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NON-ARTIFACTED SPECIMEN PREPARATION FOR TRANSMISSION ELECTRON MICROSCOPY OF SUBMICRON SOIL PARTICLES

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__ABSTRACT:__ An adapted centrifugation procedure is proposed for the preparation of Transmission Electron Microscopy (TEM) specimens of soil solutions. The centrifugation method allows to quantitatively produce non artefacted specimens of soil solutions in order to visualize colloids present within the soil. The soil of interest is a well stratified Alpin Podzol where colloids <0.45 µm were studied. The complete scheme of centrifugation is presented in this paper. The results obtained by TEM on lysimeter solutions and soil suspensions are discussed.

INTRODUCTION

Submicron mineral colloids and particles are ubiquitous in the different environmental compartments (1,2). In fresh waters, it has been shown that, despite their low mass concentration, submicron particles are supposed to intervene actively in the removal of micro-pollutants because of their high specific surface area and their relatively large number concentration (3). This behaviour might be similar in the mobile phase of soils which contains non-negligible concentrations of colloids (4). Therefore in order to understand the mechanisms of transfer of species within soils, morphological and elemental analysis of mineral colloids at the level of the individual particles is a complementary approach to their more classical analysis at the bulk level. Transmission Electron Microscopy (TEM) is precisely the technique which allows observation of these mineral entities down to the nanometer scale.

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Specimen preparation of suspensions for TEM is usually performed by resin embedding followed by ultramicrotomy or by dry deposition (5). These techniques do not systematically guarantee qualitative and quantitative representativity. On the other hand, TEM studies of fresh waters, sea water, and sediments have pointed out that direct deposition of particles onto TEM grids by ultracentrifugation produces quantitative, non-artefacted specimens and allows size speciation when coupled to a sequential centrifugation scheme (6,7).

Therefore, we propose to transfer this valuable technique to the preparation of soil solutions for TEM analysis. Optimization of the direct deposition technique has been achieved with a podzolic soil taken as a model. This type of soil is characterized by clearly stratified horizons and is thus well suited for the tentative application of the TEM preparation described below.

MATERIAL AND METHODS

Soil Characteristics

The studied soil is located at the entrance of the Arpette valley (coord. 572.61-97.725), on a 68% slope, developed on a granitic moraine. The soil is an Alpin Podzol (8) and shows a well developed E horizon. The charge of rocks increases with depth and reaches a maximum of 30% of the total weight in the BPs horizon. Its general texture is coarse.

As shown in Table 1 (9), the pH increases along the profile from 3.6 in the O horizon (0-20 cm thick) to 5.1 in the C horizon (65-90 cm); the proportion of carbon is slightly lower than expected in this type of soil. The BP horizons (h for humic and s for sesquioxides) contain aluminum (~6-6.5% of total Al) and iron (~1.5-2% of total Fe); BPh (35-40 cm) contains 2% of Natural Organic Matter (NOM), while this proportion is lower (0.6%) in the BPs (40-65 cm). The C horizon was not investigated during this study.

Only ultrapure ultrafiltered UV-irradiated water was used (Elgastat UHQ-II) for dilutions and preparation of suspensions. Manipulations were made at a laminar flow bench (Skan VFC 120) whenever possible in order to avoid contamination by particles from the atmosphere. All centrifugations were done with a Kontron Centrikon T2070 equipped with a swing-out rotor (Kontron TST-28). Observations were done on a conventionnal TEM (Zeiss EM-10, 80 kV).

Sampling and Size Fractionation

The observation of submicron mineral particles in the podzol involved: (i) their extraction from the different soil horizons with ultrapure water or their sampling

TABLE 1: Physico-Chemical Characteristics of the Alpin Podzol

Horizon	Thickness [cm]	C% of total C	C/N	pHwater	Si% of tot. Si	Fe% of tot. Fe	Al% of tot. Al
E	0-20	0.5	18	3.9	11.34	0.35	11.15
O	1-35	42.3	30	3.6	11.95	0.32	2.51
BPh	35-40	3.0	/	4.6	12.71	1.75	11.86
BPs	40-65	0.6	16	5.1	12.42	1.54	12.10
C	65-90	0.4	/	5.1	11.95	0.66	11.76

from lysimeters, (ii) their separation by centrifugation, and (iii) their deposition on TEM grids by direct ultracentrifugation.

Soil samples were extracted separately from each horizon with an auger and transported to the laboratory in clean polyethylene bags. They were kept at 4°C between manipulations.

Each horizon (10.5 g/horizon) was suspended in ultrapure water (10 mL) and stirred for five minutes without additives. A preliminary centrifugation of the highly concentrated soil suspension allowed elimination of coarse material and macroparticles >1 µm at a Relative Centrifugal Force (RCF_{max}) of 2,200 g during eight min. The upper fraction (5 mL) of the supernatant was collected, diluted with ultrapure water (25 mL), and recentrifuged (RCF_{max} = 2,200g; 13 min). This last centrifugation separates particles >0.45 µm from the microparticles to be studied. Particles <0.45 µm are usually considered as dissolved matter. This limit has been chosen in our study in order to show that microparticles are also present in suspension.

Percolated water was collected under each horizon with an open tension-free lysimeter (10,11) connected to a polyethylene bottle. The latter were installed onto lysimeters 4 weeks prior to sampling. The recovered suspensions were kept in the same conditions as the soil samples.

The lysimeter suspension (20 mL) of each horizon was fractionated as follows: a first centrifugation (RCF_{max} = 2,200g; 14 min) was applied in order to eliminate coarse material and the upper fraction (25 mL, containing particles <0.45 µm of the supernatant was recovered).

Specimen Preparation for Transmission Electron Microscopy

The complete procedure for the preparation of TEM specimen has already been described for aquatic samples (12). Its transfer to soil samples is presented below.

TEM grids (Cu 200 mesh; collodion covered, carbon coated) are slightly pasted with dry adhesive tape (Letraset®) on a flat epoxy grid holder (Iso-Tec, two components resin). The grid holder is placed at the bottom of an ultracentrifuge tube in which the suspension is then gently added. Submicron particles are finally recovered onto grids by ultracentrifugation in a swing-out rotor (RCF_{max} = 131,000g; 200 min, Figure 1). After ultracentrifugation, the supernatant is slowly discarded and grids are recovered for TEM examination.

RESULTS AND DISCUSSION

The conditions of centrifugation (duration, velocity, RCF) were calculated using a model of spherical particles, according to Equation 1 derived from Stokes' law, with a solution viscosity $\eta = 1.52 \times 10^{-2}$ [poise] for T = 5°C.

An average particle density $\rho = 2.5$ g/cm^2 was assumed as a valid approximation as it is the average true density of the soil. A spherical model and step-by-step centrifugations are appropriate for size fractionation of microparticles. Figures 2 and 3 show that most of the particles recovered onto TEM grids do not exceed the size limit of 0.45 µm, as expected with our centrifugation conditions.

Soil Suspensions

Figure 2 shows the distinct horizons obtained by direct ultracentrifugation of soil suspensions on TEM grids.

In the O horizon (organic) organic species are clearly recognized as ill-defined entities with a low electron density, in comparison to the medium to high opacity mineral particles showing a discrete boundary with their surrounding. While the E horizon (bleached horizon) contains only mineral particles, often geometrically shaped (crystalline), BP and in particular BPs (horizons of accumulation) clearly show organic networks and sponge-like structures associated to mineral colloids. These organic entities are much larger than 0.45 µm. This is explained by the fact that ρ_{NOM} is lower (close to 1.0) than $\rho_{average} = 2.5$ assumed for this podzol.

NOM is relatively difficult to visualize under the TEM, because of its poor electron density, and it usually requires staining with electron dense agents (salts of uranium or tungsten and ruthenium red) in order to be properly contrasted (13). In

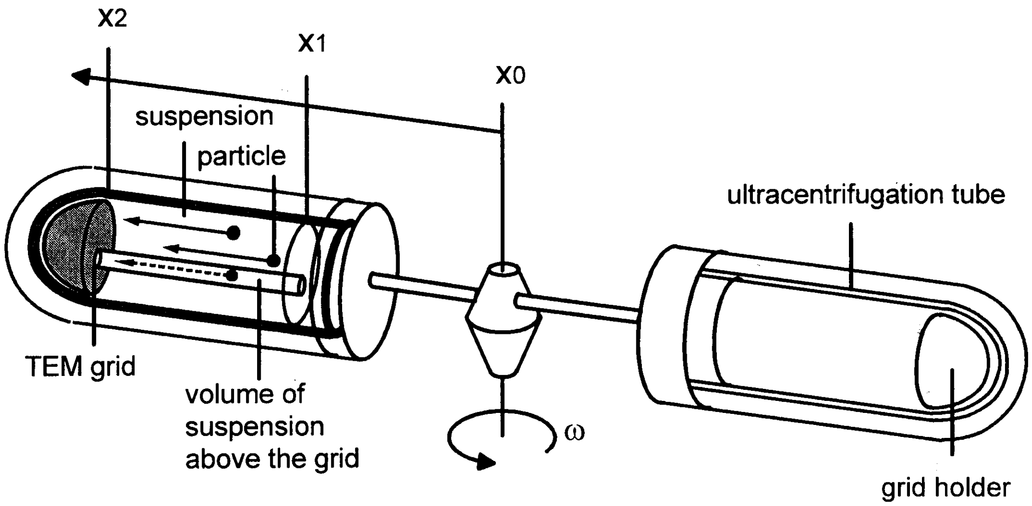
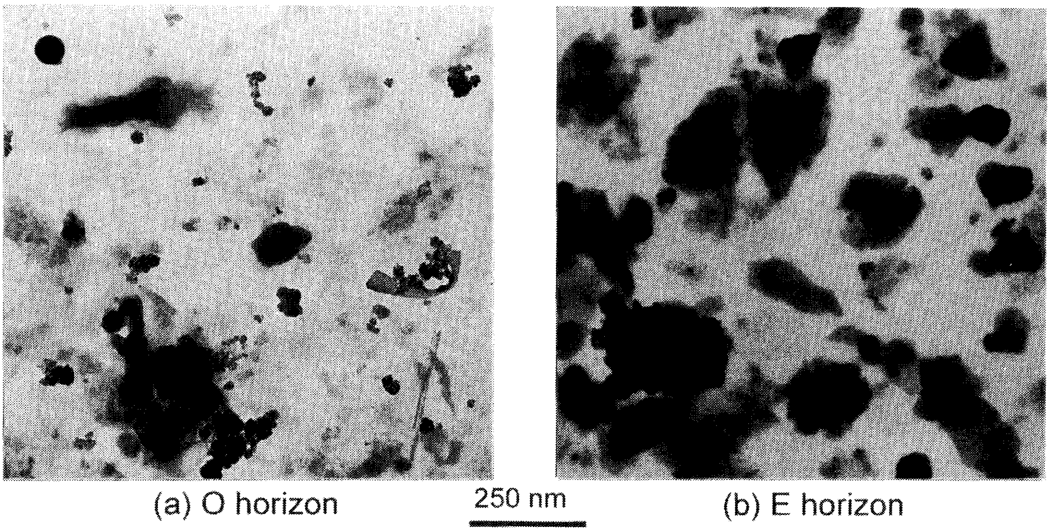


FIGURE 1 : Schematic of TEM Specimen Preparation by Centrifugation with a Swing-out Rotor

EQUATION 1 : Stokes' Law Adapted for Calculation of Centrifugation Time

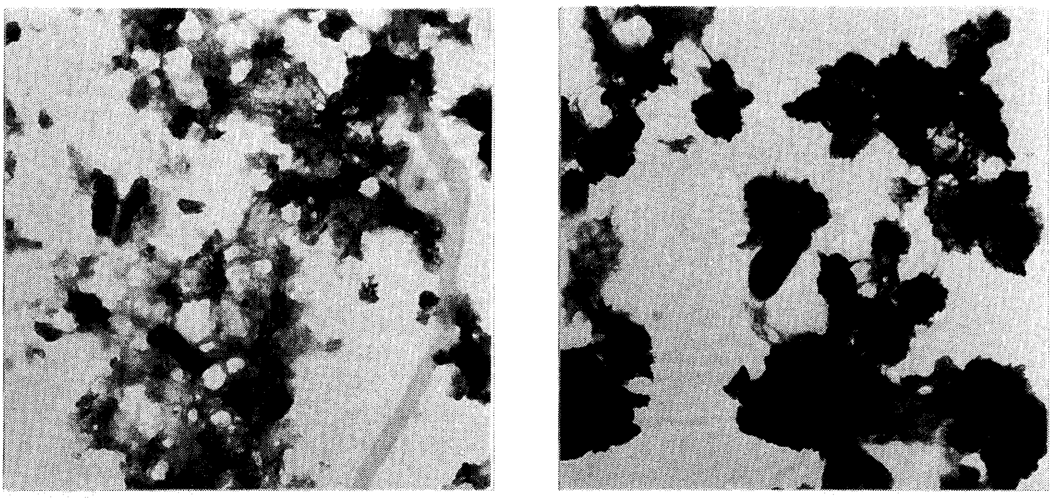
$$t = \frac{18\eta \ln(x_2 / x_1)}{(\rho - \rho_0)\omega^2 d^2}$$

where t = duration of centrifugation [s], x_1, x_2 = position of the particle in the tube [cm] (see Fig. 1), ρ_0 = solution density [g/cm^3], ρ = particle density [g/cm^3], ω = angular velocity [rad/s], η = suspension viscosity [poise], d = particle diameter [cm].



(a) O horizon

(b) E horizon



(c) BPh horizon

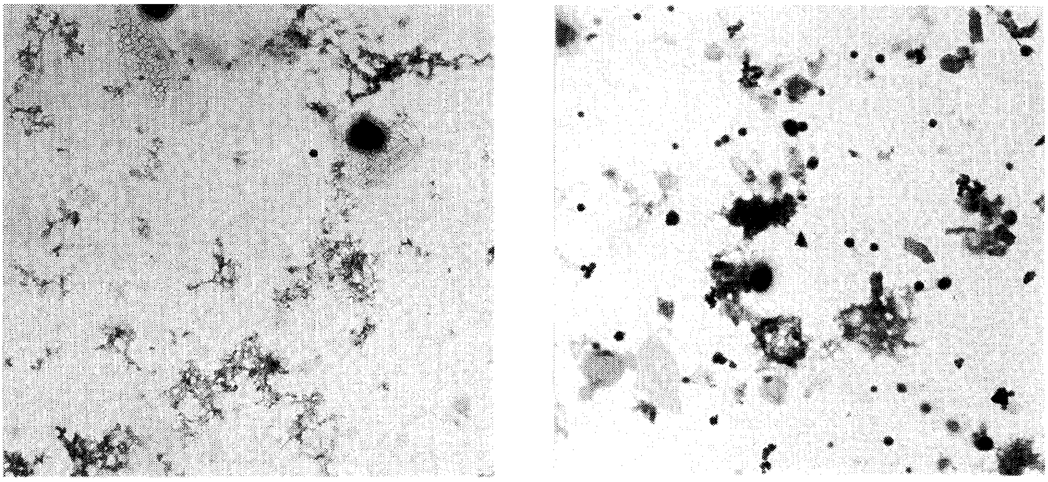
(d) BPs horizon

FIGURE 2 : TEM of Soil Suspensions Obtained from O, E, BPh and BPs Horizons

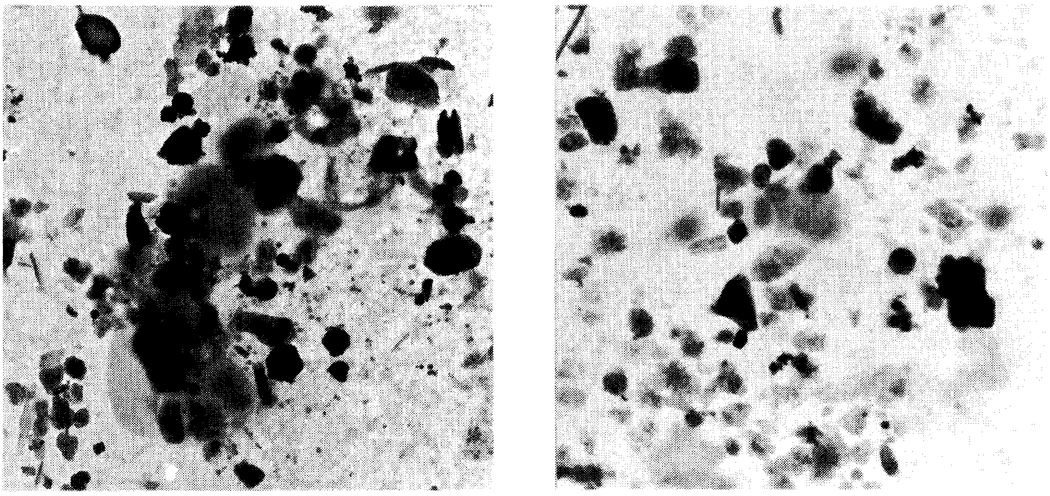
the case of this soil, NOM appears as a sufficiently contrasted material, without staining, because it may adsorb or complex cations (aluminum, calcium, and iron) present in the interstitial water.

Water Samples

Mineral colloids in the percolated water samples (Figure 3) are observed as non-aggregated entities.



(a) O horizon (b) E horizon



(c) BPh horizon (d) BPs horizon

FIGURE 3 : TEM of Lysimeter Solutions Obtained from O, E, BPh and BPs Horizons

In the sample from the lysimeter positioned under the O horizon, mineral colloids are present in different forms, either adsorbed at the surface of organic networks or as individual submicron particles; however, their number concentration is relatively negligible in this sample compared to the E, BPh, and BPs samples. Water from the lysimeter of the E horizon contains NOM which is only rarely associated to mineral particles. The latter in the E, BPh, and BPs lysimeters present a wide diversity in sizes (10 to 250 nm), morphologies (amorphous to geometrically shaped) and nature (electron densities). Furthermore, solutions from BPh and BPs lysimeters exhibit also particles larger than those present in the upper horizons.

As expected, there is not a tight adequation between samples from the soil suspensions and from the lysimeter solutions. This observation is in agreement with results obtained by classical macroanalytical methods, which show that the composition of the different horizons in a podzol does not systematically reflect the content of associated lysimeters.

According to our results, it might be possible that mineral microparticles in the smaller size range are transported through the different horizons without being trapped onto the soil matrix, as clearly exemplified in Figures 2b-d and 3b-d, while, on the opposite, NOM accumulates, as expected, in the BPh and BPs horizons during its migration within percolated water.

CONCLUSION

The preparation of soil suspensions and gravitational waters for TEM examination by direct ultracentrifugation of microparticulate species onto grids is a non-artefacted and fast method when analysis at the submicron level is required. This preparation scheme is well adapted for the morphological analysis of amorphous and crystalline oxide colloids and for the identification of organic matter. However, if the multi-layer structure of alumino-silicates has to be studied, which is often the case in soil science, direct deposition by ultracentrifugation is not recommended, because of the strong tendency for clays to fall flat on the TEM grid, thus making the recognition of their layers difficult.

At this stage of our work, we have focused on the preparation procedure and on the type and morphological identification of microparticles recovered on grids. As this method is promising, it is expected that a physico-chemical characterization (crystallinity; elemental composition) of these pedogenic entities will help understanding their transport behaviour through the different horizons of a podzol, or more generally, within soils of different origins. The coupling of the proposed preparation procedure to elemental analysis by means of Energy Dispersive Spectrometry (TEM-EDS) for heavy elements or Electron Energy Loss Spectrometry (TEM-EELS) for light elements should prove extremely useful for this task (14).

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