. A. **Water pH and the effectiveness of pesticides**. Gainesville. 2016

FOSSATI, A.; VIMERCATI, M. G.; BOZZI, M.; PASSAROTTI, C.; BANDI, G. L.; FORMENTI, A. Effects of metoxibutropate, ibuprofen and guaiacol on the gastrointestinal system. **International Journal of Tissue Reactions-Experimental and Clinical Aspects**, v. 13, n. 1, p. 45-50, 1991.

FORSTER, P.; RAMASWAMY, V.; ARTAXO, P.; BERNTSEN, T.; BETTS, R.; FAHEY, D.W.; HAYWOOD, J.; LEAN, J.; LOWE, D.C.; MYHRE, G.; NGANGA, J.; PRINN, R.; RAGA, G.; SCHULZ, M.; VAN DORLAND, R. Changes in Atmospheric Constituents and in Radiative Forcing (Chapter 2). In: SOLOMON, S.; QIN, D.; MANNING, M.; CHEN, Z.; MARQUIS, M.; AVERYT, K. B.; TIGNOR, M.; MILLER, H. L. (Eds.). **Climate Change 2007: the Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change**. Cambridge University Press; Cambridge (United Kingdom), 2007; p. 129-234; Available at http://www.ipcc.ch/pdf/assessment-report/ar4/wg1/ar4-wg1-chapter2.pdf

GONET, S. S.; DEBSKA, B. Dissolved organic carbon and dissolved nitrogen in soil under different fertilization treatments. **Plant Soil and Environment**, v. 52, n. 2, p. 55-63, 2006. DOI: 10.17221/3346-PSE

GREENWOOD-VAN MEERVELD, B.; TYLER, KUGE, OGATA. Anti-diarrhoeal effects of seirogan in the rat small intestine and colon examined in vitro. **Alimentary Pharmacology & Therapeutics**, v. 13, n. 1, p. 97-102, 1999. DOI: https://doi.org/10.1046/j.1365-2036.1999.00443.x

HANGER, M. **Potential of the slow pyrolysis products birch tar oil, wood vinegar and biochar in sustainable plant protection-pesticidal effects, soil improvement and environmental risks**. 2013. 42 f. Dissertação (Mestrado em Environmental Ecology). Faculty of Biological and Environmental Science, University of Helsinki, Lahti.

ICIS. Acetic acid prices, markets & analysis. 2017. Available: < https://www.icis.com/chemicals/acetic-acid/?tab=tbc-tab2 >.

KIM, D. H.; SEO, H. E.; LEE, S. C.; LEE, K. Y. Effects of wood vinegar mixed with insecticides on the mortalities of Nilaparvata lugens and Laodelphax striatellus (Homoptera: Delphacidae). **Animal Cells and Systems**, v. 12, n. 1, p. 47-52, 2008. DOI: https://doi.org/10.1080/19768354.2008.9647153

KOSCHORRECK, M.; CONRAD, R. Oxidation of atmospheric methane in soil - measurements in the field, in soil cores and in soil samples. **Global Biogeochemical Cycles**, v. 7, n. 1, p. 109-121, 1993. DOI: 10.1029/92GB02814

KYUMA, K. Paddy Soil Science. Kyoto University Press, 2004. 280p. Available: < https://books.google.com.br/books?id=IvjZM7jX3hEC >.

LE BERRE, C.; SERP, P.; KALCK, P.; TORRENCE, G. P. Acetic acid. In: FRITZ, U. E. A. (Ed.). **Ullmanns Encyclopedia of Industrial Chemistry**. Weinheim: Wiley-VCH, [Hoboken, N.J.], 2013. p.1-29.

LEME, D. M.; ANGELIS, D. D. F. D.; MARIN-MORALES, M. A. Action mechanisms of petroleum hydrocarbons present in waters impacted by an oil spill on the genetic material of Allium cepa root cells. **Aquatic Toxicology**, v. 88, n. 4, p. 214-219, 2008. DOI: http://dx.doi.org/10.1016/j.aquatox.2008.04.012

LENZI, E.; FAVERO, L. O. B. **Introdução à química da atmosfera: ciência, vida e sobrevivência**. Rio de Janeiro: LTC, 2009. 480p.

LI, Z.; WANG, Q.; RUAN, X.; PAN, C.; JIANG, D. Phenolics and Plant Allelopathy. **Molecules**, v. 15, n. 12, p. 8933-8952, 2010. DOI: https://doi.org/10.3390/molecules15128933>

LOO, A. Y.; JAIN, K.; DARAH, I. Antioxidant activity of compounds isolated from the pyroligneous acid, Rhizophora apiculata. **Food Chemistry**, v. 107, n. 3, p. 1151-1160, 2008. DOI: 10.1016/j.foodchem

LU, H. F.; LASHARIA, M. S.; LIU, X.; JI, H.; LI, L.; ZHENG, J.; KIBUE, G. W.; JOSEPH, S.; PAN, G. Changes in soil microbial community structure and enzyme activity with amendment of biochar-manure compost and pyroligneous solution in a saline soil from Central China. **European Journal of Soil Biology**, v. 70, p. 67-76, 2015. DOI: https://doi.org/10.1016/j.ejsobi.2015.07.005

LUCCHINI, J. J.; CORRE, J.; CREMIEUX, A. Antibacterial activity of phenolic-compounds and aromatic alcohols. **Research in Microbiology**, v. 141, n. 4, p. 499-510, 1990. DOI: 10.1016/0923-2508(90)90075-2

MA, X. H.; WEI, K.; ZHANG, S.; SHI, L.; ZHAO, Z. Isolation and bioactivities of organic acids and phenols from walnut shell pyroligneous acid. **Journal of Analytical and Applied Pyrolysis**, v. 91, n. 2, p. 338-343, 2011. https://doi.org/10.1016/j.jaap.2011.03.009

MAHMUD, K. N.; YAHAYU, M.; SARIP, S. H.; RIZAN, N. H.; MIN, C. B.; MUSTAFA, N. F.; UJANG, S. N. S.; ZAKARIA, Z. A. Evaluation on Efficiency of Pyroligneous Acid from Palm Kernel Shell as Antifungal and Solid Pineapple Biomass as Antibacterial and Plant Growth Promoter. **Sains Malaysiana**, v. 45, n. 10, p. 1423-1434, 2016.

MERCK. **2-Phenylethanol for synthesis**. 2017a. Available: <https://www.merckmillipore.com/INTERSHOP/web/WFS/Merck-BR-Site/pt_BR/-/BRL/ViewProductDocument-ReachUsesForSUBID?SUBID=100000033867 >.

MERCK. **5-Methylfurfural for synthesis**. 2017b. Available: <http://www.merckmillipore.com/INTERSHOP/web/WFS/Merck-BR-Site/pt_BR/-/BRL/ViewProductDocument-ReachUsesForSUBID

MOHAMAD, M. H.; AWANG, R.; YUNUS, W. M. Z. W. A review of acetol: Application and production. **American Journal of Applied Sciences**, v. 8, n. 11, p. 1135-1139, 2011. DOI: https://doi.org/10.3844/ajassp.2011.1135.1139.

MONTAZERI, N.; OLIVEIRA, A. C. M.; HIMELBLOOM, B. H.; LEIGH, M. B.; CRAPO, C. A. Chemical characterization of commercial liquid smoke products. **Food Science & Nutrition**, v. 1, n. 1, p. 102-115, 2012. DOI: https://doi.org/10.1002/fsn3.9.

MU, J.; UEHARA, T.; FURUNO, T. Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants II: Composition of moso bamboo vinegar at different collection temperature and its effects. **Journal of Wood Science**, v. 50, n. 5, p. 470-476, 2004. DOI: DOI:10.1007/s10086-002-0472-z

MUKHTAR, H.; LINK, C. M.; CHERNIAK, E.; KUSHNER, D. M.; BICKERS, D. R. Effect of topical application of defined constituents of coal-tar on skin and liver aryl-hydrocarbon hydroxylase and 7-ethoxycoumarin deethylase activities. **Toxicology and Applied Pharmacology**, v. 64, n. 3, p. 541-549, 1982. DOI: https://doi.org/10.1016/0041-008X(82)90251-4

MUNGKUNKAMCHAO, T.; KESMALA T.; PIMRATCH S.; TOONSAN, B.; JOTHITYANGKOON, D. Wood vinegar and fermented bioextracts: Natural products to enhance growth and yield of tomato (Solanum lycopersicum L.). **Scientia Horticulturae**, v. 154, p. 66-72, 2013. https://doi.org/10.1016/j.scienta.2013.02.020

NIU, Y. H.; CHEN, Z.; MULLER, C.; ZAMAN, M. M.; KIM, D.; YU, H.; DING, W. Yield-scaled N2O emissions were effectively reduced by biochar amendment of sandy loam soil under maize - wheat rotation in the North China Plain. **Atmospheric Environment**, v. 170, p. 58-70, 2017. DOI: https://doi.org/10.1016/j.atmosenv.2017.09.050

NÓBREGA, J. A.; ROSA, L. S.; CARVALHO, J. C.; GUERRINO, P.S.P.; BOTERO, E. R. Efeitos da interação entre hidrocarbonetos policíclicos aromáticos contaminantes e o sistema fotossintético da alface (lactuca sativa, l). **Brazilian Journal of Animal and Environmental Research**, v. 4, n. 2, p. 1582-1593, 2021. DOI: https://doi.org/10.34188

PETROVA, S.; REZEK, J.; SOUDEK, P.; VANEK, T. Preliminary study of phytoremediation of brownfield soil contaminated by PAHs. **Science of the Total Environment**, v. 599, p. 572-580, 2017. DOI: https://doi.org/10.1016/j.scitotenv.2017.04.163

POLTHANEE, A.; KUMLA, N.; SIMMA, B. Effect of Pistia stratiotes, cattle manure and wood vinegar (pyroligneous acid) application on growth and yield of organic rainfed rice. **Paddy and Water Environment**, v. 13, n. 4, p. 337-342, 2015.

RANATUNGA, T.; HIRAMATSU, K.; ONISHI, T. Controlling the process of denitrification in flooded rice soils by using microbial fuel cell applications. **Agricultural Water Management**, v. 206, p. 11-19, 2018. https://doi.org/10.1016/j.agwat.2018.04.041.

REGAZZI, A. J.; SILVA, C. H. O. Testes para verificar a igualdade de parâmetros e a identidade de modelos de regressão não-linear em dados de experimento com delineamento em blocos casualizados. **Revista Ceres**, v. 57, n. 3, p. 315-320, 2010. DOI: https://doi.org/10.1590/S0034-737X2010000300005

ROCHE, H.; BUET, A.; RAMADE, F. Accumulation of lipophilic microcontaminants and biochemical responses in eels from the Camargue Biosphere Reserve. **Ecotoxicology**, v. 11, n. 3, p. 155-164, 2002. DOI: 10.1023/a:1015418714492

SCHMID, M. H.; KORTING, H. C. Coal tar, pine tar and sulfonated shale oil preparations: Comparative activity, efficacy and safety. **Dermatology**, v. 193, n. 1, p. 1-5, 1996. DOI: 10.1159/000246189.

SCHOENY, R.; POIRIER, K. **Provisional guidance for quantitative risk assessment of polycyclic aromatic hydrocarbons**. Washington, DC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, 1993. Available: < https://www.epa.gov/sites/production/files/2015-11/documents/pah-rpfs.pdf >.

SCHOKET, B.; KOSA, H. A.; PALDEAK, L.; HEWER, A.; GROVER, P. L.; PHILIPS, P. H. Formation of dna adducts in the skin of psoriasis patients, in human skin in organ-culture, and in mouse skin and lung following topical application of coal-tar and juniper tar**. Journal of Investigative Dermatology**, v. 94, n. 2, p. 241-246, 1990. DOI: 10.1111/1523-1747.ep12874576

SCIENCEOFCOOKING. **Molecules of Taste-Acetic Acid**. Available: < http://www.scienceofcooking.com/acetic_acid.htm >.

SENEVIRATNE, G.; VAN HOLM, L. H. J. CO2, CH4 and N2O emissions from a wetted tropical upland soil following surface mulch application. **Soil Biology & Biochemistry**, v. 30, n. 12, p. 1619-1622, 1998.

SIGMA-ALDRICH. **2-Methoxy-4-methylphenol**. 2017a. Available: < http://www.sigmaaldrich.com/catalog/product/aldrich/137316?lang=pt&region=BR >.

SIGMA-ALDRICH. **5-Methylfurfural**. 2017b. Available: < http://www.sigmaaldrich.com/catalog/product/aldrich/137316?lang=pt&region=BR >.

SIGMA-ALDRICH. **Food and Cosmetic Component Standards**. 2017c. Available: < http://www.sigmaaldrich.com/analytical-chromatography/analytical-products.html?TablePage=119198418 >.

SIGMA-ALDRICH. **Guaiacol**. 2017d. Available: < http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/2/g5502pis.pdf >.

SIGUNGA, D. O.; JANSSEN, B. H.; OENEMA, O. Ammonia volatilization from vertisols. **European Journal of Soil Science**, v. 53, n. 2, p. 195-202, 2002. https://doi.org/10.1046/j.1351-0754.2002.00454.x

STEINER, C.; DAS, K. C.; GARCIA, M.; FORSTER, B; ZECH, W. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. **Pedobiologia**, v. 51, n. 5-6, p. 359-366, 2008. DOI: https://doi.org/10.1016/j.pedobi.2007.08.002

TIILIKKALA, K.; FAGERNÄS, L.; TIILIKKALA, J. History and use of wood pyrolysis liquids as biocide and plant protection product history and use of wood pyrolysis liquids as biocide and plant protection product. **The Open Agriculture Journal**, v. 4, p. 111-118, 2010. DOI: 10.2174/1874331501004010111.

VELMURUGAN, N.; CHUN, S. S.; HAN, S. S.; LEE, Y. S. Characterization of chikusaku-eki and mokusaku-eki and its inhibitory effect on sapstaining fungal growth in laboratory scale. **International Journal of Environmental Science and Technology**, v. 6, n. 1, p. 13-22, 2009. DOI: 10.1007/BF03326056.

YANAI, Y.; TOYOTA, K.; OKAZAKI, M. Effects of charcoal addition on N2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. **Soil Science and Plant Nutrition**, v. 53, n. 2, p. 181-188, 2007. DOI: https://doi.org/10.1111/j.1747-0765.2007.00123.x

YANG, J. F.; YANG, C. H.; LIANG, M. T.; GAO, Z. J.; WU, Y. W.; CHUANG, L. Y. Chemical Composition, Antioxidant, and Antibacterial Activity of Wood Vinegar from Litchi chinensis. **Molecules**, v. 21, n. 9, 2016. DOI: 10.3390/molecules21091150

ZHAI, M.; SHI, G.; WANG, Y.; MAO, G.; WANG, D.; WANG, Z. Chemical compositions and biological activities of pyroligneous acids from walnut shell. **Bioresources**, v. 10, n. 1, p. 1715-1729, 2015. DOI: 10.15376/BIORES.10.1.1715-1729.

ZHU, H. L.; YI, X.; LIU, Y. HU, H.; WOOD, T. K.; ZHANG, X. Production of acetol from glycerol using engineered Escherichia coli. **Bioresource Technology**, v. 149, p. 238-243, 2013. DOI: 10.1016/j.biortech.2013.09.062.