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The Characteristics of Diatom in Different Preservation Methods: A Comparation Study

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Abstract: Diatoms are cosmopolite microalgae with unique characteristics caused of their cells are covered by silica which is called a frustule. Diatom identification is carried out using morphological features that are contained in the frustules. There are some methods for diatom preservation including using Lugol's iodine, ethanol and formaldehyde solution. This study aims to compare those solutions. Those solutions had prepared with one control and four observations. Lugol's iodine was prepared with a dose of 1%. Ethanol was used at doses of 20% while the formaldehyde solution was used at 4%. The observed diatom characteristics including the presence of cytoplasm and ornamentation of the diatom frustules (including striae and raphes) were observed to find the distinction in those methods. the presence of cytoplasm in each cell was not too different in the treatment, with the percentage of cytoplasmic cells being 73% (ethanol and formalin) while in Lugol the percentage of cytoplasmic cells was 65% and in the control was 76%. Based on the results, it was found that there was no significant effect of differences in preservation methods on cells with the cytoplasm or not with a p-value> 0.05.

Keywords: Characteristics; Diatom; Preservation.

Introduction

Diatom is a type of microalgae that has a cell cover made of silica (Hildebrand et al., 2018). Diatoms are cosmopolitan in distribution and have very diverse roles in the environment, some of which are as bioindicators of water quality, bioremediation and paleoecological studies of the aquatic environment (Yun et al., 2017). The use of diatoms takes advantage of its characteristics, namely the cell covering called frustule, ornaments on the frustule is striae and in some types of diatoms, there are longitudinal lines on the frustule called raphe (Fu et al., 2022). The frustule is made up of two separate valves, which are arranged like a petri dish that are linked with bands namely the girdle band (Mishra et al., 2017). Those causes diatom to have two points of view when observed with a microscope, girdle view and valva view (Hwang et al., 2022). Moreover, diatoms can be differentiated based on the symmetry of the frustule, the shape of the apical axis, cell size, length and spacing between striae (Glushchenko et al., 2019). These characteristics make it easy for us to be able to distinguish between species of diatoms.

The role of diatoms which can be used as bioindicators of water quality based on their species diversity (Falah et al., 2022). In addition, diatoms can assess past water conditions using a paleolimnological approach (Soeprobowati et al., 2019). Diatoms can also be used in forensics to identify drowning victims (Zhao et al., 2015). That function makes it necessary to preserve diatoms to maintain the shape of the frustule so that it can be easily recognized (Salmaso et al, 2019).

Several preservation methods are often used for microalgae, one of which is by using a preservation solution. A preservation solution is a solution that can preserve the condition of microalgae cells so that they can be observed for a long time (Williams et al, 2016). Preservation solutions commonly used for microalgae are formaldehyde (formalin), ethanol and Lugol's iodine (Swanepoel et al, 2008). In addition, diatom preservation

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can use acidic solutions such as HCl and H_2O_2 to remove organic components and leave naked frustules (Widyanta et al., 2020). However, the use of these substances can damage Frustule so that diatoms are difficult to identify (Zhou et al., 2020). In other hand, preserving the organic components in diatoms is necessary for further research such as analysis of chlorophyll and lipid content (Hasani, 2023). Retaining the presence of organic matter such as the cytoplasm of cells can also help molecular identification (Gastineau et al., 2021).

Ethanol or also known as ethyl alcohol can be used for various purposes, one of which is as a preservative. Ethanol is recommended for long term preservation (Taylor et al., 2007). The advantage of using ethanol as a preservation solution is that it can be used for molecular analysis after the sample has been preserved. However, ethanol cannot be used as a preservative for in situ sediment trap samples (Sano et al, 2020). The use of ethanol in low concentrations cannot inhibit bacterial growth so its concentration as a preservation solution needs to be considered (Abate et al., 2023).

Formaldehyde (formalin) is one type of preservation solution that is often used. The advantage of using formalin as a preservation solution is that it is a good preserving and fixing agent for algae with more rigid cell walls, the structure of cell wall and other characteristics will be well visible and when stored properly samples preserved with formalin will remain good for a long time without the need for special handling (Anderson & Karlson, 2017). However, formalin has disadvantages, the possibility that it can be reduced and distorted some kind of microalgae and formalin is not suitable for post-preservation molecular analysis (Sano et al., 2020). The use of formalin for diatom preservation can help research to assess water quality (Audah et al., 2021).

Another solution that is often used is Lugol's iodine. There are several types of Lugol's iodine, they are acid Lugol's iodine, alkaline Lugol's and neutral Lugol's iodine. The advantage of Lugol's iodine solution is that it has an instant effect and increases the weight of the organisms reducing sedimentation time. Lugol's iodine solution will cause discolouration of some phytoplankton making identification difficult (Karlson et al, 2010). In other hand, the use of lulogl's iodine as a preservation solution can affect the biovolume of phytoplankton (Hae-Kyung et al., 2018). Moreover, the use of lugols solution also has an impact on the shrinkage of diatom cell volume. (Maden-Deuer, Lessard & Satterberg, 2001). The purpose of this study was to compare the three preservation methods on the characteristics of diatoms including the presence of cytoplasm and ornaments on the frustule (striae and raphe).

Method

Samples were taken from the Agathis Small Lake located at the Universitas Indonesia, Depok, Indonesia. The diatom samples taken are diatom samples attached to plant parts in the form of leaves submerged in a lake (Epiphithic). The leaves are put in a ziplock plastic and given water from the lake, after which the sample is squeezed so that the diatoms attached to the leaves are detached. All samples are observed on a microscope Olympus series CX23LEDRFS1 to find out what species are in the sample. Identification of diatom using Bellinger (2015).

The preservation solution to be tested is prepared, using ethanol with a concentration of 20%, formalin at 4% and Lugol's iodine with a dose of 1%. Those solutions had prepared with one control and four observations. 0,04 mL of sample from each treatment was taken using Pipette Pump Pasteur Transfering. Cells were observed with direct counting under a light microscope with a magnification of 100x to count the number of cytoplasmic cells and those that were not. The diatom frustule condition was also observed during the calculation. The data obtained were statistically analyzed using the Manova test which had previously been tested with normality and homogeneity tests using the SPSS program. The research flow scheme is presented in Figure 1.

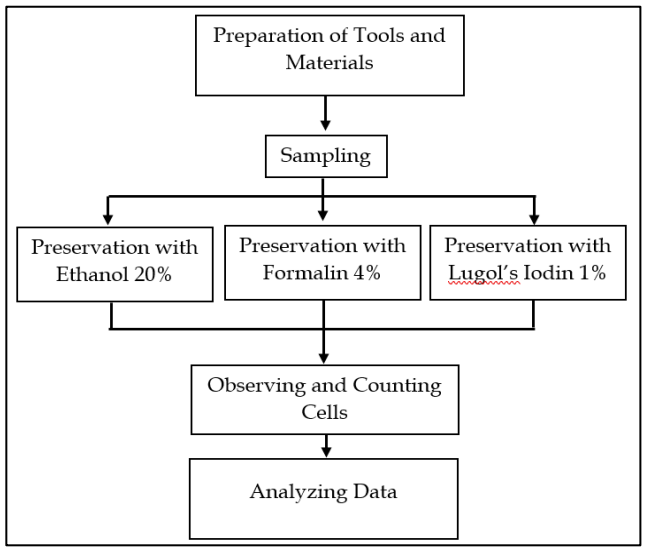


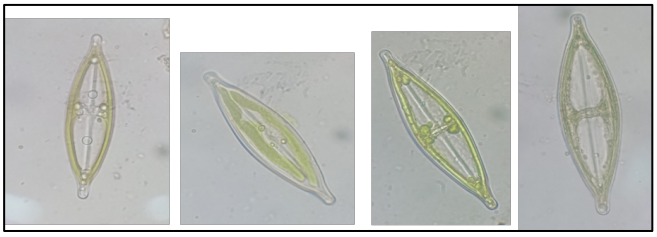
Figure 1. The Research workflow scheme

Table 1. Average percentage in frustule with cytoplasm and without cytoplasm (Naked) on different preservation methods.

Solution	Cytoplasm (%)	Naked (%)
Control	76	24
Ethanol	73	27
Lugol's iodine	65	35
Formalin	73	27

Result and Discussion

Based on observations before being given the preservation solution, the sample was dominated by two genera of diatoms, they are *Stauroneis* and *Nitzschia* accompanied by several other types of microalgae such as *Gloeocapsa*, *Oscillatoria*, *Scenedesmus* and some species of Euglenoid. After being given the preservation solution in the treatment, several changes would be immediately observable, one of them is the colour of the cytoplasm. Every preservation solution gives a different colour effect on the cytoplasm. Formalin solution gives the brightest colour compared to other preservation solutions. On the other hand, Lugol's Iodine gives the faintest colour compared to others (see Figure.1). formalin can make cells well preserved and able to display cell characteristics well (Anderson & Karlson, 2017). The concentration of 4% formalin can reduce cell size from 1-30% in pennate diatoms such as *Nitzschia bilobata*, *Pleurosigma formosa* and *synedra ulna* in long-term preservation. High formalin concentrations can inhibit microbial growth but at the same time damage preserved cell morphology (Mukherjee et al., 2014). In samples obtained from 4 observations, there was some frustule from damaged *Stauroneis*, while in other samples such as alcohol, but not as much as formalin and in Lugol's iodine, no damaged cell frustule was found. Frustule damage was only found in *Stauroneis* while in *Nitzschia* there was no frustule damage in all treatments.

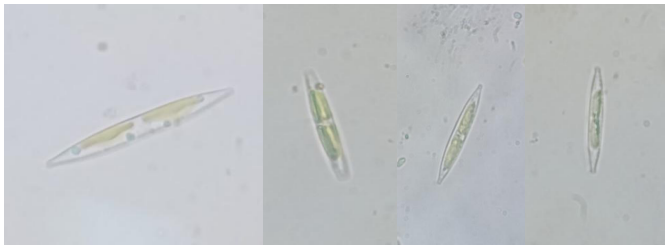


(a) (b) (c) (d)

Figure 2. Comparison of *Stauroneis* images in each treatment (a) Control View, (b) Ethanol View, (c)Formalin View, (d) Lugol's Iodine

Identification of diatoms is carried out by looking at the ornaments on the frustule inside (raphe and striae). In the sample, the presence of striae in the dominant genus *Stauroneis* is difficult to observe, this can be because it is covered with cytoplasm or the characteristics of the striae are smooth, making it difficult to observe. On the other hand, observation of raphe on *Stauroneis* can be done in each treatment. In the observation, it was found that there was no difference in the appearance of the raphe in each treatment.

Observing the characteristics of the genus Nitzschia is difficult to do because the cell size is very small so it is very difficult to see the ornaments on the frustule of this genus (see Figure 2). the observation of the cytoplasm is accompanied by a calculation of the number of cells that have cytoplasm and those that do not, this aims to be used as the dependent variable to find out whether there is an effect of different preservation methods on cells with cytoplasm or not. The percentages of cytoplasmic and non-cytoplasmic frustule are presented in Table 1.



(a) (b) (c) (d)

Figure 3. Comparison of *Nitzschia* images in each treatment (a) Control View, (b) Ethanol View, (c) Formalin View, (d) Lugol's Iodine View

The results of observations on the cytoplasmic and non-cytoplasmic frustule were tested using statistical analysis Multivariate Analysis of Variance (MANOVA). The choice of statistical analysis was because there were two dependent variables, the cytoplasmic frustule and the non-cytoplasmic frustule. Before the MANOVA test, a multivariate normality test and a variance matrix homogeneity test were carried out to ensure that the data could be applied to the MANOVA analysis. Based on the results of the normality test, it was found that the data were normally distributed in multivariate and homogeneous data was also found after the homogeneity test of the variance matrix was carried out. The result of MANOVA is presented in Table.2.

Based on the results of the MANOVA test, it was found that in each test the significance value (p-value) was >0.05. This indicates that each preservation method has no significant effect on the cytoplasmic and non-cytoplasmic frustule so that post-hoc test is not necessary.

Table 2. Result of Multivariate analysis of variance (MANOVA)

Effect		Value	F	Hypothesis df	Error df	Sig.
Methods	Pillai's Trace	.470	1.230	6.0	24.000	.326
	Wilks' Lambda	.545	1.301[b]	6.0	22.000	.298
	Hotelling's Trace	.807	1.346	6.0	20.000	.284
	Roy's Largest Root	.771	3.085[c]	3.0	12.000	.068

Conclusion

The three preservation solutions did not have a significant effect on the characteristics of the diatom frustule in the form of the presence or absence of cytoplasm. There is a difference in cytoplasmic colour in the preservation solution where formalin shows the brightest colour while Lugol's iodine shows the darkest colour. Preservation using formalin at a dose of 4% has the potential to cause damage to the frustules even though it can show sharper frustules ornaments compared to other preservation solutions. It is necessary to conduct a long-term preservation experiment to be able to determine the impact of the three preservation solutions over a longer period on the characteristics of diatoms.

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