

Adsorption of Thiamin (Vitamin B₁) on Soils and Clays

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ABSTRACT

Recent research suggests that thiamin applied to soils or coated onto seeds may stimulate plant growth. The behavior of thiamin in soils has not been investigated. Therefore, studies were carried out to determine how thiamin hydrochloride (3-[(4-amino-2-methylpyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride) is adsorbed by 17 soils and three clays. The dominate mechanism in binding of thiamin is thought to be cation exchange with clay minerals and organic matter. In soils with low to medium organic matter content, thiamin adsorption occurred primarily on clay minerals and depended particularly on the amount and composition of the clay. Adsorption is species dependent (pK_{a1} [negative logarithm of the first dissociation constant] = 4.85) and takes place principally in the acidic pH range, probably with position 1' of the pyrimidine ring. Adsorption equilibrium is attained in <30 min. Adsorption in all soils, and in kaolinite and illite clays, could be described by a one-surface Langmuir isotherm at initial concentrations ranging from 16.3 to 995 $\mu\text{mol L}^{-1}$. Adsorption by smectite in the same range was log-linearly related to the equilibrium concentration. Extending the initial concentration range from 16.3 to 9890 $\mu\text{mol L}^{-1}$ showed that a two-surface Langmuir equation more adequately described adsorption in hydroxy-interlayered vermiculitic and chloritic-illitic soils, whereas a one-surface Langmuir equation was found to be adequate in mixed layer-smectitic soils. Only in the case of smectite clays is thiamin allowed to lie in a monolayer configuration parallel to the basal plane.

MOST, IF NOT ALL, B VITAMINS are present in fertile soil (Schopfer, 1943; Lilly and Leonian, 1944; Roulet, 1948; Lochhead, 1958). Their occurrence has been ascribed to various processes such as decomposition or autolysis of plant and animal tissues and organic fertilizers (Starkey, 1942), exudation from plant roots (West, 1939; Rovira and Harris, 1961; Schönwitz and Ziegler, 1982; Strzelczyk, 1987), and synthesis by microorganisms (Van Lanen and Tanner, 1948; Lochhead, 1958). Thiamin (vitamin B₁, aneurin) is a naturally occurring compound present in soils, water, and plant and animal tissues, and is required for microbial and plant growth.

Little information exists concerning the behavior of thiamin in soils. The distribution of a chemical among various phases of the soil is of importance since this determines both mobility and biological availability. Although higher plants are autotrophic with respect to thiamin, it has been demonstrated that this capacity is not always sufficient, e.g., the number and weight of

lateral nodules on legumes could be increased by exogenous application of thiamin (Guyot, 1946). Stimulation of growth may be achieved by applying thiamin directly onto soils or coating onto seeds. Kaolinite is frequently used as a carrier to coat active ingredients onto seeds. The principle of seed coating is to use rotating drums, while gradually adding water along with a powdered blend of coating material and active ingredient to build up incremental layers around the seed. In field experiments with thiamin-coated seeds, best results were obtained with seed dressings of 3 g thiamin and 3 g kaolinite kg^{-1} seed (1991, unpublished data). In addition to uptake by the germinating seed, thiamin was adsorbed by soil particles in contact with the seed (1991, unpublished data). For these reasons, the thiamin concentrations chosen for the adsorption experiments ranged from 5 mg kg^{-1} soil, probably an amount normally encountered in soils, up to 3335 mg kg^{-1} soil.

There is no information available about the extent and intensity of thiamin adsorption on soils. Amines in general have been studied, however, and information might be obtained from related compounds. The adsorption of pyrimidines by montmorillonite was studied by Lailach et al. (1968). Adsorption by ion exchange takes place between the inorganic cations on the clay and the protonated amine group, principally in the acidic pH range. Adsorption is due mainly to cation exchange and depends particularly on pH and the pK_a of the compounds. Adsorption at pH 4.0 and greater was generally proportional to pK_a , but in all cases decreased rapidly as pH increased.

The thiamin molecule consists of one pyrimidine and one substituted thiazole ring (Fig. 1), bound by a methylene bridge. The most common commercial preparation of thiamin is the chloride-hydrochloride, usually called *hydrochloride*. Thiamin hydrochloride (molecular weight 337.3) is soluble in water (1 g mL^{-1}) and below pH 5.5 aqueous solutions are quite stable to heat and to oxidation (Machlin, 1984). The protonated salt of thiamin differs from pyrimidine and related primary amines by having two positive charges, one associated with the pyrimidine ring, and the other with the thiazole ring. The pyrimidine ring is protonated in position 1' at the ring N opposite the amino group (Cain et al., 1977). The addition of one equivalent of base will result in deprotonation of thiamin hydrochloride on the pyrimidine ring. The pK_a of this titration is 4.85 (at 25°C). The second titration step of thiamin requires two equivalents of base and occurs at $pK_a = 9.2$ to 9.3 (Cain et al., 1977).

Abbreviations: CEC, cation exchange capacity; DTA, differential thermal analysis; RCF, relative centrifugal force; XRD, x-ray diffraction. ** Significant at the 0.01 probability level.

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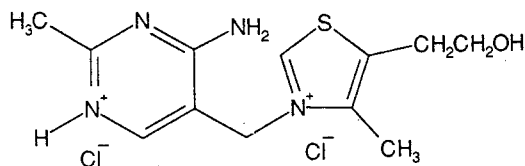


Fig. 1. The thiaminhydrochloride molecule.

The objective of this study was to determine the rate and extent of thiamin adsorption by 17 soils as well as by kaolinite, illite, and smectite. Factors that affect thiamin adsorption were evaluated and include time, pH, type and amount of clay material, and organic matter content. Binding site and steric orientation of the thiamin molecule as well as the dominant mechanism in the binding of thiamin were identified.

MATERIALS AND METHODS

Soil Properties

Oven-dry samples from surface (A) horizons of 17 soils (Table 1), 15 from various parts of the USA (Soils 1–15) and two from Switzerland (Soils 16 and 17), were screened through a 0.8-mm sieve and then stored for use in all adsorption experiments. The selection of these soils was based on previous field experiments in which thiamin was applied directly to the soil or coated on seeds (1991, unpublished data). Physical and chemical properties of the soils (≤ 0.8 -mm fraction) are listed in Table 2. Soil pH was measured in a 1:1 soil/water suspension, in a 1:1 soil/1 M KCl suspension, and in a 1:3 soil/3.29 mM thiamin solution. Clay, silt, and sand content were determined by the pipette method (Gee and Bauder, 1986). Prior to dispersion and sedimentation, carbonate was removed by acidification of the sample and organic matter was removed by H_2O_2 treatment. Organic C was determined by dichromate oxidation (Walkley and Black, 1934). Cation-exchange capacity was determined by a method similar to the procedure of Polemio and Rhoades (1977). Following saturation of the soil with 0.4 M NaOAc–0.1 M NaCl (pH 8.2), the soil was washed free of excess saturating solution with 95% ethanol, then extracted with 0.25 M $Mg(NO_3)_2$ and exchangeable Na determined with an inductively coupled plasma emission spectrometer (ICP Emission Spectrometer, Liberty 200, Varian, Basel, Switzerland). The ethylene glycol monoethyl ether method (Carter et al., 1986) was used for specific surface measurements.

Following pretreatment for mineralogical analysis to remove soluble salts, carbonates, organic matter, and free Fe oxides, sand and silt fractions were separated from the clay using gravimetric sedimentation (Kunze and Dixon, 1986). Sodium-saturated clay samples from water slurries were smeared on glass slides for XRD. X-ray diffraction patterns employing $CuK\alpha$ radiation (30 mA and 40 kV) were obtained with a Philips APD 1700 goniometer system fitted with an automatic divergence slit, a 0.2-mm receiving slit, and a diffracted-beam graphite monochromator (Philips, Almelo, the Netherlands). The clay samples were further analyzed thermoanalytically combined with the analysis of the evolved gases (Müller-Vonmoos et al., 1977). Clay samples from Soils 1, 2, 5, 7, 10, and 15 showed a characteristic water peak at 320 to 350°C with an endothermic reaction in the DTA. These changes have been attributed by Harris et al. (1992) to hydroxy-interlayered clays.

Adsorption studies were also conducted with three clays: kaolinite (China clay; Cornwall, England), Ca-illite (Massif Central, France) and Ca-Mg-smectite (bentonite Montigel; Südchemie Co., Bavaria, Germany). These clays were either used raw (untreated) or as Ca-saturated forms. Selected chemical and physical properties, as well as the mineralogical composition (Müller-Vonmoos and Kahr, 1983), are given in Table 3. Cation-exchange capacity of the clays was determined as described for the soils and additionally by exchange with 0.5 M NH_4OAc at pH 7. Aqueous suspension pH values for the untreated clays were 5.21 for kaolinite, 7.89 for illite, and 8.64 for smectite. Specific surface area was determined by glycerol adsorption on a thermobalance (Madsen, 1977). Calcium-saturated forms of kaolinite, illite, and smectite were prepared by treating 35 g of clay three times for 24 h with 200 mL of 0.5 M $CaCl_2$. The clays were then washed free of Cl by centrifugation with distilled water. After the fourth wash, they appeared Cl free by the $AgNO_3$ test and the clay suspensions were dried at 65°C, ground with pestle and mortar, and used for the adsorption experiments.

Chemical Solutions

Analytical-grade thiamin hydrochloride of specific purity $>98.5\%$ by weight was obtained from the Hoffmann-La Roche Company. Stock solutions of unlabeled thiamin were prepared daily. Analytical-grade [thiazole-2- ^{14}C]thiamin hydrochloride, specific activity 958 MBq $mmol^{-1}$, was obtained from Amersham International (Amersham, UK). [Thiazole-2- ^{14}C]thiamin hydrochloride activity was determined in a Betaszint BF 8000 liquid scintillation counter (Berthold Regensdorf, Switzerland).

Table 1. Soil identifications, classifications, and sampling locations.

Soil	Representative soil series	Classification	Location
1	Tifton	fine-loamy, siliceous, thermic Plinthic Kandiodult	Meigs, GA
2	Orangeburg	fine-loamy, siliceous, thermic Typic Kandiodult	Springfield, SC
3	Nueces	loamy, mixed, hyperthermic Aquic Arenic Paleustalf	Devine, TX
4	Ankeny	coarse-loamy, mixed, mesic Cumulic Hapludoll	Muscataine, IA
5	Rains	fine-loamy, siliceous, thermic Typic Paleaquult	Rocky Mount, NC
6	Clyde	fine-loamy, mixed, mesic Typic Haplaquoll	Cedar Falls, IA
7	Lindside	fine-silty, mixed, mesic Fluvaquentic Eutrochrept	Germansville, PA
8	Ockley	fine-loamy, mixed, mesic Typic Hapludalf	Crown Point, IN
9	Leonard	fine, montmorillonitic, mesic, sloping Vertic Ochraqualf	Leonard, MO
10	Goldsboro	fine-loamy, siliceous, thermic Aquic Paleudult	Goldsboro, NC
11	Drummer	fine-silty, mixed, mesic Typic Haplaquoll	Genesco, IL
12	Waukon	fine-loamy, mixed Mollic Eutroboralf	Hollandale, MN
13	Ontario	fine-loamy, mixed, mesic Glossoboric Hapludalf	Phelps, NY
14	Dundee	fine-silty, mixed, thermic Aeric Ochraqualf	Glen Allan, MS
15	Fuquay	loamy, siliceous, thermic Arenic Plinthic Kandiodult	Elko, SC
16	—	fine-loamy, mixed, mesic Typic Eutrochrept	Dielsdorf, Switzerland
17	—	loamy, mixed, mesic Typic Dystrochrept	Steinhof, Switzerland

Table 2. Physical and chemical properties of the soils studied.

Soil	Clay	Silt	Sand†	Organic matter‡	pH		1:3 soil/3.29 mM thiamin	Cation-exchange capacity	Specific surface	Clay mineralogy XRD peak/DTA§
					H ₂ O	KCl				
		kg kg ⁻¹		g kg ⁻¹				cmol kg ⁻¹	m ² g ⁻¹	
1	0.11	0.09	0.80	8.7	6.1	5.5	5.1	10.8	6	HIV, Q, Gt
2	0.10	0.01	0.87	7.8	6.2	5.3	4.9	2.9	14	HIV, Q, Gt, (S/I)
3	0.07	0.06	0.87	5.2	6.6	5.1	5.1	2.1	24	K, Q, I, S/I
4	nd¶	nd	nd	48.2	5.7	4.7	5.2	18.2	43	S/I, Q, C, Fs
5	0.14	0.29	0.57	20.4	6.7	5.9	5.6	5.2	17	HIV, Q, Gt
6	0.35	0.40	0.25	35.0	6.9	5.9	6.3	23.9	80	S/I, I, K, Q
7	0.28	0.51	0.21	38.5	7.1	6.3	nd	15.6	nd	I, HIV, Q, S/I, Gt, Fs
8	0.31	0.19	0.50	37.8	6.6	5.8	6.1	24.1	63	S/I, I, Q, K, C, Fs
9	0.24	0.68	0.08	22.7	7.4	6.8	7.0	16.7	95	S/I, I, Q, K
10	0.16	0.10	0.74	13.5	5.7	4.6	4.6	5.1	20	HIV, Q, I, S/I
11	0.30	0.54	0.16	43.1	6.4	5.0	5.6	29.2	84	S/I, I, Q, K
12	0.20	0.37	0.43	47.5	7.5	6.9	7.3	30.9	77	S/I, I, C, Q
13	0.13	0.14	0.73	18.9	6.2	5.3	5.6	10.9	33	C, Q, S/I, I
14	0.16	0.51	0.33	8.5	6.3	4.9	5.6	11.3	78	S/I, I, Q, K
15	0.08	0.02	0.90	8.2	6.1	5.1	4.8	2.3	35	HIV, Q, Gt
16	0.34	0.28	0.38	32.5	7.1	6.7	7.0	23.6	78	I, S/I, C, Q
17	0.19	0.35	0.46	16.8	6.0	5.2	5.2	10.2	42	C, I, S/I, Q, Fs

† Pipette method.

‡ Dichromate oxidation.

§ X-ray diffraction peaks/differential thermal analysis. Minerals are listed in order of decreasing peak intensity, most intense listed first. Trace amounts are indicated by enclosure in parentheses. C = chlorite; Fs = feldspar; Gt = goethite; HIV = hydroxy-interlayered vermiculite; I = illite; K = kaolinite; S/I = randomly interstratified smectite-illite; Q = quartz.

¶ nd = not determined.

Adsorption Experiments

For the kinetic study, 1.00-g samples of Dielsdorf clay loam (Soil 16) and Steinhof loam (Soil 17) were shaken with 3 mL of distilled water containing 988 and 98 824 $\mu\text{mol L}^{-1}$ unlabeled thiamin, respectively, and 19.3 $\mu\text{mol L}^{-1}$ labeled thiamin in 10-mL polypropylene centrifuge tubes. The samples were reacted from 1 to 60 min on a reciprocating shaker, then centrifuged for 10 min at RCF values of 7800 g. Previous experiments indicated that the highly reactive fine colloid fraction was adequately removed at this RCF. An aliquot of 0.5 mL of the supernatant solution was analyzed for ¹⁴C-thiamin. Thiamin adsorbed by the clay was calculated as the difference between the initial thiamin concentration and the equilibrium thiamin concentration. The correctness of this assumption was checked by determining the adsorbed amounts with an oxidizer (Packard Oxidizer 306, Canberra Packard, Zurich, Switzerland), using cellulose to accelerate burning of the samples. Adsorbed thiamin could quantitatively be fully recovered (data not shown) from the Dielsdorf and Steinhof soils.

The effect of temperature on adsorption was investigated with Dielsdorf and Steinhof soils in a thermostat-equipped water bath at 4, 18, and 31°C. The initial thiamin concentrations in the soil solution were 17.4, 116, and 1104 $\mu\text{mol L}^{-1}$. Adsorption was measured as described above, but centrifuga-

tion was at the same temperature as the water bath. Samples were taken by volume and corrected for changes in water density with temperature.

The influence of microbial degradation on the adsorption process was investigated by comparing adsorption on sterile and nonsterile soils. Air-dried 1.00-g samples of the Dielsdorf and Steinhof soils were weighed in 10-mL Pyrex centrifuge tubes for sterilization treatment. Preincubated, moist Dielsdorf soil (20 kPa moisture tension) was included to stimulate microbial activity before sterilization. All soils, glassware and dispensing equipment were autoclaved at 120°C and 100 kPa. Thiamin solutions were sterilized by filtration through membranes with 0.2- μm pores. All work was done under a sterilized microbiological hood. Adsorption followed the procedures described above with a reaction time of 30 min.

To determine the significance of organic matter in thiamin binding, the soils were treated with H₂O₂ to remove the labile organic matter.

The influence of pH on thiamin adsorption was investigated with samples of untreated kaolinite, illite, and smectite clays. To 4 mL of 14 292 $\mu\text{mol L}^{-1}$ labeled and unlabeled thiamin solution and 1 g of clay, various amounts of 1 M or 0.1 M HCl and H₂O were added to give a volume of 15 mL and pH values of 4, 5, 6, and 7. Adsorption was measured as described above.

Table 3. Mineralogical composition and chemical and physical properties of the clays used (Müller-Vonmoos and Kahr, 1983).

Clay	Mineralogical composition	Cation-exchange capacity		Exchangeable cations	Specific surface§	Area per electron charge¶	Charge density (σ)
		Method 1†	Method 2‡				
	%	cmol kg ⁻¹		%	m ² g ⁻¹	Å ²	esE cm ⁻² 10 ⁻⁴
Kaolinite	Kl (94)#, Qz (6)	5.0¶	9.1#	Ca, Na, K	13	43	11.1
Ca-illite	Il (75), Fs (25)	22.8¶	21.7#	Ca, (Na)	105	76	6.3
Ca-Mg-smectite	Mo (66), Mu (14) Qz (8.3), Fs (3) Cn (3.8), Kl (2)	62.0¶	64.8#	Ca (61), Mg (36) K + Na (3.2)	493	132	3.6

† Exchange with 0.5 M NH₄OAc at pH 7.

‡ Exchange with 0.4 M NaOAc-0.1 M NaCl at pH 8.2.

§ Glycerol sorption on a thermobalance.

¶ Area per electron charge = specific surface area/(CEC × Avogadro's number).

The numbers in parentheses indicate weight percentages; Kl = kaolinite, Qz = quartz, Il = illite, Fs = feldspar, Mo = montmorillonite, Mu = muscovite, and Cn = carbonate.

Calcium-saturated forms of kaolinite, illite, and smectite were used to compare measurable free Ca^{2+} ions with adsorbed thiamin. Calcium-saturated clay samples of 0.5 g were mixed with 1 mL of labeled thiamin hydrochloride solution ($40 \mu\text{mol L}^{-1}$) and 3 mL unlabeled thiamin hydrochloride solution containing 988.7 to $9887.3 \mu\text{mol L}^{-1}$ thiamin in 10-mL polypropylene centrifuge tubes and shaken for 30 min. The pH values of the initial thiamin solutions were 4.23 and 3.71, respectively. The amount of adsorbed thiamin was determined as the difference between the amount of thiamin added and that remaining in solution. The amount of displaced Ca was measured with an inductively coupled plasma emission spectrometer.

The nature of the expanded state of the smectite under the conditions of the adsorption experiments with 0.5-g samples of clay in equilibrium with 3 mL of $9887 \mu\text{mol L}^{-1}$ thiamin solution was examined by XRD as dried oriented samples on glass slides.

For the adsorption isotherm study, 1.00-g samples of the 17 soils and 0.5-g samples of the three untreated clays were mixed with 1 mL of labeled thiamin hydrochloride solution ($19.2 \mu\text{mol L}^{-1}$; specific activity 18.5 MBq L^{-1}), 1 mL unlabeled thiamin hydrochloride solution containing 29.6 to $29\,647 \mu\text{mol L}^{-1}$ thiamin, and 1 mL distilled water in 10-mL polypropylene centrifuge tubes and shaken for 30 min. Prior experiments indicated that adsorption was not influenced by the order in which the liquids were added. The pH of the solution was not adjusted during the reaction. The pH values of the initial thiamin solutions, with concentrations of 16.3, 105, 995, and $9890 \mu\text{mol L}^{-1}$, were 5.30, 4.72, 4.21, and 3.7, respectively. The final soil pH measured in a 1:3 soil/3.29 mM thiamin solution was between the values measured in a 1:1 soil/water suspension and a 1 M KCl suspension (Table 2), except in Soils 1, 2, 5, and 15, which showed values 0.4 pH units lower.

Isotherms were run at four initial concentrations at 20°C . The samples were then centrifuged for 20 min at RCF values of 7800 g and 0.5 mL of supernatant was removed for [thiazole- $2\text{-}^{14}\text{C}$]thiamin analysis. [Thiazole- $2\text{-}^{14}\text{C}$]thiamin activity was determined in a liquid scintillation counter. Thiamin adsorbed was calculated as the difference between the initial thiamin

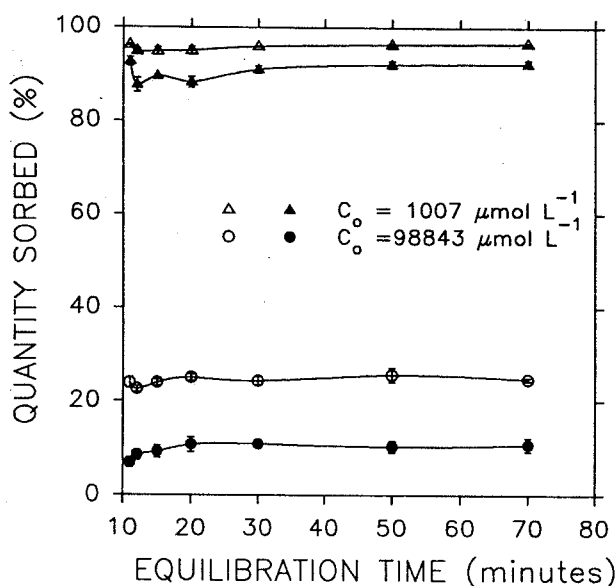


Fig. 2. Time dependence of thiamin adsorption at initial concentrations (C_0) of 1007 and $98\,843 \mu\text{mol L}^{-1}$, respectively, for Dielsdorf (open symbols) and Steinhof (closed symbols) soils. Vertical error bars represent the standard deviations. Error bars fit within the plot symbol if not shown.

concentrations, ranging from 16.3 to $9890 \mu\text{mol L}^{-1}$, and the equilibrium thiamin concentrations. The Langmuir equation was used to interpret the equilibrium adsorption data:

$$x/m = kbC/(1 + kC) \quad [1]$$

where C is the equilibrium thiamin concentration ($\mu\text{mol L}^{-1}$), x/m is the amount of thiamin adsorbed per unit mass of adsorbent ($\mu\text{mol kg}^{-1}$ soil), b is the thiamin adsorption maximum ($\mu\text{mol kg}^{-1}$ soil), and k is a constant related to the energy of adsorption ($\text{L } \mu\text{mol}^{-1}$ thiamin).

All results shown are means of three replications, if not stated otherwise.

RESULTS AND DISCUSSION

Preliminary experiments with the Dielsdorf and Steinhof soils showed that equilibrium was attained in <30 min (Fig. 2), and that thiamin adsorption decreases with increasing temperature (Fig. 3). To prevent biological degradation of labile chemicals, a common practice is to sterilize soil samples. The sterilization procedures most frequently used, however, may alter important soil properties and hence the behavior of the organic chemical in the treated soil samples (Dao et al., 1982). Thiamin adsorption by air-dried soil samples, however, was not modified by oven drying or autoclaving (data not shown). Preincubation of the soil samples to stimulate microbial activity prior to the sterilization treatment did not affect the adsorption of thiamin. These observations show that no decomposition of the adsorbate occurred during the 30-min equilibration time used for the rest of the soils.

Equilibrium concentrations and adsorbed quantities of thiamin as found in the 17 soils at initial concentrations of 16.3 to $9890 \mu\text{mol L}^{-1}$ are shown in Fig. 4. Clay varied between 0.07 and 0.35 kg kg^{-1} and organic matter content varied from 5.2 to 48.2 g kg^{-1} in the 17 soils. In general, the greater the content of clay and organic matter, the greater the amount of thiamin adsorbed (Table 2, Fig. 4). Because contents of clay and organic matter are frequently autocorrelated ($r = 0.82^{**}$), the individual contribution of clay and organic matter in binding of thiamin must be determined separately. Adsorption

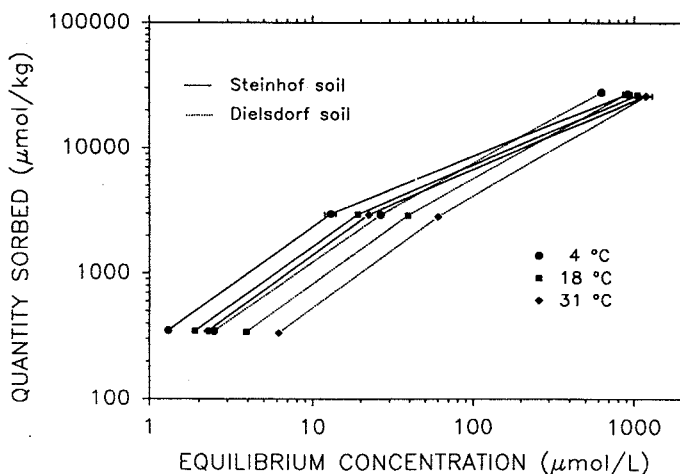


Fig. 3. Adsorption isotherms of thiamin on Steinhof and Dielsdorf soils at various temperatures. Vertical and horizontal error bars represent the standard deviations. Error bars fit within the plot symbol if not shown.

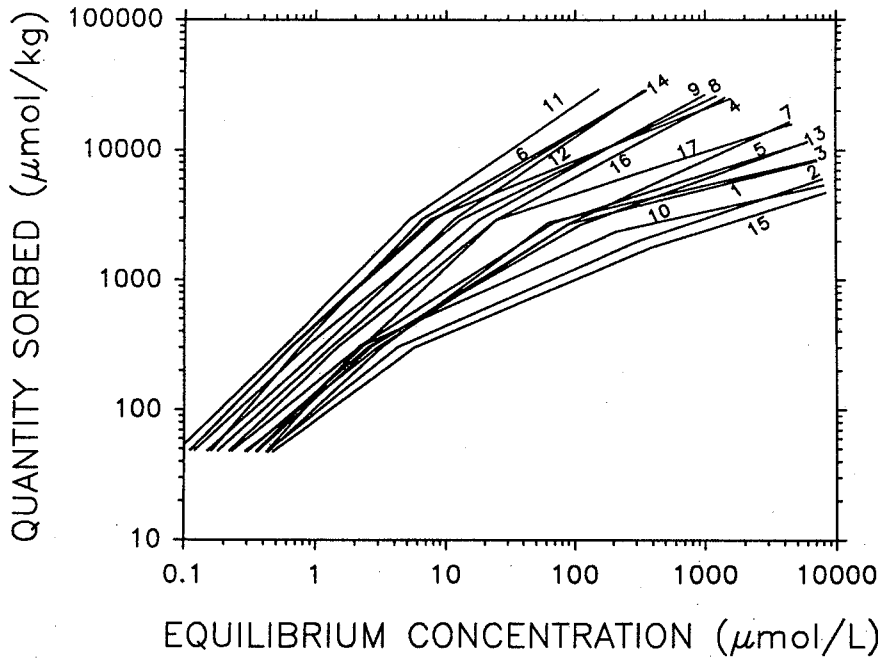


Fig. 4. Equilibrium concentrations and adsorbed quantities of thiamin as found in the 17 soils investigated at initial concentrations of 16.3 to 9890 $\mu\text{mol L}^{-1}$. Means of three replications. Numbers refer to soils listed in Table 1.

of thiamin by most of the soils was not modified to a significant extent by removing the labile organic matter (Fig. 5). Adsorption of thiamin was decreased in Soils 1, 2, and 13, and to a lesser degree in Soil 7, after organic matter removal. Chemical treatment of soil clay to remove organic matter inevitably alters the clay surface, and it may not always be prudent to interpret the differences in adsorption as being due to the contributions from components removed by treatment (Bailey and White, 1970). The removal of organic matter can lead to a decrease in poorly crystallized Fe and Al hydrous oxides on the surface of the clays, which may cause a reduction in thiamin adsorption. If CEC is assumed to be of importance in thiamin adsorption, organic matter might play a greater role in binding thiamin at increasing

pH. The pH will also influence speciation and stability of the thiamin molecule, both of which are pH dependent. The results of this study show that adsorption of thiamin occurs primarily on clay minerals in these soils, which have considerably more clay than organic matter.

With increasing thiamin concentration, the pH of the soil solution decreases. In the adsorption experiments, pH values in the soil solutions were well encompassed by the $\text{pH}(\text{H}_2\text{O})$ and $\text{pH}(1\text{ M KCl})$ values, which ranged from 5.7 to 7.5 and 4.7 to 6.9, respectively (Table 2). Soils 1, 2, 5, and 15 showed lower pH values (by ≈ 0.5) compared with the 1 M KCl solution at the highest investigated thiamin concentration. The influence of pH on thiamin adsorption was investigated with samples of kaolinite, illite, and smectite clays. In the pH 4 to 7 range

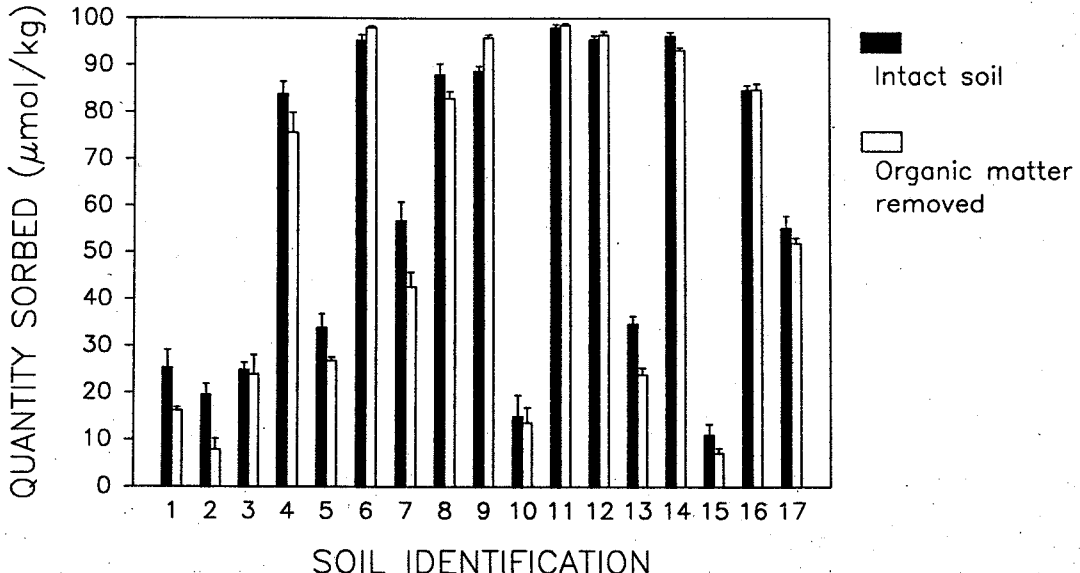


Fig. 5. Adsorption of $98\ 827\ \mu\text{mol L}^{-1}$ thiamin in intact soils and the same soils treated with H_2O_2 (organic matter removed). Vertical error bars represent the standard deviations.

of the experiments, the thiazole ring remains protonated, whereas the pyrimidine ring is deprotonated at the higher pH values ($pK_{a1} = 4.85$). Adsorption significantly decreases for illite at pH values >5.5 whereas only a slight decrease is observed for kaolinite and smectite between pH 5 and 7 (Fig. 6). The decreased adsorption of thiamin by illite at pH values >5.5 probably indicates a binding of position 1' of the pyrimidine ring onto clay surfaces. At the lowest initial thiamin concentration ($16.3 \mu\text{mol L}^{-1}$), with the correspondingly highest soil-solution pH, the amount adsorbed varied between 97.1 and 99.4% in the 17 soils. The pH values of the soil solutions were ≈ 5.3 to 7.3. Adsorption is not significantly influenced in kaolinitic and smectitic soils in this pH range. Figure 6 shows that in illite pH must be ≤ 5.6 to attain adsorption of $>97\%$. We concluded that the adsorption process in the soils depended to a greater extent on the amount and composition of the clay than on the pH of the soil solution. In Soils 11 and 14, which are characterized by relatively high and low organic matter contents, respectively, thiamin percentages adsorbed were not affected by changes in the soil solution pH resulting from initial thiamin concentrations of 16.3 to $995 \mu\text{mol L}^{-1}$. Impacts of speciation are therefore more important for the binding of thiamin than are pH-dependent CEC effects. This confirms a previous finding that thiamin adsorption was greater in the inorganic soil fraction than in the organic matter.

The process of interaction of organic molecules with the soil involves more than one mechanism, although a single mechanism may dominate. Many organic cations are positively charged because of protonation of an amine group as in the case of alkyl amines. Such organic cations will be adsorbed on clay mineral surfaces by ion exchange with cations, which neutralize the negative electrical charges responsible for the CEC of the mineral (Mortland, 1970). The protonated salt of thiamin can have two positive charges, one associated with the thiazole ring and the other with the pyrimidine ring. Protonation

does not occur with the amine group of the pyrimidine molecule, but in position 1' (Cain et al., 1977).

Calcium-saturated clay suspensions were used to measure the amount of exchanged Ca and adsorbed thiamin. The ratio between thiamin adsorbed and Ca desorbed was close to 1 in kaolinite at nonsaturating levels (Fig. 7). The somewhat lower ratio between thiamin adsorbed and Ca replaced found in illite and smectite can probably be attributed to an excess of initial Ca. Removal of excess Ca in preparing Ca-saturated clays was apparently incomplete. A parallel behavior between thiamin adsorbed and Ca desorbed, as found in illite and smectite, also suggests a stoichiometric exchange. The accordance of thiamin adsorption and exchange of Ca^{2+} suggests that the adsorption process occurs mainly by an exchange mechanism. This is further supported by desorption experiments where adsorbed thiamin could be efficiently replaced by different salt solutions (data not shown).

The Langmuir equation was used to describe thiamin adsorption over an initial concentration (C_0) range of 16.29 to $9890 \mu\text{mol L}^{-1}$ thiamin. Equilibrium concentrations and adsorbed amounts are shown in Fig. 4. The Langmuir constants b and k were evaluated for all soils. When only the coefficients of determination were considered (e.g., for Soils 1 and 16 the R^2 values were 0.9870 and 0.9999, respectively), the Langmuir equation appeared to be successful in describing the adsorption of thiamin. However, in the extended concentration range, goodness of fit was not satisfactorily explained even by R^2 close to 1. Based on these results, the soils could be classified into two groups. The first group, Soils 4, 6, 8, 9, 11, 12, 14, 16, and 17, showed that the adsorption process was fairly well described by a Langmuir-type equation. These soils were characterized as mixed layer-smectitic soils (Table 2). The Langmuir b and k constants and the R^2 values for the relationship between x/m and C for this group of soils are shown in Table 4. In the second group, Soils 1, 2, 3, 5, 7, 10, 13, and 15, the fitted Langmuir equation underestimated adsorption in

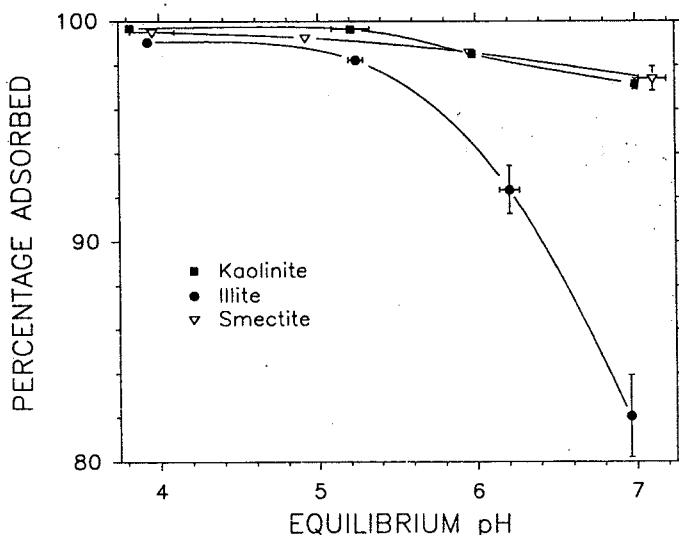


Fig. 6. Percentage of thiamin adsorbed in kaolinite, illite, and smectite vs. equilibrium pH. Vertical and horizontal error bars represent the standard deviations. Error bars fit within the plot symbol if not shown.

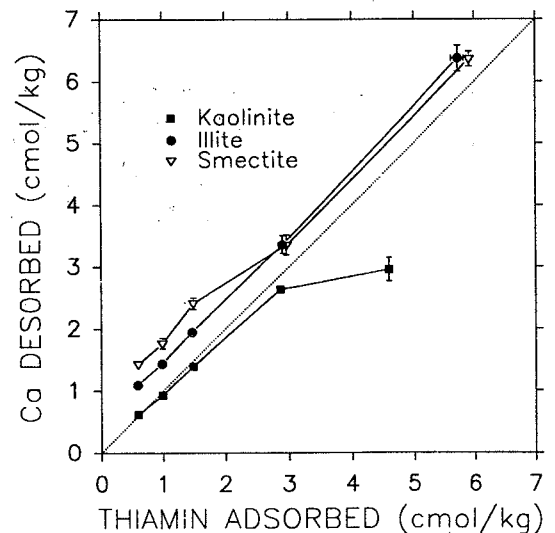


Fig. 7. Thiamin adsorbed by and Ca^{2+} liberated from Ca-kaolinite, Ca-illite, and Ca-smectite. Vertical and horizontal error bars represent the standard deviations. Error bars fit within the plot symbol if not shown.

Table 4. Langmuir one-surface and two-surface coefficients k and b for the sorption of thiamin by 17 soils and kaolinite (K), illite (I), and smectite (S) at initial concentrations of 16.3 to 9890 $\mu\text{mol L}^{-1}$. Two-surface coefficients are indicated for soils where the goodness of fit was improved as compared with a one-surface model.

Soils†	k_1 L μmol^{-1}	b_1 $\mu\text{mol kg}^{-1}$	k_2 L μmol^{-1}	b_2 $\mu\text{mol kg}^{-1}$	$R^2‡$
1	0.00297	7205	0.08435	1355	0.9880**
2	0.00105	6215	0.31646	467	0.9958**
3	0.00401	6532	0.03699	2127	0.9977**
4	0.01720	25856			0.9984**
5	0.00210	10640	0.08290	1716	0.9991**
6	0.01116	35610			0.9992**
7	0.00130	18222	0.11480	906	0.9998**
8	0.00890	28395			0.9999**
9	0.00580	31264			0.9999**
10	0.08732	1883	0.00081	4034	0.9983**
11	0.01420	42707			0.9999**
12	0.00800	39103			0.9975**
13	0.00160	11779	0.14420	968	0.9991**
14	0.01500	28593			0.9995**
15	0.00102	4783	0.31000	425	0.9854**
16	0.00460	29281			0.9999**
17	0.00840	16297			0.9998**
K	0.03030	31606			0.9985**
I	0.00250	78939			0.9969**
S	0.00100	852880			0.9976**

** Significant at the 0.01 probability level.

† Soils are described in Table 1.

‡ Coefficients of determination (R^2) for the relationship between x/m and C are based on four concentrations \times three replications.

the low concentration range. Langmuir isotherms representative of the fit exhibited by the first and second groups of soils are shown in Fig. 8a and b. Deviations from the Langmuir equation may be attributed to nonuniformity of the adsorbing surfaces and to the occurrence of multilayer adsorption. Agreement between measured and calculated adsorption could be improved in the second group of soils either by restricting the concentration range from 16.3 to 995 $\mu\text{mol L}^{-1}$ (Fig. 8b) or by fitting data to the two-surface Langmuir equation (Fig. 8b, Table 4):

$$x/m = [k_1 b_1 C / (1 + k_1 C)] + [k_2 b_2 C / (1 + k_2 C)] \quad [2]$$

where subscripts refer to two energetically different categories of adsorption sites. Almost all soils in this group were characterized by hydroxy-interlayered vermiculitic and chloritic-illitic clays (Table 2), which was also reflected in reduced b values (Table 4). Goodness of fit is, therefore, dependent on the concentration range, soil clay type, and the model used to describe the adsorption.

The Langmuir constants k and b can be used for comparing the adsorption capacity of the soils. Langmuir one-surface b values ranged from 5112 $\mu\text{mol kg}^{-1}$ for Soil 15 to 42707 $\mu\text{mol kg}^{-1}$ for Soil 11. This difference can be explained by the clay type and the higher amount of clay in Soil 11 relative to Soil 15. Linear correlation coefficients were calculated to relate one-surface Langmuir b values to soil properties. The results indicate that Langmuir b values were positively correlated with specific surface (0.92**), CEC (0.88**), silt content (0.84**), and clay content (0.78**).

Adsorption of thiamin by kaolinite and illite can be well described by a Langmuir equation at initial concentrations ranging from 16.3 to 9890 $\mu\text{mol L}^{-1}$ (Table 4,

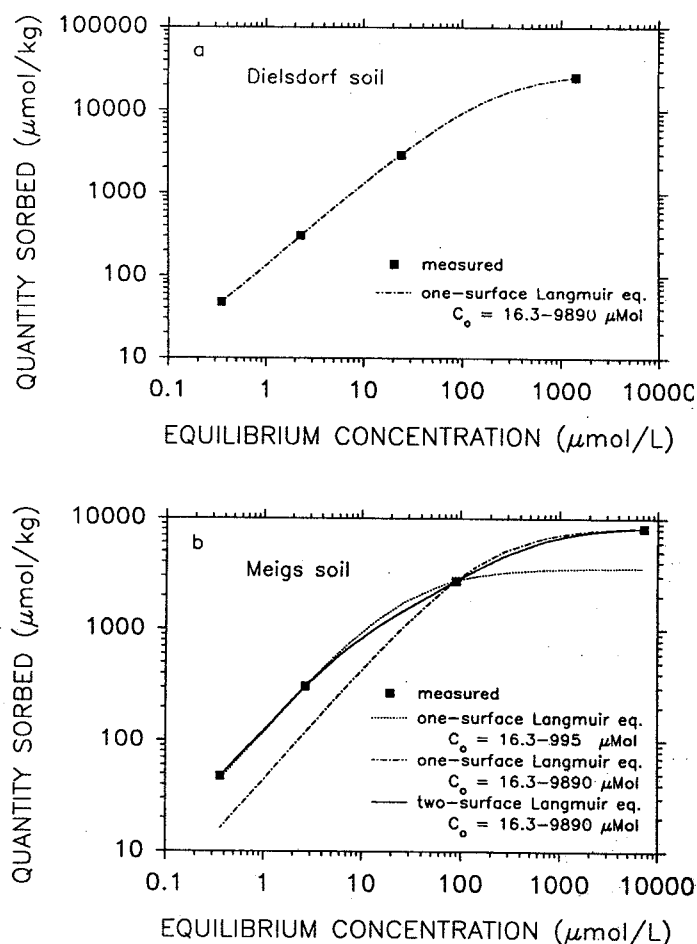


Fig. 8. (a) Langmuir isotherm for thiamin adsorption on Dielsdorf soil at initial concentrations (C_0) ranging from 16.3 to 9890 $\mu\text{mol L}^{-1}$; (b) Langmuir one-surface and two-surface isotherms for thiamin adsorption on Meigs soil at C_0 ranging from 16.3 to 995 and 9890 $\mu\text{mol L}^{-1}$.

Fig. 9). Adsorption of thiamin by smectite in the same concentration range is log-linearly related to the equilibrium concentration. At higher concentrations, a Langmuir type of adsorption was observed. At initial thiamin concentrations of 16.3, 105, and 995 $\mu\text{mol L}^{-1}$, the percentage adsorbed was 99.46, 99.47, and 99.24 for kaolinite, 97.01, 96.87, and 96.45 for illite, and 99.13, 99.10, and 99.11, for smectite, respectively. These data agree with Fig. 6.

Determination of adsorption maxima, based on calculated b values, may lead to considerable underestimations (Harter, 1984). Adsorption maxima obtained with kaolinite at initial concentrations of 995 and 9890 $\mu\text{mol L}^{-1}$ were 5922 and 31397 $\mu\text{mol kg}^{-1}$, respectively. The maxima calculated at initial concentrations of 995 and 9890 $\mu\text{mol L}^{-1}$ were 5909 and 31606 $\mu\text{mol L}^{-1}$. Thus adsorption maxima can only be calculated reliably if the experiments are conducted at saturation concentrations. At initial thiamin concentrations of 98822 $\mu\text{mol L}^{-1}$, adsorption on kaolinite, illite, and smectite amounted to 7.3, 15.3, and 46.0 cmol kg^{-1} , respectively. With the exception of kaolinite, adsorption in the clays and soils was not conducted at saturation levels. Hence b values derived from the Langmuir adsorption isotherms cannot be used to calculate the effective adsorption area.

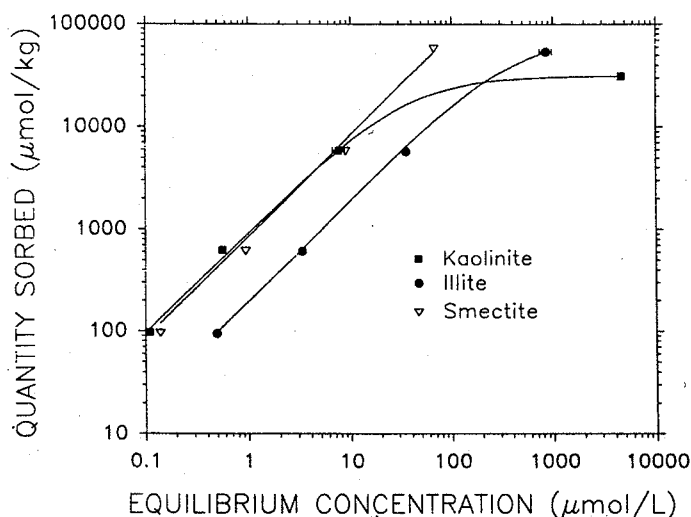


Fig. 9. Adsorption of thiamin by kaolinite, illite, and smectite. Vertical and horizontal error bars represent the standard deviations. Error bars fit within the plot symbol if not shown.

Minimum and maximum molecular cross-sectional areas of the thiamin molecule are 36 and 132 Å² as calculated from crystallographic data. The unit cell of thiamin hydrochloride crystals is monoclinic with $a = 11.77$, $b = 20.59$, and $c = 6.99$ Å and $\beta = 114.0^\circ$ (Kraut and Reed, 1962) and contains four molecules. The area per electron charge is 43 Å² for kaolinite, 76 Å² for illite, and 132 Å² for smectite (Table 3). Only in the case of smectite clays is thiamin allowed to lie in a monolayer configuration parallel to the basal plane. With the illite and kaolinite, higher charge density clays, thiamin must have a nonplanar steric orientation. Comparing this with the specific surface areas determined for the three clays (Table 3) shows that, with the minimum molecular cross-sectional area, 6 and 49 cmol kg⁻¹ univalent thiamin could be adsorbed onto kaolinite and illite, respectively, and with the maximum molecular cross-sectional area 62 cmol kg⁻¹ could be adsorbed onto smectite. These values are reduced by one-half if thiamin is bivalent. Table 3 shows that CEC values for the same clays were, depending on the method chosen, between 5.0 and 9.2, 22.8 and 21.7, and 62.0 and 64.8 cmol kg⁻¹, respectively. The experimentally determined "highest" adsorption values in kaolinite and illite suggest that, assuming thiamin is adsorbed as a monolayer, thiamin is oriented with the minimum molecular cross-sectional area facing the clay surface. In smectite the interlayer distance should be ≈ 14 Å (the assumed thickness of the aluminosilicate layer is ≈ 10 Å and of thiamin ≈ 3.5 Å), if the molecule is not keyed into the hexagonal depressions of the O surfaces, which would result in an apparent contraction of the thickness of the thiamin molecule on intercalation by smectites. Maximum adsorption may exceed CEC, because H bonds may allow multilayer adsorption of thiamin on external clay surfaces. The maximum adsorption values are far from realistic in natural situations, but furnish valuable information for the coating of thiamin on clay material as used in seed dressings. Monolayer or eventually multilayer adsorption as well as steric orientation in smectite was checked by XRD. The x-ray results for thiamin adsorp-

tion in smectite for the initial concentration of 9887 µmol L⁻¹ indicate a basal plane spacing of ≈ 14.2 Å corresponding to single sheets of thiamin molecules aligned parallel to d_{001} . The basal spacing in the untreated hydrated smectite clay was 14.8 Å, pointing to an apparent contraction of the thickness of the thiamin molecule. The amount adsorbed, ≈ 6 cmol kg⁻¹, is roughly equivalent to 10% of the CEC. In the smectite clay $\approx 12\%$ of the CEC is attributed to outer surfaces (Kraehenbuehl et al., 1987). Evidence for interlamellar adsorption of thiamin and apparent contraction in smectite clay at an even lower initial thiamin concentration of 2500 µmol L⁻¹ was also obtained from desorption experiments (unpublished data). In these experiments, thiamin could be desorbed efficiently with various extractants (e.g., 0.1 M NH₄CH₃COO or 0.1 M CaCl₂) from kaolinite and illite, but not from smectite. However, intercalated thiamin could be desorbed from smectite with tetramethylammoniumchloride. These ions act as "pillars" that hold the aluminosilicate sheets permanently apart.

CONCLUSIONS AND SUMMARY

The results of this study show that thiamin is strongly adsorbed by clay and, therefore, hardly leachable. In the soils with a low to medium content of organic matter, adsorption occurs primarily on the clay fraction. With increasing pH, a more significant contribution of organic matter to the binding of thiamin might be expected due to the greater contribution of organic matter to the CEC. At lower soil solution pH, however, thiamin is adsorbed to a greater extent. Thiamin adsorption is species dependent ($pK_{a1} = 4.85$). Adsorption probably occurs by electrostatic forces with position 1' and not with the amino group of the pyrimidine ring. A stoichiometric exchange of Ca²⁺ by thiamin suggests that the adsorption process occurs mainly by an exchange mechanism. Based on this investigation, highly efficient extractants could be developed to recover thiamin quantitatively from the soil (unpublished data). The biological availability of thiamin might be reduced in smectitic soils because thiamin could be intercalated into the smectite interlayers. Thiamin availability may also be reduced in acidic soils because thiamin is bound more tightly to clays at low pH than at high pH. Thiamin adsorbed in soil solution systems is chemically more stable than has been reported for aqueous solutions above pH 5.5. Knowledge of the extent of thiamin adsorption by clay material enables the development of thiamin seed coatings using clay as the carrier material. Thus, thiamin can be applied exogenously to stimulate plant growth.

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