Damage of diatoms through long term storage in formaldehyde

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Abstract

The long term storage of plankton in formaldehyde will destroy the silicated cell walls of diatoms due to lowering of pH with time. Buffering with hexamethylentetramine can prevent this.

It is often necessary to keep samples of plankton for quite a long time. If you can take them from the shelf, students can investigate the changes of the biocoenosis during the seasons of the year after they found out the current state of the lake by microscopic examination. Also to document the development of a water body preserved samples are needed. So material is still present to answer questions which may arise later.

Therefore a part of every plankton draught collected at the ecological field station at the YH Sorpesee has been preserved and stored since 2001. For preservation we have been applying two methods which are recommended in standard textbooks:

Both samples are kept in a polypropylene vial (type N 100/15SPPn of KABE Labortechnik). To the first formaldehyde is added until a final concentration of 2-3% is reached. To the second Lugol's solution containing 10% acetic acid is added "until the colour of cognac is reached". Both methods accord to Schwoerbel (1994, p. 71 resp. 81). He warns that with Lugol's colonies may disintegrate into single cells and therefore recommends formaldehyde for long term storage.

Studying the changes in population structure of diatoms we examined samples that dated back until january 2002. Due to Schwoerbel's warning we were not surprised that after 18 months in Lugol's the greater part of the stellate diatom *Asterionella formosa* had separated into single cells, so we examined the plankton preserved with formaldehyde. Under the microscope we saw totally new shapes of *Asterionella*. The cell walls were thinner than usual, the valves had separated from each other and formed bends in opposite directions (Fig. 1). We also witnessed changes in *Melosira italica*. This diatom had partly lost its cell walls with the protoplasts still being connected (Fig.2).

What had happened?

The search in the literature remained resultless but we got some hints from colleagues. As long term storage of plankton might be interesting for a lot of microscopists we do not want to withhold their remarks.

The basic problem seems to be the loss of silica from the cell walls, which is primarily independent of the preservation medium. We had chosen the plastic vial for its greater tightness compared to plastic topped glass bottles. But glass loses silica which counters the dissolving of diatomic valves.

This process is reinforced by formaldehyde because it reacts slowly into small amounts of formic acid, which is responsible for the said phenomenon. Measurement of pH in samples of different age (made from the same stock solution) yielded values up to 2.7 (Fig 3). The influence of this acid has been described in the scarce literature. Krammer and Lange-Bertalot (1986) state that, according to Kolbe, solutions with a low concentration of formaldehyde could destroy the delicate structure of diatoms through formic acid. They add that, according to Rie-

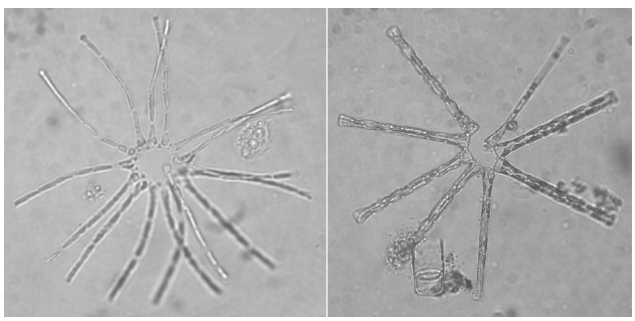

Fig 1: Left: Asterionella formosa *after 2 yrs in 2% formaldehyde; Right: the same alga freshly caught*

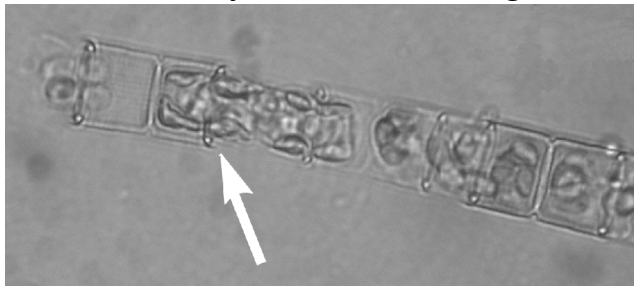

Fig 2: Melosira italica after 2 yrs in 2% formaldehyde. The arrow points to the considerably corroded edge of the cell wall. The wall of the neighbouring cell on the right is already missing.

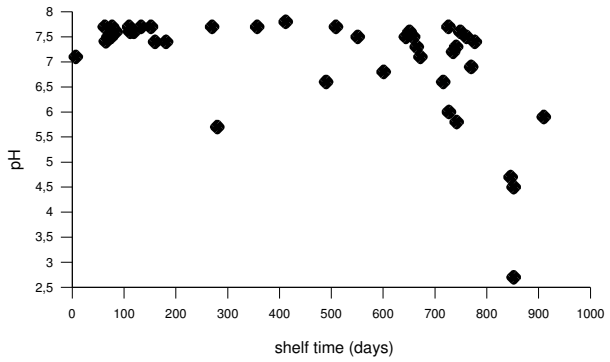

Fig. 3: pH of formaldehyde preserved plankton samples of different age

mann's observations, the amount of the damage would be so tiny that after 21 months in formaldehyde solutions with a concentration up to 10% no alterations were visible with the electron microscope at all. In contrast to this our observations show that the effect may be considerable.

What can be done against this?

1.) A remedy for the silicate shortness in plastic vials are glass beads that are added by some investigators. The problem with this is that those glass beads accelerate the distruction of the fragile organisms. A way out of this dilemma is the use of traditional glass bottles if you regularly check their tightness.

2.) It is recommended to buffer formaldehyde with hexamethylentetramine to reach a pH of 7.5. If the initial pH of the sample is relatively high (as with the samples examined in fig. 3, which originally had a pH of 7.5 - 8) you should check the pH from time to time and add some hexamethylentetramine. In this way the nascent formic acid is neutralized and is not harmful any more. Gerlach (1977) recommends to put some pieces of marble in the stock solution to achieve neutralization.

Preserved plankton samples are kind of a valuable document of the momentary situation of an ecosystem. It would be desirable to keep them not only for years, but for future generations of scientific workers.

Thanks

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