

Interaction of a Recombinant Prion Protein with Organo-Mineral Complexes as Assessed by FT-IR and CPMAS ^{13}C NMR Analysis

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Abstract: Prion proteins are considered as the main agents for the Transmissible Spongiform Encephalopathies (TSE). The misfolded form, PrP^{Sc}, which is also indicated as the etiological agent for TSE, exhibits high resistance to degradation in environmental processes. Soil contamination by prion proteins is a real environmental issue since contaminated soils can become potential reservoir and diffuser for TSE infectivity. In this work, the interaction of prion protein with organo-mineral complexes was studied by using a recombinant non pathogenic prion protein and a model soil system. This latter was represented by a soil manganese mineral coated with polymerized catechol. FT-IR spectra showed amide I and II signals which revealed protein involvement in catechol polymers coating manganese oxide surface. CPMAS ^{13}C -NMR was applied to follow the complexation of the protein to the soil-like system. All the signals were attributed to C-N in peptidic bonds, alkyl chains and methyl groups. The NMR spectrum of the prion protein interacting directly with birnessite revealed disappearance of signals due to the paramagnetic nature of manganese oxide or protein abiotic degradation, while the presence of organic matter strongly reduced the disappearance of prion protein signals.

Keywords: Prion protein, TSE diseases, Soil Organo-Mineral Complexes, FT-IR, CPMAS ^{13}C NMR.

INTRODUCTION

Transmissible Spongiform Encephalopathies are fatal, neurodegenerative diseases also known as prion diseases including bovine spongiform encephalopathy, human Creutzfeldt-Jakob disease, kuru, sheep scrapie, and chronic wasting disease of deer, elk and moose [1]. PrP^{Sc} is a misfolded isoform of the normal cellular prion protein (PrP^C) considered as the main, if not the sole, agent of TSE [2]. PrP^{Sc} causes pathogenesis in the central nervous system with the formation of amyloid aggregates and a consequent spongiosis in the brain of humans and animals. These diseases, nowadays considered incurable, lead slowly but inexorably to death.

PrP^{Sc} is protease resistant and exhibits a remarkable resistance to degradation and inactivation by standard decontamination procedures [3].

Dispersion of PrP^{Sc} contaminated material in soil can occur from meat and bone meal storage plants, use of fertilizers augmented with meat and bone, decomposition of animal carcasses buried in soil, liquid and solid waste fragments from abattoirs, and wastes from TSE-infected animals [4]. As firstly evidenced by Brown and Gajdusek [5], buried infectious prion protein can persist in soil even for years. Seidel *et al.* [6] found that in soil, scrapie agent remained in an infectious form after 29 months. Moreover, infected soil

was supposed to preserve TSE capability to transmit the disease to healthy grazing animals after 16 years from the contamination event [7].

Either the infective or the benign forms of prion proteins are able to strongly bind to mineral [8-12] and organic soil components [13-15]. PrP^{Sc} can be displaced from environmental compartments to soil, but this does not necessarily decrease its infectivity [10,16]. Prion protein and other soil exogenous proteins can also easily interact with organo-mineral complexes that likely are in soil more abundant than the mineral or organic components alone. Soil has been suggested to act as a reservoir of TSE infectivity [17,18] and even to enhance the transmissibility of prion disease by oral uptake of soil complexed PrP^{Sc} [16]. While biotic PrP^{Sc} degradation by some proteases can be reduced because of persistent PrP^{Sc} aggregates, its inactivation may arise by abiotic oxidative reactions. In fact birnessite, a manganese oxide common in soil, is able to degrade the PrP^{Sc} in aqueous suspensions [19]. The presence of organic matter coating reactive soil mineral surfaces could hinder abiotic degradation processes with different effects on prion stability.

Interaction of PrP^{Sc} with organic matter can take place either with forming or pre-formed humic substances (HS). Interaction with already-formed HS could lead to superficial protein sorption, while entrapment phenomenon could arise if the protein is involved in the humification process [13]. In general, immobilized proteins may result more stable than free forms; for instance enzymes in humic complexes are more resistant to thermal denaturation and proteolysis than the respective free enzymes [20].

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In soil, prion proteins can interact with soil constituents and remain immobilized in soil colloids. Since in soil environment prion protein contamination can likely occur at the surface layers, the infectious agent can result more easily available for free-ranging animals promoting the disease diffusion in the environment. It is, therefore, important to understand how prion proteins interact with soil components present in the surface layers and how the soil organic matter and its transformation in humic substances could affect the stability of prions, possibly protecting them from environmental degradation.

The aim of the present work was to study through different spectroscopic techniques, such as FT-IR and CPMAS ^{13}C -NMR, the complexation of a recombinant ovine prion protein (recPrP) with soil organo-mineral complexes obtained by oxidative polymerization of catechol mediated by birnessite. The birnessite-catechol complexes resembling soil organo-mineral complexes were prepared in the presence of recPrP and by following two different sequences of addition of recPrP, before and after catechol polymerization, in order to obtain either entrapment or sorption, respectively.

MATERIALS AND METHODS

Chemicals

Reagent grade catechol (Cat) (>99 % purity) and HPLC grade solvents were purchased from Sigma Aldrich (Germany). Birnessite (Bir) was synthesized according to McKenzie [21] using KMnO_4 and HCl . X-ray spectra diffraction analyses performed on the synthesized MnO_2 confirmed the birnessite poorly crystalline mineral structure with characteristic peaks as reported in McKenzie [21]. Birnessite has a point of zero charge of 1.81 [22], and pH-dependent negative charge at typical soil pHs.

A purified recombinant ovine ARQ genetic variant prion protein (recPrP) with a MM of 23 kDa (residues 23-234) was prepared according to Rezaei *et al.* [23] and kindly furnished by Virologie et Immunologie Moléculaires lab of INRA (Jouy-en-Josas, France). The protein with an isoelectric point of 9.2 [23] is constituted by a well-folded C-terminal domain (residues 125-234) and a flexible N-terminal arm (residues 23-124) [24].

Preparation of recPrP-Organic-Mineral Complexes

Organic-mineral-recPrP complexes used in this study were prepared according to the methodology described by Rao *et al.* [13]. Briefly, either 3 or 5 mM catechol (Cat3 and Cat5, respectively), 0.5 mg ml^{-1} recPrP and 5 mg ml^{-1} birnessite in 0.1 M sodium acetate buffer at pH 5.5 were incubated in different systems. Two ternary samples (catechol-birnessite-recPrP) were produced: Cat-Bir-recPrP, where Cat, Bir and recPrP were incubated for 4 h at 25 °C, and Cat-Bir+recPrP, where Cat and Bir were incubated for 2 h before adding recPrP and incubating for further 2 h at 25 °C. Binary systems, Cat-Bir, Cat-recPrP and Bir-recPrP, and sample with only Cat, recPrP or Bir were also produced and served as controls.

All trials were incubated for a total of 4 h at 25 °C. Supernatants and insoluble precipitates were separated by centrifugation (30 min at 10,000 rev min^{-1} and 4 °C). Residual catechol and recPrP concentrations were evaluated by HPLC

analysis [13] with a Shimadzu instrument equipped with UV-Vis variable-wavelength absorbance detector, set at 280 and 222 nm respectively.

The pellets were washed twice with 0.02 M NaCl, twice with double-distilled water, freeze-dried and stored at 4 °C [13].

FT-IR Analyses

The Fourier transform infrared spectra of insoluble products were recorded by the Universal Attenuated Total Reflectance (UATR) method using a Perkin Elmer FT-IR spectrometer. Each spectrum represents a collection of 24 scans recorded at a 4 cm^{-1} resolution.

CPMAS ^{13}C -NMR Analyses

CPMAS ^{13}C NMR measurements were performed on a Bruker Avance-II 400 spectrometer (Bruker Biospin, Milan, Italy) operating at 100.6 MHz on carbon-13 and equipped with a 4 mm standard bore solid state probe. Samples were packed into 4 mm zirconia rotors with Kel-F caps and the rotor spin rate was set at 13,000 \pm 1 Hz. In total, 4 k data points (TD) were collected over an acquisition time (AQ) of 50 ms, a recycle delay (RD) of 2.0 s, and 30,000 scans (NS). Contact time was 1 ms, while a ^1H RAMP sequence was used to account for possible inhomogeneity of the Hartmann-Hahn condition at high rotor spin rates [25]. Precise ^1H 90° pulse calibration for CP acquisition was obtained as reported in Conte and Piccolo [26]. Bruker Topspin® 2.0 software was used to acquire all the spectra. Spectra elaboration was conducted by Mestre-C software (Version 4.9.9.9, Mestrelab Research, Santiago de Compostela, Spain). All the FIDs (free induction decays) were transformed by applying first a 4 k zero filling and then an exponential filter function with a line broadening (LB) of 100 Hz. Fully automatic baseline correction using a Bernstein algorithm was applied for baseline corrections [27].

RESULTS

FT-IR Analyses

Infrared spectra were recorded for polymeric products of catechol adsorbed on birnessite surface and their complexes with recPrP added under different sequences (Fig. 1).

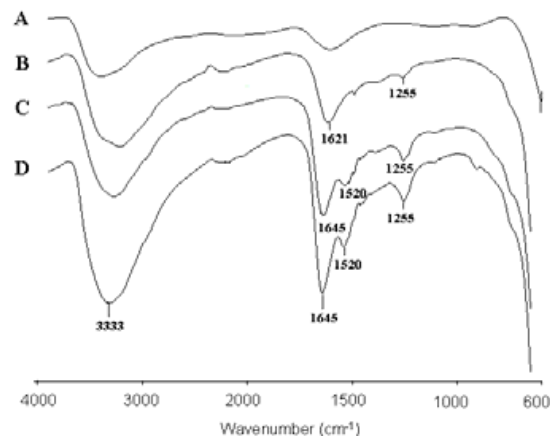


Fig. (1). FT-IR spectra of **A.** Bir; **B.** Bir-Cat3; **C.** Bir-Cat3+recPrP; **D.** Bir-Cat3-recPrP.

Table 1. Location of Relevant Indicator Bands in recPrP and Organo-Mineral Complexes and the Assignment to Functional Groups

Wavenumber (cm ⁻¹)	Sample	Assignment
3333	Cat3-Bir Cat3-Bir-recPrP Cat5-Bir+recPrP	OH, bonded through intermolecular H bonds
1621	Cat3-Bir	Aromatic C=C, H-bonded C=O or alkenes in conjugation with C=O
1645	recPrP Cat3-Bir-recPrP Cat3-Bir+recPrP	C=O stretching (amide I)
1520	recPrP Cat3-Bir-recPrP Cat5-Bir+recPrP	N-H bending (amide II)
1255	recPrP Cat3-Bir Cat3-Bir-recPrP Cat5-Bir+recPrP	C-O stretching and aromatic C=C; amide III

The most characteristic bands and their assignments related to samples obtained with 3 mM Cat as well as 5 mM Cat (data not shown) are summarized in Table 1.

All samples showed a broad band at 3333 cm⁻¹ attributable to OH groups bound through intermolecular H bonds [28]. Spectral bands derived from vibrations of aromatic carboxylates (R-COO⁻) and/or aromatic C=C structures (1650-1620 cm⁻¹) were also present. FT-IR spectra revealed the presence of organic material attributed to the presence of Cat polymerization products, (1621 and 1255 cm⁻¹). Complexes containing recPrP showed typical signals of amide I and amide II (1645 and 1520 cm⁻¹, respectively); a reinforcement of the weak signal at 1255 cm⁻¹ was attributed to amide III. Similar FT-IR spectra were recorded for catechol-birnessite-PrP complexes obtained at 5 mM catechol [13].

CPMAS ¹³C NMR Analyses

RecPrP was analysed by CPMAS ¹³C NMR (Fig. 2A). The spectrum revealed a typical NMR signal pattern for proteins in the solid state [29]. In fact, a signal attributable to C=O groups was observed at 178 ppm, while signals at 161, 133, 120 ppm were assigned to aromatic moieties having different substitution degrees [30]. All the signals comprised between 90 and 0 ppm were attributed to C-N in peptidic bonds (80 ppm), alkyl chains (47 ppm) and methyl groups (34 ppm), respectively. The spectra of the birnessite with prion protein or catechol (Bir-recPrP, Bir-Cat5 and Bir-Cat3, respectively, Fig. 2B,C,D) appeared resolution-less, whereas those of samples obtained by interaction of birnessite, phenol and prion protein added before and after catechol polymerization process (Bir-Cat3+recPrP and Bir-Cat3-recPrP, respectively, Fig. 2E,F) revealed the same signal pattern of recPrP alone as in Fig. (2A), but with a larger signal width.

DISCUSSION

The recPrP was completely removed from the solution by sorption or entrapment in catechol-birnessite soil-model systems confirming the high affinity of prions to soil organo-

mineral colloids. The presence in the insoluble catechol-protein polymeric products of humic-like compounds and of the protein was confirmed by FT-IR signals typical of humic compounds and proteins (Table 1).

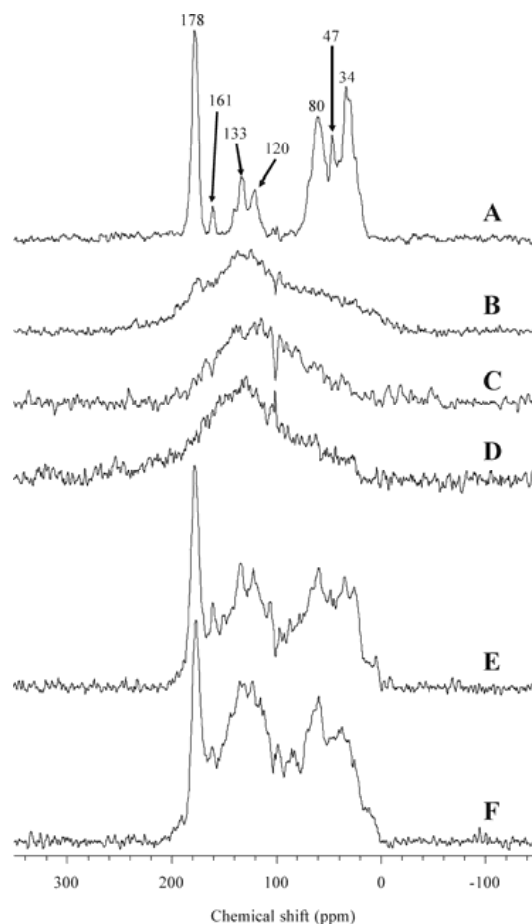


Fig. (2). CPMAS ¹³C-NMR spectra of A. recPrP; B. Bir-recPrP; C. Bir-Cat5; D. Bir-Cat3; E. Bir-Cat3+recPrP; F. Bir-Cat3-recPrP.

Catechol transformation by birnessite started to produce soluble compounds as detected in the supernatants by UV-Vis analyses. They were more abundant in the samples with 3 mM catechol than with 5 mM catechol [13]. When the protein was added before catechol polymerization (Cat-Bir-recPrP) as well as when catechol was preventively polymerized and partially adsorbed on birnessite surfaces (Cat-Bir+recPrP) recPrP interacted with birnessite-catechol complexes and was completely removed from solution, as demonstrated by HPLC analysis [13]. Conversely in Bir-recPrP sample, 33% of recPrP was detected as free in the supernatant. The stability of recPrP in the humic-like complexes was confirmed by the negative release of the protein after extractions with several, even strong, extractants [13].

As reported in literature, a partial recPrP abiotic degradation mediated by MnO₂ could be not excluded [19]. However, in the presence of catechol the degradation of recPrP should have been reduced because birnessite active surfaces were covered by organic catechol polymers [31,32]. After catechol polymer deposition, a reduced birnessite activity is reasonable because the birnessite-mediated reaction is reported to be chemically surface-controlled and occurring by associative ligand substitution mechanism with a surface complex formation on Mn-oxide sites [22]. According to this mechanism, during the preparation of the complexes, catechol produced large polymers located at the surface of birnessite and capable of promoting the formation of large pores among the enclosed birnessite particles [15].

As also reported in Rao *et al.* [13], UV-Vis spectra and elemental analyses of the samples produced with 3 mM catechol solution showed a larger soluble polymeric content while samples produced with a 5 mM catechol solution had a dominant insoluble catechol polymeric fraction. The weaker bands of amide I and II observed in Cat3-Bir+recPrP than those of Cat5-Bir+recPrP confirmed their different behaviour. In any case, the initial catechol concentration and the sequence of the addition of the protein had no effect on protein immobilization that resulted always completely removed from the solution.

Acquisition of solid state (SS) NMR spectra is strongly affected by paramagnetism [33]. In fact, the presence of paramagnetic systems (PS) alters NMR signal shape and intensity, thereby providing no signal at all when the amount of paramagnetic species is larger than that of the NMR investigated nucleus. It is reported, for example, that iron (III) in soil samples prevents reliable ¹³C NMR spectra acquisition when the Fe/C mass ratio is »1 [25].

NMR signal disappearing is attributed to the interactions between the magnetic field generated by the paramagnetic centers and the applied magnetic field [34]. These interactions fasten both longitudinal (T₁) and transversal (T₂) relaxation times so that NMR signals are both broadened and lowered [25]. In the solid state, an additional problem is the mismatching of the fundamental SS NMR condition, T_{CH}«T_{1ρ}(H), which usually guarantees obtainment of quantitative NMR spectra [25]. However, from a qualitative point of view, when a spectrum is acquired, the relative intensities of the various resonances do not reflect the real distribution present in the samples since some functional groups may be more affected by the presence of the paramagnetic centers

[35]. In fact, all the groups directly interacting with the paramagnetic ions relax faster than those placed far away from PSs. As a consequence, the NMR signals of the quick relaxing nuclei disappear more rapidly than the resonances of the remaining others.

Fig. (2A, B) reports the CPMAS ¹³C NMR spectra of the recombinant prion protein before (Fig. 2A) and after (Fig. 2B) absorption on birnessite (Bir). Due to the presence of the paramagnetic manganese, all the signals of recPrP were broadened. However, a broad signal around 130 ppm due to aromatic structures and the resonance at 178 ppm due to carboxyls were still visible. Only the signals between 90 and 0 ppm completely disappeared (Fig. 2B). A possible explanation for such behaviour can be related to the preferential interactions of recPrP with the surface area of birnessite through C-N and alkyl moieties.

The effect of natural organic matter on recPrP absorption on birnessite was estimated by analyzing the complexes obtained with catechol at the two different concentrations (3 and 5 mM, respectively) (see Materials and Methods). Fig. (2C, D) shows that the humic-like organic substances produced by the incubation of catechol are adsorbed on birnessite. In fact, the signals of the incubated catechol disappeared and only a very large band around 130 ppm became visible. When the incubation of catechol-birnessite mixture was conducted also in the presence of recPrP, added either after or before catechol polymerization, the spectra in Figs. (2E and 2F) were obtained, respectively. Both spectra resembled that in Fig. (2A), thereby indicating that only a humic-like mediated interaction between recPrP and birnessite was possible.

CONCLUSIONS

The interaction of recPrP with catechol-birnessite complexes occurred at all phenol concentrations used in the present study. Moreover, the interactions took place regardless of the sequence used to add the natural-organic-matter-like (NOM-like) system to the mineral phase. In fact, recPrP appeared to be bound to the birnessite through NOM-like mediated bindings which were observed by comparing the CPMAS ¹³C NMR spectra of different birnessite-recPrP complexes. FT-IR analysis contributed to confirm the presence of recPrP in the insoluble polymeric products, already at the lowest catechol concentration. Both FT-IR and NMR studies were helpful to highlight the important role of humic-like substances formed by abiotic catalysis from phenolic compounds in adsorbing and/or entrapping recPrP, and the prevalent involvement of alkyl moieties rather than aromatic or carboxylic groups.

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ABBREVIATIONS

recPrP = recombinant prion protein
Cat-Bir-recPrP = recPrP added before catechol polymerization

Cat-Bir+recPrP	= recPrP added to catechol preventively polymerized and partially adsorbed on birnessite surfaces
CPMAS ¹³ C NMR	= cross polarization magic angle spinning carbon-13 nuclear magnetic resonance
FT-IR	= Fourier transform infrared spectroscopy
PS	= paramagnetic systems
SS NMR	= solid state nuclear magnetic resonance
T _{CH}	= cross polarization time
T _{1ρ} (H)	= proton longitudinal relaxation time in the rotating frame
T ₁	= longitudinal relaxation time
T ₂	= transverse relaxation time
UV-Vis	= ultraviolet visible spectrophotometry

REFERENCES

- Watts JC, Balachandran A, Westaway D. The expanding universe of prion diseases. *PLoS Pathog* 2006; 2: e26.
- Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science* 1982; 216: 136-44.
- Taylor DM. Inactivation of transmissible degenerative encephalopathy agents: a review. *Veter J* 2000; 159: 10-7.
- Andr oletti O, Lacroix C, Chabert A, *et al.* PrP(sc) accumulation in placentas of ewes exposed to natural scrapie: influence of foetal PrP genotype and effect on ewe-to-lamb transmission. *J Gen Virol* 2002; 83: 2607-16.
- Brown P, Gajdusek DC. Survival of scrapie virus after 3 years' interment. *Lancet* 1991; 337: 269-70.
- Seidel B, Thomzig A, Buschmann A, *et al.* Scrapie agent (strain 263K) can transmit disease *via* the oral route after persistence in soil over years. *PLoS One* 2007; 2: 435-42.
- Georgsson G, Sigurdarson S, Brown P. Infectious agent of sheep scrapie may persist in the environment for at least 16 years. *J Gen Virol* 2006; 87: 3737-40.
- Vasina EN, D ejardin P, Rezaei H, Grosclaude J, Quiquampoix H. Fate of prions in soil: adsorption kinetics of recombinant unglycosylated ovine prion protein onto mica in laminar flow conditions and subsequent desorption. *Biomacromolecules* 2005; 6: 3425-32.
- Revault M, Quiquampoix H, Baron MH, Noinville S. Fate of prions in soil: trapped conformation of full-length ovine prion protein induced by adsorption on clays. *Biochim Biophys Acta* 2005; 1724: 367-74.
- Johnson CJ, Phillips KE, Schramm PT, McKenzie D, Aiken JM, Pedersen JA. Prions adhere to soil minerals and remain infectious. *PLoS Pathog* 2006; 2: 296-302.
- Rigou P, Rezaei H, Grosclaude J, Staunton S, Quiquampoix H. Fate of prions in soil: adsorption and extraction by electroelution of recombinant ovine prion protein from montmorillonite and natural soils. *Environ Sci Technol* 2006; 40: 1497-503.
- Ma X, Benson CH, McKenzie D, Aiken JM, Pedersen JA. Adsorption of pathogenic prion protein to quartz sand. *Environ Sci Technol* 2007; 41: 2324-30.
- Rao MA, Russo F, Granata V, Berisio R, Zagari A, Gianfreda L. Fate of prions in soil: Interaction of a recombinant ovine prion protein with synthetic humic-like mineral complexes. *Soil Biol Biochem* 2007; 39: 493-504.
- Polano M, Anselmi C, Leita L, Negro A, De Nobili M. Organic polyanions act as complexants of prion protein in soil. *Biochem Biophys Res Commun* 2008; 367: 323-9.
- Pucci A, D'Acqui LP, Calamai L. Fate of prions in soil: interactions of RecPrP with organic matter of soil aggregates as revealed by LTA-PAS. *Environ Sci Technol* 2008; 42: 728-33.
- Johnson CJ, Pedersen JA, Chappell RJ, McKenzie D, Aiken JM. Oral transmissibility of prion disease is enhanced by binding to soil particles. *PLoS Pathog* 2007; 3: 874-81.
- Miller MW, Williams ES. Prion disease: horizontal prion transmission in mule deer. *Nature* 2003; 425: 35-6.
- Schramm PT, Johnson CJ, Mathews NE, McKenzie D, Aiken JM, Pedersen JA. Potential role of soil in the transmission of prion disease. *Miner Geochem* 2006; 64: 135-52.
- Russo F, Johnson CJ, Johnson CJ, McKenzie D, Aiken JM, Pedersen JA. Degradation of the pathogenic prion protein by a manganese mineral prevalent in soils. *J Gen Virol* 2009; 90: 275-80.
- Nannipieri P, Ceccati B, Grego S. Ecological significance of biological activity in soil. In: Bollag J-M, Stotzky G, Eds. *Soil biochemistry*. Marcel Dekker: New York 1990; Vol 6: p. 293.
- McKenzie RM. Manganese oxides and hydroxides. In: Dixon JB, Weed SB, Eds. *Minerals in soil environments*, 2nd ed. SSSA, Madison, WI 1989; p. 439.
- Matocha CJ, Sparks DL, Amonette JE, Kukkadapu RK. Kinetics and mechanism of birnessite reduction by catechol. *Soil Sci Soc Am J* 2001; 65: 58-66.
- Rezaei H, Marc D, Choiset Y, *et al.* High yield purification and physico-chemical properties of full-length recombinant allelic variants of sheep prion protein linked to scrapie susceptibility. *Eur J Biochem* 2000; 267: 2833-9.
- Eghiaian F, Grosclaude J, Lesceu S, *et al.* Insight into the PrPc - PrPsc conversion from the structures of antibody bound ovine prion scrapie-susceptibility variants. *Proc Natl Acad Sci USA* 2004; 101: 10254-9.
- Conte P, Spaccini R, Piccolo A. State of the art of CPMAS ¹³C-NMR spectroscopy applied to natural organic matter. *Prog Nucl Magn Reson Spectrosc* 2004; 44: 215-23.
- Conte P, Piccolo A. Precise measurement of ¹H 90° pulse in solid-state NMR spectroscopy for complex and heterogeneous molecular systems. *Anal. Bioanal Chem* 2007; 387: 2903-9.
- Brown DE. Fully automated baseline correction of 1D and 2D NMR spectra using Bernstein algorithm. *J Magn Reson A* 1995; 114: 268-70.
- Schnitzer, M. Characterization of humic constituents by spectroscopy. In McLaren AD, Skujins J, Eds. *Soil biochemistry*. Marcel Dekker: New York 1971; Vol. 2: p. 60.
- Watts A, Straus SK, Grage SL, Kamihira M, Lam YH, Zhao X. Membrane protein structure determination using solid-state NMR. *Methods Mol Biol* 2004; 278: 403-73.
- Wilson MA. *NMR Techniques and applications in geochemistry and soil chemistry*. 1st ed. London: Pergamon Press 1987.
- Naidja A, Huang PM, Bollag J-M. Comparison of the reaction products from the transformation of catechol catalysed by birnessite or tyrosinase. *Soil Sci Soc Am J* 1998; 62: 188-95.
- Majcher EH, Chorover J, Bollag J-M, Huang PM. Evolution of CO₂ during birnessite-induced of ¹⁴C-labeled catechol. *Soil Sci Soc Am J* 2000; 64: 157-63.
- Preston, CM. Application of NMR to soil organic matter analysis: history and prospects. *Soil Sci* 1996; 161: 144-66.
- Keeler, J. *Understanding NMR spectroscopy*. Chichester, England: Wiley & Sons 2009.
- Smernik RJ, Oades MJ. Paramagnetic effects on solid state carbon-13 nuclear magnetic resonance spectra of soil organic matter. *J Environ Qual* 2002; 31: 414-20.

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