DOI:10.1021/jf9030162

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Interaction of Phenol, *o*-Cresol, and *p*-Cresol with a Clay-Rich Soil Sample

Rafael Garrett Dolatto,[†] Iara Messerschmidt,[†] Betânia Fraga Pereira,[‡] Talita de Oliveira,[†] Clênio Nailto Pillon,[§] and Gilberto Abate*,[†]

[†]Departamento de Química, Universidade Federal do Paraná, C.P. 19081, 81531-990, Curitiba, PR, Brazil, [‡]FAPEG/EMBRAPA CLIMA TEMPERADO, BR 392, km 78, C.P. 403, 96001-970, Pelotas, RS, Brazil, and [§]EMBRAPA CLIMA TEMPERADO, BR 392, km 78, C.P. 403, 96001-970, Pelotas, RS, Brazil

The present paper describes an interaction study of phenol, *o*-cresol, and *p*-cresol with a rich-clay soil sample (clay content of 62.3%). Experiments performed using long contact times, in concentrations of 50.0 mg L^{-1} showed practically no signal of phenol, *o*-cresol, and *p*-cresol after 48, 72, and 120 h, respectively, suggesting a sorption process. Sorption experiments in the period of 24 h were carried out with the phenolic compounds in concentrations between 5.00 and 500.0 mg L^{-1}, and negligible interaction between the phenolic species and the soil was observed. Additional experiments were carried out using $HgCl_2$ or NaN_3 solution as biodegradation inhibitors. After 10 days of contact time in the presence of inhibitors, no alterations in the concentrations of the three compounds studied were observed, and the results suggest no sorption process, with the compounds being almost entirely biodegraded by the soil sample, or possibly the formation of nonextractable residues could occur.

KEYWORDS: Soil; phenolic compounds; sorption; biodegradation

INTRODUCTION

Phenol and phenolic compounds are an important class of environmental contaminants for soil and bodies of water. Natural sources of these compounds include animals' urine, decay of vegetation (*1*), degradation of lignin and humic substances (*2*, *3*), and the photochemical decomposition of natural substrates from plants cell walls by UV irradiation (*4*). However, anthropogenic sources of phenols comprise the major method of soil and water contamination. Among the principal sources of phenol wastes in the environment are oil refineries and the pharmaceutical and polymer industries (*5*−*7*). The appearance of phenol and its derivatives in the environment may be associated with the use of these compounds as intermediates in the synthesis of plastics, dyes, and pesticides (*8*).

Processes concerning phenol sorption and its derivatives are very important to the whole soil or its fractions, since they may serve as indicators of the impact degree, for the soil and to the groundwater. Phenol is a common constituent of contaminated soil and groundwater, besides being toxic to plants and aquatic life (*9*). Several mechanisms of association between phenolic compounds and soil minerals can occur, especially hydrogen bonding and van der Waals interactions (*10*). Although these compound classes are acidic molecules, they are weaker than carboxylic groups, with pK_a values above 9. Thus, the sorption process of the anion form of these species is generally not significant on soil organic matter (SOM) and layer silicate clays, because of the electrostatic repulsion between the molecules and the negative charge of the soil particles. On the other hand, the neutral form can be weakly sorbed by physical processes (*11*, *12*). These characteristics of phenolic species, as well as the high water solubility of most compounds (*1*) and low octanol−water partition coefficient (log K_{ow}) values, provide to phenolic compounds a high mobility and susceptibility to leaching in soils. A study performed with a clay-rich soil sample in the presence of an initial phenol concentration between 5 and 500 mg L^{-1} showed adsorption isotherms with a linear behavior. In this study, the authors observed a removal near 15% from the initial phenol concentrations. In spite of the great solubility in aqueous medium, the authors verified that, the greater the soil hydrophobicity, the higher phenol adsorption, in accordance with results observed for organically modified soil samples (*12*).

The high water solubility of organic compounds with polar groups such as phenolic −OH causes much more susceptibility to microbial degradation (*11*). As a result, microbial decomposition is responsible for the apparent enhancement of phenol adsorption by soils with the increase of the contact time (*13*). According to Viotti et al. (*14*), phenol is an organic pollutant that presents processes of adsorption, biodegradation, and volatilization. Therefore, special attention should be dedicated in order to distinguish the type of interaction process involved among a soil sample and the phenol compounds. An appropriate procedure is autoclaving the soil to avoid the microbial degradation of phenol during the sorption experiments (*15*). Also, sodium azide (*15*, *16*) or $HgCl_2$ (*14*, *16*) can be used to circumvent the microbial activity in soils during the sorption experiments. Shaw et al. (*17*) studied the effect of autoclaving on selected soil physical and chemical properties and compared the adsorption of 2,4-dichlorophenol in autoclaved and untreated soils. Khan and Anjaneyulu (*6*) report the influence of soil components on adsorption−desorption of

*Corresponding author. E-mail: gilberto@quimica.ufpr.br. Fax: 55 41 33613186.

 Published on Web 01/22/2010

phenol, *p*-nitrophenol, 4-chloro-2-nitrophenol, and 2,4-dichlorophenol on soils from an industrial area. The authors showed a reduction of 67.5%, 53.8%, and 24.2% in the adsorption capacity in the absence of organic matter, clay, and Fe/Al oxides, respectively, when compared to untreated soils. According this paper, desorption isotherms exhibited hysteresis at higher initial concentrations, suggesting a great degree of irreversibility. Shibata et al. (*18*) report a study related to the microbiological degradation of phenol and some of its alkyl-derivatives with Japanese paddy soil samples under aerobic and anaerobic conditions. Half-lives between 24 and 260 days for phenol and between 11 and 740 days for *p*-cresol were verified under anaerobic conditions. Under aerobic conditions, such as an aquatic environment, phenol and alkylphenols were degraded within several days. Some papers have been dedicated to discuss the methods of soil sterilization to study the interaction with chemicals (*15*−*17*).

Another approach dedicated to understand the behavior of phenolic compounds in soils is the influence of oxidoreductive enzymes. Phenolic compounds may be transformed by an oxidative coupling reaction, and the formation of less soluble compounds takes place with higher molecular weight than that of the parent compounds (*19*). Under this condition, the enzymatic oxidation products may interact with phenolic constituents from humic substances by cross-coupling reactions (*19*−*21*). In this case, the products formed may be strongly retained in the hydrophobic soil fraction, avoiding the bioavailability of phenolic compounds, due to the low aqueous solubility of the polymeric compounds (*22*). This subject has provided several studies concerning the use of enzymes in remediation of soil contaminated with phenolic compounds (*20*−*23*). According to Barriuso et al. (*24*), phenols are between the reactive groups that tend to yield a larger proportion of nonextractable residues (NER) in soils, and a small percentage of NER may be released from the soils.

The aim of this work was to study the interaction of phenol, *o*-cresol, and *p*-cresol with a soil sample rich in clay fraction that was collected in an experimental area near a shale industry. A comparison of mercuric chloride and sodium azide methods was performed to investigate the existence of phenolic compounds biodegradation. A reasonable water volume is generated in the oil shale industrialization, arising from the shale pyrolisis process. This water can be used as soil conditioner, due to being rich in micronutrients important to plants and crops. Nevertheless, a high content of other not desirable compounds, such as phenol and the derivatives *o*-cresol and *p*-cresol, can be present in this water. Although the water would be diluted previously to the application, it is very important to be aware of the phenolic compounds' behavior in the presence of the soil sample in order to prevent and avoid the leaching to groundwater.

MATERIALS AND METHODS

Soil Sample. The soil sample, classified as Ultisol, was collected in the experimental area of Embrapa Clima Temperado in São Mateus do Sul, 150 km distant from Curitiba City, Paraná State, Brazil. The region has no history of the application of herbicides or other chemicals. The soil samples were collected at depths between 0 and 20 cm from several different points and mixed to compose a sample. The soil was air-dried for three days. The sample was composed considering that 34.98%, 23.52%, and 41.49% of the sample passed through the sieves of 9 mesh (2 mm), 16 mesh (1 mm), and 60 mesh (0.25 mm), respectively. Such aggregate distribution represents the natural distribution of soil aggregate. Approximately 2 kg was kept in an oven at 60 °C for 24 h and stored in a desiccator to be employed in all experiments. Some characteristics of the sample are shown in **Table 1**.

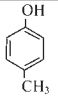
Table 1. Main Characteristics of the Soil Sample

characteristics	results
pH (0.01 mol L^{-1} $CaCl_2$ solution)[a]	4.50 ± 0.01
loss on ignition (%)[a]	19.79 ± 0.04
textural characteristics (%)	
sand	2.6
silt	31.1
clay	62.3
elemental analysis (%)[a]	
C	3.7 ± 0.3
H	2.2 ± 0.2
N	0.10 ± 0.02
CEC ($cmol_c$ kg^{-1})[a]	31.4 ± 0.3

[a]The results express the medium value of three experiments.

Apparatus and Reagents. An UV 2401 PC, Shimadzu spectrophotometer, with a 1.0 cm quartz cell was employed to obtain the spectra of the phenolic compounds in the ultraviolet (UV) and visible (VIS) regions and to quantify the remaining concentrations after the sorption experiments. A Gehaka PG-2000 potentiometer (resolution of 0.01 pH units) coupled to an Ag/AgCl combination glass electrode was utilized for all pH measurements and adjustments, when necessary. A Perkin-Elmer elemental analyzer CHN 2400 was utilized to determine the C, H, and N composition of the soil. The content of the main oxides present in the soil sample was estimated employing a Philips Analytical PW 2400/00 X-ray fluorescence (XRF) spectrometer equipped with a sampler changer 2510. The identification of the clay minerals was carried out by basal spacing using X-ray diffraction with a Philips Analytical X-ray diffractometer PW-1830. A flame photometer Digimed, DM-61, was utilized to determine the cation exchange capacity (CEC) by the sodium saturation method (*12*, *25*, *26*). Other auxiliary equipment, such as an orbital shaker, an oven, a centrifuge, and micropipets, was employed to perform all the experiments. Water used in all experiments was distilled and deionized using the Simplicity system from Millipore coupled to an UV lamp. The phenol standard solutions and all the analytical reagents were supplied by Merck, Aldrich, and Carlo Erba or similar companies. **Table 2** shows the selected properties of the phenol compounds under study. Phenol, *o*-cresol, and *p*-cresol solutions were prepared in concentrations of 1000.0 mg L^{-1}, standardized by the bromide-bromate method (*27*), and stored in stoppered amber glass bottles at 4 °C.

Table 2. Selected Properties of the Target Chemicals

Properties	*phenol*	*o-cresol*	*p-cresol*
Structure			
Molecular Weight (g mol^{-1})	94.11	108.14	108.14
Vapor Pressure (mmHg)	0.357 (20°C)	0.31 (25°C)	0.13 (25°C)
Water Solubility at 25°C (g L^{-1})	93	25	23
pK_a at 25°C	9.89	10.20	10.17
log K_{OW}	1.46	1.95	1.94

Sorption Experiments. The soil mass for all sorption experiments was estimated according to OECD (ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT) (*28*). One gram (±0.1 mg) of the dried soil sample was transferred to ten amber glass flasks with 60 mL of capacity. Increasing volumes of 1000.0 mg L^{-1} phenol solution in ionic medium of 0.01 mol L^{-1} $CaCl_2$ was transferred to the flasks, between 0.00 mL (blank experiment) and 15.0 mL (500.0 mg L^{-1}). The volume of all flasks was completed to 30.0 mL with 0.01 mol L^{-1} $CaCl_2$ solution, providing the following final phenol concentrations: 0.00, 5.00, 10.00, 25.00, 50.00, 100.0, 200.0, 300.0, 400.0, and 500.0 mg L^{-1}. A buret was utilized to complete the volumes. An additional five flasks containing 30.0 mL of phenol standard in similar concentrations in 0.01 mol L^{-1} $CaCl_2$ medium were prepared, in order to evaluate possible sorption in the glass walls or volatilization. All flasks were closed, protected from light, and kept under gentle agitation for 24 h in an orbital shaker at 170 rpm.

After that, all the suspensions were centrifuged, the supernatant phases were carefully removed and filtered under vacuum in a 0.45 μm cellulose nitrate membrane, and the phenol concentration was determined by spectrophotometry in the UV region (269 nm) for phenol and *o*-cresol or in the 277 nm region for *p*-cresol. In parallel, a triplicate assay was done using the blank extract of the soil under the same experimental conditions.

Contact Time. Five grams (±0.1 mg) of the dried soil sample was transferred to a 200 mL amber glass flask, and 150.0 mL of 50.0 mg L^{-1} phenol solution was added in 0.01 mol L^{-1} $CaCl_2$ solution. A control experiment was carried out, under identical conditions, but in the absence of soil, as well as a blank experiment without phenol. The flasks were closed, protected from light, and kept under moderate agitation in an orbital shaker at 170 rpm. After regular time intervals, 4.00 mL aliquots were taken, just after a manual vigorous homogenization of the suspension, with this volume being collected as quickly as possible, employing a micropipet. Next, the suspensions were centrifuged and filtered in a 0.45 μm cellulose nitrate membrane. This routine was done for 48 h. The remaining phenol concentration in each aliquot was quantified by spectrophotometry at 269 nm, being compared with the control experiment. For *o*-cresol and *p*-cresol, the experiments were performed under 72 and 96 h of contact time, and the concentrations of *o*-cresol and *p*-cresol were determined by spectrophotometry in the UV region at 269 and 277 nm, respectively. All experiments were done in triplicate.

Desorption Experiments. One gram (±0.1 mg) of the dried soil sample was transferred to amber glass flasks with 60 mL of capacity. Thirty milliliters of 50.0 mg L^{-1} phenol solution in an ionic medium of 0.01 mol L^{-1} $CaCl_2$ was added to the flasks. All flasks were closed, protected from light, and kept under gentle agitation for 120 h in an orbital shaker at 170 rpm. Next, the suspensions were centrifuged, and 25.0 mL of the supernatant phases was filtered under vacuum in a 0.45 μm cellulose nitrate membrane, and the phenol concentration was determined by spectrophotometry employing the 4-aminoantipyrine method (4-AAP) (*12*, *27*). First, the desorption process was assessed employing 0.01 mol L^{-1} $CaCl_2$ solution. Next, the evaluation of the desorption process was done by addition of 25.0 mL of 0.10 mol L^{-1} NaOH solution (*3*) to the flasks containing the soil and 5.0 mL of remaining phenol solution. The flasks were maintained under agitation for 1 h and centrifuged, and the pH of the supernatant phases was adjusted to 2.0 by HCl additions before the 4-AAP method was employed. This step was performed in order to obtain the precipitation of humic acid. With the purpose of verifying the influence of humic substances, some additional experiments were performed by adding phenol in the soil blank alkaline extracts followed by the acid treatment, centrifugation to remove humic acid, and determination of phenol in the supernatant phase by the 4-AAP method. All the steps of desorption procedure were repeated to *o*-cresol and *p*-cresol. The determination of *p*-cresol was made using the UV region in 277 nm.

A spike of phenol was added to the supernatant blank extracts to provide 10.0, 30.0, and 60.0 mg L^{-1} phenol concentration. The flasks were maintained under agitation for 15 min, and the phenol concentration was determined using the 4-AAP method, in order to assess the phenol recovery.

Microbial Activity Inhibition. An analogous experiment as described in contact time was performed, but in the presence of 100 mg L^{-1} $HgCl_2$ solution or 10 g L^{-1} sodium azide solution. In both situations, a 4.00 mL aliquot of soil suspension was sampled for phenol, *o*-cresol, or *p*-cresol monitoring, and the suspensions were centrifuged and filtered as already explained. The phenolic compounds' concentration was determined using UV absorption at 269 nm for phenol and *o*-cresol and at 277 nm for *p*-cresol. When the phenolic compounds were not detected in the supernatant phases, an appropriate spike was done, in order to provide nearly 50 mg L^{-1} of the respective compound. After that, other aliquots of the soil suspension were sampled to continue the monitoring of the compounds. All experiments of microbial activity inhibition were performed in triplicate.

RESULTS AND DISCUSSION

Soil Characterization. The results of the main characteristics of the soil sample are shown in **Table 1**. An unusually high CEC value of 31.4 ± 0.3 $cmol_c$ kg^{-1} was observed. According to Melfi et al. (*29*), this region of Brazil presents CEC values less than 10 $cmol_c$ kg^{-1} or between 10 and 25. This high CEC value is in good agreement with the high organic carbon (OC) content (3.7 ± 0.3), as well as the clay percent (62.3%), which can contribute to an enhancement of the CEC value, as observed for Meng et al. (*12*) for a soil sample with a CEC value of 28.09 $cmol_c$ kg^{-1}, a clay content of 53.04%, and a pH of 8.24. However, it should be emphasized that the sodium acetate method is more appropriate for calcareous soils and not for acidic ones. In addition, Dümig et al. (*30*) verified high values of OC and clay contents for many soil samples, with CEC values between 18.3 and 155.2 $cmol_c$ kg^{-1}. According to the authors, the higher values of organic carbon are related to the greater CEC values. A high loss of mass on ignition of almost 20% was determined, as a result of the hydroxyl-rich secondary minerals and organic matter present in the clay fraction that is characteristic of clayey soils in South America (*31*). Considering soil organic matter (SOM) as 1.724 × OC % (*15*, *32*), approximately 6.4 of the soil mass is related to SOM, and as a consequence, the mass depletion on the order of 13.6% may be attributed to the structural hydroxyl of the mineral clay fraction, such as kaolinite, gibbsite, and goethite (*31*). In spite of the fact that these minerals have not been quantified, the XRD confirms their presence, that is corroborated by the soil mineral composition of the south region of Brazil (*30*, *33*−*35*). It is important to notice that the results of textural properties and carbon content are in good accordance with those for other soils from the metropolitan region of Curitiba city (*34*). The pH value of 4.50 ± 0.01 in a 0.01 mol L^{-1} $CaCl_2$ solution is characteristic of the southern Brazilian soils, as shown by Bortoluzzi et al. (*33*), who investigated 75 soil samples, with results ranging from 3.5 to 5.4. The pH value shows that the soil sample is acidic, probably due to the Brazilian soils being rich in aluminum and iron content. The results of XRF are shown in **Table 3**, expressed as oxides, and confirm the high aluminum oxide content of 27.4% and iron oxide of 12.9%, with the sum of these two oxides being higher than the SiO_2 content of 34.1%. The Al_2O_3 content is comparable to the results obtained by Melo et al. (*35*) for 40 samples of soils from the metropolitan region of Curitiba, with Al_2O_3 contents between 19.5 and 39.9%. Also, for the Fe_2O_3 content, the result of the present paper is greater in comparison with the maximum value of 8.9% (*35*), justifying the small pH value.

Table 3. Oxides Content of the Soil Sample

oxide	SiO_2	Al_2O_3	Fe_2O_3	TiO_2	CaO	MgO	Na_2O	K_2O	MnO	P_2O_5
content (%)	34.1	27.4	12.9	1.6	0.23	0.71	0.04	0.85	0.04	0.25

Analytical Methods. The spectra of the supernatants after filtration in a 0.45 μm cellulose nitrate membrane are shown in **Figure 1**. These experiments were done to evaluate if the quantification method would be employed for the soil extract analysis. The phenol solution shows a maximum absorbance signal at λ = 210 and 269 nm according to spectrum **A**. Although not shown, *o*-cresol and *p*-cresol had similar spectra with small differences in λ maximum. In spite of filtration through a 0.45 μm filter, the supernatant phase absorbs in all the UV region (spectrum **B**). Thus, the study was carried out with a 0.01 mol L^{-1} $CaCl_2$ solution, which does not absorb at 210 or 269 nm (spectrum **C**). According to **Figure 1** (spectrum **D**), it is not possible to use λ = 210 to monitoring the phenolic compounds, which explains the use of 269 nm for phenol and *o*-cresol and 277 nm for *p*-cresol. The analytical curves were done between 5.00 and 80.00 mg L^{-1}.

Also, the official method of 4-AAP (*12*, *27*) in λ = 510 nm for soil extract analysis was utilized, which provides an absorbance signal almost ten times the signal in 269 nm and better selectivity. In this case, the analytical curves were performed between 0.50 and 8.00 mg L^{-1}. For *p*-cresol, only the λ of 277 nm was

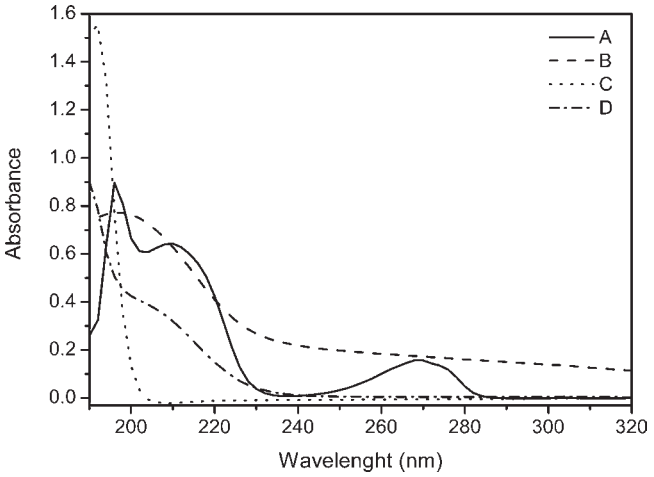


Figure 1. Absorption spectra in the UV region. (**A**) 10.0 mg L^{-1} phenol solution in deionized water; (**B**) soil extract after 24 h of contact time with 1.0000 g of soil in deionized water; (**C**) 0.01 mol L^{-1} $CaCl_2$ solution; (**D**) soil extract after 24 h of contact time with 1.0000 g of soil in 0.01 mol L^{-1} $CaCl_2$ solution.

employed, since there is no significant reaction with 4-AAP (*27*). A recovery experiment was performed in order to verify if the 4-AAP method could be applied to soil extract analysis, although this method had been employed for comparable situations (*12*). The phenol concentrations employed in this experiment were 10.0, 30.0, and 60.0 mg L^{-1}, and the recovery for triplicate analysis was 10.4 ± 0.3, 29.9 ± 0.5, and 59.3 ± 0.5, respectively, suggesting that the method was appropriate to determine phenol in soil extracts, showing no interference due to the complexity of the soil matrix, with little standard deviation.

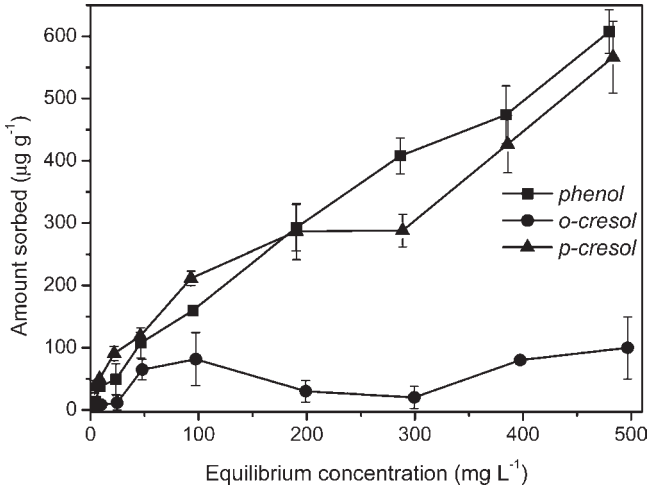


Figure 2. Sorption isotherms of the phenolic compounds after 24 h of contact time with 1.0000 g of soil in 30.0 mL of 0.01 mol L^{-1} $CaCl_2$ solution. The initial concentration of the phenolic species is between 5.00 and 500.0 mg L^{-1}. The points denote the medium result of three experiments.

Sorption Experiments. In this study, the UV method was utilized to carry out the quantification of phenol, *o*-cresol, and *p*-cresol. Although the first concentration of the phenolic species for the analytical curves or sorption experiments was 5.00 mg L^{-1}, it is important to notice that the LOQ observed for this method was near 1.00 mg L^{-1}. The isotherms of the phenolic species removal for initial concentrations between 5.00 and 500.0 mg L^{-1} are shown in **Figure 2**. For phenol and *p*-cresol, the curves showed a very similar behavior and are almost linear, and a removal between 10 and 4% was verified for phenol and one of between 25 and 4% for *p*-cresol, for the first and the last point of the sorption isotherms, respectively. For *o*-cresol the sorption was not significant, being near 3% for the first point of the curve and 0.7% for the last point. This behavior was not in good agreement with the literature (*18*, *22*, *23*), since the sorptions of phenol and *o*-cresol in these references were very similar. In addition, characteristics such as the log K_{OW} and water solubility of both cresols are analogous, which would suggest a similar sorption process. The source of this different behavior is not clear at this time, indicating the necessity of additional studies. The sorption decreasing for the three compounds suggests a saturation of the soil sorption sites, probably due to the high phenolic compounds' concentration. For an initial concentration of 50.0 mg L^{-1}, the concentrations of phenol, *o*-cresol, and *p*-cresol were close to 46, 48, and 46 mg L^{-1}, respectively, after 24 h of contact time. Meng et al. (*12*) showed a superior sorption process results for a study between a soil sample and phenol, under the same contact time and initial phenol concentration of 50.0 mg L^{-1}. According to this study, nearly 38 mg L^{-1} was maintained in equilibrium after the contact time. Although the soil characteristics, such as clay, sand, silt, organic matter, iron and aluminum oxides, pH, and CEC, of this study (*12*) are very different in comparison with those of the present study, the phenol removal was quite similar. The soil under study is rich in clay fraction (62.3%), as well as organic carbon (OC) (3.7%), according to **Table 1**. Three different compositions of soils were studied by Khan and Anjaneyulu (*6*), with clay contents of 7, 10, and 28.3% and OC contents of 0.93, 1.5, and 2.4%, demonstrating a moderate interaction between the soil samples and phenol. For the same initial concentration of 50.0 mg L^{-1}, these authors verified an equilibrium phenol concentration between 40 and 43 mg L^{-1} after 24 h of contact time. In this study was employed 60 mg L^{-1} of soil suspension, while in the study of Meng et al. (*12*) was utilized 100 mg L^{-1}, and in the present study, nearly 33 g L^{-1} of soil suspension was used. Therefore, this indicates that a different relation between the liquid and solid phases exerts a significant influence on the sorption process due to an augment of the sorption sites. These remarks suggest that phenolic compounds have negligible sorption by soil samples with distinct characteristics, and consequently, a great leaching potential may take place for this class of compounds.

Contact Time Experiments. Usually, a contact time of 24 h is employed in this kind of sorption studies. Despite this, a new step was performed to investigate if this contact time would be more appropriate for the experiments and in order to attain the apparent equilibrium time. An initial concentration of 50.0 mg L^{-1} was utilized to estimate the ideal equilibrium time. The curves for phenol, *o*-cresol, and *p*-cresol, being the first points indistinguishable up to 5 h of contact time, are showed in **Figure 3**. The phenol concentration after 24 h of contact time was close to 36 mg L^{-1}, that is, quite different in comparison to the result shown in **Figure 2**, near 46 mg L^{-1}. On the other hand, for *o*-cresol and *p*-cresol, similar results were obtained after the period of 24 h, in comparison to the results from the isotherm (**Figure 2**). After 48 h, practically no phenol was detected, while for *o*-cresol and *p*-cresol, similar behavior was observed, but after 72 and 120 h, respectively, suggesting a complete sorption after these contact times. After 48 h of contact time, the phenol concentration was smaller than 5.00 mg L^{-1}, that is, the first concentration of phenol standard solution from the analytical curve. Nevertheless, the LOD of the method for the three phenolic compounds was near 1.00 mg L^{-1}, indicating at least 98% of phenol removal. The study with *o*-cresol and *p*-cresol showed similar behavior, although no significant removal was observed to *o*-cresol until 24 and 72 h for *p*-cresol, according to **Figure 3**. It is important to

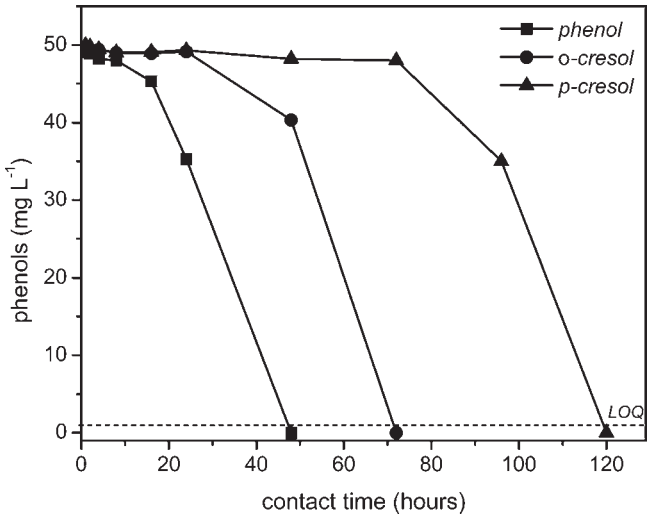


Figure 3. Contact time study between 5.0000 g of soil and 150.0 mL of the phenolic compounds at an initial concentration of 50.0 mg L^{-1} in 0.01 mol L^{-1} $CaCl_2$ medium. The dotted line represents an estimative of the LOQ value. The points denote the medium result of three experiments.

notice that the results suggested a complete removal of phenol, *o*-cresol, and *p*-cresol, and this experiment provided no realistic information about the apparent equilibrium time. Also, no significant sorption in the internal glass walls or volatilization of the three compounds was observed, based on the results from the control experiments. On the one hand, the physicochemical characteristics of the phenolic compounds under study (**Table 2**) suggest that the phenolic compounds have a hydrophilic behavior. The octanol−water partition coefficient (log K_{OW}) and water solubility (g L^{-1}) for the phenolic species are as follows: phenol (1.46 and 93), *o*-cresol (1.95 and 25), and *p*-cresol (1.94 and 23), respectively. Xu and Bhandari (*22*) verify that the sorption process of phenol, *o*-cresol, and 2,4-dichlorophenol is higher according to the lesser water solubility and the greater log K_{OW} values. The water solubility (4.5 g L^{-1}) and log K_{OW} (3.20) of the 2,4-dichlorophenol showed the greatest extent in the sorption process, when compared to the other two phenolic compounds. In the present study, the pK_a values of the species are near 10 (**Table 2**), and the pH of the soil suspensions under the experimental conditions was close to 4.5; that is to say, under this condition, the phenol is in neutral form, which could provide a sorption process by hydrogen bonding with the soil surfaces (*15*). According to Khan and Anjaneyulu (*6*), sorption isotherms with three soil samples showed a clear influence of the augment of phenol removal due to the enhancement of carbon content. In addition, the authors showed a study with these soils, but with the previous removal of organic matter from three soil samples, verifying a significant reduction in the adsorption capacity (67.5%) in comparison with the case of untreated soil. An augment of the hydrophobicity seems to be responsible by the enhancement in the phenol sorption by organically modified soils (*12*), as well as for organically modified clay minerals (*9*, *36*). From this point of view, the soil under study showing great carbon content (3.7%), consequently, should provide a high phenol removal.

Desorption Study. On the basis on the complete apparent removal of phenol, *o*-cresol, and *p*-cresol, a desorption study was performed, in order to evaluate the phenol recovery. After 120 h of contact time, between the 50.0 mg L^{-1} phenol, *o*-cresol, or *p*-cresol solution in 0.01 mol L^{-1} $CaCl_2$ medium and the soil sample, the supernatant phases showed no measurable concentrations of the three compounds. This is in good accordance with the experiments of contact time, suggesting total removal of the three compounds. After the sorption process, several desorption experiments with 0.01 mol L^{-1} $CaCl_2$ solution were done, but the concentrations in the supernatant phases were lower than the LOQ of the method, suggesting a totally irreversible process. On the contrary, Khan and Anjaneyulu (*6*) presented 71.5, 60.2, and 53.2% of desorption for an initial phenol concentration of 5.00 mg L^{-1} and 63.9, 50.7, and 48.7% for an initial phenol concentration of 25.0 mg L^{-1}, for carbon contents of 0.93, 1.5, and 2.4%, respectively. This hysteresis effect may be attributed to the degradation of the compounds during the contact time (*6*, *18*) or also by the physical or chemical properties of soil solution system (*6*). Furthermore, the soil particles' micropores can trap the sorbate due to cooperative sorption, and as a consequence, the desorption is not expected to follow the same path as in sorption, with the hysteresis process occurring (*15*). Because no desorption of the phenolic compounds was observed, a new series of experiments was performed using a 0.10 mol L^{-1} NaOH solution as extractor solution. After the contact time between the soil sample and the 0.10 mol L^{-1} NaOH solution, the supernatant phase was analyzed, and no phenol, *o*-cresol, or *p*-cresol was detected in the solution. It is important to notice that, in this desorption step, the 4-AAP method was employed in order to provide a small LOQ (except for *p*-cresol, which was quantified at 277 nm), and only noises were observed in the baseline of the spectra. In spite of the great number of steps in the 4-AAP method, according to recovery experiments, no interferences in the determination of the phenolic species were observed. This corroborates that phenolic compounds were not present in the soil or were not desorbed by the alkaline medium, although NaOH solutions have been employed to extract phenol from soils, providing quantitative recoveries. According to Crespin et al. (*3*), a 0.10 mol L^{-1} NaOH solution provided recoveries of 90% and 60% for phenol and *p*-cresol, respectively, for only 1 min of contact with soil samples. The authors stated that soil particles under higher pH values are negatively charged, as well as the phenolic compounds, and consequently desorption of phenolic compounds is favored, while the sorption is hindered. Because the NaOH solution is frequently used to extract humic substances from soil samples, the addition of HCl solution to reduce the pH value could provide phenol removal with humic acid, mainly due to the compounds to be protonated. Thus, other series of experiments were done by the addition of the phenolic compounds in the concentrations 1.00 mg L^{-1} (phenol and *o*-cresol) and 5.00 mg L^{-1} (*p*-cresol) in the alkaline supernatants, followed by the precipitation of the humic acid with HCl solution. For the three compounds, the recoveries (%) after the whole process were as follows: phenol, 93 ± 1; *o*-cresol, 91 ± 1; and *p*-cresol, 110 ± 3, indicating no interaction between humic acid and the phenolic compounds, at least under these conditions. Although the analysis had been carried out in the VIS region (except for *p*-cresol), it is relevant to report that, after the alkaline desorption, no UV absorption was verified in the spectra, indicating that no absorbent species in the UV region were formed in the alkaline supernatants. Therefore, these remarks suggest that other processes may be involved, but not sorption or volatilization, especially due to the nonoccurrence of desorption in alkaline medium. Maybe an irreversible sorption process such as nonextractable compounds may take place or even the degradation of the phenolic compounds may be arising (*15*, *24*).

Microbial Activity Inhibition. The results of biodegradation experiments for phenol are shown in **Figure 4**. The monitoring for the period of ten days showed a reduction in the phenol concentration near 2.0 mg L^{-1} after two days of contact time.

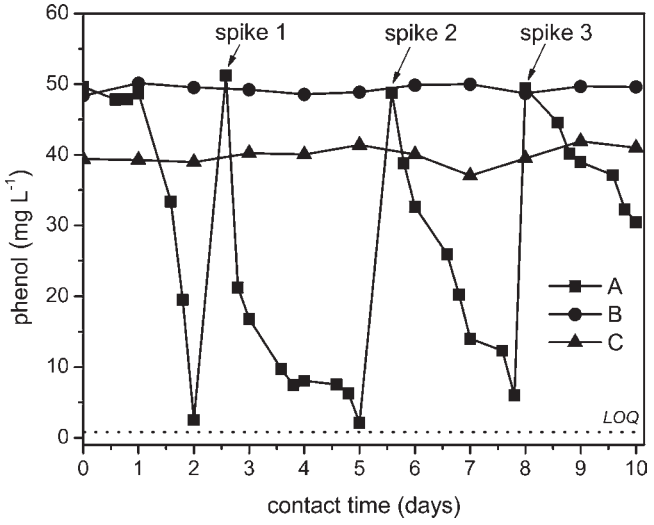


Figure 4. Monitoring of phenol concentration versus time in the absence of inhibitor (**A**), in the presence of a 100 mg L^{-1} $HgCl_2$ solution (**B**), and in the presence of 10 g L^{-1} azide solution (**C**). Initial phenol concentration and spike values, 50.0 mg L^{-1}; soil mass, 5.0000 g; initial volume, 150.0 mL; ionic medium, 0.01 mol L^{-1} $CaCl_2$ solution. The points denote the medium result of three experiments.

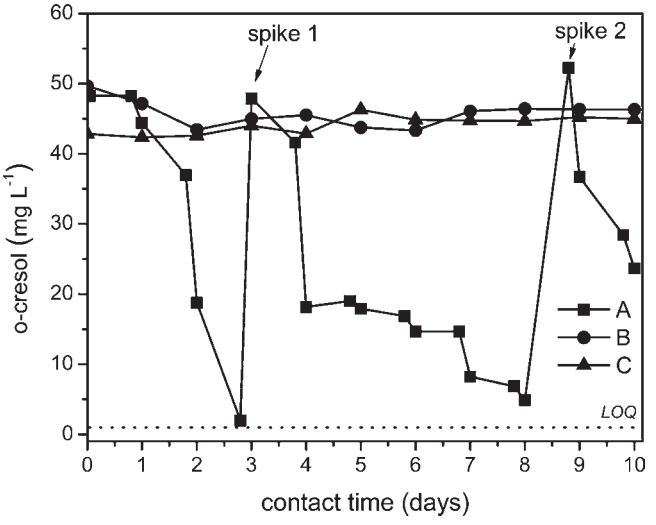


Figure 5. Monitoring of *o*-cresol concentration versus time in the absence of inhibitor (**A**), in the presence of 100 mg L^{-1} $HgCl_2$ solution (**B**), and in the presence of 10 g L^{-1} azide solution (**C**). Initial *o*-cresol concentration and spike values, 50.0 mg L^{-1}; soil mass, 5.0000 g; initial volume, 150.0 mL; ionic medium, 0.01 mol L^{-1} $CaCl_2$ solution. The points denote the medium result of three experiments.

This is in good agreement with the data from **Figure 3**. A spike was made in order to provide a phenol concentration near 50.0 mg L^{-1}, and again the concentration was decreasing until 2.0 mg L^{-1}, after an additional three days of contact time. According to **Figure 4**, after the second and third spike, the behavior of the system was very similar. Comparable results were obtained by Viotti et al. (*14*), but the soil and initial phenol concentrations were 60 g L^{-1} and 200 mg L^{-1}, respectively, with eight days being necessary to proportionate a total consumption of phenol, and the authors showed a faster depletion in phenol concentration after each new phenol spike. The experiments done in the presence of a 100 mg L^{-1} $HgCl_2$ solution or a 10 g L^{-1} NaN_3 solution also are shown in **Figure 4**. In both cases, the concentration remained practically constant during ten days, although for NaN_3 inhibitor, an abrupt reduction of the phenol concentration was observed at the first point, near 39 mg L^{-1}. This aspect is not clear, suggesting a partial sorption of phenol in the soil sample, close to 20%. On the other hand, for $HgCl_2$, no partial sorption was observed, although Viotti et al. (*14*) showed a great diminution in the phenol concentration during the 18 days of the experiment. Analogous profiles were verified for *o*-cresol in **Figure 5**. Nevertheless, only two spikes were made during the 10 days of the experiments, in the absence of azide or $HgCl_2$ inhibitors. For *o*-cresol, an initial diminution in the concentration was observed until 43 mg L^{-1} in the presence of azide, remaining constant until the last day of the assay, while for $HgCl_2$ a gradual depletion in *o*-cresol concentration occurred until two days, and after that the concentration remained approximately constant. For *p*-cresol (**Figure 6**) the curves were very similar to those for *o*-cresol, but the initial concentration for the three situations was near 45 mg L^{-1}, and as observed for *o*-cresol, the *p*-cresol concentration continued constant until the end of the experiment. Both *o*-cresol and *p*-cresol showed a higher contact time to reduce totally their concentrations in comparison with phenol, in the absence of the inhibitors, and thus three spikes were made against two spikes for the cresols. This feature may be related to the different molecular structure of the cresols, which could provide a longer lifetime to degradation. According to **Figures 4**−**6** for the three phenolic compounds, practically no sorption was verified,

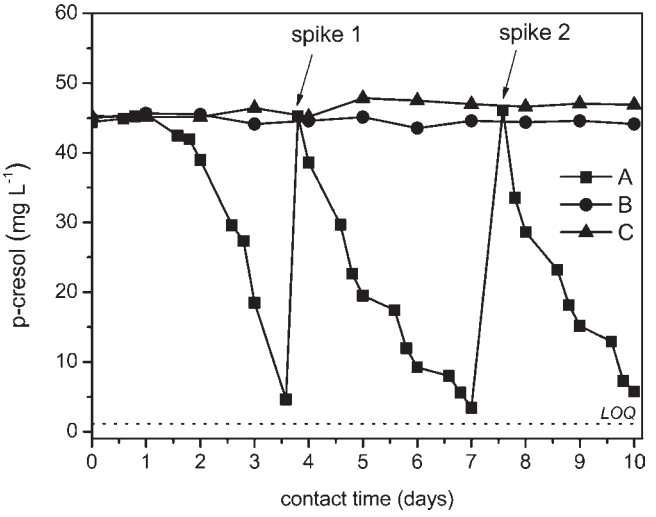


Figure 6. Monitoring of *p*-cresol concentration versus time in the absence of inhibitor (**A**), in the presence of 100 mg L^{-1} $HgCl_2$ solution (**B**), and in the presence of 10 g L^{-1} azide solution (**C**). Initial *p*-cresol concentration and spike values, 50.0 mg L^{-1}; soil mass, 5.0000 g; initial volume, 150.0 mL; ionic medium, 0.01 mol L^{-1} $CaCl_2$ solution. The points denote the medium result of three experiments.

in the presence of both inhibitors, since the concentrations practically did not vary.

Concluding Remarks. Based on the results previously discussed, one can speculate that the phenolic species here studied have negligible interaction with the clay-rich soil sample, for a period of 24 h, at least for this specific soil sample, which represents the region under study, with the characteristics mentioned in **Table 1**. On the other hand, at higher contact times, the results suggest a complete sorption process after 48, 72, and 120 h, for phenol, *o*-cresol, and *p*-cresol, respectively, which was not confirmed by recovery experiments in the presence of 0.01 mol L^{-1} $CaCl_2$ or by 0.10 mol L^{-1} NaOH solution as extractors. Biodegradation studies with two different inhibitors indicate that an insignificant sorption process occurs with the three species, and between 2 and

4 days their concentration is very low. Thus, two approaches are feasible, the biodegradation or the formation of nonextractable residues. Notwithstanding this, one would suppose a major likelihood of a biodegradation process, on account of neither in $HgCl_2$ nor in NaN_3 for 10 days of contact time has been observed depletion in the concentration of the phenolic species, conversely expected for the generation of nonextractable compounds. Briefly, the utilization of water containing these species in soil could attain the groundwater, if great volumes of water are employed, or due to leaching by rainfall. Otherwise, the remaining phenolic species in these soil samples, with the characteristics described, would be biodegraded or even continue to be present as nonextractable residues, and for this reason, no risks of groundwater contamination could take place.

ACKNOWLEDGMENT

The authors acknowledge the financial and logistic support of EMBRAPA Clima temperado, Pelotas, RS, PETROBRAS/SIX—São Mateus do Sul, PR, and of LAMIR, Curitiba, PR, for the X-ray fluorescence analysis.

LITERATURE CITED

(1) Vidic, R. D.; Suldan, M. T.; Brenner, R. C. Oxidative Coupling of Phenols on Activated Carbon: Impact on Adsorption Equilibrium. *Environ. Sci. Technol.* **1993**, *27*, 2079–2085.

(2) Stevenson, F. J. *Humus Chemistry, Genesis, Composition, Reactions*. John Wiley & Sons, Inc.: New York, 1994.

(3) Crespin, M. A.; Gallego, M.; Valcarcel, M. A. Semiautomatic Module for the Direct Leaching and Determination of Sixteen Phenols in Agricultural Soils. *Anal. Chem.* **1999**, *71*, 2687–2696.

(4) Michałowicz, J.; Bukowska, B.; Duda, W. The differences in phenolic content in rivers exposed and non-exposed to anthropogenic contamination. *Chemosphere* **2008**, *71*, 735–741.

(5) Wake, H. Oil refineries: a review of their ecological impacts on the aquatic environment. *Estuarine, Coastal Shelf Sci.* **2005**, *62*, 131–140.

(6) Khan, Z.; Anjaneyulu, Y. Influence of soil components on adsorption-desorption of hazardous organic-development of low cost technology for reclamation of hazardous waste dumpsites. *J. Hazard. Mater.* **2005**, *B118*, 161–169.

(7) Kamble, S. P.; Mangrulkar, P. A.; Bansiwal, A. K.; Rayalu, S. S. Adsorption of phenol and *o*-chlorophenol on surface altered fly ash based molecular sieves. *Chem. Eng. J.* **2008**, *138*, 73–83.

(8) Dabrowski, A.; Podkościelny, Z.; Hubicki, Z.; Barczak, M. Adsorption of phenolic compounds by activated carbon − a critical review. *Chemosphere* **2005**, *58*, 1049–1070.

(9) Richards, S.; Bouazza, A. Phenol adsorption in organo-modified basaltic clay and bentonite. *Appl. Clay Sci.* **2007**, *37*, 133–142.

(10) Sposito, G. *The Chemistry of Soils*; Oxford University Press, Inc.: Oxford, U.K., 1989.

(11) McBride, M. B. *Environmental Chemistry of Soils*; Oxford University Press, Inc.: Oxford, U.K., 1994.

(12) Meng, Z.-F.; Zhang, Y.-P.; Zhang, Z.-Q. Simultaneous adsorption of phenol and cadmium on amphoteric modified soil. *J. Hazard. Mater.* **2008**, *159*, 492–498.

(13) Scott, H. D.; Wolf, D. C.; Lavy, T. L. Apparent adsorption and microbial-degradation of phenol by soil. *J. Environ. Qual.* **1982**, *11*, 107–112.

(14) Viotti, P.; Papini, M. P.; Stracqualursi, N.; Gamba, C. Contaminant transport in an unsaturated soil: laboratory tests and numerical simulation model as procedure for parameters evaluation. *Ecol. Modell.* **2005**, *182*, 131–148.

(15) Site, A. D. Factors Affecting Sorption of Organic Compounds in Natural Sorbent/Water Systems and Sorption Coefficients for Selected Pollutants. A Review. *J. Phys. Chem. Ref. Data* **2001**, *30*, 187–439.

(16) Trevors, J. T. Sterilization and inhibition of microbial activity in soil. *J. Microb. Activity Soil* **1996**, *26*, 53–59.

(17) Shaw, L. J.; Beaton, Y.; Glover, L. A.; Killham, K.; Mehang, A. A. Re-inoculation of autoclaved soil as a non-sterile treatment for xenobiotic sorption and biodegradation studies. *Appl. Soil Ecol.* **1999**, *11*, 217–226.

(18) Shibata, A.; Inoue, Y.; Katayama, A. Aerobic and anaerobic biodegradation of phenol derivatives in various paddy soils. *Sci. Total Environ.* **2006**, *367*, 979–987.

(19) Gianfreda, L.; Sannino, F.; Rao, M. A.; Bollag, J.-M. Oxidative transformation of phenols in aqueous mixtures. *Water Res.* **2003**, *37*, 3205–3215.

(20) Bollag, J.-M. Decontaminating soil with enzymes. *Environ. Sci. Technol.* **1992**, *26*, 1876–1881.

(21) Canfora, L.; Iamarino, G.; Rao, M. A.; Gianfreda, L. Oxidative Transformation of Natural and Synthetic Phenolic Mixtures by *Trametes versicolor* Laccase. *J. Agric. Food Chem.* **2008**, *56*, 1398–1407.

(22) Xu, F.; Bhandari, A. Retention and Extractability of Phenol, Cresol, and Dichlorophenol Exposed to Two Surface Soils in the Presence of Horseradish Peroxidase Enzyme. *J. Agric. Food Chem.* **2003**, *51*, 183–188.

(23) Bhandari, A.; Xu, F. Impact of Peroxidase Addition on the Sorption-Desorption Behavior of Phenolic Contaminants in Surface Soils. *Environ. Sci. Technol.* **2001**, *35*, 3163–3168.

(24) Barriuso, E.; Benoit, P.; Dubus, I. G. Formation of Pesticide Nonextractable (Bound) Residues in Soil: Magnitude, Controlling Factors and Reversibility. *Environ. Sci. Technol.* **2008**, *42*, 1845–1854.

(25) Hesse, P. R. *A Textbook of Soil Chemical Analysis*; John Murray Publishers: London, 1971.

(26) Abate, G.; Masini, J. C. Adsorption of Atrazine, Hydroxyatrazine, Deethylatrazine, and Deisopropylatrazine onto Fe(III) Polyhydroxy Cations Intercalated Vermiculite and Montmorillonite. *J. Agric. Food Chem.* **2005**, *53*, 1612–1619.

(27) APHA—American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, 19th edition; Water Environment Federation, American Water Works Association, Water Pollution Control Federation: Washington, DC, 1995.

(28) OECD—ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT. Guideline for the testing of Chemicals. *Adsorption−Desorption Using a Batch Equilibrium Method*; OECD/OCDE 106 Adopted: 21st January **2000**.

(29) Melfi, A. J.; Montes, C. R.; Carvalho, A.; Forti, M. C. Use of pedological maps in the identification of sensitivity of soils to acidic deposition: application to Brazilian soils. *An. Acad. Bras. Cienc.* **2004**, *76*, 139–145.

(30) Dümig, A.; Schad, P.; Kohok, M.; Beyerlein, P.; Schwimmer, W.; Kögel-Knabner, I. A mosaic of nonallophanic Andosols, Umbrisols and Cambisols on rhyodacite in the southern Brazilian highlands. *Geoderma* **2008**, *145*, 158–173.

(31) Marques, J. J.; Schulze, D. G.; Curi, N.; Mertzman, S. A. Major element geochemistry and geomorphic relationships in Brazilian Cerrado soils. *Geoderma* **2004**, *119*, 179–195.

(32) Allison, L. E. *Methods of Soil Analysis*; Black, A., Ed.; Series of Agronomy, Part 2; ASA: Madison, Wisconsin, **1965**.

(33) Bortoluzzi, E. C.; Tessier, D.; Rheinheimer, D. S.; Julien, J. L. The cation exchange capacity of a sandy soil in southern Brazil: an estimation of permanent and pH-dependent charges. *Eur. J. Soil Sci.* **2006**, *57*, 356–364.

(34) Pires, A. C. D.; Melo, V. F.; Lima, V. C.; Motta, A. C. V. Major Soil Classes of the Metropolitan Region of Curitiba (PR), Brazil: I—Mineralogical Characterization of the Sand, Silt and Clay Fractions. *Braz. Arch. Biol. Technol.* **2007**, *50*, 169–181.

(35) Melo, V. F.; Barbar, L. C.; Zamora, P. G. P.; Schaefer, C. E.; Cordeiro, G. A. Chemical, physical and mineralogical characterization of soils from the Curitiba Metropolitan Region for forensic purpose. *Forensic Sci. Int.* **2008**, *179*, 123–134.

(36) Rytwo, G.; Kohavi, Y.; Botnick, I.; Gonen, Y. Use of CV- and TPP-montmorillonite for the removal of priority pollutants from water. *Appl. Clay Sci.* **2007**, *36*, 182–190.

Received for review August 27, 2009. Revised manuscript received December 21, 2009. Accepted December 22, 2009.