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Instruments and Methods

Quantitative diatom analyses—a faster cleaning procedure

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Abstract

Laboratory techniques employed for cleaning marine sediments for quantitative diatom analyses are time consuming and expensive. In an attempt to reduce preparation time, the method in use in our laboratory, has been compared to six other different methods, which derive from Barron's procedure for rapid sample preparation at sea.

Based on the statistical analyses of the results all the methods in which centrifugation was used, were eliminated. From the two methods that did not show differences from the control method, the cleaner and better preservation of the diatom specimens observed in the slides produced by method M2 lead us to elect this procedure as the best. This method distinguishes itself from other techniques in using of a non-dried sample dispersed before the chemical attack.

Keywords: Diatoms; Cleaning procedure; Quantitative analyses; Paleoproductivity; Paleoceanography; Paleoclimatology

1. Introduction

Diatoms constitute the basis of the food chain and are the dominant phytoplankton in the most productive regions of the world's oceans, the upwelling areas (both coastal and equatorial). In papers recently published, Falkowsky (Falkowski et al., 1998; Falkowski, 2002) calls the attention for the importance that phytoplankton and diatoms in particular may have in climate regulation in the future as major players in the sequestration of CO_2 from the atmosphere. The need to understand their distribution, abundance and species composition in past oceans, as well as their relation/reaction to past climate change is therefore, of primary importance. At present, coring technology is capable of retrieving long sedimentary sequences from marginal regions with sedimentation rates high enough to resolve past climate variability at a decadal scale. However, current laboratory preparation methodologies, quantitative microscopic counting, and

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observation techniques are very laborious and time-consuming and prevent diatomists from fulfilling the need of simultaneously obtaining long and high-resolution diatom records. A number of procedures have been employed to clean siliceous microfossils (cf. Schrader and Schuette, 1968; Schrader and Gersonde, 1978; Fenner, 1982; Scherer, 1994). Abrantes (1988) has combined and adapted Fenner's cleaning procedure (Fenner, 1982) with Battarbee's technique for quantitative slide preparation (Battarbee, 1973). The method has been successfully used in several distinct diatom studies over the last several years (cf. Abrantes, 1988; Nave et al., 2001). However, the cleaning procedures for clay and/or organic carbon-rich sediments, keep samples in the laboratory for long periods of time, with the danger of loss of siliceous material by dissolution during preparation. In an attempt to reduce the laboratory preparation time, Barron's procedure for rapid sample preparation at sea (Barron, 1985) was used as the basis for the new approaches. In order to control the representativeness of the concentration/absolute abundances of microfossils as estimated from sample aliquots, marker microspheres (ECRC divinylbenzene microsphere solution) were added to a set of 25 samples randomly selected from the samples/areas under study at the INETI's Marine Geology Laboratory.

This paper presents the results obtained with the tests of various laboratory methods, as well as with the test of the counting procedure, and, proposes a new, faster and more efficient laboratory methodology.

2. Materials and methods

2.1. Sample cleaning procedures

Seven different methods were tested on a single sample (GeoB 6003-1 35–36 cm). The differences introduced are restricted to the cleaning methodology; all other phases of the quantitative estimation were maintained, according with the routine protocol. One of the methods, the control, followed the cleaning procedure used routinely until now (Abrantes, 1988) and the other 6 consisted in modifications of the rapid procedure (Barron, 1985).

2.1.1. The control method—control

The method in use in our laboratory (Abrantes, 1988) is a follow up of the method of Fenner (1982) and includes the following steps:

- Weight a known volume of sample (about 2 cc), dry over night at 40 °C and weight again.
- Place the material in 250 ml beakers and attack for carbonate and organic matter destruction with 25 ml 10% hydrochloric acid (HCl) and 25 ml 35% hydrogen peroxide (H_2O_2–110 V). Let the reaction take place at room temperature, when finished, put the beakers over a hotplate at 120 °C until reaction stops.
- Add distilled water and leave to settle for about 8 h and then gently remove the excess liquid (correspondent to a 9 cm height) with the help of a vacuum pump. Repeat this operation until the solution has a neutral pH.
- To remove the clay fraction, fill the beakers with a 0.5% sodium pyrophosphate solution and leave for 8 h, then remove the excess liquid of the suspension with the help of a vacuum pump. Add distilled water, let rest for another 8 h and gently remove excess liquid with the help of a vacuum pump. Repeat sodium pyrophosphate/distilled water washing until no clay remains in suspension.

2.1.2. New methods

The new methods followed from modifications of the rapid cleaning procedure proposed by Barron (1985) for fast sample preparation at sea, and start with a bulk non-dried sediment sample:

2.1.2.1. Method 1—M1 (Barron's laboratory procedure)

- Weight about 1 g of bulk sediment and place it in 50 ml centrifuge tubes.
- Attack carbonate with 25 ml 10% HCl.
- Decant excess acid.
- Attack organic matter with 25 ml 30% H_2O_2.
- Clean off excess acid and H_2O_2 through centrifuging 2 min at 1200 rpm with distilled water.