Review of Palaeobotany and Palynology 215 (2015) 46–56

Contents lists available at ScienceDirect

Review of Palaeobotany and Palynology

journal homepage: www.elsevier.com/locate/revpalbo

Freeze-dried is as good as frozen: Evaluation of differential preservation of pollen grains in stored lake sediments

Diana Tirlea [a,*,1], Alwynne B. Beaudoin [a], Rolf D. Vinebrooke [b]

[a] Royal Alberta Museum, 12845-102 Avenue, Edmonton, Alberta T5N 0M6, Canada
[b] University of Alberta, Department of Biological Sciences, Room 2-271 CCIS, Edmonton, Alberta T6G 2R3, Canada.

ARTICLE INFO

Article history:
Received 18 September 2013
Received in revised form 31 July 2014
Accepted 7 December 2014
Available online 13 December 2014

Keywords:
Alpine
Palynology
Paleolimnology
Pollen preservation
Freeze-drying

ABSTRACT

In Quaternary studies, sediment storage methods (e.g., freezing, freeze-drying, vacuum-drying) vary across paleoecological disciplines. Typically, palynologists store sediment cores in a freezer or refrigerator; however, sediments already collected and stored (freeze-dried) for paleolimnological studies are available and potentially useful for pollen analysis. Here, we investigate the effect of freeze-drying on pollen grain structure compared to pollen recovered from frozen sediments. Pollen was recorded for eight deterioration categories for paired samples of freeze-dried and frozen sediment obtained from a small alpine lake (Sentinel Lake) in Banff National Park, Canada. No statistically significant differences in pollen deterioration were detected between storage methods for identified pollen taxa and groups (*Pinus*, *Picea*, *Abies*, *Alnus*, non-arboreal, arboreal). There were also no statistically significant differences between freeze-dried and frozen samples and their mean pollen sum, number of indeterminable pollen grains and number of taxa detected per sample. Instead, deterioration of pollen for both storage methods increased with the depth of sample taken from the core. These results demonstrate freeze-drying as a useful method for storing sediments intended for palynological research. Our findings highlight how limnologists and palynologists can collaboratively share freeze-dried archival sediment samples to reduce field costs and strengthen interpretation of data through analysis of multiple proxies.

1. Introduction

In Quaternary studies, sediment storage methods vary across paleoecological disciplines; paleolimnologists often store sediments in freezers or by freeze-drying, whereas palynologists generally freeze or refrigerate sediments (Faegri et al., 1989; Leavitt and Hodgson, 2001; Reuss et al., 2005). In both disciplines, storage conditions are optimized to reduce possibilities of degradation for the target indicators, including algal pigments, diatoms, and pollen. Increasingly, limnologists and palynologists are working on collaborative projects in which sharing of the same sediment cores would avoid duplicating field-efforts, maximize the use of recovered material, and strengthen interpretation of data through analysis of multiple proxies.

Limited information exists on the effects of different sediment storage methods (i.e., freezing or freeze-drying (vacuum-drying)) on pollen preservation. Freeze-drying rapidly removes water (through sublimation) from cells using a two-step process of freezing and vacuum-drying, while freezing alone results in the formation of intracellular ice crystals (Matthews and Kraus, 1981; Adams, 2007). Therefore, mechanical stresses associated with processing sediment cores for storage may damage (e.g., tear, crumple) pollen grains. The few studies (e.g., Lechterbeck and Schwark, 2003; Guo et al., 2012) that use freeze-dried sediment for pollen analysis do not present detailed and qualitative analysis on the effects of freeze-drying on pollen deterioration. To the best of our knowledge, there are no studies that explore whether mechanical damage from freeze-drying of sediments leads to a greater proportion of deteriorated pollen grains compared to pollen recovered from frozen sediments. It is this question that the present study was designed to explore.

This study was conducted at the University of Alberta and the Quaternary Environments Laboratory, Royal Alberta Museum, where standard practices include freezing or freeze-drying sediment cores for long-term storage. Refrigerated samples are typically stored for short-term use only. We examined the deterioration of pollen grains obtained from paired frozen and freeze-dried sediment samples to determine if there was a significant difference in the pollen assemblage data between storage methods. Specifically, we investigated whether freeze-drying sediments leads to a greater proportion of deteriorated pollen grains because of suspected mechanical breakage of the exine. By comparing the number of indeterminable pollen grains between storage methods, we investigated which method provided the least amount of information loss due to pollen deterioration. Our objectives were to

* Corresponding author at: Quaternary Environments, Royal Alberta Museum, Culture 12845 - 102 Avenue, Edmonton, AB T5N 0M6. Tel.: +1 780 453 9192; fax: +1 780 454 6629.
E-mail addresses: diana.tirlea@gov.ab.ca (D. Tirlea), Alwynne.Beaudoin@gov.ab.ca (A.B. Beaudoin), rolf@ualberta.ca (R.D. Vinebrooke).
[1] Present address of author. Actual work completed at the Royal Alberta Museum and the University of Alberta (co-supervised).

http://dx.doi.org/10.1016/j.revpalbo.2014.12.001
0034-6667/

determine if there are significant differences in a) overall pollen preservation between sample storage methods; b) the number of palynomorphs observed in subsamples processed from frozen versus freeze-dried sediments; and c) frequency of indeterminable pollen grains between sediment storage methods. If freeze-drying does not significantly affect the pollen assemblage data, then there is the potential to use samples already collected for paleolimnological studies for pollen analysis as well.

2. Methods and materials

2.1. Study site and field collection

A sediment core (35 cm length) was obtained from the deepest (6.7 m) depth of Sentinel Lake (51°20′ N, 116°13′ W; 2424 a.s.l.) a small (<0.1 ha) alpine lake located in Banff National Park, Alberta, Canada. The surrounding forest is composed of *Picea engelmanni* Parry ex Engelmann (Engelmann spruce), *Abies bifolia* A. Murray (Rocky Mountain subalpine fir) and *Larix lyallii* Paralatore (alpine larch). The immediate area is composed of bare rock and sediment, open meadows with low-lying vegetation and sparsely distributed krummholz. Dominant low-lying vegetation in the area includes *Phyllodoce glanduliflora* (Hooker) Coville (yellow heather) and *Cassiope mertensiana* (Bongard) G. Don (mountain heather). Other commonly encountered vegetation includes *Salix* L. species (willow) and *Dryas octopetala* L. (white mountain avens). Refer to Holland and Coen (1983) for a comprehensive list of local and regional vegetation of the area.

Coring location was selected using a bathymetric map of Sentinel Lake (Mayhood and Anderson, 1976) and a depth sensor. The core was obtained by deploying a Glew gravity piston corer (Glew, 1989) from an anchored inflatable boat in August 2007. The sediments were extruded in the field and sectioned by placing the piston vertically on a stand and subsequently lowering the piston at a set increment and scraping the sediment with a spatula into a pre-labeled sealed sterile plastic bag. Sectioning commenced at the top of the core (sediment surface) with 0.25 cm sections from 0 cm to 10 cm down the core, 0.5 cm sections from 10 cm to 20 cm down the core, and 1.0 cm sections from 20 to 30 cm down the core. Samples were kept in a cooler with ice during transportation.

2.2. Sediment subsampling and laboratory processing

To optimize preservation of algal pigments and pollen, samples were frozen and stored at −80 °C in a freezer, until freeze-drying (Faegri et al., 1989; Moore et al., 1991; Leavitt and Hodgson, 2001). Samples were removed from the freezer, thawed for approximately half an hour and manually homogenized (stirred) for 5 min. Ten frozen subsamples were taken from 10 sections along the core's length. Ten subsamples for freeze-drying were then taken from the same levels as those used for frozen samples (Table 1). These subsamples were vacuum freeze-dried using the VirTis Freeze Mobile 24 Freeze Dryer for approximately 24 h at approximately −60 °C or until samples were dried (based on visual inspection). Subsample weights and volumes varied due to sample storage method (dry versus wet samples) and limited material available from the core.

Frozen and freeze-dried sediment subsamples were chemically processed to concentrate pollen and spores following Faegri et al. (1989). Processed subsamples were stained with safranin to enhance structural contrasts, and suspended in silicone oil. Suspensions were stirred for 5 min and small subsamples were mounted on slides for counting.

Using a transmitted light microscope, equally spaced transverses were counted at magnifications of 400×, with 1000× and oil-immersion used for critical identifications, for each of the 10 frozen and 10 freeze-dried subsamples (Table 1). For each subsample, an entire slide was counted to eliminate bias from differential sorting on the slide (Brookes and Thomas, 1967), rather than setting a standardized count size. The approach typically involved counting one or two subsamples (slides) at each level, providing reliable estimates of pollen percentages across multiple samples from the same level, especially for major components (Moore et al., 1991; Beaudoin and Reasoner, 1992). The pollen sum of paired frozen and freeze-dried samples differed by less than 100 pollen grains (Table 1). For frozen samples 4, 6, 7, 8, and 10, additional slides were required to increase the sum and reduce the difference between frozen and freeze-dried sample counts. Counting of these additional slides ended when the difference was less than 100 pollen grains between the paired samples. Raw count data are included in Tirlea (2011).

2.3. Pollen identification and taxonomy

Taxonomic identifications of pollen grains were made using several keys and publications, including Kapp et al. (2000) and McAndrews et al. (1973), and pollen reference collections at the Royal Alberta Museum (Quaternary Environments) and the University of Alberta (Palynology Laboratory, Department of Anthropology), supplemented by additional collections made from local site vegetation. Most pollen grains were identified at the genus or family level. Plant taxonomy for this pollen study follows Moss (1983).

2.4. Deterioration categories

Each pollen grain counted and identified was assigned to one or more of the eight deterioration categories (D1–D8) (Fig. 1, Plate I). Pollen grains that were indeterminable due to deterioration or concealment were also recorded. Pollen percentages for each

Table 1
Summary of samples and counts for frozen and freeze-dried samples.[a]

Sample ID	Core depth (cm)	Frozen					Freeze-dried				
		Pollen sum	AP	NAP	Indet.	Taxa	Pollen sum	AP	NAP	Indet.	Taxa
1	5.5–5.75	267	261	6	19	9	320	307	13	11	12
2	9.5–9.75	307	287	20	12	12	350	340	10	15	11
3	12.5–13	668.5	650.5	18	17	13	667.5	656.5	11	24	8
4	14–14.5	722	704	18	21	14	713	692	21	17	13
5	15–15.5	426.5	413.5	13	8	12	498.5	488.5	10	22	12
6	17–17.5	857.5	848.5	9	17	10	856	835	21	12	14
7	18.5–19	550	536	14	17	8	567	554	13	13	14
8	23–24	521.5	499.5	22	19	19	541	514	27	7	12
9	29–30	266.5	261.5	5	16	16	216	213	3	23	6
10	32–33	536.5	529.5	7	16	16	524	509	15	7	10

[a] Pollen sum comprises total determinable grains counted per sample. All determinable grains were assigned to one or more deterioration categories (Fig. 1, Plate I). AP = arboreal pollen. NAP = non-arboreal pollen. Indet. = indeterminable grains, i.e., could not be identified due to deterioration or concealment. Indeterminable grains were assigned to one of the indeterminable categories (corroded/degraded, crumpled, broken, or concealed). Taxa category indicates the number of unique taxa identified per sample.