

Diagnosis And Classification For Some Species Of Diatomes In Iraqi Water By Depending Silica Structure

Shaimaa Satae M.A.¹, Zahraa Hussain², Halla Abdul-Hadi CHabuk³, Bassam AL-yassin⁴

¹Environmental studying and research center, University of Babylon, Iraq ^{2,3,4}Biology Dept, College of Science, University of Babylon, Iraq

Abstract: Current study aims to diagnosis some species from diatoms in Iraqi water by using silica skeleton in their bodies because these properties of silica helpe to classification, and depending on this method in classification because it best to differentiation the species, according to it diagnosis some species (Nit dissipata, Diploneis, syndra ulna, stephanodiscus astrea, Nitechia acuta, cocconeis pediculus.).the method using in preperation (culturing media, chemical treatment, Baking treatment. In future studies we can depending on silica skeleton in classification and in nanotechnology studies

1. INTRODUCTION

The classification of algae into taxonomic groups is based upon the same rules that are used for the classification of land plants, but the organization of groups of algae above the order level has changed substantially since 1960Algae are an extremely large and diverse group of simple, phototrophic, organisms ranging in their structure from unicellular to multicellular, complex plant body called the thallus [1]. It was Linnaeus who used the term "Algae" for the first time as one of the orders in the class Cryptogamia, but included only the genera Conferva Ulva, Fucus and Chara of the algae as presently understood. Linnaeus proposed his artificial sexual system of classification and popularized the binomial (or binary) system of nomenclature. He divided the plant kingdom into twentyfour classes. His last Class is named as Cryptogamia ("plants with a hidden marriage") which included the flowerless plants like Algae, Fungi Lichens, Bryophytes and Pteridophytes. Linnaeus acknowledged that these genera' were for artifical convenience as he had little interest or expertise in 'them. Several other taxa presently regarded as algae, such as Corallina, were, later placed by Linnaeus in classes in between plants and animals (Linnaeus) Linnaeus's total of 80 species in ten genera represents about 47 modern [2] Eichler (1886) proposed a 5-group system of classification for algae and divided them into Cyanophyceae, Diatomeae, Chlorophyceae Phaeophyceae and Rhodophyceae. Fritsch (1935) considered algae as a group and designated as a division which is further divided into eleven classes on the basis of characteristics such as pigmentation, flagellar arrangement, reserve food Despite major advances in algal systematics, relationships between the various groups were rarely considered as each appeared internally coherent but externally unique. A lack of shared characteristics made phylogenetic speculation difficult, and it is only quite recently that methods like Electron microscopy, DNA barcoding etc. have become available whereby realistic appraisals of ancestral relationships can be proposed. Electron microscopy has had a significant impact on the higher-level classification and phylogeny of virtually all groups of algae. Examples



include: the arrangement of the flagellar root assemblages and cell division processes in the Chlorophyta, which led to the erection of many new classes processes in the Chlorophyta, which led to the erection of many new classes and the refinement of our understanding of the evolutionary pathway that gave rise to the higher plants [3]. Diatoms: are a major group of algae, specifically microalgae, found in the oceans, waterways and soils of the world. Living diatoms make up a significant portion of the Earth's biomass: they generate about 20 to 50 percent of the oxygen produced on the planet each year [4]. Diatoms are divided into two groups that are distinguished by the shape of the frustule: the centric diatoms and the pennate diatoms. Pennate diatoms are bilaterally symmetric. Each one of their valves have openings that are slits along the raphes and their shells are typically elongated parallel to these raphes. They generate cell movement through cytoplasm that streams along the raphes, always moving along solid surfaces. Centric diatoms are radially symmetric. They are composed of upper and lower valves – epitheca and hypotheca – each consisting of a valve and a girdle band that can easily slide underneath each other and expand to increase cell content over the diatoms progression. The cytoplasm of the centric diatom is located along the inner surface of the shell and provides a hollow lining around the large vacuole located in the center of the cell. This large, central vacuole is filled by a fluid known as "cell sap" which is similar to seawater but varies with specific ion content. The cytoplasmic layer is home to several organelles, like the chloroplasts and mitochondria. Before the centric diatom begins to expand, its nucleus is at the center of one of the valves and begins to move towards the center of the cytoplasmic layer before division is complete. Centric diatoms have a variety of shapes and sizes, depending on from which axis the shell extends, and if spines are present.[5][6]. Eukaryota **Domain** : Heterokonta : Superphylum:

Ochrophyta **Phylum :** Bacillariophyceae:**Class Dangeard, [5]**

2. MATERIAL AND METHODS

Preparation of ditom sample 1-

fresh water sample Were collacted from different sites from Al-hilla river contain different algae . order to selected the ditoms from these sample , by series is relief to samples , and there for ease of operation culturing to ditomes [7].



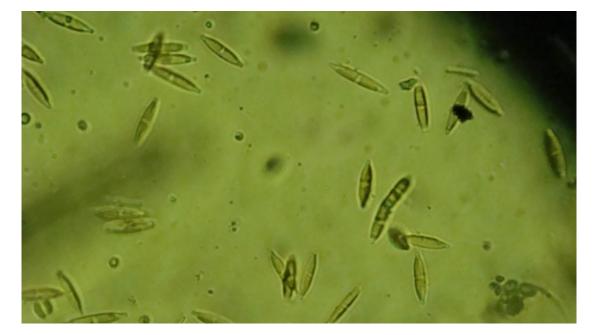


Figure (1) : Live Diatoms living

Culturing Media: 2-

Media are consist of three components: macronutrients, traceelements, and vitamins; there are predominatingly making ready as stock solutions. the media , which is (Freshwater Bacillariophyceae) is called gutin [8] According to the table

Reagents	Per Lite
Ca(NO3)2* 4H2O	20 mg
KH2PO4	12.4 mg
MgSO4* 7H2O	25 mg
NaHCO3	15.9 mg
EDTA FeNa	2.25 mg
EDTA Na2	2.25 mg
H3BO3	2.48 mg
MnCl2* 4H2O	1.39 mg
(NH4)6Mo7O24* 4H2O	1.0 mg
Biotin (Vitamin H)	0.04 mg
Thiamine HCl (Vitamin B1)	0.04 mg
Cyanocobalamin (Vitamin B12)	0.04 mg
Na2SiO3* 9H2O	57 mg
pH ¼ 6.9	

From all these materials taken stock solutions in quantities of 100 mL to 1 liter are prepared at a nutrient concentration of 100 to 1,000 times that desired. For use, some amount is elimination antiseptic and used. Stock solutions are useful for multiple reasons. Refined single weighting of chemicals is time- exhaustion and errors in weighting (e.g., mistaking mg for mg) may occur. The stock solution is made some time, and once made, it supplies an



simple and consistent source. That is, if a liter stock solution is prepared, generally where is used 10 mL from each liter of final medium, then can be used to make 1 liters of medium from the stock solution [9]. Sterile cultures of ditomes may be obtained from specialized culture collections (figure 2). After then addition 50 ml from algae (ditome) to the media (150 ml) order to obtain 250 ml mature, after 7-9 days another addition from media 250 ml to the previous mixture, to become 500 ml. After another 7-9 days too addition 500 ml from media, to obtain 1 litter. In all stage ago we operate ventilation to culturing. under aeration phase, the end of stationary phase. The gather diatom solution was centrifuged at 4 000 r/min for 10 min (figure 3). The deionized water were re-suspended are collected by centrifuged under the same condition for three times figure (4). the diatom pellets were dried in refrigerated sequeeze air dryer The come by dry biomass samples were conserve in drying bowl for the devising experiments[10]. The specie which were show (*Nualgi Aquarium, cocconeis pediculus*, *Nit dissipata*), Continued to grow Chemical treatment. Will be removal organic mass from diatom was carried out by acid solution washing process [11] and the acide (HCL) was chosen as common solvent[12].

Baking treatment

After acid washing process, the color of remain was dark green, It is better there may be other organic mass residue in the washed diatom biomass, the washed diatom biomass needs to be more over process to remove the remaining organic wickedness. There were announce solvents effective in organic material lifting [13]. So, by 2% HCl washed diatom biomass after exposure under different temperatures will be mass removal and the remaining is dark green diatom samples. It is plain that the mass removal average of 2% HCl washed diatom biomass at 600°C was up to 88.31%, which is Almost about that under 800°C process at 600°C for 0.5 h is enough for degrade the remnants of sinfulness of 2% HCl washed diatom biomass (figure 5). Ending test: scanning electron microscope order to silica porse .

3. THE RESULTS AND DISSECTION

These picture from stepes isolation Diatoms: After extraction of (Biosilica) was in the form of (bowder) mg /500 ml), which is the last stage before the examination.





Figure (2): Culturing of ditom after growthing



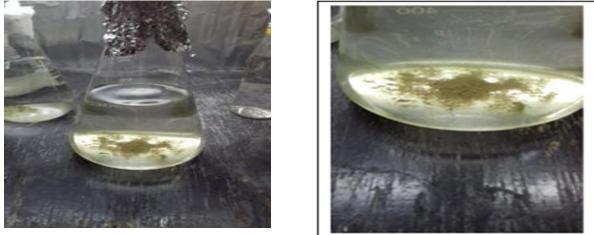


Figure (3) : Culturing of ditom after growthing



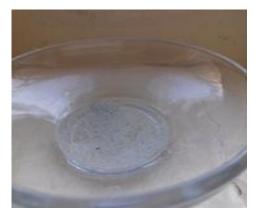


Figure (4) after treatment (chemical and backing)

Then they went to solvent this bowder in ethanol and It is placed on the metal plate Special for the electron microscope to take SEM image (Quanta 450 FEI USA) in (University of babylon, College pharmacy), according to the results and readings and images of the electron microscopy. It was figures of biosillica Differentiation and varied in shape and size from diameter prose (104.1,100.5, 106.2 nm). The length of the component plates for Diatom. Where the diameter of small plates nm 243.4, 409.2 the reason of difference in size and shape returned to type of ditom which returned to The reproductive speeds of different diatoms in collection samples (figure 1). Results showed Existence three type from diatoms (*Achnanthes, Diploneis, Navicula, Cyclotella meneghiniana, Pinularia, cocconeis pediculus*) order to electronmicroscope image [15]. Depending on those pictures below we can classification those species diatoms: by Taxonomic foundations from daimintion skeletal of silica [16] [17].



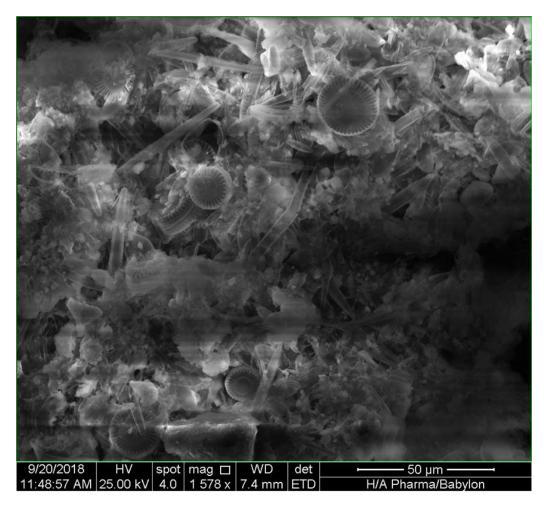


Fig.5: SEM images of (Achnanthes, *Diploneis*, *Navicula*, *Cyclotella meneghiniana*, *Pinularia*, *cocconeis pediculus*) raw diatom from fresh wate

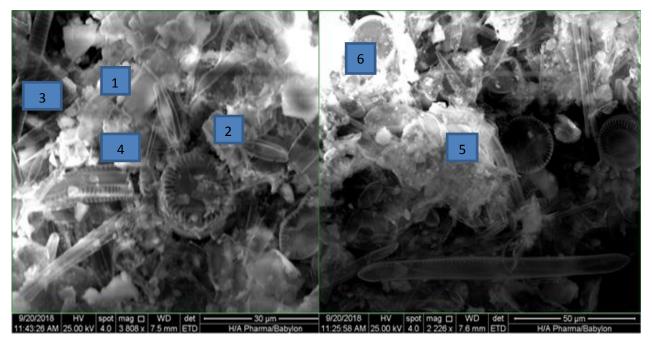
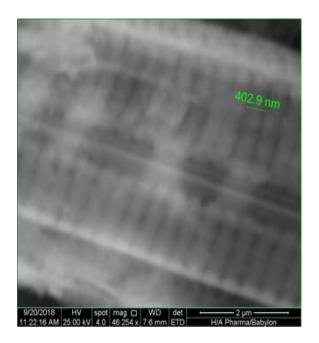




Fig.6 : SEM images of (1- Achnanthes), (2- Diploneis) 3-Navicula 4- Cyclotella meneghiniana 5-Pinularia 6- cocconeis pediculus) raw diatom from fresh wate



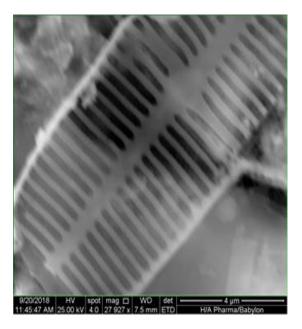


Fig.7 : SEM images of biosilica in *Pinularia* by Small diameter unit 402.9nm

Order : Naviculales

Family : Pinnulariaceae

:Genus : Pinnularia

Fig.8 : SEM images of biosilica in *Navicula ulna* by Small diameter unit



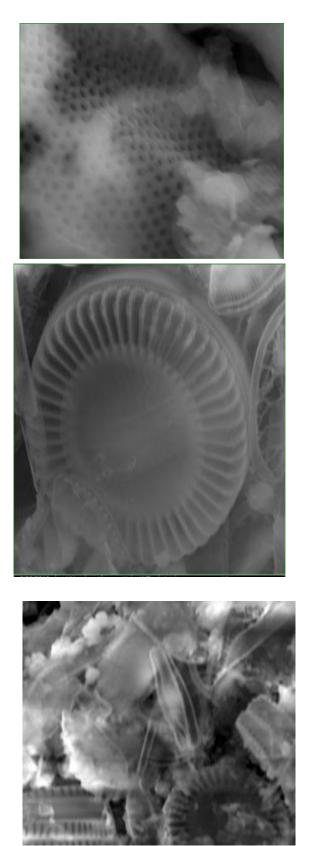


Fig.9 : SEM images of biosilica in *coscinoconus* by Small diameter unit

Fig.10 : SEM images of biosilica in Cyclotella meneghiniana by Small diameter unit

Fig.11 : SEM images of biosilica in *Achnanthes* by Small diameter unit



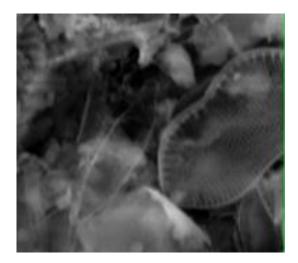
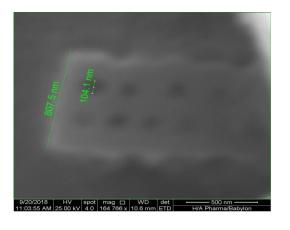
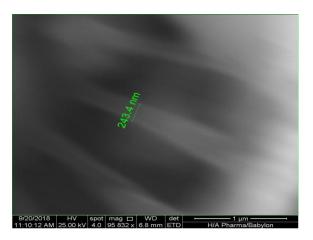


Fig.12 : SEM images of biosilica in *Diploneis* by Small diameter unit

Acorrding all above we can classification theses species order to shape skeletal of silica which backing to these species .There are the diameter of skeletal of silica and the distance between the pores in the same skeletal , became biomarker to diagnosis the algae at in the below:





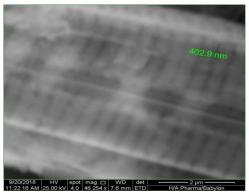


Fig.12 : The diameter of skeletal silica



According to scanning electron microscope, the SiO 2 morphologies of having microspores and fibers in the surface of 59.81 m 2 /g. All results refer that the basilica specimens get found in this study. The prepared basilica material having Porous structures which its ability, it can be used in other industrial applications.

REFERENCES

- [1] A.D. Steinman1, G.A. Lamberti2, P.R. Leavitt3 and D.G. Uzarski . 2017. Biomass and Pigments of Benthic Algae, Elsiever. 12-19.
- [2] Silva, P.C. 1980. Names of classes and families of living algae. Regnum vegetabile 103: 1-156.
- [3] LB Van den Hoek, T De Jong Astronomy 1995. adsabs.harvard.edu. Astron. Astrophys. 293, 38 1-395 (1995) ASTRONOMY AND ASTROPHYSICS The evolution of galactic carbon stars MAT Groenewege.
- [4] Diatoms of North America. Archived from the original on 25 January 2020. Retrieved 28 January 2020.
- [5] Mann, David G. (2005). "The species concept in diatoms: Evidence for morphologically distinct, sympatric gamodemes in four epipelic species". Plant Systematics and Evolution. 164 (1/4): 215–37. doi:10.1007/BF00940439. JSTOR 23675282. S2CID 37684109.
- [6] Diatomophyceae Rabenhorst, 1864.
- [7] Guillard, R R L. 1975. Culture of phytoplankton for feeding mari invertebrates. In: Smith W L, Chanley M H eds. Culture of Marine Invertebrate Animals. Plenum Press, New York USA. p.26-60.
- [8] Andersen, P. and Throndsen, J., Estimating cell numbers, in Hallegraeff, G. M., Anderson, D. M., and Cembella, A. D., Eds., Manual on Harmful Marine Algae, IOC Manuals and Guides, No. 33, UNESCO Publishing, Paris, 2003, pp. 99– 129.
- [9] Hassenteufel, W., Jagitsch, R., and Koczy, F. F. 1963. Impregnation of glass surface against sorption of phosphate traces. *Limnol. Oceanogr.* 8:152–6
- [10] QI Yarong, WANG Xin, CHENG Jay Jiayang ,2017. Preparation and characteristics of biosilica derived from marine diatom biomass of *Nitzschia closterium* and *Thalassiosira*, Chinese Journal of Oceanology and Limnology.
- [11] Alyosef H A, Ibrahim S, Welscher J, Inayat A, Eilert A, Denecke R, Schwieger W, Münster T, Kloess G, Einicke W D, Enke D. 2014. Eff ect of acid treatment on the chemical composition and the structure of Egyptian diatomite. *International Journal* of Mineral Processing, 132: 17-25.
- [12] Mazumder N, Gogoi A, Kalita R D, Ahmed G A, Buragohain A K, Choudhury A. 2010. Luminescence studies of fresh water diatom frustules. *Indian Journal of Physics*, 84 (6): 665-669.
- [13] De Stefano L, Rotiroti L, De Stefano M, Lamberti A, Lettieri S, Setaro A, Maddalena P. 2009a. Marine diatoms as o ptical biosensors. *Biosens Bioelectron*, 24 (6): 1580-1584.
- [14] Wang Yu, Zhang Deyuan, Cai Jun, Pan Junfeng, Chen Mingli, Li Aobo, Jiang Yonggang (2012) Biosilica structures obtained from Nitzschia, Ditylum, Skeletonema, and Coscinodiscus diatom filtrationaided acid cleaning method, BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING, 95:1165– 1178.



- [15] Barsanti, laura .Gualtieri ,Paolo.2006 ,Algae Anatomy ,biochemistry and biotechnology ,published by CRC press is an imprint of taylor and francis group.
- [16] Boyd, P. W., Watson, A. J., Law, C. S., Abraham, E. R., Trull, T., Murdoch, R., et al. (2000). A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. Nature, 407, 695–702.
- [17] Harwood, D. M. (1999). Diatomite. In E. F. Stoermer & J. P. Smol (Eds.), The diatoms: Applications for the environmental and earth sciences (pp. 436–443). Cambridge, UK: Cambridge University Press.
- [18] Harwood, D. M., & Gersonde, R. (1990). Lower Cretaceous diatoms from ODP leg 113 site 693 (Weddell Sea). Part 2: Resting spores, chrysophycean cysts, an endoskeletal dinoflagellate, and notes on the origin of diatoms. In P. F. Barker & J. P. Kennett et al. (Eds.), In: Proceedings of the Ocean Drilling Program, Scientific Results (Vol. 113, pp. 403–415