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A Survey of Soil Diatoms

By JOHN M. W. HAYEK AND ROBERT L. HULBARY

Although extensive work is being done in stream surveys and in the taxonomic study of aquatic forms of diatoms, very few papers have appeared relative to diatoms in soil. Petersen (1935) made studies of algae in soils of Denmark and East Greenland in which he included a consideration of the diatoms present. Thirty-seven species of diatoms were listed as being present in the soils of Denmark and East Greenland.

The work of J. W. G. Lund (1945, 1946) seems to be the only fairly complete study of soil diatoms He reported 13 genera and 52 species from his observations of 66 British soils.

This investigation was undertaken to determine qualitatively the complement of diatom species in six different soils in the vicinity of Iowa City, Iowa. Separating diatoms from the various soils and cleaning them presented a problem requiring the trial of several existing techniques.

The diatoms were collected in test tubes in quantities of 20 cc. of soils scraped from the surface of the ground at six stations. The pH of these soil samples had a range of 6.7 to 7.9.

Collections were taken from the following areas:

1. *Path soil* from a path between University Experimental Schools and the east bank of the Iowa River, Iowa City, Ia. Station no. 1. Soil pH 7.9. Seven samples studied.
2. *Sandy soil* from a fill near a bridge at the south duck pond at City Park, Iowa City, Ia. Station no. 2. Soil pH 6.2. Seven samples studied.
3. *Duff and humus soil* from open deciduous forest in City Park, Iowa City, Ia. Station no. 3. Soil pH 7.3. Seven samples studied.
4. *Field soil* from an alfalfa field near City Park woods, Iowa City, Ia. Station no. 4. Soil pH 7.4. Seven samples studied.
5. *Garden soil* from a lawn at 114 E. Market St., Iowa City, Ia. Station no. 5. Soil pH 6.7. Seven samples studied.
6. *Loessial-clay soil* from the bluffs on the west bank of the Iowa River, Iowa City, Ia. Station no. 6. Soil pH 7.4. Seven samples studied.

All samples were collected in sterile test tubes at irregular intervals in the late fall and early spring, when the development of the diatom flora was thought to be most luxuriant.

After bringing collections into the laboratory, the samples were air-dried in open plates for three days. A pH check of each of these samples was carried out by moistening the three-day soil with distilled water to the consistency of thick cream as recommended by Daubenmire (1947) for the measurement of pH in soils. The actual measurements were made by placing the moistened soil under the electrodes of a Beckman pH meter, model N.

All of each 20 cc. sample was then transferred to 109 ml. of 10% hydrochloric acid in order to produce a preliminary disinte-

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gration of organic materials present. The mixture was shaken vigorously for about five minutes and then allowed to set for at least 24 hours. The sample was shaken vigorously again for an additional five minutes and strained through #20 bolting cloth in order to remove larger soil particles. There was no evidence to indicate that any diatoms were being lost in the straining process as the largest shells measured were approximately 60 microns long whereas the dimensions of the holes in the mesh were found to be 235 by 270 microns. The filtrate was then left to settle for a least 12 hours. After the excess liquid was decanted, the sediment was boiled by the sulfuric acid-potassium dichromate method utilized by Hohn (1951). It was found that chromic acid could be substituted for potassium dichromate with comparable cleaning effect. The mixture containing the cleaned diatoms was then poured into a liter of distilled water and left to settle for 12 hours. After settling the excess liquid was poured off and the sediment was washed. This solution was allowed to settle and decanted again. The sediment was centrifuged for ten minutes in order to separate the solid from the liquid part. The solid material was then transferred to a test tube containing 95% ethyl alcohol as a preservative.

Permanent slides of sediment containing the diatoms were prepared by placing a droplet of material on a cover slip and allowing it to dry. The cover slip was then fixed to a slide with "Permount." When bubbles appeared in a mount, they were removed by applying heat to the slide very gently according to the recommendations of Hohn (1951).

Identification to species and often to genus was found to be extremely difficult with living material because of the smallness of soil diatoms and the presence of the chromatophores. Therefore, temporary water mounts containing fresh material were prepared only to ascertain the presence and general nature of diatoms in the soil being collected and to check the possibility that diatoms were lost in the process of cleaning.

Most of the frustules encountered on permanent slides were measured to the nearest .5 micron. For purposes of references, the location of the frustules measured was made possible by a specially prepared grid-marked slide on which each grid square was numbered. This device was found to be particularly helpful in the study of species infrequent in occurrence. In spite of the fact that the material on the slide had been diluted by a small drop of water, observations were made difficult frequently by the presence of siliceous and mineral fragments which hid some frustules partially, if not almost completely, from view.

Synopsis of Species Encountered

All except one of the diatoms found in this study were members of the order Pennales, those with bilateral symmetry. The single occurrence of one frustule of a member of the Centrales makes its presence in the soil seem conspicuous. All the pennate forms found in this study are considered to have at least one true raphe with the exception of the species of *Eunotia*. Each of the 42 samples studied was found to contain diatoms. In the various samples diatoms ranged from scant in number of species and individuals to abundant in both. Of all soils studied the sandy soil was found to be by far the most productive; the loessial-clay and the lawn soils were found to have the poorest development.

Vouchers for each were prepared as microslides and are deposited in the State University of Iowa Herbarium, Iowa City, Ia.

Division: Chrysophyta.
Class: Bacillariophyceae.
Order I: Centrales.
Family:—Coscinodiscaceae.

Cyclotella Kütz.

1. *Cyclotella kützingiana* Thw.
   Van Heurck (1896, plate 22, fig. 656); Hohn (1951, p. 30, fig. 4). 17 μ (diameter).
   Valve with fine, rather than robust, marginal striae; center with few fine punctae, scattered (not radiant from center) unlike *C. meneghiniana* Kütz. in Van Heurck (1896, p. 447).
   Only one such frustule was found in the collections from the duff-humus soil of the deciduous forest. Microslide no. 301-1-b.

Order II:—Pennales.
Family:—Eunotiaceae.

Eunotia Ehrenberg

2. *Eunotia diodon* Ehrenb.
   Van Heurck (1896, plate 30, fig. 829-830). 25 μ x 4 μ. Dorsal margin of valve with two rather slight rounded ridges with obtuse, rounded apices appearing as the illustrations cited above show.
   A very few frustules of this species were found in the duff-humus soil of the deciduous forest.
   Microslide no. 301-1-a.

3. *Eunotia* spp.
   Some specimens observed seemed to be of a species near *E. tenella* (Grun.) Hust. They were similar to those studied by Lund (1946, pp. 57, 105, fig. 18 F-I) in that they showed little or no decrease in width of the valves toward the apices. Positive identification to species was not possible as all specimens studied were broken, lacking one apex. Some other frustules studied seemed to belong to a species near *E. major* (W. Sm.) Rabenh.; Van Heurck (1896, plate 9, fig. 366), Hohn (1951,

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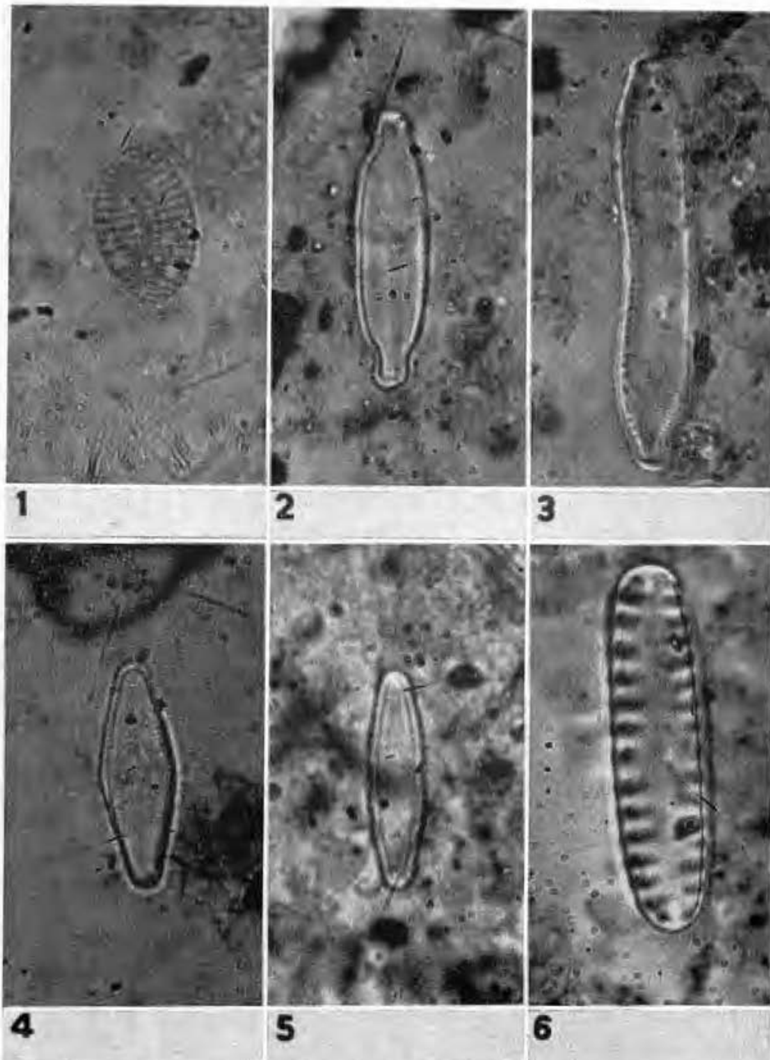

Figure 1: *Cocconeis scutellum.*
Figure 3: *Hantzschia amphioxys.*
Figure 5: *Pinnularia microstauron.*
Figure 2: *Navicula dicephala.*
Figure 4: *Navicula seminulum.*
Figure 6: *Surirella ovata.*

p. 30, fig. 12) and *E. gracilis* (Ehrenb.) Rabenh.; Van Heurck (1896, plate 9, fig. 368), Hohn (1951, p. 30, fig. 11). These shells had arcuate, elongate valves with parallel margin somewhat capitate. Broken ends on these specimens did not permit further separation of species with certainty.

These assorted frustules were found in the sandy soil, duff and humus soil of the deciduous forest and in the field soil of the alfalfa plot. None of them occurred in quantity.

Five species of *Eunotia* were reported in soils by Lund (1945, 1946) of which *E. tenella* was one. Petersen (1935, p. 140) reported *E. gracilis* as a dubious hydro-terrestrial on rocks among mosses.

Microslides nos. 201-2-b, 301-2-b, 301-2-a.

Family:—Achnanthaceae.

Achnanthes Bory

4. *Achnanthes minutissima* Kütz.

Van Heurck (1896, plate 8, fig. 334); Lund (1946, p. 60, fig. 2 A-K). 14-15 μ x 4-5 μ.

Valves very narrowly lanceolate; striae delicate, very slightly radiant; central striae shortened.

Present in the sandy soil, but not in large numbers. Lund (1946) reported the presence of this species in six soils examined by him. Petersen (1935) found this species as well as *A. lanceolata* Bréb. and *A. coarctata* Bréb. only the latter of which he considered euterrestrial.

Microslide no. 202-1-a.

Achnanthidium Cleve

5. *Achnanthidium flexellum* (Kütz.) Cleve.

Van Heurck (1896, p. 276, fig. 60; plate 8, fig. 322). 10-11 μ x 4-5 μ.

Valves elliptic, inflated at median portion; raphe sigmoid; upper valve with a pseudoraphe; striae faint, somewhat radiant.

Found only in sandy soil and never in abundance.

This genus was not reported in soils either by Lund (1945, 1946) or by Petersen (1935).

Microslides nos. 202-1-a, 202-3-b.

Cocconeis Ehrenberg

6. *Cocconeis scutellum* Ehrenb. Fig. 1.

Van Heurck (1896, plate 8, fig. 338). 13-15 μ x 8-9 μ.

Valve broadly oval; upper valve with pseudoraphe; lower valve with a straight raphe; valve striae on distinct puntae.

Found rarely in sandy soil and on duff-humus soil of the deciduous forest.

This genus not reported in soils by Lund (1945, 1946) or Petersen (1935).

Microslides nos. 202-1-b, 301-2-b.

Family:—Naviculaceae.

Navicula Bory

7. *Navicula minima* Grun.

Van Heurck (1896, plate 5, fig. 229); Lund (1946, p. 70, fig. 5 A-H) 13-19 μ x 4-5.5 μ.

Valves linear to elliptical; apices rounded; striae fine, slightly radial and almost to the raphe except for the central region where the axial field is very wide. The valves of some slightly inflated.

Found in sandy, duff-humus and loessial-clay soils.

Microslides nos. 202-1-a, 401-1-a, 503-2-a.

8. *Navicula seminulum* Grun. Fig. 4.

Van Heurck (1896, plate 5, fig. 228); Lund (1946, p. 70, fig. 5 I-GG, II-YY). 13-18.5 μ x 4-7 μ.

Valve variable in shape from linear-elliptic to lanceolate and rhomboidal. The majority of shells found in this investigation were of the latter two shapes. Lund (1946) reported variations in form among the ones studied by him. Striae definite to faint; the axial field expanded in the central region into a rectangular to butterfly-shaped area. Variation in shape and faintness of markings sometimes made its identification from *N. minima* difficult.

Found in abundance in path, sandy, duff-humus and less frequently in loessial-clay soil.

This species was reported in the studies of Lund (1946) of British soils.

Microslides nos. 101-2-b, 202-3-b, 207-2-a, 401-1-a, 401-2-b, 503-2-a.

9. *Navicula mutica* Kütz.

Van Heurck (1896, plate 4, fig. 167); Lund (1946, p. 72, fig. 6 A-H); Hohn (1951, p. 34, fig. 122).

13-14 μ x 4-4.5 μ.

Valves variable between elliptic to lanceolate in shape; apices weakly but definitely capitate; striae definitely punctate and almost to the raphe, faint in some; central region of the axial field variable from almost circular to unequally rectangular.

Observed frequently on sandy soil and rarely on duff-humus soil.

Reported by Lund (1946) as one of the commonest of all soil diatoms, occurring on soils which are relatively alkaline. Petersen (1935) found many forms of this species on Danish soils.

Microslides nos. 202-3-b, 301-1-a.

10. *Navicula muralis* Grun.

Lund (1946, p. 84, fig. 8 A-I). 13-14.5 μ x 5-5.5 μ.

A few valves elliptical, somewhat rhomboid with a very narrow axial field widening into a barely visible circular central area and with radial striae were found and seemingly belong to this species as described by Lund (1946, p. 83-84), although the frustules seen in this study were appreciably larger than those studied by him (valves 5-8 μ x 2-3 μ) in British soils. Petersen (1935) considers it mainly hydrophytic.

Observed in path, sandy, and duff-humus soils.
Microslides nos. 101-2-b, 202-1-a, 301-1-a.

11. *Navicula dicephala* (Ehrenb.) W. Sm. Fig. 2.
Van Heurck (1896, plate 3, fig. 138); Van Heurck (1880-81, vol. 2, plate 8, fig. 34); Hohn (1951, p. 36, fig. 137). 12-23 μ x 6-7.5 μ.
Valves linear, somewhat narrow, with apices definitely rostrate-capitate; striae radial and shortened around the central nodule. Some frustules appeared to be somewhat elongate in comparison with the other "average" individuals.
Found in sandy and duff-humus soils.
This species was not reported in soils by Lund (1945, 1946) or Petersen (1935).
Microslides nos. 202-1-a, 303-1-b.

Pinnularia Ehrenberg

12. *Pinnularia microstauron* (Ehrenb.) Cleve. Fig. 5.
Lund (1946, p. 91, fig. 11, A-E, P-T); Hohn (1951, p. 34, fig. 196). 17 μ x 3.5-8 μ.
Valves linear to elliptic; striae definitely of costae unequal in length, strongly radial centrally and converge towards the apices; central portion of axial field greatly expanded; apices slightly capitate.
Observed in sandy and duff-humus soils only and then never in abundance.
This species was described by Lund (1946) as being one of the commonest and, sometimes, one of the most abundant soil diatoms on well-cultivated soils.
Microslides nos. 201-2-b, 202-1-b.

Stauroneis Ehrenberg

13. *Stauroneis anceps* Ehrenb.
Van Heurck (1896, plate 1, fig. 56); Lund (1946, p. 62 fig. 3 I-J); Hohn (1951, p. 32, fig. 85).
15-16 μ x 3-4 μ.
Valves elliptic to lanceolate; apices capitate; raphe straight; central nodule expanded into a stauros; striae faint, approximately parallel and slightly radial.
A few frustules found only in the sandy soil. This species was reported by Lund (1946) in larger sizes (26-34 μ x 7-8 μ) on two (2) woodland and one (1) garden soils. Petersen (1935) reported a small form of this species from eastern Greenland soil.
Microslide no. 202-1-a.

14. *Stauroneis acuta* W. Sm.
Van Heurck (1896, plate 1, fig. 51); Hohn (1951, p. 32, fig. 84). 15 μ x 3-4 μ.
Valves lanceolate; apices obtuse and show a distinct lumen;

undiscernable as whether or not the raphe is formed by a double line. Some shells resembled *S. thermicola* (Boye Pet.) as described by Lund (1946, plate 61; p. 62, fig. 3 K-AA) which seemingly has no apical lumina.

A very few specimens were found on the sandy soil.

Microslide no. 201-2-b.

Family:—Epithemiaceae.

Epithemia Brébisson

15. *Epithemia ? hyndmanni* W. Sm.

Van Heurck (1896, plate 9, fig. 350); Van Heurck (1880-81, vol. 2, plate 31, fig. 8). 44-60 μ x 11-12 μ.

Valve with dorsal and ventral surfaces arcuate; apices obtuse; costae radial with two rows of robust punctae between each successive one.

Observed in sandy, duff-humus and field soils. Many frustules with apices broken or missing. Some broken pieces resembled *E. turgida* (Ehrenb.) Kütz, but could not be identified as such with any degree of certainty. This genus was not reported in soils by Lund (1946) or Petersen (1935).

Microslides nos. 202-1-a, 301-1-a, 405-3-a.

Family:—Nitzschiaceae.

Hantzschia Grunow

16. *Hantzschia amphioxys* (Ehrenb.) Grun. Fig. 3.

Van Heurck (1896, plate 15, fig. 488b); Lund (1946, p. 95, fig. 13 A-D, F); Hohn (1951, p. 38, fig. 192).

valve: 15-47.5 μ x 4-8 μ..

girdle: 15:5-42 μ x 3.5-9 μ.

Valve arcuate; apices somewhat prolonged, capitate; striae definite to faint; size and shape variable.

This species was found to be present on all soils in abundance except path soil, where it was present in smaller numbers.

A number of workers have found this species widely distributed in soils. Lund (1946) reported the presence of this diatom on 55 soils and described it as the most ubiquitous of soil diatoms. Petersen (1935) and Patrick (1948 believed it to be the most widespread soil diatom.

Microslides nos. 401-1-a, 503-2-b.

Family:—Surirellaceae.

Surirella Turpin

17. *Surirella ovata* Kütz. Fig. 6.

Lund (1946, p. 105, fig. 18 K-DD); Hohn (1951, p. 38, fig. 201. 12-37.5 μ x 4.5-8.5 μ.

Valves variable from elongate-ovoid to lanceolate; apices nearly equal in size and shape, approximating obtuse; broad costae extend inward from margins; striae faint to invisible (absent ?). Found in path, duff-humus, alfalfa field and lawn soils.

Reported by Lund (1946) as being a common soil diatom especially on well-cultivated soils, rarely occurring in large numbers. This genus was not reported on Danish soils by Petersen (1935)

Microslides nos. 101-1-b, 201-1-a, 203-2-a, 301-1-b.

18. *Surirella ? verrucosa* Pant.

Cleve-Euler (1952, vol. 5, fig. 1547).

Valve elliptic; costae robust extending inward from margins. Only one frustule identifiable to this species was found in sandy soil. *S. verrucosa* must therefore be treated as a very doubtful soil diatom.

This species was not reported in the soils observed by Lund (1946) or Petersen (1935).

Microslide no. 202-1-b.

A critical examination of the literature has shown that a number of species identified in this study previously have not been reported in soils. The species heretofore not reported in soils are as follows:

1. *Cyclotella Kützingiana.*
2. *Eunotia diodon.*
3. *Achnanthidium flexellum.*
4. *Cocconeis scutellum.*
5. *Navicula dicephala.*
6. *Stauroneis acuta.*
7. *Epithemia hyndmanni.*
8. *Surirella verrucosa.*

Table I

The distribution of species studied in the various soils:

species:	stations: path	sandy	duff-humus	field	lawn	loessial-clay
1. *Cyclotella kützingiana*	–	–	+	–	–	–
2. *Eunotia diodon*	–	–	+	–	–	–
3. *Eunotia* spp.	–	+	+	+	–	–
4. *Achnanthes minutissima*	–	+	–	–	–	–
5. *Achanthidium flexellum*	–	+	–	–	–	–
6. *Cocconeis scutellum*	–	+	+	–	–	–
7. *Navicula minima*	–	+	+	–	–	+
8. *Navicula seminulum*	++	++	++	–	–	+
9. *Navicula mutica*	–	++	+	–	–	–
10. *Navicula muralis*	+	+	+	–	–	–
11. *Navicula dicephala*	–	+	+	–	–	–
12. *Pinnularia microstauron*	–	+	+	–	–	–
13. *Stauroneis anceps*	–	+	–	–	–	–
14. *Stauroneis acuta*	–	+	–	–	–	–
15. *Epithemia ? hyndmanni*	–	+	+	+	–	–
16. *Hantzschia amphioxys*	+	++	++	++	++	++
17. *Surirella ovata*	+	–	+	+	+	–
18. *Surirella verrucosa*	–	+	–	–	–	–

Key: ++ = abundant; + = not abundant; – = absent.

Discussion and Conclusions

Some species of diatoms studied have been reported both from aquatic and terrestrial habitats. After the frustules of these species were measured, they were found generally to be in a size range smaller than that of aquatic individuals of the species. Lund made a similar observation in his investigation of diatoms on British soils (Lund, 1945, 1946). This observation seems to support the theory (Lund, 1945) that the availability of water to the diatom cells tends to regulate the range of sizes among individuals of a species and therefore, limits diatoms on soils to sizes smaller than those of aquatic habitats. Of the diatoms encountered, the largest frustules belonged to the species *Epithemia ? hyndmanni* and *Hantzschia amphioxys*.

Except for *Cyclotella,* the diatoms of the soils studied were found to be in the Pennales. All species of this order found in the soils of this investigation had at least one true raphe except those of *Eunotia*. This fact clearly suggests that soil diatoms are nearly always motile.

Of all soils studied, the sandy soil, from which fifteen species were identified (Table I), was found to have the most luxuriant growth of diatoms both in number of species and individuals. The duff and humus soil of the deciduous forest was found to be second in number of species (13) encountered, but was far behind the sandy soil in number of individuals. Very few species of diatoms were encountered in the path (4), field (4), lawn (2), and loessial-clay (3) soils.

It is apparent from this investigation that the distribution of species may be related to the texture of the soils. This relationship is striking in the case of the sandy versus loessial-clay soils. However, this association is not so apparent in other soils. The relative abundance of diatoms, both in number of species and individuals, in the sandy soil is probably due to optimal conditions for aeration in that soil. This soil was found to be very porous consisting of a mixture of sand and a little humus. The porosity of the soil readily allowed for optimum aeration. The "soil crumbs" of humus throughout the topsoil, in addition to containing many mineral nutrients and organic substances absorbed water droplets, thus retaining moisture which was available to organisms in the soil. In contrast, loessial-clay soil is not at all porous, being very compact in texture. Furthermore, the particles of clay strongly hold water by adsorption, thus keeping it from soil organisms. As a result of poor aeration and lack of available water, loessial-clay soils seem to have fewer plants and animals and lack diatoms.

Hantzschia amphioxys was found to be relatively few in number in path soil. In all other soils it was found to occur in equal abundance. The presence of this species in all soils supports the belief (Petersen, 1935; Lund, 1946; and Patrick, 1948) that it is the commonest of soil diatoms.

A large number of specimens could not readily be identified to species, particularly in the genus *Navicula* as minute variations among frustules made it appear that there is a high degree of intergradation between species. This observation makes the need for additional taxonomic work rather obvious in the genera *Eunotia* and *Pinnularia* as well as *Navicula.* A few frustules could not be identified because of their extremely small size and consequently faint markings.

Many difficulties were encountered in the examination of frustules among the numerous particles of undestroyed soil matter in "cleaned material." This is a strong indication for the need of a new technique for separating frustules from soil. The occurrence of many broken diatom shells and shell fragments, mostly of *Eunotia,* and the apparent absence of faint striae and other markings which in the literature are reported to be present may be the result of severe treatment with strong acids. The method of cleaning used in this investigation may have resulted in processes of etching and erosion by the strong acids and potassium dichromate. These facts strongly suggest that a milder means of separating and cleaning frustules would be more satisfactory in a study of the soil diatoms which are seemingly more fragile than the aquatic forms. Perhaps the substitution of a weak acid or distilled water in place of concentrated acids and postassium dichromate may be a more desirable means of preparing soil diatoms for study.

Summary

1. Seven samples of soil were scraped from the surface at each of six collection stations. Soils studied were path, sandy, duff and humus of a deciduous forest, field soil from an alfalfa field, garden soil from a lawn and loessial-clay soil.

2. The soil samples were cleaned by the sulfuric acid-potassium dichromate technique used by Hohn (1951) or a modification thereof. The cleaned material was centrifuged and washed several times.

3. Permanent slides of each sample were prepared with "Permount" mounting medium. Surplus sample materials were stored in ethanol in test tubes.

4. Among species studied reported from both aquatic and terrestrial habitats, the species showed a strong tendency to grow smaller in the terrestrial habitat.

5. For the most part, diatoms studied belonged to the order Pennales, most of which possess at least one true raphe.

6. Of all soils studied, the sandy soil had a far more luxuriant growth of diatoms both in number of species (15) and individuals

than any other. The loessial-clay soil had the poorest growth of frustules.

7. The presence of *Hantzschia amphioxys* in all soils, sometimes in abundance supports the theory that this is the most common of soil diatoms.

8. The difficulty in separating some specimens identified to species, particularly in the genus *Navicula,* because of an apparent intergradation of species points out the need for additional taxonomic work.

9. Observational difficulties added to a prevalence of broken frustules indicates the need for a new means for separating diatoms from the soil and cleaning them for study.

10. This investigation has revealed the occurrence of a number of species of diatoms which heretofore have not been reported in soils.

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