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Particulate Formation Due to Freezing Humic Waters

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Water samples were collected from a soft water highly humic pond, and all particulates of >0.15-μm nominal diameter were removed by continuous flow centrifugation. Freezing caused the formation of brown particulates which could be removed by centrifugation. The particulates can be dispersed by sonification, but the size distribution of organic fractions is permanently altered by freezing.

Naturally occurring colored organic compounds are found at varying concentrations in almost all surface waters [*Lamar*, 1968; *Midwood and Felbeck*, 1968; *Schnitzer and Khan*, 1972]. Humic materials are of worldwide distribution, and their major properties are independent of their source [*Zajicek and Pojasek*, 1976]. These refractory polyphenolic compounds are major constituents of soil and sediment organic matter [*Otsuki and Hanya*, 1966] and are thought to be the result of chemical polymerization and microbial decomposition of plant components such as lignin and synthesis [*Flaig*, 1964; *Felbeck*, 1971]. Humic substances have been operationally separated into four fractions on the basis of their solubility [*Schnitzer and Khan*, 1972]: humins (insoluble in H_2O, base, and acid), humics (soluble in base, insoluble in acid), hymatomelanic acids (humics soluble in alcohol), and fulvics (soluble in H_2O, acid, and base).

While much research has been conducted into the chemistry and structure of these compounds, they are still poorly defined. However, it is well established that nonlabile organics can form stable complexes, which determine the form, movement, and availability of trace metals in water [*Jenne*, 1968; *Beck et al.*, 1974; *Elder*, 1975]. Humic substances are important in determining the toxicity of metals [*Prakash and Rashid*, 1968; *Brown et al.*, 1974; *Whitfield and Lewis*, 1976] and availability of micronutrients [*Giesy*, 1976] to plants and animals.

The solubility of humic substances in water is dependent on their size or degree of polymerization. Larger organics, such as humins and humics, are colloidal in water [*Zajicek and Pojasek*, 1976]. Trace metal availability is also dependent upon the size of the organic fraction with which it is associated [*Stevenson and Ardakani*, 1972; *Giesy and Paine*, 1977]. The molecular size distribution of humics varies seasonally and temporally [*Giesy and Briese*, 1978]. Thus any change in the form of naturally occurring organics may affect the complex chemistry involving these organics. This study was conducted to determine the effects of freezing on naturally occurring organic compounds.

METHODS

Water was collected from Skinface Pond on the U.S. Energy Research and Development Administration's Savannah River Plant Reservation, near Aiken, South Carolina, in July of 1976. Samples were collected, transported, and stored in polyethylene bottles. Particulates of >0.15-μm nominal diameter were removed using a Sorvall SS-1 centrifuge equipped with a KSB continuous flow system (Sorvall, Norwalk, Connecticut). After centrifugation, samples were divided into 1-l aliquots. One fraction was immediately passed serially through XM-300, PM-10, and UM-05 ultrafilters, under an N_2 atmosphere in a model 402 stirred cell (Amicon Company, Lexington, Massachusetts) [*Giesy and Paine*, 1977]. The other fraction was frozen at −18°C for 5 days and thawed and ultrafiltered. Total organic carbon (TOC) was determined by using a Beckman model 915 carbon analyzer equipped with a ceramic combustion chamber.

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RESULTS AND DISCUSSION

Particle settling in a gravitational field is dependent on particle density and diameter and fluid viscosity, which are related by the Svedberg coefficient, with particles having similar coefficients settling together. If particles are assumed to have the density of kaolinite clay (2.66 g/cc) [*Grim*, 1968], which is the dominant clay type in our area, particles sedimented together under a rotor speed of 7000 rpm and a flow rate of 11.0 l/hr would nominally be 0.15 μm in diameter. This diameter is often used as the boundary between colloids and particulates.

The TOC in Skinface Pond was 15.2 mg/l (Table 1), which is approximately 2 times the world average for rivers [*Garrels and MacKenzie*, 1971] and is typical of southeastern aquatic systems [*Beck et al.*, 1974]. More than 85% of the TOC was in the fraction less than 10,000 molecular weight. These lower molecular weight organics are probably fulvic in nature [*Beck et al.*, 1974].

Freezing caused the formation of irregular brown particles (Figure 1). After freezing, more than 90% of the TOC was retained by the XM-300 ultrafilter (Table 1). Sonification dispersed some of the particles, resulting in a TOC molecular diameter distribution more similar to that of unfrozen water. However, sonification still resulted in a molecular diameter distribution skewed toward the larger sizes. Less than 50% of the TOC could be resolubilized by 0.5 *N* NaOH. No particulates were formed in samples maintained at room temperature for 3 months. Many investigators have used freezing to concentrate and preserve naturally occurring refractory organics [*Midwood and Felbeck*, 1968; *Martin and Pierce*, 1971; *Schindler and Alberts*, 1974]. Organics isolated from surface waters have also been frozen before molecular weight determinations [*Davies et al.*, 1969; *Stevenson*, 1976] and chelation studies [*Rashid and King*, 1969; *Zajicek and Pojasek*, 1976; *Cleveland and Rees*, 1976]. *Povoledo et al.* [1972] lyophilized humic compounds before studying their elution patterns on various chromatographic supports. *Gjessing and Gjerdahl* [1972] found that freezing humics changed their isoelectric focusing pattern indicating a change in molecular charge.

The formation of particulates due to freezing is important in determining acceptable sample-handling protocols. Freezing of samples containing humic substances should be avoided, since the chemical and physical properties of these organics

TABLE 1. Distribution of TOC in Nominal Size Fractions Before and After Freezing and Sonification

Ultrafilter	Pore Diameter, μm	Nominal Molecular Weight Range	Unfrozen		Frozen: Unsonified		Frozen: Sonified	
			mg C/l	%	mg C/l	%	mg C/l	%
XM-300	0.0183	$F > 300{,}000$	1.9	12.4	13.7	92	7.3	47.9
PM-10	0.0032	$300{,}000 > F > 10{,}000$	0.33	2.2	0	0	0.97	6.3
UM-05	0.009	$10{,}000 > F > 500$	3.0	19.1	0.022	0	1.8	12.0
UM-05	0.009	$F < 500$	10.0	66.0	1.2	8	5.3	34.0
Total			15.2		14.9		15.4	

may be altered. Particulate formation due to freezing may be important in determining the concentrations of mobile organics in soils and surface waters which can undergo complexation reactions with metals. Humic substances are more prone to be aggregated into particulates in soft water [*Lush and Hynes*, 1973], such as that in the southeastern United States, where the *p*H of the pond water studied was 5.5. *Chen and Schnitzer* [1976] reported on *p*H dependent fulvic particle formation in soils and presented scanning electron micrographs showing particles similar to those reported on in this study. Stream and swamp waters of the southeastern coastal plain have high humic contents, which are important in determining the chemical environment of these black waters. Water in swamps and sloughs is subject to numerous cycles of freez-

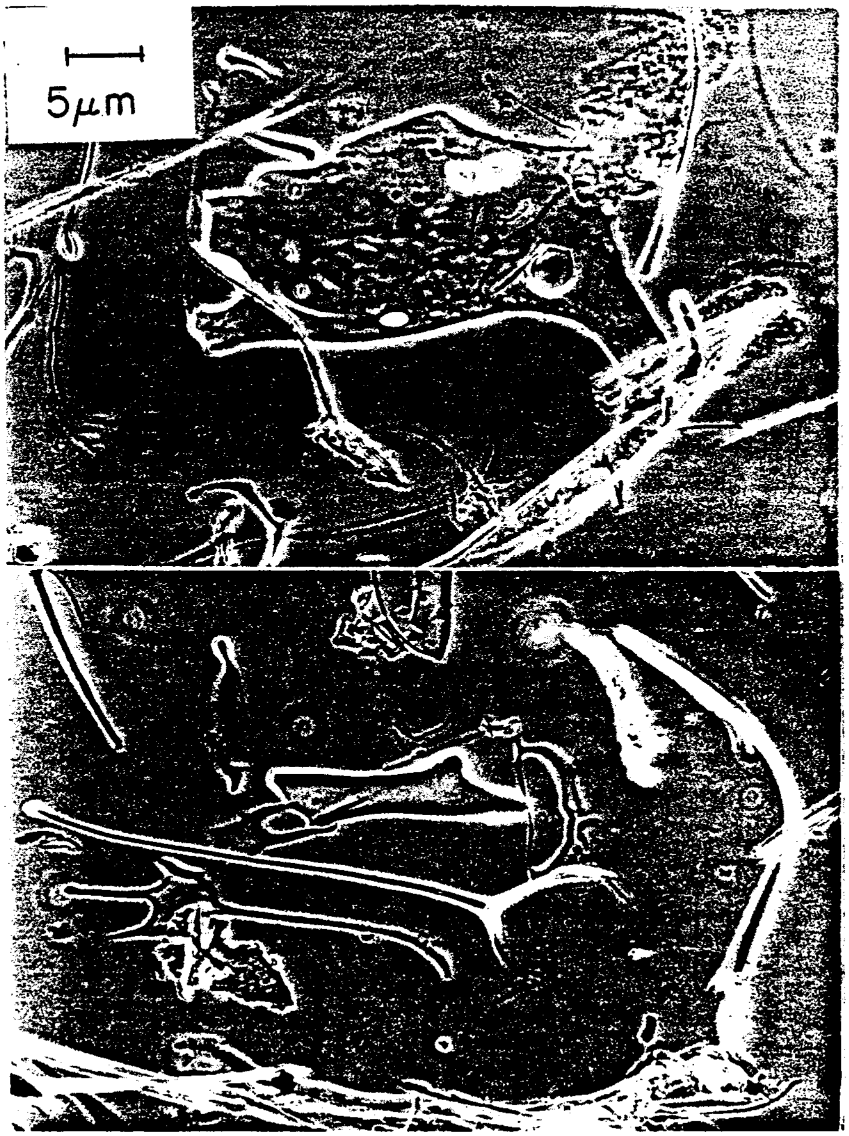

Fig. 1. Phase micrograph of particulates formed by freezing.

ing and thawing during the winters which may affect the humic content and size distribution of organics. Humics in the soil solution may also be subject to changes due to freezing.

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