

POLLEN, STARCH, PARASITE, AND PROTEIN RESIDUE ANALYSIS OF SEDIMENT FROM THE EVANSTON CHINATOWN HISTORIC ARCHAEOLOGICAL SITE, 48UT1749, WYOMING

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INTRODUCTION

One sediment sample was collected from sediments at the Evanston Chinatown historic site (48UT1749). This sample was examined for evidence of pollen and starch granules that might identify both modern vegetation and food, for parasite eggs that might indicate parasite infestation, and for protein residue to identify animals present.

METHODS

Pollen

A chemical extraction technique based on flotation is the standard preparation technique used in this laboratory for the removal of the pollen from the large volume of sand, silt, and clay with which they are mixed. This particular process was developed for extraction of pollen from soils where preservation has been less than ideal and pollen density is low.

Hydrochloric acid (10%) was used to remove calcium carbonates present in the soil, after which the sample was screened through 150 micron mesh. The sample was rinsed until neutral by adding water, letting it stand for 2 hours, then pouring off the supernatant. A small quantity of sodium hexametaphosphate was added to the sample once it reached neutrality, then the beaker was again filled with water and allowed to stand for 2 hours. The sample was again rinsed until neutral, filling the beaker only with water. This step was added to remove clay prior to heavy liquid separation. At this time the sample was dried then pulverized. Zinc bromide (density 2.1) was used for the flotation process. The sample was mixed with zinc bromide and centrifuged at 1500 rpm for 10 minutes to separate organic from inorganic remains. The supernatant containing pollen and organic remains was decanted and diluted. Zinc bromide was again added to the inorganic fraction to repeat the separation process. After rinsing the pollen-rich organic fraction obtained by this separation, the sample received a short (one hour) treatment in hot hydrofluoric acid to remove any remaining inorganic particles. The sample was then acetolated for 3 minutes to remove any extraneous organic matter.

A light microscope was used to count the pollen to a total of 100 to 200 pollen grains at a magnification of 400-600x. Pollen preservation in this sample varied from good to poor. Comparative reference material collected at the Intermountain Herbarium at Utah State University and the University of Colorado Herbarium was used to identify the pollen to the family, genus, and species level, where possible.

Pollen aggregates were recorded during identification of the pollen. Aggregates are clumps of a single type of pollen, and may be interpreted to represent pollen dispersal over short distances, or the introduction of portions of the plant represented into an archaeological setting. Aggregates were included in the pollen counts as single grains, as is customary. The presence of aggregates is noted by an "A" next to the pollen frequency

on the pollen diagram. The entire microscope slide was scanned to look for the presence of parasite eggs.

Indeterminate pollen includes pollen grains that are folded, mutilated, and otherwise distorted beyond recognition. These grains are included in the total pollen count, as they are part of the pollen record.

Parasite eggs are known to be recovered and preserved in the pollen sample using this extraction method. If parasite eggs are contained in the sample, they are expected to be present in the microscope slide examined.

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Protein Residue

Artifacts sent for protein residue analysis were tested against prepared animal antisera obtained from Organon Teknika Corporation and Sigma Chemical Company, and against agave and bison antisera raised under the direction of Dr. Richard Marlar at the Thrombosis Research Laboratory in the Denver VA Medical Center and the University of Colorado Health Sciences Center. Samples were tested using a technique referred to as cross over immunoelectrophoresis (CIEP or COE). The method for CIEP is based on forensic work by Culliford (1964, 1971) with changes made by Newman (Newman and Julig 1989) following the procedure used by the Royal Canadian Mounted Police Serology Laboratory in Toronto, Canada. Further changes were made at Paleo Research Labs following the advice of Dr. Richard Marlar.

Artifacts were washed using 1-2mL of a 0.02M Tris hydrochloride, 0.5M sodium chloride, and 0.5% Triton X-100 solution. Artifacts were placed in an ultrasonic bath for 30 minutes, then on a rotating mixer for 30 minutes. One-half of the grinding surface was washed using 20mL of a 0.02M Tris hydrochloride, 0.5M sodium chloride, and 0.5% Triton X-100 solution. Dirt was removed using centrifugation, and the resulting solution was concentrated down to approximately 1mL using a Centriprep-10 centrifugal concentrator device with a 10,000 molecular weight cut-off membrane. Because soils contain compounds such as bacteria and animal feces that can cause false positive results for artifacts buried in the soil, soil controls also were tested. One gram of soil was added to 1mL of the Tris/NaCl/Triton solution, then refrigerated for several days prior to testing. The extracted solutions were stored at 4C using polypropylene microcentrifuge tubes.

CIEP was performed using agarose gel as the medium. The samples were first tested against pre-immune goat serum (serum from a non-immunized animal) to detect non-specific binding of proteins. Non-specific binding is absent if a negative result is obtained. Samples were electrophoresed in Barbital buffer (pH 8.6) for 45 minutes at a voltage of 130v. Samples were then pressed and rinsed in 1M saline solution overnight to remove extraneous proteins.

The next morning, the gel was washed, pressed, dried, stained in a Coomassie Blue solution, then destained. Gels were observed to determine if non-specific binding was occurring. Positive reactions appear as a line of precipitation between the two wells. All samples tested negative against pre-immune serum and were then tested against the various antisera. Appropriate positive and negative controls were run for each gel. A positive control consists of the blood of each species tested, and a negative control consists of the blood of the species in which the antiserum was raised. Gels were electrophoresed, pressed, washed, dried, stained, and de-stained as before.

Positive reactions were re-tested with dilute antisera to determine between true and false positives. Antisera were diluted to increase specificity of reactions, usually 1:10 or 1:20. Positive reactions obtained after this step were reported.

Identification of animals represented by positive results is usually made to the family level. All mammalian species have serum protein antigenic determinations in common; therefore, some cross reactions will occur between closely and sometimes distantly related animals (Gaensslen 1983:241). For example, bovine antiserum will react with bison blood, and deer antiserum will react with other members of the Cervidae (deer) family, such as elk and moose.

DISCUSSION

A single sediment sample (170) from test unit 2.115-2.26S/11.83-12.0W (Table 1) in Evanston Chinatown was examined for its pollen and starch record of plants and for protein residue. The pollen record reflects local vegetation dominated by Artemisia (sagebrush). A moderate quantity of High-spine Asteraceae pollen probably reflects rabbitbrush in the local vegetation. Small quantities of Alnus and Betula pollen represent alder and birch growing in a riparian zone, probably along a nearby drainage. Small quantities of Juniperus and Pinus pollen probably reflects juniper and pine trees growing on outcrops or other elevated areas.

Small quantities of Low-spine Asteraceae and Chenopod pollen (Figure 1, Table 2) probably represent weedy plants such as ragweed and goosefoot growing in Evanston. Small quantities of Phlox-type, Poaceae, and Rhus-type pollen probably represent the presence of phlox, grass, and sumac or squawbush shrubs growing in Evanston. Ephedra pollen is noted to travel long distances on the wind, and probably represents the presence of Mormon tea within the regional vegetation surrounding Evanston. A single starch granule was recovered that looks like those produced by grass seeds and probably represents the deterioration of grass seeds in this area. There is no evidence in the pollen record of specific activities involving plants or food processing. No parasite eggs were noted during pollen analysis, nor during the scan of the rest of the microscope slide. Absence of parasite eggs might reflect an absence of parasites in the population living in this area, or an absence of human fecal material in this area, which is necessary to transport parasite eggs into the sediment.

Antisera used for protein residue analysis is listed in Table 3. Protein residue analysis yielded positive results to goat and horse antisera (Table 4), indicating that goats and horses frequented this area. Proteins are present in all body fluids and tissues. Immunological studies on coprolites and modern animal dung have shown that CIEP will identify what animal produced the feces (Newman et al. 1993).

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SUMMARY AND CONCLUSIONS

Combined pollen and phytolith analysis of a sediment sample from Evanston Chinatown indicate that the local vegetation was very similar to that of the surrounding area today and was dominated by sagebrush. The pollen record yielded no evidence of plant processing in the area sampled. No evidence of parasite eggs was found. Information concerning the exact provenience of the sample was lacking, so it is difficult to determine whether or not human feces or night soil are expected for this area. They are necessary as a transportation agent for parasite eggs. Protein residue analysis yielded evidence of the presence of goats and horses in the area sampled. Since proteins are present in urine and feces, positive results are expected in areas where animals are kept or where they frequent, as a result of protein introduced into the sediments from urine and/or feces.

TABLE 1

PROVENIENCE DATA FOR A SINGLE SAMPLE FROM SITE 48UT1749

Sample No.	Description/ Provenience	Analysis
170	2.115-2.26S/11.83-12.0W; Sediment from ...	Pollen/Starch/Parasite Protein Residue

TABLE 2

POLLEN TYPES OBSERVED IN SAMPLES FROM 48UT1749

Scientific Name	Common Name
ARBOREAL POLLEN:	
<u>Alnus</u>	Alder
<u>Betula</u>	Birch
<u>Juniperus</u>	Juniper
<u>Pinus</u>	Pine
NON-ARBOREAL POLLEN:	
Asteraceae:	Sunflower family
<u>Artemisia</u>	Sagebrush
Low-spine	Includes ragweed, cocklebur, etc.
High-spine	Includes aster, rabbitbrush, snakeweed, sunflower, etc.
Cheno-am	Includes amaranth and pigweed family
<u>Ephedra nevadensis</u> -type	Mormon tea
<u>Phlox</u>	Phlox

Poaceae	Grass family
<u>Rhus</u>	Sumac, squawbush
Indeterminate	Too badly deteriorated to identify

TABLE 3

LIST OF ANTISERA USED IN TESTING ARTIFACTS FROM SITE

ANTISERA	SOURCE
Bovine	Sigma Chemical Company
Cat	Sigma Chemical Company
Catfish	Sigma Chemical Company
Chicken	Sigma Chemical Company
Dog	Sigma Chemical Company
Goat	Sigma Chemical Company
Guinea pig	Sigma Chemical Company
Horse	Organon Teknika Corporation
Human	Organon Teknika Corporation
Mouse	Sigma Chemical Company
Pig	Organon Teknika Corporation
Rabbit	Sigma Chemical Company
Rat	Sigma Chemical Company
Sheep	Sigma Chemical Company
Turkey	Sigma Chemical Company

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TABLE 4

POSITIVE PROTEIN RESIDUE RESULTS FOR SAMPLES FROM SITE 48UT1749

Sample No.	Description	Positive Result (Antiserum Type)	Possible Animal(s) Represented
170	Sediment	Goat, Horse	Goat, Horse

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** Paleo Research Laboratories: Denver, Colorado, Paleo Research Labs Technical Report 98-19
Prepared For Western Wyoming Community College Rock Springs, Wyoming, October 1998*