

SHORT COMMUNICATION

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Sorption of L-methionine-sulphoximine on humic acids and clay minerals

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Abstract The sorption of L-methionine-sulphoximine (MSX) on soil-extracted humic fractions (MW > 20,000 Da), and mined clays (kaolinite and montmorillonite) was studied by a reversed-phase high-performance liquid chromatography method. The molecule, acting as an inhibitor of glutamine synthetase activity, was recently used in soil incubation assays for the determination of gross rates of N mineralization. Equilibrium data of MSX adsorbed on humic fractions and clay minerals were described by both the Freundlich and Langmuir sorption isotherms. It was observed that humic fractions were more effective than mined clay minerals in removing MSX from water. Most isotherms were of Langmuir type, indicating a higher affinity of MSX for sorbing sites which become increasingly saturated at higher equilibrium concentrations. Analysis of Langmuir empirical constants revealed that different adsorbing mechanisms took place. Results showed that when MSX is applied in soil incubation assays, sorption of MSX on soil colloids considerably reduces the availability of the inhibitor to target microorganisms, and renders the method unsuitable for the determination of the gross rate of N mineralization, especially when high levels of both organic matter and clay minerals are present in the soil.

Key words L-Methionine-sulphoximine · Humic fraction · Clay minerals · Sorption isotherms

Introduction

N mineralization is a key process which determines the amount of soil N made available for plant uptake and for other microbial and chemical transformations. Estimating gross N mineralization rates is difficult as the microbial processes of NH_4^+ production via mineralization of organic N, and consumption via immobilization (incorporation into amino acids), occur simultaneously.

Nannipieri et al. (1994) proposed a method for determining gross rates of N mineralization by using L-methionine-DL-sulphoximine (MSX), an inhibitor of glutamine synthetase (GS) activity. The enzyme is part of the GS-glutamate synthase system for inorganic N incorporation into organic compounds (Magasanik and Neidhardt 1987), and is thought to play a key role in the process of N immobilization by microorganisms at the NH_4^+ levels commonly found in soil (< 0.1 mM) (McCarty 1995). The hypothesis for this approach was that, provided there were no significant NH_4^+ losses in short-term experiments, a complete inhibition of GS activity would block microbial N immobilization and thus would allow the determination of gross mineralization rates from net mineralization rates. However, in previous studies increases in NH_4^+ concentration were accompanied by increased evolution of CO_2 , which raised the possibility of MSX mineralization (Hopkins et al. 1995; Landi et al. 1995). The application of an ^{15}N -isotopic-dilution technique showed an increased gross N mineralization rate in the presence of the inhibitor (Landi et al. 1999). In addition, it was hypothesized that MSX was only partially effective at blocking N immobilization due to its possible involvement in sorption processes on soil particle surfaces and/or microbial breakdown. In another study the recovery of MSX was negatively correlated with cation exchange capacity (CEC) values and total C and N contents of soil (Gelsomino et al. 1999). No information is available on the mechanisms and the factors affecting MSX sorption by clay and soil organic matter. In order to investigate

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the contribution of soil colloids to the sorption of MSX, we carried out sorption measurements of MSX by humic acids (HA) extracted from soil and by mined clays, namely montmorillonite and kaolinite.

Materials and methods

HA from four surface soils (0–20 cm depth), whose chemical and physical properties are reported in Table 1, were extracted and purified according to the IHSS method (Rouiller et al. 1994). Purified HA extracts were dispensed in 5-ml aliquots and stored at -20°C . HA from the B_h horizon of an alpine podzolic soil (Table 1) were extracted and purified as described by Cacco et al. (1974), and stored after freeze-drying. Chemical characteristics of the HA are shown in Table 2. Total acidity and the levels of carboxyl groups were determined by the $\text{Ba}(\text{OH})_2$ method and the Ca-acetate exchange method, respectively (Stevenson 1982). The content of phenolic groups was assumed to be the difference between the total acidity and the content of carboxyl groups. The degree of polymerization was evaluated by the ratio of the absorbance at 465 and 665 nm. The ash content was determined by combustion at 550°C (Stevenson 1982).

A Twiggs County (Georgia, USA) kaolinite and a Clay Spur (Wyoming, USA) montmorillonite with CEC values of 9.56 and 70.27 cmol kg^{-1} , respectively, were obtained from Ward's Natural Science Establishment (Rochester, N.Y.). MSX was purchased from Sigma. (St. Louis, Mo.), *o*-phthalaldehyde (OPA) of chromatographic grade from Pickering (Mountain View, Calif.); boric acid and β -mercaptoethanol of analytical grade were purchased from Carlo Erba (Milan); LiChrosolv HPLC grade methanol, HPLC grade tetrahydrofuran, 100% glacial acetic acid and sodium acetate were from Merck (Darmstadt, Germany).

Sorption measurements on HA were performed at a pH ranging from 5.6 to 6.0. Duplicate samples of 2.85 mg HA were equilibrated in centrifuge polyallomer tubes with 6 ml aqueous MSX solution ranging from 18 to 1034.5 μM . The tubes were shaken

for 14 h at $23 \pm 2^{\circ}\text{C}$, then the suspensions were centrifuged at 35,000 g for 15 min. The supernatant was stored at -20°C before purification and reversed-phase high-performance liquid chromatography (RP-HPLC) analysis. Sorption isotherms on mined kaolinite and montmorillonite were determined on duplicate 50-mg samples of air-dried clay in centrifuge polyallomer tubes with 5 ml aqueous MSX solution ranging from 50 to 566 μM . The tubes were shaken for 14 h at $23 \pm 2^{\circ}\text{C}$, then the suspension was centrifuged at 35,000 g for 15 min. The MSX concentration in the supernatant was determined, after purification, by a RP-HPLC method after pre-column derivatization with OPA, followed by UV detection of MSX derivatives as previously described (Gelsomino et al. 1999). The extent of sorption was estimated as the difference between the initial and final MSX concentration in the solution. Sorption data were fitted to either the Freundlich adsorption equation

$$x/m = Kf Ce^{1/n} \quad (1)$$

or the Langmuir adsorption equation

$$x/m = \frac{Kl \cdot b \cdot Ce}{(1 + Kl \cdot Ce)} \quad (2)$$

where the amount adsorbed per mass of sorbent (x/m , $\mu\text{mol g}^{-1}$) is plotted vs. the equilibrium concentration (Ce , μM). In Eq. 1, Kf and $1/n$ are empirical constants describing the sorption capacity and sorption intensity, respectively. In Eq. 2, Kl and b are empirical constants related to bonding energy and the sorption maximum, respectively. The fitting of the data was performed by non-linear regression software (TableCurve 2D v4.0; Jandel Scientific, San Rafael, Calif.) using the Levenberg-Marquardt optimization algorithm. Mean values of three data points were reported in the isotherms.

Results and discussion

Sorption isotherms of MSX on either soil-extracted humic fractions, or mined montmorillonite and kaolin-

Table 1 Physical and chemical properties of the soils. *Sa* Sand, *Sa-L* sandy loam, *L-Sa* loamy sand

Sampling site	Vegetation cover	Soil type ^a	Particle distribution (%)			Texture ^b	pH		Total C (%)	Total N (%)	CEC (cmol kg^{-1})
			Sand	Silt	Clay		(H_2O)	(KCl)			
Passo del Mercante ^c	Beech forest	Haplic phaeozem	61.0	32.0	7.0	Sa-L	5.8	4.6	8.11	0.680	45.6
Passo del Mercante ^c	Pine forest	Haplic phaeozem	76.5	15.0	8.5	Sa-L	5.6	4.6	2.47	0.166	17.5
Pistoia ^d	Horticultural	Eutric fluvisol	63.2	17.6	19.2	Sa-L	6.7	6.1	3.89	0.280	15.2
Romola ^d	Fallow	Haplic luvisol	90.7	3.6	5.7	Sa	7.2	5.9	1.05	0.098	16.9
Passo del Giovo ^c	Alpine meadow	Ferric podzol	81.7	13.1	5.2	L-Sa	4.6	nd	4.70	nd	36.2

^a FAO classification

^b USDA classification

^c Reggio Calabria (southern Italy), 850 m altitude

^d Tuscany (central Italy)

^e Bolzano (northern Italy), 2200 m altitude

Table 2 Some chemical characteristics of soil humic matter. E_4/E_6 Ratio of the absorbance at 465 and 665 nm

Soil sample	Total acidity	COOH	Phenolic OH	E_4/E_6	Ash (g kg^{-1})
	(cmol kg^{-1})				
Passo del Mercante ^a	6.60	2.00	4.60	5.24	31.0
Passo del Mercante ^b	9.60	2.00	7.60	4.84	54.0
Pistoia	12.00	3.50	7.50	4.77	9.9
Romola	13.60	4.50	9.10	6.25	6.7
Passo del Giovo	10.60	1.25	9.35	2.90	87.3

^a Beech forest soil

^b Pine forest soil

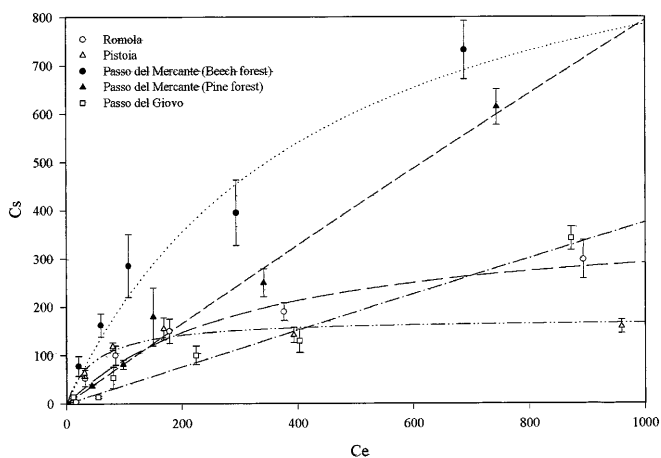


Fig. 1 Langmuir sorption isotherms of L-methionine-sulphoximine (MSX) on soil-extracted humic acids (HA). Error bars are SEs. C_e Equilibrium concentration (μM), C_s $\mu\text{mol MSX adsorbed g}^{-1}$ HA

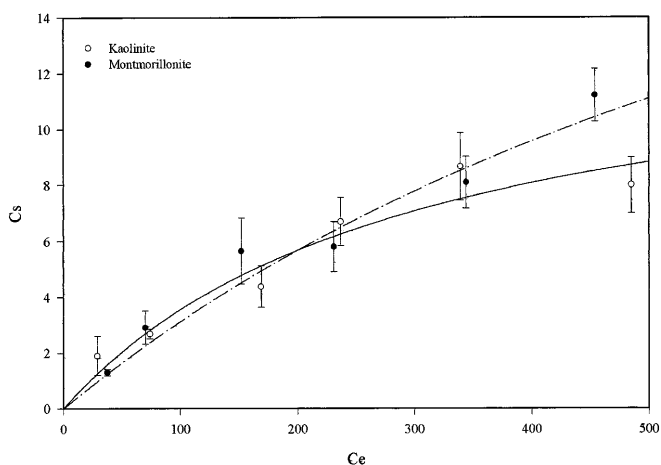


Fig. 2 Langmuir adsorption isotherms of MSX on kaolinite and montmorillonite. Error bars are SEs. C_e (μM); C_s $\mu\text{mol MSX adsorbed g}^{-1}$ clay

ite, are shown in Figs. 1 and 2. The isotherms were classified as Langmuir type according to the system devised by Giles et al. (1960, 1974). This implied that the solute was preferentially and initially adsorbed compared to the solvent, and this sorption decreased as more solute was sorbed. The experimental data were fitted to the relationships based on the empirical Freundlich and Langmuir adsorption equations. We found that the Langmuir isotherm gave the best fits for the description of MSX sorption. The r^2 values together with Langmuir empirical constants are given in Table 3.

The most prominent feature of these results was the different affinity of the inhibitor for the adsorbent surfaces. The humic fractions showed a marked adsorbing capacity (Fig. 1) whereas the clay minerals provided a smaller degree of sorption (Fig. 2). Humic fractions from Passo del Mercante beech forest soil and from Pis-

Table 3 Langmuir constants (Kl and b are empirical constants related to bonding energy and sorption maximum, respectively) for the sorption of L-methionine-sulphoximine on soil humic acids and clay minerals

Sample	Kl	b	r^2
Passo del Mercante ^a	2.30×10^{-3}	1127.42	0.918
Passo del Mercante ^b	0.06×10^{-3}	14997.24	0.904
Pistoia	21.03×10^{-3}	175.38	0.746
Romola	3.09×10^{-3}	385.50	0.948
Passo del Giovio	0.02×10^{-3}	18807.54	0.847
Kaolinite	3.40×10^{-3}	14.04	0.905
Montmorillonite	1.14×10^{-3}	30.67	0.861

^a Beech forest soil

^b Pine forest soil

toia soil showed the highest and the lowest adsorbing capacity, respectively. The Langmuir constants, Kl , and b , indicating the bonding energy and the maximum sorption capacity, respectively, differed from sample to sample, suggesting that various mechanisms of sorption may occur (Table 3). MSX is an amino acid analogue that has three acidic sites: the carboxyl group, the α -amino group and the imino group, which dissociate at pH 2.2, 9.3 and 10.2, respectively. Being an amphoteric compound its ionization into cationic, zwitterion or anionic molecules depends on the pH of the solution. In the pH range investigated (5.6–6.0), only the cationic form was present in solution. The ionic interaction occurring between the cationic form of MSX and the negatively charged humic surface may account for the strong sorption of the inhibitor by humic fractions. However, while sorption via ion exchange can be considered to be of major importance, other binding and trapping mechanisms cannot be excluded in the interaction between MSX and the humic fraction. Schulten and Schnitzer (1997) suggested that in soil proteins, peptides and amino acids can be partially trapped in the voids of three-dimensional HA structures or, to a greater extent, physically or chemically retained by the HA surfaces. Amino acids of N-rich humic fractions have been monitored (Sorge et al. 1993) and ca. 40% of total soil N was found to be present as amino acid N after acid hydrolysis (Schulten and Schnitzer 1998; Senwo and Tabatabai 1998). Lack of significant correlations between the sorption constants (Table 3) and the chemical characteristics of humic fractions (Table 2) could have been due to the simultaneous occurrence of different mechanisms for the sorption of MSX on HA.

Among soil components, clay minerals may also adsorb and bind a range of organic molecules (Stotzky 1986), including amino acids (Greenland et al. 1965a,b; Dashman and Stotzky 1982). Since MSX is an amino acid analogue, it is therefore not surprising that it could be adsorbed on clay minerals. However, the extent of sorption on mined montmorillonite and kaolinite was much lower than that on the humic fractions (Figs. 1, 2), probably because ion exchange was the only mechanism involved in sorption by clays.

These results provide evidence of the action of MSX in soil. However, although assessing the role of each adsorbing material in soil is important, in situ the situation is more complex because, for example, organic matter is intimately bound to clay minerals (Stevenson 1982). Thus, the contribution of clay minerals and organic fractions to sorption will depend upon the extent to which their surfaces are coated with other materials. Possible microbial breakdown of MSX cannot be excluded when it is present at lower concentrations in soil incubation assays (Landi et al. 1999). However, the fact that an equilibrium state was reached in most isotherms accounts for a predominant sorption process. The amount of MSX available for biochemical interaction with the microbial enzyme, GS, can therefore be very low in soil rich in organic matter and/or clay minerals.

In conclusion, it is worth considering that soil microbial populations are significantly less sensitive to effects of biochemical inhibitors than the same organisms in pure culture (Prosser 1997). The difference is due to possible degradation of biochemical inhibitors within the soil or, as shown in the present study, by their sorption on active surfaces of soil colloids.

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References

- Cacco G, Maggioni A, Ferrari G (1974) Electrofocusing: a new method for characterization of soil humic matter. *Soil Biol Biochem* 6:145–148
- Dashman T, Stotzky G (1982) Adsorption and binding of amino acids on homoionic montmorillonite and kaolinite. *Soil Biol Biochem* 14:447–456
- Gelsomino A, Landi L, Cacco G, Nannipieri P (1999) Determination and depletion kinetics of L-methionine-sulphoximine in soil. *Soil Biol Biochem* 31:561–566
- Giles CH, MacEwan TH, Nakhwa SN, Smith D (1960) Studies in adsorption. Part XI. A system of classification of solution adsorption isotherms, and its use in diagnosis of adsorption mechanisms and in measurement of specific surface area of solids. *J Chem Soc*, pp 3973–3993
- Giles CH, D'Silva AP, Easton IA (1974) A general treatment and classification of the solute adsorption isotherms. Part II. Experimental interpretation. *J Colloid Interface Sci* 47:766–778
- Greenland DJ, Laby RH, Quirk JP (1965a) Adsorption of amino acids and peptides by montmorillonite and illite. Part 1. Cation exchange and proton transfer. *Trans Faraday Soc* 61:2013–2023
- Greenland DJ, Laby RH, Quirk JP (1965b) Adsorption of amino acids and peptides by montmorillonite and illite. Part 2. Physical adsorption. *Trans Faraday Soc* 61:2024–2035
- Hopkins DW, Anderson L, Scott SE (1995) N and C mineralization in soil amended with the N immobilization inhibitor, methionine sulphoximine. *Soil Biol Biochem* 27:377–379
- Landi L, Badalucco L, Nannipieri P (1995) Changes in inorganic N and CO₂ evolution in soil induced by L-methionine-sulphoximine. *Soil Biol Biochem* 27:1345–1351
- Landi L, Barraclough D, Badalucco L, Gelsomino A, Nannipieri P (1999) L-Methionine-sulphoximine affects N mineralisation-immobilisation in soil. *Soil Biol Biochem* 31:253–259
- Magasanik B, Neidhardt FC (1987) Regulation of carbon and nitrogen utilization. In: Neidhardt FC, Ingraham JL, Low KB, Magasanik B, Schaechter M, Umberger HE (eds) *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society of Microbiology, Washington, D.C., pp 1318–1325
- McCarty GW (1995) The role of glutamine synthetase in regulation of nitrogen metabolism within the soil microbial community. *Plant Soil* 170:141–147
- Nannipieri P, Badalucco L, Landi L (1994) Holistic approaches to the study of populations, nutrients pools and fluxes: limits and future research needs. In: Ritz K, Dighton J, Giller KE (eds) *Beyond the biomass*. Wiley, Chichester, pp 231–238
- Prosser JI (1997) Microbial processes within the soil. In: van Elsland JD, Trevors JT, Wellington EMH (eds) *Modern soil microbiology*. Marcel Dekker, N.Y., 33:183–213
- Rouiller J, Souchier B, Bruckert S, Feller C, Toutain F, Védry JC (1994) Méthodes d'analyses des sols. In: Duchaufour P, Souchier B (eds) *Pédologie*, 2nd edn, vol 2. Masson, Paris, pp 630–631
- Schulten H-R, Schnitzer M (1997) Chemical model structures for soil organic matter and soils. *Soil Sci* 162:115–130
- Schulten H-R, Schnitzer M (1998) The chemistry of soil organic nitrogen: a review. *Biol Fertil Soils* 26:1–15
- Senwo ZN, Tabatabai MA (1998) Amino acid composition of soil organic matter. *Biol Fertil Soils* 26:235–242
- Sorge C, Schnitzer M, Schulten H-R (1993) In-source pyrolysis-field ionization mass spectrometry and Curie-point pyrolysis-gas chromatography/mass spectrometry of amino acids in humic substances and soils. *Biol Fertil Soils* 16:100–110
- Stevenson FJ (1982) *Humus chemistry*. Genesis, composition, reactions. Wiley, New York
- Stotzky G (1986) Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. In: Huang PM, Schnitzer M (eds) *Interactions of soil minerals with natural organics and microbes*. SSSA special publication no. 17. SSSA, Madison, Wis., pp 305–428