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Diatoms in lung tissue: first investigation in Brazil in proving death by drowning

N.P.M. Carneiro [a,*], L.C. Torgan [b], M. Vaz [c], L.P. Utz [d]

[a] *Pontifícia Universidade Católica do Rio Grande do Sul, Rio Grande do Sul (RS), Brasil*
[b] *Fundação Zoobotânica do Rio Grande do Sul, Museu de Ciências Naturais, Porto Alegre (RS), Brasil*
[c] *Departamento Médico Legal-RS, Pontifícia Universidade Católica do Rio Grande do Sul (RS), Brasil*
[d] *Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul (RS), Brasil*

**Endereço de e-mail para correspondência: nickpetry@hotmail.com. Tel.: +55-51-98610-7741.*

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Resumo

As diatomáceas são microalgas amplamente distribuídas em águas continentais e marinhas e durante o afogamento são inaladas juntamente com a água, dispersando-se pela circulação sanguínea para diversos órgãos internos da vítima. A investigação objetivou avaliar as diatomáceas extraídas de tecidos de pulmão de um indivíduo com diagnóstico de morte por afogamento, comparando com as que são encontradas no ambiente, com o fim de comprovar a causa-mortis e o local de afogamento. Um fragmento de pulmão de 17,9 gramas retirado da vítima foi oxidado e preparado para identificação e quantificação das diatomáceas ao microscópio invertido. Um total de 133 valvas de diatomáceas foram encontradas, sendo 85% pertencentes a formas penadas e 15% a cêntricas. *Pinnularia*, um gênero bentônico, apresentou maior número de valvas, seguido por *Fragilaria*, *Actinocyclus*, *Cocconeis* e *Encyonema*. Esses resultados revelaram que a vítima afogou-se no fundo, inalando água e sedimento com diatomáceas. Trata-se de primeira investigação dessa natureza realizada no Brasil.

Palavras-Chave: Diatomáceas; Microalgas; Afogamento; Pulmão.

Abstract

Diatoms are microalgae widely distributed in inland and marine waters and during drowning they are inhaled together with water, passing through the bloodstream to various internal organs of a victim. Our investigation aimed to evaluate the diatoms extracted from the lung tissues of an individual with drowning diagnosis and compare them to those found in the environment, in order to prove the cause of death and the drowning site. A left lung fragment of 17.9 grams removed from the victim was oxidized and prepared for diatoms identification and quantification using inverted microscope. A total of 133 diatoms valves was found, being 85% belong to pennate forms and 15% to centric forms. *Pinnularia*, a bentonic genus, had greatest number of valves, followed by *Fragilaria*, *Actinocyclus*, *Cocconeis* and *Encyonema*. These results revealed that the victim drowned at the bottom of the lake, inhaling both water and sediments with diatoms. This is the first investigation of this nature carried out in Brazil.

Keywords: Diatoms; Microalgae; Drowning; Lung.

1. INTRODUCTION

The use of diatoms to diagnose drowning began in Europe in the nineties with investigations by coroners [1-7]. They found that human body parts such as the femur, brain, heart and lung used in the autopsy of victims by drowning had diatoms, a microalga that has cell wall (valves) with silica, thus resistant to degradation. These organisms are widely distributed in inland and marine waters, and during the drowning process, are inhaled together with the water passing through the bloodstream to various internal organs of the victim.

Successful evidences of diatoms in the pulmonary cavities, tissues and bone marrow greatly depends on their concentration in the water. The first investigations by Timperman [7] were not successful because in some rivers of Belgium, planktonic diatoms were sparse.

Investigations advanced in Europe encouraging diatomologists to study this theme and several case studies can be found in the book of Ludes & Coste [8].

Some researchers in forensic medicine, non-diatom specialists, questioned the value of the diatom test due to detecting these algae in peripheral organs of people who did not drown or their absence in cases of drowning [9]. The absence can be explained by lack or shortage of diatoms in certain water bodies and the presence of these algae in the gut of people and animals can originate from raw water intake and not completely cleaned food containing diatoms. For a true diagnosis of drowning, these aspects must be observed, as well as possible external contamination on the autopsy table during the extraction of the body parts or during the storage and material preparation in the laboratory [10,11].

In recent years, drowning diagnostic studies using diatoms have advanced with the use of new methods of preparation and material analysis [12-14]. A review about the diagnosis of death by drowning using diatoms can be found in Donadel *et al*. [15]. For future applications and automatic use of diatoms in forensic medicine, studies mapping water bodies in India and the inventory of diatoms in major rivers in China are already being undertaken [16,17].

This study aimed to evaluate diatoms extracted from lung tissues of an individual with drowning diagnosis and comparing them to those found in the environment, in order to prove the cause of death and drowning site. This is the first reported case associating diatoms to death by drowning in Brazil.

2. MATERIAL AND METHODS

2.1. Fact Description

The victim, a 20 year old male, was found on the shores of Sailors' Island in Guaiba Lake. Upon arriving at the Medical Legal Department (DML) of Porto Alegre, Brazil, he had a wound on left eyebrow, according to witnesses, by a hitting his head on a stone at the time he dove, a fact that probably led to a partial loss of consciousness and subsequent drowning. The diagnosis given by the medical examiner on duty on the date of November 25, 2012, was death by drowning.

2.2. Sample preparation

A left lung fragment of the victim located near the windpipe was collected and stored in a flask with formalin 4%. In the laboratory the fragment was weighed in a precision electronic weighing scale and oxidized with concentrated nitric acid in a glass Becker directly on fire. After fifteen minutes of preparation, the degraded material was placed in test tubes for washing and acid removal. We washed the material 12 times with distilled water, using a centrifuge (2,500 rotations/minute) for ten minutes. Later using a Pasteur pipette, the sample was distributed in sedimentation chambers with 2 ml capacity, allowed to settle for two hours for observation under an inverted microscope Zeiss (640 X magnification).

2.3. Analysis and Identification of diatoms

We analyzed all material in 12 chambers for identification and valves quantification. The results was expressed in numbers of valves per 17.9 grams. The diatoms were measured and recorded in digital imaging with equipment attached to the microscope (Power Shot G10).

The identification of genera and species was based on the reference collection (iconography) of the diatoms from Rio Grande do Sul at the Herbarium of the Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul.

3. RESULTS AND DISCUSSION

The analysis of 17.9 grams of oxidized lung fragment showed 133 diatoms. The valve concentration can be considered fairly high compared with literature [18-20]. One can consider the diatom test as positive, since the minimum amount required for diagnostic confirmation is five valves per 10 grams sample of cadaveric tissue [12]. Of the total number of taxa present, 85% belong to pennate forms of bilateral symmetry and 15% to centric forms of radial symmetry (Fig. 1). The latter occurs mainly in plankton (suspended in the water column) while the other originates from the benthos (sediment) or periphyton (marginal zone of the lake). Regarding the taxonomic composition (Fig. 2), the genera that had greatest number of valves were *Pinnularia* (38 specimens), which are benthic (epipelic) organisms [21], followed by *Fragilaria* (14), *Actinocyclus* (12), *Cocconeis* (5) and *Encyonema* (4).

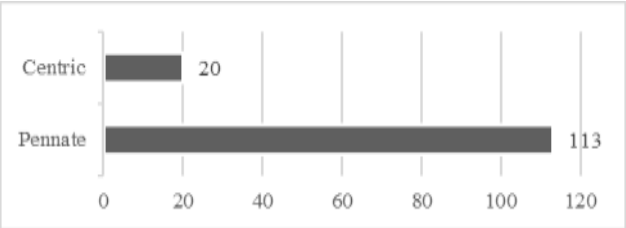

Fig. 1. Number of diatoms observed in the victim's lung fragment.

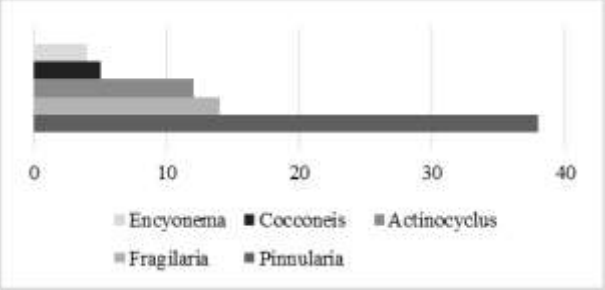

Fig. 2. Valve numbers of the main genera observed in the victim's lung fragment.

In the lung fragment, we found 32 genera, 6 species and 11 morphotypes, which due to its position in the chamber could not be identified. The taxa, in general, are known to be present in the Guaiba Lake Basin where the diatom community has been extensively studied [22-24] as well as in some rivers that flow into this lake [24-27]. This knowledge allowed us to identify some abundant species (Plate 1, Figures 1-4) and some pennate diatoms belonging to several genera (Plate 2, Figures 5-16). Comparative analyzes of diatoms in lungs with those found in the water samples were also used successfully in rivers from France to indicate drowning sites [27].

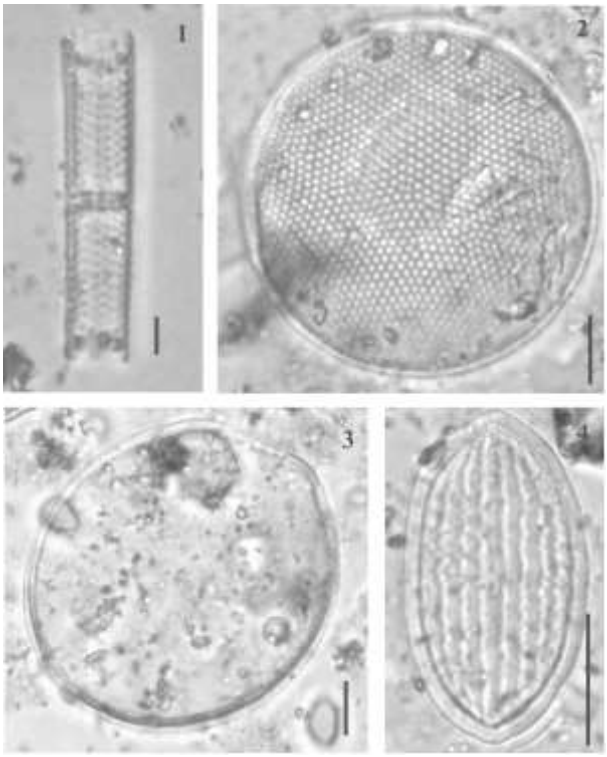

Plate 1. Figures 1-4. 1. Aulacoseira granulate (Ehrenberg) Simonsen, 2. Actinocyclus normanni (Gregory) Hustedt, 3. Pleurosira laevis (Ehrenberg) Compere, 4. Cocconeis placentula var. acuta Meister

Our investigation revealed that the victim drowned at the bottom of the lake, inhaling both water and sediments with diatoms. The presence of the diatoms from Guaíba Lake in the victim's lung fragment confirms the drowning site.

This research has unprecedented character in Brazil and supports the use of diatom tests in forensic medicine.

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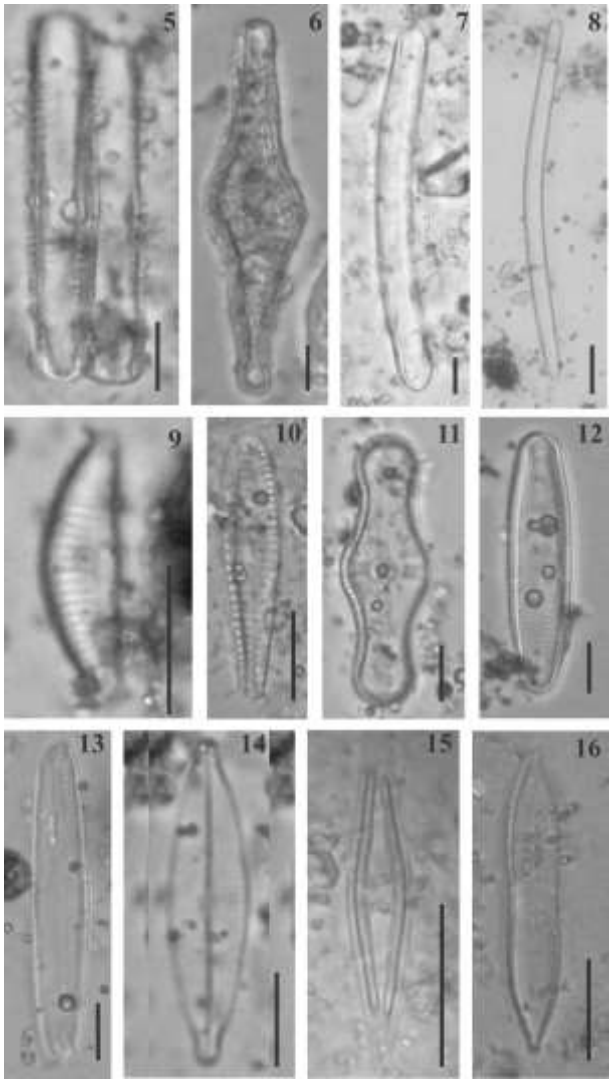

Plate 2. Figures 5-16. 5. Fragilaria, 6. Fragilariforma, 7, 8. Eunotia. 9. Encyonema, 10. Gomphonema, 11. Achnanthes, 12. Pinnularia, 13. Pinnularia, 14. Frustulia, 15. Navicula, 16. Nitzschia.

REFERENCES

[1] V. Revenstorf. Der Nachsweis der aspirierten Ertränkungflussigkeit als Kriterium des Todes durch Ertrinken. *Vjschr gerichtl Med*. **27**, 274-299, 1904.

[2] B. Mueller. Zur Frage der Diagnostik des Ertrinkungstodes. *Dtsch Z. gerichtl Med*. **41**, 400-404, 1952.

[3] F. Thomas; W. Van Hecke; J. Timperman. The Detection of Diatoms in the Bone Marrow as Evidence of Death by Drowning. *J. Forensic Med*. **8(3)**, 142-144, 1961.

[4] F. Thomas; W. Van Hecke; J. Timperman. Diagnostic médico-légal de la mort par submersion par la mise en évidence de diatomées dans la moelle des os longs. *Ann. Méd. lég*. **42(4)**, 1-7, 1962.

[5] F. Thomas; W. Van Hecke; J. Timperman. The Mediocolegal Diagnosis od Deadth by drowning. *J. Forensic Sci*. **8(1)**, 1-14, 1963.

[6] J. Timperman. The detection of Diatoms in the Marrow of the Sternum. *J. Forensic Med.* **9(4)**,134-136, 1962.

[7] J. Timperman. Medico-Legal Problems in Death by Drowning. *J. Forensic Med.* **16(2)**, 45-75, 1969.

[8] B. Ludes; M. Coste. *Diatomées et medicine légale*. Tec & Doc Lavoisier, Paris, p. 115-120, 1996.

[9] A. J. Peabody. Diatoms and Drowning - A Review. *Med. Sci. Law.* **20(4)**, 254-261, 1980.

[10] N. N. I. Maidana. *El uso de las diatomeas en la medicina forense en Argentina*. Anais do 57º Congresso Nacional de Botânica. p. 258-260, 2006.

[11] N. I. Maidana. El test de diatomeas em el diagnóstico de muerte por sumersión. *Acta Nova* **6(1-2)**, 70-81, 2013.

[12] M. Ming; X. Meng; E. Wang. Evaluation of four digestive methods for extracting diatom. *Forensic Sci. Int.* **170(1)**, 29-34, 2007.

[13] S. Hu; C. Liu; J. Wen; W. Dai; S. Wang; H. Su; J. Zhao J. Detection of diatoms in water and tissues by combination of microwave digestion, vacum filtration and scanning electron microscopy. *Forensic Sci. Int*. **226**, 48-51, 2013.

[14] N. Fucci. A New Procedure for Diatom Extraction in the Diagnosis of Drowning. *Clin Exp Pharmacol* **2(2)**, 1-2, 2012.

[15] L. Donadel; N. Cardoso; A. Hoenisch; L.R.P. Utz. Revisão sobre o diagnóstico de afogamento com o uso do plankton, teste de diatomáceas e de PCR. *Rev. Bras. Crimin.* **3(2)**, 17-23, 2014.

[16] R. Singh; R. Kaur. Diatomological mapping of water bodies - A future perspective. *J. Forensic Leg. Med.* **20**, 622-625, 2013.

[17] J. Zhao; Y. Wangb; Y. Zhangc; S. Hua; C. Liu. Types of diatoms in China's three major rivers and the possible application for an automatic forensic diatom test. *Austr. J. Forensic Sci*. **47(3)**, 268-274, 2005.

[18] A.J.Peabody. Diatoms and Drowning- A Review. *Med Sci Law* **20(4)**, 254-261, 1980.

[19] J.C.M. Macchiavello. Aplicación de las Diatomeas en Medicina Forense. *Contribuciones en Diatomología* **1**, 1-8, 2003.

[20] S. Krstic; A. Duma; B. Janevska; Z. Levkov; K. Nikolova; M. Noveska. Diatoms in forensic expertise of drowning - a Macedonian experience. *Forensic Sci Int.* **127**, 198-203, 2002.

[21] F.E. Round; R.M. Crawford; D.G. Mann. *The diatoms.* Biology & Morphology of the genera. Cambridhe Univesity Press, Cambridge p. 556, 1990.

[22] L.C. Torgan; L.W. Aguiar Diatomáceas do "rio" Guaíba, Porto Alegre, Rio Grande do Sul, Brasil. *Iheringia, Sér. Bot*. **23**, 19-63, 1978.

[23] S.E. Salomoni; L.C. Torgan. Epilithic diatoms as organic contamination degree indicators in Guaiba lake, Southern Brazil. *Acta Limnol. Bras*. **20(4)**, 313-324, 2008.

[24] L.C.Torgan; S.E. Salomoni; A.B.Bicca. Diatomáceas sobre Limnoperna fortunei Drunker molusco introduzido no Lago Guaíba, sul do Brasil. *Rev. Bras. Bot.* **32(1)**, 23-31, 2009.

[25] L.Martau; L.W. Aguiar; V.L.M. Callegaro Diatomáceas do rio dos Sinos, Rio Grande do Sul. *Iheringia, Sér. Bot*. **22**, 45-83, 1977.

[26]D. Bes; L.Ector; L.C. Torgan; E.A. Lobo Composition of the epilithic diatom flora from subtropical river, Southern Brazil. *Iheringia, Sér. Bot*. **67(1)**, 93-125, 2012.

[27] M. Laux; L.C. Torgan. Diatomáceas com plastídeos no plâncton da foz dos rios do Delta do Jacuí, sul do Brasil, um complemento a taxonomia tradicional. *Iheringia, Sér. Bot*. **66(1)**, 109-132, 2011.

[28] B. Lulodes; M. Coste; N. North; S. Doray; A. Tracqui Diatom analysis in victim´s tissues as an indicator of the site of drowning. *Int. J. Legal Med*. **112**, 163-166, 1999.