Research Article

An improved method for diatom sample preparation

Ali Naseri [*1] **and Mostafa Noroozi**[2]

[1] Environment Organization, Department of Environment, Alborz Province, Iran
[2] Department of Biotechnology, University of Alzahra, Tehran, Iran

*Corresponding author: Ali Naseri, Ph.D. nasseriaza@yahoo.com

Received 27th August 2020 Revised 28th November 2020 Accepted 28th January 2021

Abstract

Introduction and aim: Diatoms are small phototrophic organisms with an essential role in the food chain in terrestrial and aquatic environments. The purpose of the present study was to optimize and improve the methods for diatom sample preparations.

Methods: The difference between the improved method with the old ones is based on the 3-interval time of the supernatant collection. Also, the centrifuge is not necessary for this improved method.

Results: The average number of cells in our proposed method was 338% higher than the other methods.

Conclusion: The preparation of specimen suspension and caution in a sampling of stations, is the most important factor for obtaining a maximum sample of diatoms.

***Keyword*:** Diatom, Improved method, Taleghan river

Introduction

Diatoms are small phototrophic organisms with an important role in the food chain in terrestrial and aquatic environments. Members of this algae group have unique morphological characteristics with various ornamentations on the valves of siliceous cell walls which have been used to identify them by the diatom specialists. The features such as valve shape, central node, fibulae, fultopotula, alveoli, conopeum, costa, and raphe are the main characteristics that help to diatom identification .

The presence of organic matters on the outer side of frustule and also the organelles inside the cell, make it difficult to observe the ornamentations. Therefore, it is necessary to remove these materials to see the bared siliceous valve. There are different ways to burn these organic components which most of which include treatment of the cells with invasive solvents and acids. The acidic solvent may destroy the diatom's ornamentations by dissolving the carbonate materials, so it is better to remove the organic maters using hydrogen peroxide .

Kelly *et al.* (1998) strongly recommended the standardization of methods that use for the sampling of benthic diatoms for studying the water quality in Europe. Taylor *et al.* provided

protocol for the collection, preparation, and enumeration of diatoms from riverine habitats for water quality monitoring in South Arica (Taylor *et al.*, 2005). The commonly used methods for diatoms identification are based on the methods proposed by Welsh (Welsh, 1964), Hasle (Hasle, 1978), Lohman (Lohman, 1972), McBride (McBride, 1988), Krammer and Lange- Bertalot (Krammer, 2000); and Taylor et al. (Taylor *et al.*, 2005). Sampling conditions at sampling sites (e.g. shade, depth, and water flow intensity, light, and entry of organic matter) should be considered as important conditions during harvesting of diatom samples.

The purpose of the present study was to optimize and improve the methods for diatom sample preparations.

Methods

To collect epipelic samples, a plastic syringe was inserted into the substrate, and 170 ml of sediment was collected from each station in three replicates. The sampling was carried out from 1 cm of the sediment surface. The sediment samples were transferred to 200 ml plastic bottles, fixed with 4% formaldehyde solution, and then transferred to the laboratory.

The difference of the improved method with the old ones is based on the three interval time of supernatant collection; 10 ml of samples were prepared as follows: at the beginning, any solvent (such as 60% sulfuric acid in a ratio of one-fifth) were added to the initial sample (raw sample) and was simultaneously mixed, then 50 ml suspension (supernatant) was prepared in 3 steps. After 60 second (20 ml), 30 second (20 ml), and 10 second (10 ml). Finally, 5 to 10 ml of this sample were prepared for the next steps.

2-5 ml of prepared samples based on the above-improved method, were treated with 25-30 ml of hydrogen peroxide (H_2O_2) for 1.5 h at 100 °C, then 10 ml hydrochloric acid (HCl) (2 h at 120 °C) were added to remove organic materials (Van der Werff, 1953).

The samples were kept in the lab for 24 h. Then two-thirds of the supernatant was taken. 5 ml distilled water was added to the mixture and was mixed again and supernatant discharged to a bottle. This step is repeated at least 5 times (Fig.1a-b). The centrifuge is not necessary for this method.

A single drop of ammonium chloride (NH_4CL; 10%) was added for every 10 mL of diluted diatom suspension to neutralize electrostatic charges on the suspended particles and reduce aggregation (McBride, 1988). A drop of diatom suspension was put on coverslips and adhered to slides using Naphrax glue. The abundance of species was estimated by counting 300 valves of the slides, which had been prepared using the samples of random transects.

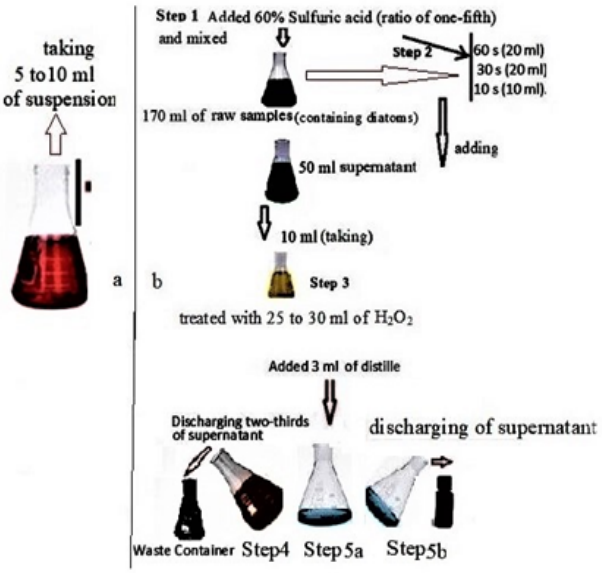

Figure 1. (a): Schematic of sample preparation to some methods; (b): Schematic of sample preparation based on the improved method. Step 1: added acid (ratio of one-fifth) for release stalked of pennate diatoms). Step 2: 15 ml of suspension (supernatant) after mixing was prepared at intervals to make more extraction chances. Step 3: 5-10 ml of 50 ml supernatant was prepared by added H_20_2 and left without movement for 24 h; Step 4: discharging two-thirds of supernatant in a waste container (without any sediment); Step 5: add 3-5 ml of distilled water; discharging the supernatant in a working bottle after mixing.

Results

The number of valves was 5-11 in conventional methods and 18-30 in triplicate counting improved method (Table 1). To evaluate the efficiency of the new method, the diatom cells of epipelic sample in station 3 was compared with the conventional method. The average number of cells in triplicate cell count in improved method were 338% higher than old method (Table 1). The result of the improved method was compared with some other studies (Table 2).

Table 1. The results of number valve in 3 replicate of 2-5 ml suspension based on improved & other methods.

Replicate	Improved Method	Other Methods
R-1	30	11
R-2	18	5
R-3	23	6

Table 2. The result of the improved method compared with some other studies

Study subject	Number taxa	Number sampling site	Time of study (months)	Reference
Kan River, Iran. MSc Thesis.	39	8	12	(Alaghehband, 2007)
Kan River, Iran, MSc Thesis.	46	7	12	(Adl *et al.*, 2020)
Ecbatana Dam & River, Iran MSc Thesis.	107	10	12	(Mohaghegh, 2010)
Amir Kabir Dam and Karaj River, Iran (Dissertation)	128	6	12	(Kheiri *et al.*, 2018)
Helleh River, Iran	20	6	12	(Farhadian *et al.*, 2015)
Balikhli River (Dissertation)	109	6	12	(Panahi Mirzahasanlou *et al.*, 2018)
Lake Neure, Iran A Study on diatoms	76	6	12	(Nejadsattari, 2005)
Anzali Lagoon Iran (Dissertation)	150	10	12	(Nejadsattari *et al.*, 2005)
National Botanical Garden, Iran (a study)	68	8	12	(Nezhadsatari *et al.*, 2007)
River Gharasou, Iran (Dissertation)	150	10	12	(Atazadeh *et al.*, 2007)
Phytoplankton of the Anzali lagoon (N Iran) and Caspian Sea,(Dissertation)	97	8	12	(Ramezanpoor, 2004)
Streams in Ramsar, Iran (Dissertation)	155	12	12	(Soltanpour-Gargari *et al.*, 2011)
Shahrood River, Iran (Dissertation)	35	8	12	(Soltanpour-Gargari *et al.*, 2011)
Estonian rivers, Republic of Estonia	130	3	12	(Vilbaste, 2001)
Ismailia Canal, Egypt	167	60	12	(Abd El-Karim, 2014)
Diatoms in small rivers in Northwestern Russia	388	68	120	(Komulaynen, 2009)

Periphytic diatoms of Nakaikemi Wetland, Japan	297	22	12	(Kiahara *et al.*, 2015)
Checklist of Algal Flora in Iraq	68-196	all ecosystems	various	(Maulood *et al.*, 2013)
Cali River, Colombia	82	6	12	(Heinrich *et al.*, 2019)
Diatoms from distinct habitats São Paulo in southeastern Brazil	67	51	12	(Costa *et al.*, 2017)
Diatom flora of headwater streams from the Czech Republic.	307	40	24	(Veselá and Johansen, 2009)
Epilithic diatoms in the Agnéby River, Ivory Coast	159	10	12	(N'Guessan *et al.*, 2018)
Taleghan River & Dam, Iran*	203	8	12	(Naseri, 2020)

*: The improved method was used only Taleghan study.

Discussion

Common diatom sampling methods, which have been performed by most diatomists, are the addition of hot HNO_3/H_2SO_4 from sample sin calcareous sites. In the old method, the sample is shaken well and 5-10 ml is poured in a heat-resistant container depending on the concentration of suspension matters, however, all diatoms cannot enter into the container. Hot HCL and $KMnO_4$ method is in line with a method (Hasle, 1978), and was introduced by Round (Round *et al.*, 1990). The hydrogen peroxide method is a milder method than acid and is useful for samples that require little cleaning. It is more useful in SEM studies (Karthick *et al.*, 2010). In the hot H_2O_2 method, 20 ml of oxygenating water should be added to 5 ml of diatom suspension.

In most common methods after mixing the sample, 5-10 ml of the top of the sample container must be removed, and maybe some diatoms removed accidentally. However, in this study, 5-10 ml of suspension from the whole sample was not taken. In this study most of the methods were used for six months, but very little taxon was obtained. Therefore, it is necessary to make fundamental changes in the preparation method.

Naseri, achieved a total of 203 taxa in a sample from the Taleghan river, based on the improved method which was the maximmume diatoms detection from epipelic samples (Naseri, 2020). However, 45 of taxa were achieved in the same sampling sites of Taleghan river (Shoja-Tashan, 2015). Although, their sampling was done on periphytic diatoms (epipelic and epilithic diatom), while our study was done only on epiploic diatom. Base on this comparing study, most of the studies have not reported a similar number of taxa as our study on the same scale (8 stations during one year). Some studies have more detected taxon, but the number of sampling sites and duration of study is longer, for example, Komulaynen, reported 388 taxa in 68 rivers during10 years (Komulaynen, 2009); or Kihara *et al*. achieved 297 taxa in 25 sites sampling (Kiahara *et al.*, 2015).

Conclusion

The most important specifications of this improved method are the addition of a solvent to the initial sediment (raw sample) at a ratio of one-fifth of total sediment in the preparation of suspension, the way of preparing 10-15 ml of the initial sample (raw sample), as well as the precision of preparing 10 ml of diatom supernatant. The preparation of specimen suspension in a sampling of stations, especially consideration of environmental factors such as sampling places of rivers, wastewater pollution, shade, light, and deep were the most important factors in obtaining maximum diatoms of a sample. 3 steps at intervals (60, 30, 10 s) for preparation of samples is based on personal experience and it can be changed at more steps

and intervals that those will be more careful to achieving of taxa of a raw sample.

Acknowledgments

The financial support of the Science and Research Branch of Islamic Azad University is gratefully acknowledged.

References

Abd El-Karim, M.S. 2014. Epipelic algal distribution in ismailia canal and the possible use of diatoms as bioindicators and a biomonitoring tool. The Egyptian Journal of Aquatic Research, 40(4): 385-393.

Adl, M.M., Iranbakhsh, A., Noroozi, M., Asri, Y. and Saadatmand, S. 2020. Epipelic diatoms flora of kordan river, alborz province in iran.

Alaghehband, H. 2007. Investigation of the flora of epipelic diatoms of can river and the structure of diatom communities. In: Biology. Science and Research Branch, Islamic Azad University.

Atazadeh, I., Sharifi, M. and Kelly, M. 2007. Evaluation of the trophic diatom index for assessing water quality in river gharasou, western iran. Hydrobiologia, 589(1): 165-173.

Costa, L.F., Wengrat, S. and Bicudo, D.C. 2017. Diatoms from distinct habitats of a highly heterogeneous reservoir, billings complex, southeastern brazil. Hoehnea, 44(4): 559-579.

Farhadian, O., Pouladi, M., Vazirizadeh, A. and Sedaghat, R. 2015. A study of diatoms seasonal distribution and biodiversity in helleh river estuary, persian gulf. Environmental Studies of Persian Gulf, 2(1): 32-44.

Hasle, G. 1978. Some specific preparations: Diatoms,[in:] phytoplankton manual. UNESCO, Paris.

Heinrich, C.G., Palacios-Peñaranda, M.L., Peña-Salamanca, E., Schuch, M. and Lobo, E.A. 2019. Epilithic diatom flora in cali river hydrographical basin, colombia. Rodriguésia, 70.

Karthick, B., Taylor, J.C., Mahesh, M. and Ramachandra, T. 2010. Protocols for collection, preservation and enumeration of diatoms from aquatic habitats for water quality monitoring in india. IUP Journal of Soil & Water Sciences, 3(1).

Kheiri, S., Solak, C.N., Edlund, M.B., Spaulding, S., Nejadsattari, T., Asri, Y. and Hamdi, S.M.M. 2018. Biodiversity of diatoms in the karaj river in the central alborz, iran. Diatom Research, 33(3): 355-380.

Kiahara, Y., TSUDA, K., ISHII, C., ISHIZUMI, E. and OHTSUKA, T. 2015. Periphytic diatoms of nakaikemi wetland, an ancient peaty low moor in central japan. Diatom, 31: 18-44.

Komulaynen, S. 2009. Diatoms of periphyton assemblages of small rivers in north-western russia. Studi trentini di scienze naturali, 84: 153-160.

Krammer, K. 2000. The genus pinnularia. Diatoms of Europe: Diatoms of the European Inland Waters and Comparable Habitats 1.

Lohman, K.E. 1972. A procedure for the microscopical study of diatomaceous sediments. Verlag nicht ermittelbar.

Maulood, B.K., Hassan, F.M., Al-Lami, A.A., Toma, J.J. and Ismail, A.M. 2013. Checklist of algal flora in iraq. Ministry of Environment, Baghdad.

McBride, T.P. 1988. Preparing random distributions of diatom valves on microscope slides. Limnology and oceanography, 33(6part2): 1627-1629.

Mohaghegh, T. 2010. A study on community structure of epipelic diatoms in ecbatana dam. Science and Research Branch, Islamic Azad University.

N'Guessan, K., Aboua, B., Tison-Rosebery, J., Ouattara, A. and Kouamelan, E. 2018. Biodiversity and ecology of epilithic diatoms in the agnéby river, ivory coast. African Journal of Aquatic Science, 43(2): 131-140.

Naseri, A. 2020. A study on community structure of epipelic diatoms and their relationship with physicochemical factors of taleghan river & dam, alborz province, iran, and ability of certain species to uptake of heavy metals. . Science and Research Branch, Islamic Azad University.

Nejadsattari, T. 2005. The diatom flora of lake neure, iran. Diatom Research, 20(2): 313-333.

Nejadsattari, T., Noroozi, M. and Fallahi, M. 2005. The composition and seasonal distribution of epiphytic algae in anzali lagoon, iran. Cryptogamie. Algologie, 26(4): 387-398.

Nezhadsatari, T., SHARIAT, M.Z. and Jamzad, Z. 2007. A study on diatoms of the artificial ponds and lakes of the national botanical garden, iran.

Panahi Mirzahasanlou, J., Nejadsattari, T., Ramezanpour, Z., NAMIN, J.I. and Asri, Y. 2018. The epilithic and epipelic diatom flora of the balikhli river, northwest iran. Turkish Journal of Botany, 42(4): 518-532.

Ramezanpoor, Z. 2004. Ecological study of phytoplankton of the anzali lagoon (n iran) and its outflow into the caspian sea. Fottea, 4(1): 145-154.

Round, F.E., Crawford, R.M. and Mann, D.G. 1990. Diatoms: Biology and morphology of the genera. Cambridge university press.

Shoja-Tashan, S. 2015. Floristic study of epiphytic diatoms epipel and epilith) in taleghan river, alborz province, iran. Science and Research Branch, Islamic Azad University.

Soltanpour-Gargari, A., Lodenius, M. and Hinz, F. 2011. Epilithic diatoms (bacillariophycae) from streams in ramsar, iran. Acta Botanica Croatica, 70(2.): 167-190.

Taylor, J.C., de la Rey, P.A. and van Rensburg, L. 2005. Recommendations for the collection, preparation and enumeration of diatoms from riverine habitats for water quality monitoring in south africa. African Journal of Aquatic Science, 30(1): 65-75.

Van der Werff, A. 1953. A new method of concentrating and cleaning diatoms and other organisms. Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen, 12(1): 276-277.

Veselá, J. and Johansen, J.R. 2009. The diatom flora of ephemeral headwater streams in the elbsandsteingebirge region of the czech republic. Diatom research, 24(2): 443-477.

Vilbaste, S. 2001. Benthic diatom communities in estonian rivers. Boreal environment research, 6(3): 191-203.

Welsh, H. 1964. A method of cleaning diatoms and the preparation of permanent slides for ecological survey work. Newsletter of the Limnological Society of Southern Africa, 1(1): 39-47.