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## Fire Impact on Several Chemical and Physicochemical Parameters in a Forest Soil

Andrea Rubenacker, Paola Campitelli, Manuel Velasco and Silvia Ceppi  
*Departamento de Recursos Naturales, Facultad de Ciencias Agropecuarias,  
 Universidad Nacional de Córdoba, Córdoba  
 Argentina*

### 1. Introduction

Cordoba is a Mediterranean State, with semiarid climate, dry autumn and winter, in which the wild fire can take place, especially at the end of dry season. Forest fires happen frequently in the mountain zones of the province of Córdoba, Argentina, which are located at west and south-west region. The vegetation, in the south-west zone, are principally *Pinus halepensis* Mill.; *Pinus elliottii* implanted and the native vegetation cover is *Stipa caudata*, *Piptochaetium hackelii*, *P. napostaense* y *Briza subaristata*, between others. Taxonomically the soil corresponds to an Ustorthent.

In the west area the native vegetation is principally *Acacia caven*, *Festuca hieronymi*, *Stipa*, *Poa stukerti*, between others. The soil is an Argiustoll.

Forest wild fires constitute a serious environmental problem, not only due to the destruction of vegetation but also because the degradation that may be induced in a soil as a consequence of the change produced in its properties. Wild fire can strongly modify the abiotic and biotic characteristics of soil, altering its structure, chemical and physicochemical properties, carbon content and macronutrient levels. The degree of the alteration produced depends on the frequency and intensity of fire, all these modifications being particularly important in the surface horizons.

Organic matter is a key factor for forest soil. It has a direct and /or an indirect influence on all physical and chemical characteristics of the soil. While low severity fires, such as those prescribed for forest management, have been reported to have transient but positive effect on soil fertility, severe wildfire result in significant losses of soil organic matter, and nutrient, and deterioration of the overall physical-chemical properties of soil that determine its fertility, such as porosity, structure among others (Certini, 2005).

Fire may directly consume part or all of the standing plant material and litter as well as the organic matter in the upper layer of the soil. One of the most important soil change, during the burning is the alteration in the organic matter content therefore, the nutrient contained in the organic matter are either more available or can be volatilized and lost from the site. The soluble nutrient would be loss for erosion or leaching if they are not immediately absorbed by plants or retained by soil.

Humic substances are one of the most important fractions of the organic matter and are considered the most abundant organic component in nature and largely contribute to soil structuring and stability, to its permeability for water and gases, to its water holding

capacity, to the nutrient availability, to the pH buffering and to the interaction with metal ions (Schnitzer 2000; Hayes & Malcom, 2001, Campitelli & Ceppi 2008)

Depending on the fire severity the organic matter may change not only their level, but also, their different fractions, i.e. the humic substances (humic and fulvic acids) content and their principal characteristics (Vergnoux et al., 2011a, 2011b; Duguy & Rovira 2010).

The fire could induce transformation in the solubility of humic substances in different media, alkali or acid, and of their fraction (humic and fulvic acids). Thus, it could mean that the humic and fulvic acids suffer structural modification, provably of the peripheral chains and the oxygenated moieties.

The study of the burned soil is necessary to analyze the soil degradation, not only to estimate the modification of the nutrient content but either the physicochemical characteristics of the organic matter, specially analyzing the humic substances and their fractions.

Humic acids have an important role in soil structure and nutrient capacity due to their surface charge development which may change due to the fire event. Some researchers have mainly shown changes in the aromaticity and in the oxygen-containing functional groups (Almedros et al., 2003; Gonzalez-Vila & Almendros 2009; Kniker et al., 2005; Vergnoux et al., 2007).

Most of the researches are focusing in the nutrient content, carbon content and their fraction, the fate of nutrient after fire, the effect of erosion, in general, the effect of fire disturbance of the soil properties. Moreover, a single parameter is insufficient to give an accurate evaluation of soil alteration. That is why several parameters need to be taken into consideration (Vergnoux et al., 2009).

Because the abundance and importance in soil of the organic matter and their fraction, mainly the humic acids, is necessary to focus the study not only onto the principal nutrient content, but also, on the characteristics of the humic acids and compare it with those extracted from the same unburned soil.

The objective of this research was to study, through different analytical techniques, i) several soil parameters related to chemical soil fertility and ii) chemical and physicochemical properties of organic carbon and their different fractions, focusing mainly, on the humic acids extracted from the forest soil after the fire event in order to compare it with the humic acids from the unburned soil.

## 2. Materials

### 2.1 Study site and sampling

The area selected was the south-west area of the province of Cordoba, named as San Agustín (Departamento de Calamuchita).

The annual average rainfall is about 600-800 mm. The mean temperature in the area fluctuates from 9 °C in winter to 20 °C in summer.

The tree stratum are principally *Pinus halepensis* Mill.; *Pinus elliotii* implanted and native vegetation cover is *Stipa caudata*, *Piptochaetium hackelii*, *P. napostaense* y *Briza subaristata*, between others. Three composite samples (10 subsamples) were taken from the upper layer (0-10 cm) of soil, some days after the wildfire occurrence and before any rainfall event. The samples were taken from the burned (BS) and adjacent unburned soil (UBS), at the same sampling moment. Litter and the ash were not removed from the soil surface before

sampling in the unburned and burned soil, respectively. The soil was taxonomically characterized as Ustorthent. The samples were air-dried, crushed and passed through a 2 mm sieves before all the analytical analysis

The humic acids (HA) analyzed were extracted from the burned (HA-BS) and unburned soil (HA-UBS)

## 2.2 Methods

The samples of burned and unburned soil were analyzed for pH at a rate 1:2.5 (w:v), electric conductivity (EC), total nitrogen content (TN) by the Kjeldahl method, phosphorus available (P) by Bray & Kurtz method (1945), total organic carbon (TOC) by combustion at 540 °C for 4 h (Abad, et al., 2002) and oxidable carbon (Cox) by the methodology proposed by de Richter & Von Wistinghausen (1981). Organic light fraction (OLF) were determined according the method proposed by Janzen et al., 1992, the C and N content of the OLF by dry combustion using a Perkin Elmer CN Elemental Analyzer.

The carbon content of humic substances (CHS), humic acids (CHA) and fulvic acids (CFA) were determined according to the technique proposed by Syms & Haby 1971. The carbon content of each fraction (CHS, CHA and CFA) were calculated as percentage of the TOC, therefore, the % CHA correspond to the Humification Index (HI) (Roletto et al., 1985; Ciavatta et al., 1988).

The apolar or free lipidic fraction (FLF) were extracted with petroleum ether (40-60 °C) in 250 ml Soxhlet loaded with 50 g of soil; the extraction phase was renewed every 12h. The total extract was dehydrated with anhydrous Na<sub>2</sub>SO<sub>4</sub> evaporated under reduced pressure to approximately 50 ml, dried under N<sub>2</sub> stream at room temperature (20-25 °C), and finally weighted, following the methodology proposed by Zancada et al., 2004.

The spectroscopy characteristics of the alkaline extract of both soil samples, the absorbance at different wavelength (280, 470 y 664 nm), were determined by the methodology proposed by Sapeck & Sapeck (1999). The ratio E2/E6, E4/E6 and E4/E6 were calculated from the corresponding absorbance value of the alkaline extract. The measures were determined using Spectronic 20 Genesys Spectrophotometer.

### 2.2.1 Humic Acids isolation

HA from burned and unburned soil were extracted with NaOH 0.1 mol L<sup>-1</sup>, purified with HCl:HF (1:3) and dried at low temperature until constant weight, according to the procedure recommended by Chen et al. (1978). All solutions were prepared with tridistilled water and all the reagents were ACS reagent grade.

### 2.2.2 Humic Acids analyses

HA ash content was measured by heating it at 550 °C for 24 h. The elemental composition for C, H, N, S was determined by an analyzer instrument Carlo Erba 1108, using isothiurea as standard. Oxygen was calculated by difference: O% = 100 - (%C+%H+%N+%S) (ash and moisture-free basis).

### 2.2.3 Spectroscopic characteristics

The absorbance of the extracted HA were measured on a solution containing 3.0 mg of each HA in 10 mL of 0.05 mol L<sup>-1</sup> NaHCO<sub>3</sub> at different wavelength (280, 470 y 664 nm)

according to the methodology proposed by Kononova, 1982; Zbytniewski & Buszewski, 2005; Sellami et al., 2008. From the absorbance value then were calculated the E2/E4, E2/E6 and E4/E6 ratio

#### 2.2.4 Potentiometric titration

Potentiometric titrations were carried out according to the technique proposed by Campitelli et al. (2003), which is briefly: HA solution of each samples were prepared by dissolving HA ( $\approx 50$  mg) with minimum volume of NaOH solution ( $0.1 \text{ mol L}^{-1}$ ) and adding water up to the final volume (50 ml). An aliquot containing the desired amount of HA ( $\approx 7\text{--}8$  mg) was transferred to the titration flask containing 10 mL of tridistilled water. The titrant ( $\text{HCl} = 0.05 \text{ mol L}^{-1}$ ) was added from an automatic burette (Schott Geräte T80/20) at a titrant rate of  $0.1 \text{ ml}/40 \text{ s}$ . This rate was chosen taking into consideration that the variation of pH values should range between 0.02 and 0.04 pH units. The pH values were measured with an Orion Research 901 pH meter equipped with a glass-combined electrode (Orion 9103 BN). All titrations were performed in  $\text{KCl } 0.01 \text{ mol L}^{-1}$  as background electrolyte. The same titration was followed in absence of HA (reference or blank titration) for each titration curve, in order to subtract it from the raw data titration, and thus obtain the charge developed by the HA sample. Each HA solution, with the corresponding blank solution, was titrated by triplicate and the reported data representing the average values. All the reagents were ACS reagent grade.

#### 2.2.5 Capillary zone electrophoresis

Capillary zone electrophoresis (CZE) experiments were performed on an Agilent Technology Capillary electrophoresis system equipped with a diode array. Operation of the instrument, data collection and analysis were controlled by Agilent ChemStation software. The polarity was negative, voltage of  $-30 \text{ kV}$ , temperature  $25^\circ \text{C}$ , total run time 30 min (for time migration higher than 30 min no significant peak were observed). The samples were injected hydrodynamically using pressure of  $5000 \text{ Pa}$  for 20 s. The absorbance was monitored at four different wavelengths (210 nm, 230 nm, 260 nm and 450 nm) and 260 nm was selected to report.

Each HA electropherogram was carried out by triplicate and the reported data representing the average values. The dimensions of the fused-silica capillary were  $75 \text{ }\mu\text{m}$  internal diameter; 73.0 cm total length and 64.5 cm effective length. All the solutions and background electrolyte (BGE) were prepared from analytical (p.a. or HPLC) chemicals and ultra pure water. BGE was buffer borate  $20 \text{ mmol L}^{-1}$  at  $\text{pH}=9.3$ , the concentration of the HA solutions were 1000 ppm. At the beginning of daily work, the capillary was washed for 5 min with  $0.1 \text{ mol L}^{-1}$  NaOH solutions, followed by 5 min washing with ultra pure water and 20 min with BGE at  $25^\circ \text{C}$  and  $10^4 \text{ Pa}$ . At the end of the daily work, the capillary was rinsed with BGE for 5 min and water for 10 min, at the same temperature and pressure condition.

The capillary was treated before each sampling injection, as following, pre-condition: 2 min with NaOH  $0.1 \text{ mol L}^{-1}$  at  $10^4 \text{ Pa}$ , followed by washing with BGE for 3 min at  $10^4 \text{ Pa}$ , and finally waiting for 1 min. Post-run conditions were: 1 min with NaOH  $0.1 \text{ mol L}^{-1}$  at  $10^4 \text{ Pa}$ , followed by 5 min with water at the same pressure.



3. Results and discussion

3.1 Soil characterization

3.1.1 Main properties

The results of the principal chemical parameters are shown in table 1. The concentration of the cations, such as Na<sup>+</sup> and K<sup>+</sup> were not altered by fire, Ca<sup>2+</sup> content slightly increase after fire, probably due to their release from the litter layer and Mg<sup>2+</sup> decrease. The increase observed in the availability of Ca<sup>2+</sup>, may be remarkably in a fire event, but ephemerally (Certini, 2005).

Sample	pH	EC	TN	P	TOC	Cox	TOC/TN	Na	K	Ca	Mg	CIC
Unburned soil (UBS)	6.20a	0.60a	6.6a	23.0a	105a	24.2a	15.9a	0.22a	1.15a	10.25a	2.25b	23.5a
Burned soil (BS)	6.53a	1.19b	7.1a	52.4b	128b	24.9a	18.0b	0.22a	1.03a	11.1a	1.5a	23.6a

EC: dSm<sup>-1</sup>; P: mg kg<sup>-1</sup>; TOC, Cox: g kg<sup>-1</sup>; CIC, Na, K, Ca Mg: cmol kg<sup>-1</sup> Different letters (a-b) in the same column indicate significant differences (p<0.05) according to Tukey test.

Table 1. Principal chemical characteristic of burned and unburned soil

The effect of burning onto soil Total Nitrogen (TN) content present a paradox, which have been debated for years (Neary et al., 1999; Knicker & Skjemstad,, 2000). Fisher & Binkley (2000) found that the immediate response of soil N to heating is a decrement because some loss through volatilization; Certini (2005), suggested that organic N could be volatilizes and in part mineralized to ammonium. Santin et al. (2008) found that the TN after fire increase and González-Vila et al. (2009) suggest that wildfire promote the accumulation of recalcitrant organic-N forms. The N, would be as NH<sub>4</sub><sup>+</sup> or NO<sub>3</sub><sup>-</sup>, the NH<sub>4</sub><sup>+</sup> could be adsorbed onto negative charge of mineral and/or organic surface, but with time transformed to NO<sub>3</sub><sup>-</sup>. Nitrate, without any plant uptake, will be lost from the ecosystem either by denitrification or leaching (Certini, 2005; Knicker, 2007).

The TN content increased slightly in the burned soil, but this change is not statistically significant; this behavior may be due to the nitrogen supplied by the burned litter and/or the ash contained in the sample.

The forest fires have not necessarily the same impact on soil P as on N, because losses of P through volatilization or leaching are small. The combustion of vegetation and litter causes modification on biogeochemical cycle of P. Burning convert the organic pool of soil P to orthophosphate, which is the form of P available to biota. Furthermore, the peak of P bioavailability is around pH 6.5. These could be the reason for which an enrichment of P is observed in the studied burned soil, but this enrichment will decline soon, because it precipitates as slightly available mineral forms (Certini, 2005; Cade-Menun et al., 2000). In agreement with this suggestion, the increase in the available P content in this burned soil could be due to the soil pH value (table 1).

Cation Exchange Capacity (CIC), on average, decrease after a fire event due to the loss of organic matter (Certini 2005; Badía & Martí, 2003), in this soil CIC was not changed, probably because the Cox content is the same before and after fire.

In general the soil pH increase by soil heating as a result of organic acids denaturalization, this increase take place when the temperatures are higher than 450 or 500°C, in coincidence with the complete combustion of fuel and the bases release (Arocena & Opio 2003; Knicker,

2007; Certini 2005). For the soil analyzed, the increasing observed in soil pH after the fire event was slight (around 5%), this is in agreement with the cation (Na, K, Ca, Mg) content which were not largely modified by the heating soil, suggesting that the temperature did not raise up to 450°C or greater.

The electric conductivity (EC) increase in the burned soil, it could be assigned to the release of inorganic ions from the combusted organic matter present as litter or ash; this increase could be temporary (Kutiel & Imbar 1993; Hernandez, et al., 1997; Certini, 2005).

### 3.1.2 Organic matter

The most intuitive expected change in the soils during a fire event is the loss of organic matter. This change depends on the fire severity, vegetation type, soil texture and even slope. The impact on the organic matter consist of slight distillation (volatilization of minor constituents), charring or complete oxidation. Substantial consumption of organic matter begins in the 200-250 °C range to complete at around 450-500 °C (Fernandez et al., 1997; Giovannini et al., 1988; Certini, 2005; Knicker, 2007).

The influence of fire on the organic matter content have been reported a wide range of effects, showing even contrasting results (Gonzalez-Perez et al., 2004; Czimczik et al., 2005; Dai et al., 2005; Knicker et al., 2005; Alexis et al., 2007)

The oxidable organic carbon (Cox) content was not altered by fire, but, total organic carbon (TOC) increase around 21%, this behavior could be attributed to the accumulation of recalcitrant hydrophobic fraction of organic matter (Gonzalez-Perez et al., 2004; Santin et al., 2008).

The organic fraction extracted with petroleum ether, the soil free lipids, represents a diverse group of hydrophobic substances ranging from simple compounds such as fatty acids, to more complex substances as sterols, terpenes, polynuclear hydrocarbons, chlorophylls, fats, waxes and resins. The hydrophobic fraction extracted (FLF) from the sample after fire was greater ( $\approx 38\%$ ) than that quantified for the sample of the control soil (table 2), in agreement with those found by Almendros et al. (1988), for a soil under *Pinus pinea*. Although, such compounds occur in fire unaffected soil, their abundance is increased by fire due to greater stability of lipids and lignin derivatives but also due to the neoformation of aromatic polymers (Almendros et al., 2003; Fernandez et al., 2004; Knicker et al., 2005a).

The high TOC content before and after fire event could be due to the sampling methodology, taking the soil sample with all the litter and grass in soil before fire and litter from decaying fire affected vegetation. The increase in the TOC content suggests that this fire event contributes to an enhancement of the organic matter, through the incomplete combust vegetation and thus contributes to a soil TOC increase. With the time residence in the soil this unstable organic matter could be incorporated to the stable pool of organic matter, this behavior is related to the process of accumulation of organic compounds in soil controlled by their chemical affinity with the native organic matter. The randomness of the process and the heterogeneity of the organic molecules, probable produced by fire, lead to the accumulation of organic matter in which hydrophilic association may be contiguous with hydrophobic domains or contained in one other, and thus the native organic matter pool could behave as sink of the decaying fire affected vegetation. (Spaccini et al., 2000; Santin et al., 2008; Gonzales-Perez et al., 2004; Knicker et al., 2005).

The increase in the TOC/TN ratio (Table 1) after the fire event is due, principally, to the TOC increase more than to the TN change after fire. This could confirm the accumulation of

incompletely burnt plants necromass, or a post-fire enhancement of the litter from decaying fire-affected vegetation production (Knicker et al., 2005a; 2007; Gonzalez-Perez et al., 2004; Santin et al., 2008)

The light fraction (LF) content (Table 2), which represents all residues, with a density value lower than 1.7 g ml<sup>-1</sup>, on the top soil before and after the wildfire event, could be the reason for the high value of the TOC observed. This fraction (LF) increases after fire in the same way as the TOC, around 28%; which represent one possible source of organic material (incomplete burnt plants) that would be incorporated to the native pool of soil organic matter and thus a way to a progressive stabilization of the different organic compounds produced by the fire effect, such as, aliphatic compounds, polysaccharides, peptides of plant and microbial origin and other organic compound generated by fire. The carbon content slightly increases and nitrogen content decreases significantly ( $\approx$  27%) after fire in the LF. The C/N ratio indicate that this fraction is formed by an unstable organic fraction, composed by debris with incomplete combustion, thus, it could produce a nitrogen immobilization during the stabilization and the incorporation to the native soil organic matter.

3.2 Organic matter fractions analysis

The carbon content of each fraction (CHS, CHA and CFA) were calculated as a percentage of the TOC, therefore, the % CHA correspond to the Humification Index (HI) (Roletto et al., 1985; Ciavatta et al., 1988).

Vergnoux et al.(2011a, 2011b), found that the different fraction of the humic substances decrease after fire, in agreement with Almendros et al.(1990); Fernandez et al.(1997); Gonzalez-Perez et al.(2004); Kincker et al.(2005). Other studies suggest that during the wildfire a humic-like fraction can be produced from burned plant biomass and thus it would be extractable in alkaline solution. In general, medium heating, i.e. temperatures not higher than 250°C, leads to increase complexity of the organic matter: newly formed compounds, oxidation and thermal fixation of alkyl moieties, etc. (Almendros et al., 1992; Gonzalez-Perez et al., 2004).

The organic carbon content of each fraction (CHS, CHA, and CFA) of the burned and unburned soil is shown in table 2.

Sample	CHS	CFA	CHA(HI)	CHA/CFA	LF	N%	C%	C/N	FLF
Unburned soil (UBS)	1.92a	0.68a	1.23b	1.80b	56.7a	1.54b	17.35a	11.2a	0.24a
Burned soil (BS)	1.84a	0.79b	1.06a	1.34a	66.9b	1.12a	18.37a	16.4b	0.39b

CHS, CFA, CHA, LF and FLF: expressed as % in function of 100 g of TOC Different letters (a-b) in the same column indicate significant differences (p< 0.05) according to Tukey test.

Table 2. Carbon content in each humic substances fraction (CHS, CFA, and CHA), carbon light fraction content (LF), nitrogen and carbon content in the carbon light fraction, and the free lipidic fraction (FLF) content in burned and unburned soil

The variation in the CHS content after the wildfire is not statistically significant; this could be due to the original humic materials transformations into an alkali-insoluble macromolecule material (Gonzalez-Perez et al., 2004; Fernandez et al., 2004), which is in agreement with the amount of hydrophobic fraction (FLF) found in both soil samples (table 2).



The CFA increases around 15% after fire and CHA decrease around 12% in the soil exposed to high temperatures. The increase of the CFA content indicate the newly formed compounds, with more aliphatic chains, in general, with less molecular size, produced by the breakup of the more aggregated structures of the humic acids and thus, the carbon humic fraction decrease. The Humification Index (HI) (Table 2) is reduced about 12% indicating, also, the alteration in the humic substances by wildfire.

The ratio CHA/CFA (Table 2), also known as “degree of polymerization or polymerization index”, decrease around 25% in the burned soil, reflecting the breakdown of the complex and more aggregated structures of unheated soil humic fraction, indicating that the wildfire lead to an important change in the structure and the properties of the humic substances fraction (Debano et al., 2000; Shakeesby & Doerr 2006).

### 3.3 Spectroscopic properties of soil alkaline extracts

The scattering of monochromatic light in a diluted solution of macromolecules or colloidal particles is closely related to weight, size, aggregation and interaction of particles in solution. The UV-Visible absorption of humic substances was used to evaluate the condensation degree of the aromatic compounds (Chen et al., 1977; Stevenson, 1982; Polak et al., 2009).

Sutton & Sposito (2005), suggest that the apparent size of humic materials do not change due to tight coiling (or uncoiling), but instead change due to disaggregation (or aggregation) of clusters of small molecules.

The absorption at 280 nm was also introduced to represent total aromaticity, because the  $\pi$ - $\pi^*$  electron transition occurs in this UV region, for phenolic arenes, benzoic acids, aniline derivatives polyenes and polycyclic aromatic hydrocarbon with two or more rings (Uyguner & Bekbolet, 2005).

The absorption at 470 nm is related with the fragment produced for the depolymerization or disaggregation of the supramolecular structure or material with a low humification degree (Sellami et al., 2008; Zbytniewski & Buszewski, 2004).

The absorbance at 664 nm is characteristics of high oxygen content, aromatic compound, strongly humified material with a high degree of condensed groups (Sellami et al., 2008).

Lipski et al.(1999) defined E2/E4 ratio (the ratio of absorbance at 280 and 400 nm) to characterize the degradation of phenolic/quinoid core of humic acids to simpler carboxylic aromatic compounds. This ratio may represent an alternative parameter for the elucidation of the photocatalytic degradation efficiency.

The value of the quotient E4/E6 (the ratio of absorbance at 400 and 665nm) and E2/E6 (the ratio of absorbance 280 and 665 nm) coefficient are related with aromatic condensation; suggest the aggregation level, phenolic and benzene-carboxylic group content, among other characteristics. A low ratio reflects a high degree of aromaticity, aggregation and high humification level; large values are associated with the presence of smaller size organic molecules, more aliphatic structures, high content of functional groups, high disaggregation level (Chen et al., 1977; Pertusati & Prado, 2007, Zbytniewski & Buszewski, 2004).

The value of the coefficient E2/E4 for CHS and CFA (Table 3) obtained in the alkaline extracts for the burned and unburned soil, don't have a great variation, suggesting that the degradation of core structure of humic substances, depolymerization or the disaggregation of the supramolecular structure was not significant, probably several aggregate disruption was produced by heating the soil (Uyguner & Bekbolet, 2005; Sutton & Sposito, 2005).

The values of the quotient E2/E6 and E4/E6 are around 20-30% greater for both fraction (CHS and CFA) in the burned soil than in the unburned (Table 3). This variation suggest that the temperatures reached during the fire event, probably around 250-300°C, produced some degree of disaggregation effect and also, the increasing in the quotient value could be due to the newly organic compounds produced by the litter and vegetal residues burned during the wildfire.

Sample	E2/E4	E2/E6	E4/E6		E2/E4	E2/E6	E4/E6
	CHS	CHS	CHS		CFA	CFA	CFA
Unburned soil (UBS)	7.7a	0.67a	5.2a		28.65a	0.63a	18.1a
Burned soil (BS)	7.5a	0.84b	6.33b		29.4a	0.81b	23.9b

Different letters (a-b) in the same column indicate significant differences (p<0.05) according to Tukey test.

Table 3. Alkaline extracts Absorbance ratio of burned and unburned soil samples

The greater content obtained for the CFA (table 2) is in agreement with the disaggregation observed through the E2/E6 and E4/E6 values after the fire event.

3.4 Humic Acids characterization

3.4.1 Elemental composition

Elemental composition (ash and moisture-free basis) O/C, H/C (atomic ratios) and E2/E4, E2/E6 and E4/E6 ratio of the HA extracted from unburned and burned soil are shown in Table 4.

The increase in the carbon content after fire could be produced by the incorporation of the incompletely burned necromass to the original supramolecular structure. The decrease in the oxygen content after fire suggests that the environment could have reducing properties.

The atomic ratio of O/C and H/C are often used to monitor structural changes of humic substances (Gonzalez-Perez et al., 2004; Adani et al., 2006).

Sample	C	H	N	O	S	O/C	H/C	E280	E460	E660	E4/E6
HA-UBS	49.67a	5.46a	4.97a	39.46b	< 0.4a	0.59b	1.32b	1.78a	0.34a	0.09a	3.77a
HA-BS	53.89b	5.28a	4.93a	35.45a	< 0.4a	0.49a	1.18a	2.15b	0.47b	0.12b	3.92a

Different letters (a-b) in the same column indicate significant differences (p<0.05) according to Tukey test.

Table 4. Elemental composition (ash and moisture-free basis) O/C, H/C (atomic ratios) and E2/E4, E2/E6 and E4/E6 ratio of the HA studied

The decrease in the atomic H/C ratio observed for HA-BS, suggest a diminution in the peripheral aliphatic chains with low thermal stability and thus, an increase in the aromaticity because this domains was found resistant to the effects of fire. The decrease in the O/C ratio indicates a substantial loss of oxygen-containing functional groups. The mains change observed in HA heated in laboratory or in natural fire are the dehydration and

decarboxylation which explain the progressive alteration in the colloidal properties of soil affected by fire (Gonzalez-Perez et al., 2004).

### 3.4.2 Spectroscopic properties

UV-Visible spectra were recorded for both HA analyzed, the specific absorbance decreases steadily with increasing wavelength. The spectra are close to those presented in other studies related to the chemical nature of humic acids (Senesi et al., 1989; Fuentes et al., 2006). The absorption properties are conventional and versatile for the characterization and were used to evaluate the condensation degree of the humic aromatic nuclei. Various absorption wavelengths at 270, 280, 300, 400, 465 nm, among other, and their ratios have been cited for the spectral differentiation of humic substances (Sellami et al., 2008; Uyguner et al., 2005).

By analyzing the absorption spectrum of UV-Visible, three important regions were observed at 280, 460 and 660 nm. The absorbance at 280 nm (E280) is related to lignin, aniline derivatives, polyenes and polycyclic aromatic hydrocarbon with two or more rings (Uyguner & Bekbolet, 2005). The absorbance at 460 nm (E460) is the result of organic macromolecules with a low polymerization degree, and the absorbance at 660 nm (E660) is characteristic of high oxygen content, aromatic compound, high size and molecular weight (Sellami et al., 2008; Uyguner et al., 2005).

The absorbance of the HA extracted from the burned soil is greater than the absorbance of the HA isolated from the unburned soil, similar to those obtained for Vergnoux et al. (2011a). This behavior indicates that the HA isolated from the soil exposed to high temperatures have greater content of different fraction of organic compounds. The increase of the absorption at 280 nm (Table 4) indicates the presence of fraction like lignin derivatives and compounds with aliphatic chains; the absorption at 460 nm (Table 4) suggests the increment of compounds with a low polymerization degree or less condensed structural domains and the increment of the absorption at 660 nm (Table 4) suggests the increase of aromatic compounds with great microbial and/or chemical resistance, structures that have refractory character (Vergnoux et al. 2011a; Sellami et al., 2008; Santin et al. 2008; Gonzalez-Perez et al., 2004).

The growth observed in the content of all these fractions could be due through the incorporation of the compounds produced by an incomplete combustion of the vegetation, and therefore, a considerable amount of newly formed C forms were adding together to the thermal modified C forms previously existing in the ecosystem (Cofer et al., 1997; Gonzalez-Perez et al., 2004). Through the E4/E6 value for both HA, burned and unburned HA, (3.92 and 3.77 respectively), in general, is possible to suppose that the nuclei of the macromolecule of HA, the aromaticity, the size, the weight were not disrupted by the temperature reached in this event fire, instead, the wildfire could have enough energy to produce a disruption onto the linkage which retain together the small fraction of the supramolecular structure and thus a disaggregation could take place; this behavior is shown through the increment of the absorbance values.

### 3.4.3 Potentiometric titration: Acid base properties and charge evolution

The charges-pH curves (-Q versus pH) of the HA isolated, between pH 3 and 11, obtained from potentiometric titration, corrected for blank solution and fitted with sixth degree polynomial according to Machesky (1993) and Campitelli & Ceppi (2008), are shown in the Figure 1a. This smoothing function was selected for their simplicity.

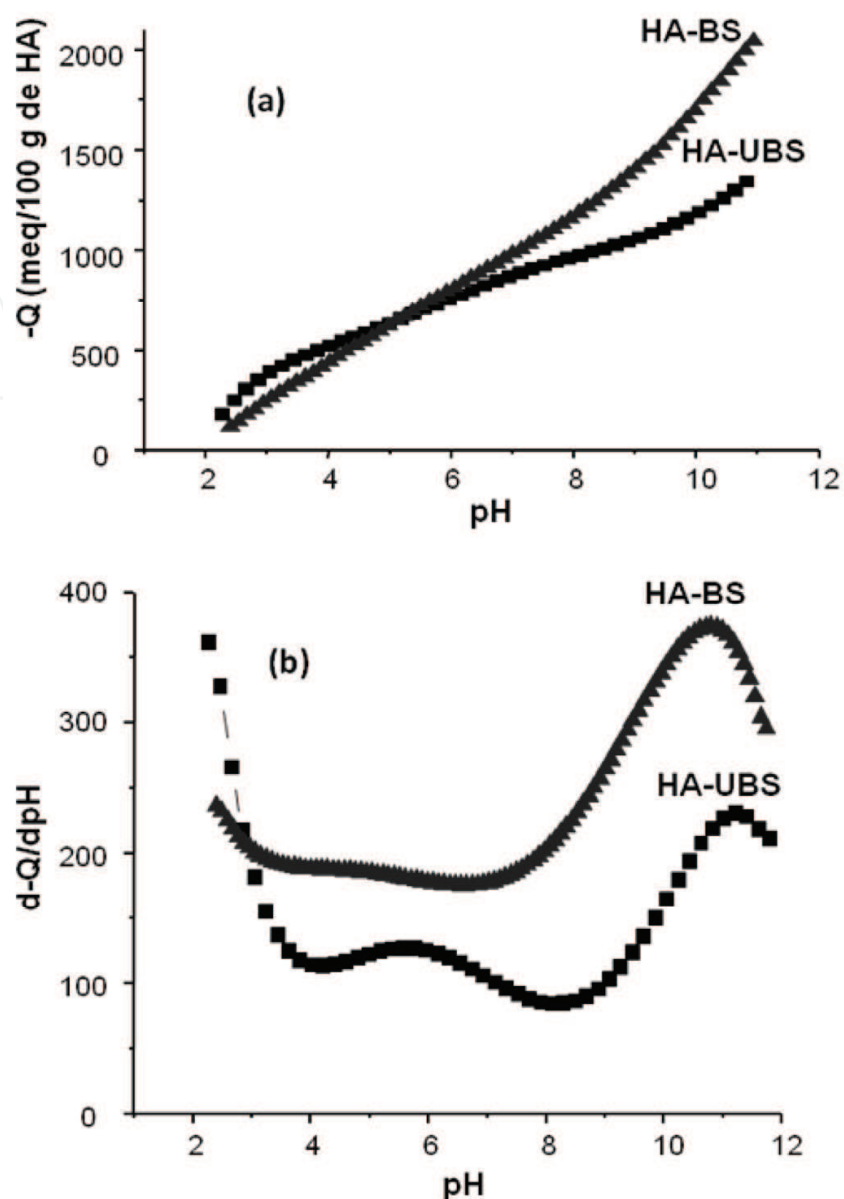


Fig. 1. (a): Charge-pH curves of humic acids extracted from burned (HA-BS) and unburned soil (HA-UBS). The charge developments were calculated on the basis of the sixth polynomial equation (with  $R^2$  values exceeding 0.999 in all cases). (Charge development were calculated taking into account the ash content); (b): Apparent proton-affinity distribution of humic acids extracted from from burned (HA-BS) and unburned soil (HA-UBS) obtained from the first derivatives through charge-pH curves  $[d(-Q)/d(pH)]$  smoothing with sixth degree polynomial equation through the experimental data in the range of 3–10

The charge development of HA isolated from the burned soil (HA-BS) is greater than for the humic acids extracted from unburned soil (HA-UBS) in the region of pH 6 to 11 and lower at the more acidic region (3 to 6). Total acidity is about 60% greater in the HA extracted from burned soil than those of the unburned soil. In the acidic pH region (3 to 6) the lower charge development for HA isolated from burned soil could be due to the loss of strong acidic sites produced by the disruption of the supramolecular structure.

The disaggregation produced by temperature could be the reason for the increment of the negative charge development up to pH 6, because the negative charged groups increase as the size of the fractions decrease (Tombacz, 1999). This behavior is in agreement with that observed through the spectroscopic analysis.

Through the first derivative of the  $-Q$  versus pH curves ( $-dQ/dpH$ ) obtained from the titration curves smoothed with the polynomial equation (Figure 1b), is possible: i) to obtain the average of apparent proton-dissociation constant ( $pK_{aap}$ ) of each set of acidic groups, ii) to analyze the chemical heterogeneity of each class of acidic group present in the HA macromolecule, iii) to estimate the concentration of each set of acidic groups by the calculus of the area under each peak and iv) to estimate the buffer capacity developed by each class of acidic site (Nederlof et al., 1994; Koopal et al., 2005; Campitelli et al., 2006; Campitelli & Ceppi, 2008). In this way, is possible to follow how the acid-base characteristics, i.e., the evolution in quantity and quality for the principal acidic groups (carboxylic and phenolic), were changed for the fire event.

The number of site classes (set of acidic groups) is then equal to the number of peaks and the peak position could be used as an average of the apparent dissociation constant ( $pK_{aap}$ ) (de Wit et al., 1993).

The samples of HA extracted from unburned soil (HA-UBS) show two main peaks, the first would be assigned to the carboxylic groups (strong acidic sites) and the second to the phenolic groups (weak acidic sites). For the HA isolated from burned soil (HA-BS) the first peak is only a shoulder and the second peak is well defined. In both HA samples (HA-BS and HA-UBS), also is observed, a small or developing peaks at more acidic pH values ( $\leq 4$ ), indicating, probably, a presence of stronger acidic sites; this behavior is more clear in HA-BS. This is in agreement with previous results obtained studying HA extracted from soil (Campitelli et al., 2006; Campitelli & Ceppi, 2008).

HA isolated from burned soil (HA-BS) presents the first peaks or shoulder, not well defined, with the maxima at around pH 3.5 and the second with a maximum at pH 10.8; the first could be assigned to strong acid sites (carboxylic groups) and the second to weak acidic sites (phenolic groups). The peak at pH=3.5 was wider than the peak at pH=10.8. The minimum was not well defined, and the partial overlapping of peaks indicate that there is no significant differences among the acidic sites in the surface, in terms of proton dissociation strength. This results suggest a large chemical heterogeneity on the HA present or the production of small organic compounds during the fire event.

These small organic compounds could be produced by the incomplete combustion of the vegetation present; Knicker et al. (2007) suggested that around 250 °C new molecular structures are produced; the principal structures could be aliphatic C; phenol and/or furan C; Sharma et al. (2004) suggested that some decarboxylation could occur at higher temperatures ( $>250^{\circ}\text{C}$ ) but the aromatic rings still remain essentially intact. This behavior could justify the decrease in the negative charge development at pH values lower than 6 and their increase at higher pH values ( $\text{pH} > 6$ ).

HA isolated from unburned soil (HA-UBS) have two well defined peaks, the first with the maximum at pH 5.6 and the second at pH 11.2, these values are similar to other obtained for soil derived humic acids (Campitelli et al., 2006; Campitelli & Ceppi 2008)

The  $pK_{aap}$  for the carboxylic and phenolic groups in the HA derived from the burned soil (HA-BS) are lower than the corresponding for HA extracted from unburned soil (HA-UBS), this could be due to the disruption of the supramolecular structure of the humic acids, and



in this way the carboxylic groups that remains in the surface are those with very strong acidic characteristics, probably those in the aromatic structures, like *o*-COOOH or in greater fractions, and the phenolic groups are those in the small fraction produced by the disaggregation (Table 5) (Knicker et al., 2007; Sharma et al., 2004). For both type of acidic groups (*o*-COOH and OH-Phenolic), the contribution could be from the partial combustion of vegetation and then extracted with the alkaline media, without discrimination (Adani et al., 2004).

Humic acids	<i>o</i> -COOH	pKaap	-COOH	pKaap	phenolic-OH	pKaap
HA-UBS	320a	2.3	473	5.6	567a	11.2
HA-BS	588b	3.5	---	---	1318b	10.8

Acidic groups: cmol kg<sup>-1</sup> Different letters (a-b) in the same column indicate significant differences (p<0.05) according to Tukey test.

Table 5. Acidic functional groups content (*o*-carboxylic, carboxilic and phenolic) content calculated by integration of the area under each maximum of the curves (d-Q/ dpH) obtained through the first derivative of smoothed experimental data. The pKaap values correspond to the maximum of each peak

The fire event altered the concentration of acidic sites (Table 5) and therefore the buffer capacity. For the burned soil, the buffer capacity of HA was neglectable at soil pH value around 6 (Table 1) and for pH value ranging between  $\approx 3 - 7$ . This can be attributed to the great heterogeneity of HA in this pH range and to the lost of carboxylic groups with pKaap values around 5.

In the zone up to pH 8 (weak acidic sites) the buffer capacity is greater than that observed for HA from unburned soil, but this groups, in both cases, are not dissociated at soil pH values, thus they have not a significant contribution to the soil buffer capacity. The fire event produced important changes in the acid-base properties, principally in the buffer capacity of the HA.

The loss of carboxylic groups onto this HA structure produced by fire event (Table 5), i.e. the decrease of negative charge development below pH 6, cause a deficiency of charged site to make linkage between the inorganic and organic fraction through cation-bound; and thus, the formation of soil aggregates. In this way, this characteristic could be the key factor promoting soil erosion (Mill & Fey 2004). The fire event could generate important modification in the physicochemical properties of the HA

At the lowest pH measured (sites domains below 4), the HA-UBS shows a developing peak (Fig 1b) indicating that very acidic sites could be present in the macromolecule, in HA-BS it seems that this sites are the only present (Table 5). The minimum around pH 4, which could be considered as a separation of both type of acidic sites (like COOH) from the very acidic sites (like *o*-COOH), is clearer in the HA extracted from unburned soil (HA-UBS) than in the HA from the burned soil (HA-BS), this indicate, also, the heterogeneity of the acidic groups present in the HA extracted from soil exposed to high temperatures, due to the disaggregation produced by the temperature developed during the wildfire.

3.4.4 Capillary zone electrophoresis

The main characteristics of HA are the occurrence of acidic site with different strength, the principal groups are the strong (carboxilic groups) and weak (phenolic groups) acidic site.

For these HA analyzed the average  $P_{ka}$  value are around 3.5 – 5.5 for the carboxylic groups and 10 – 11 for the phenolic groups (Table 5).

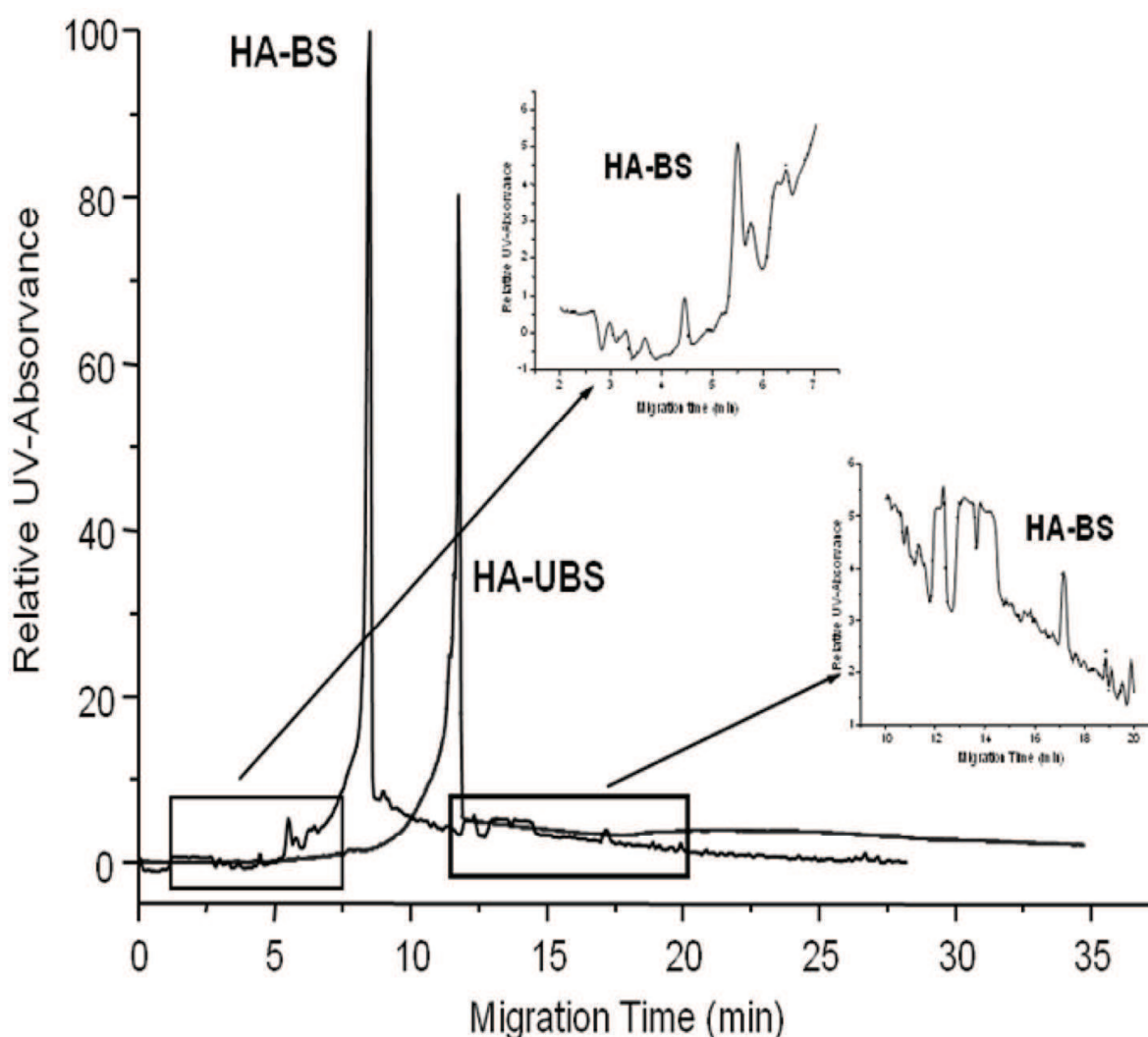


Fig. 2. Electropherograms of acids extracted from burned (HA-BS) and unburned soil (HA-UBS) in buffer borate 20 mmol L<sup>-1</sup> (pH=9.3), temperature 25°C, the concentration of the AH solutions were 1000 ppm. CZE conditions: voltage of -30kV, injection hydrodynamic 5000 Pa for 20 s, detection at 260 nm, fused-silica capillary, 73 cm total length, 75 µm i. d. (effective length 64.5 cm). Total run time 30 min (for time migration higher than 30 min no significant peak were observed)

At the experimental condition (pH ≈ 9) all of the strong acidic groups and approximately, the half of the weak acidic groups of HA are deprotonated (negatively charged). The presence of negative charges permit to separate HA by electrophoresis in an electrical field (+) to (-) in which the EOF (electro osmotic flow) is responsible for the movement of the analyte (Peuravouri et al., 2004).

The electropherogram of HA extracted from unburned soil (HA-UBS) (fig 2) shows a principal and well defined peak at time migration 11.73 min and the characteristic hump at time migration around 7 – 8 min, just before the main peak, is shown as a tail; at migration time higher than 12 min no peaks were distinguished.

The electropherogram of HA isolated from burned soil (HA-BS) presents the main peak at lower time migration (8.40 min) than in HA of unburned soil (HA-UBS), and several peaks are detected before and after the main peak (fig 2); at migration time higher than 25 min no peaks were distinguished.

The peak at 11.73 min observed in the electropherogram corresponding to the HA-UBS, could indicate that in these experimental conditions the macromolecule migrate as a unbroken entity, the tailing observed at lower time migration, could be assigned to some structure with low mass/charge ratio difficult to be separated; i.e. the macromolecule is not easy to be separated in subfraction with different electrokinetic properties, similar behavior was observed for Fetch & Havel (1998); Pokorna et al.(2000); Peuravouri et al.(2004).

The different time migration for the principal peak of the HA from burned soil (HA-BS) and the peaks detected at both side of the peak at 8.40 min could indicate changes in the macromolecule structure and the presence of subfraction.

The BGE, borate, could react with phenols, phenols carboxylic, polycarboxylic acids, dihydroxy or perihydroxy groups present in the solution and thus the separation of each fraction would be improved ( Fetsch & Havel 1998). The phenolic groups present in HA isolated from burned soil (HA-BS) is greater than that quantified in HA extracted from unburned soil (HA-UBS), this characteristic could produce the interaction between the BGE and this acidic groups and enhance the separation.

The electropherogram profile of the HA extracted from burned soil (AH-BS) indicates the presence of distinct subfraction, which could be produced by the disaggregation of the macromolecule of HA and/or the formation of newly small carbon compounds after heating, suggesting that the temperature reached during the fire event, breaks, disaggregates or creates new structure, with lower and higher mass/charge ratio and diverse electrokinetic mobility. This behavior confirms the large heterogeneity, the disaggregation and the new carbon compound produced for the wildfire and are in agreement with those observed through the other different analytical techniques used to study these HA.

#### 4. Conclusions

The temperature reached in the fire event was enough to produce several changes in the organic matter characteristics, i.e. changes in the quantity and/or quality of their fraction: light fraction, humic acids, fulvic acids, free lipidic fraction.

The fire event produced important changes in the structure of the macromolecule of humic acids, like break and/or disaggregation which generate compound with lower size, weight, mass/charge ratio and/or newly formed carbon compounds originated by the incomplete combustion of the vegetal materials.

The fire event could generate important modification in the physicochemical and acid-base properties of the HA.

The amount of acidic functional group was changed: the COOH sites were decreased and the OH phenolic sites were increased by the fire event. The pKaap values were modified, in general, the acidic site are stronger after fire than in the unburned soil. The COOH groups with pKaap value about 5 were lost after fire. The buffer capacity is lower or practically missing at soil pH ( $\approx 6$ ) after fire.

The negative charge development decrease significantly at field pH ( $\approx 6$ ) after the fire event, producing a deficiency on sites to make linkage between the organic and inorganic soil

fraction, and in this way a reduction of aggregates formation. This characteristic could be the key factor promoting soil erosion.

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Soils play multiple roles in the quality of life throughout the world, not only as the resource for food production, but also as the support for our structures, the environment, the medium for waste disposal, water, and the storage of nutrients. A healthy soil can sustain biological productivity, maintain environmental quality, and promote plant and animal health. Understanding the impact of land management practices on soil properties and processes can provide useful indicators of economic and environmental sustainability. The sixteen chapters of this book orchestrate a multidisciplinary composition of current trends in soil health. Soil Health and Land Use Management provides a broad vision of the fundamental importance of soil health. In addition, the development of feasible management and remediation strategies to preserve and ameliorate the fitness of soils are discussed in this book. Strategies to improve land management and relevant case studies are covered, as well as the importance of characterizing soil properties to develop management and remediation strategies. Moreover, the current management of several environmental scenarios of high concern is presented, while the final chapters propose new methodologies for soil pollution assessment.

### **How to reference**

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### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

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