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RED EUGLENOID BLOOMS: A BIOMARKER OF ENVIRONMENTAL IMPACT IN FLOODED RICE FIELDS

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ABSTRACT

The nutrient load introduced into flooded rice fields is one of the main factors that impact this type of ecosystem, contributing to the development of blooms of pigmented euglenoids. This study was carried out to investigate the behavior of red euglenas, a group of pigmented euglenoids, forming blooms in this type of environment. Sedimented spores in the soil after water drainage, as well as water samples from vegetative cells in living blooms were collected. The collected material was inoculated into a culturing medium for microalgae and incubated for three weeks. The cultures grown in nutrient medium were used for morphometric analysis to identify the species. In order to characterize the water environment, chemical and physical parameters were also monitored "in situ". The results indicated *Euglena sanguinea* Ehr. as the bloom-forming species and nitrogen as a key element in the behavior of this species in this type of ecosystem. It was suggested, however, that more studies are needed to indicate the use of red euglenas as biomarkers of nitrogen overload in flooded rice fields.

Keywords: *Euglena sanguinea;* environmental contamination flooded; ecology.

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INTRODUCTION

One of the major concerns of the modern world refers to water quality and environmental impact caused by anthropogenic activities (Grützmacher *et al.*, 2008). The exponential increase in world population generates a need to increase food production and thus, increase farm income.

The demand for an increment in crop production is forcing farmers to produce more in a reduced area, with the use of more resources. Therefore, the utilization of fertilizers and pesticides in a large scale is generating an excess of these substances in the environment. Also, it can be leached into shallow waters causing eutrophication of rivers and lakes. This makes agriculture a major source of environmental contamination and possible modifier of the environment.

Eutrophication occurs due to an over population of algae and bacteria which use nutrients as the base of their metabolism (Von Sperling, 2006). A clear example of the impact occurs in rice production, which utilizes a large amount of water and is exposed to several chemicals.

The cultivation of rice is an activity of great importance in agriculture worldwide. In Brazil, more than 1.5 million hectares of flooded rice are cultivated annually, over one million hectares of which, are cultivated on Rio Grande do Sul State, representing 62.7% of the national production (Empresa Brasileira de Pesquisa Agropecuária, 2009).

Irrigated crops, when poorly planned and conducted, can result in negative environmental impacts to natural ecosystems, damage to physical and chemical properties of soil, and reduce the quantity and quality of water resources (National Water Agency, 2009).

Rice fields are agroecosystems with a high biological productivity and develop plankton communities that have important roles in soil nutrient fixation and recycling (Roger *et al.*, 1991). However, management and human alteration, with the purpose of establishing an increasing agricultural production, make this environment very different from natural ecosystems, presenting their own characteristics. One of the changes observed in these environments is the occurrence of blooms of cyanobacteria and algae, among them the euglenas, which can be indicators of environmental impact.

Red euglenas and many species of cyanobacteria may produce toxins and thus jeopardize the survival of other more complex organisms naturally present in this type of ecosystem (Vasconcellos, 2010). For instance, Zimba *et al.* (2010) have recently described euglenophycin an ichthyotoxin produced by *Euglena sanguinea*, in cultures isolated from a pond.

The inorganic forms of nitrogen (N) and phosphorus (P) in anaerobic conditions such as flooded rice fields predominate and facilitate the assimilation by euglenas and other algae, being a possible cause of the blooms. Euglenas present a high degree of heterotrophy, living in waters rich in organic matter such as rice fields where they have the ability to degrade organic matter, directly absorb ammonia and contribute to the assimilation of organic carbon. Thus, they act as bioindicators of water quality, and are primarily related to the degree of eutrophication of an aquatic environment (Araújo *et al.*, 2007). The experiments described herein were designed in order to describe the responses of red euglenoids in paddy rice fields.

This study was conducted with the objective to study the main chemical and physical factors and conditions which favour de development of red euglenoid blooms in paddy rice fields, as well as identify the morphotypes present in the blooms.

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MATERIAL AND METHODS

Study site

The experiment was carried out in an experimental area of the Department of Plant Science, Federal University of Santa Maria (UFSM), located in Camobi, Santa Maria – RS, Brazil. The monitoring of red blooms euglenas was conducted in four experimental plots of rice with dimensions of 3 x 5 m.

The area is currently intended for experiments of undergraduate and graduate courses, with lines of research devoted to the cultivation of irrigated rice and other plant species. The soil is classified as typic Planossol Haplic eutrophic hapludalf (EMBRAPA, 2006), belonging to Unit Vacacaí mapping.

The experimental plots were located in the vicinity of a water reservoir, where fish farming is carried out. This location allowed for flood water to drain into the experiment, which may contribute with the nutrient load in the experimental blocks.

The cultures prevailing in the region receive fertilizers normally, with the excess remaining deposited in the upper soil layers.

Sampling methods and light microscopy

Soil sampling took place in April 2011, after water drainage of the rice crops. Collection of samples was done by removing the surface layer (0-5 cm), where the euglenoid spores were sedimented (Figure 2a). Soil samples were air dried, crushed and sieved into a 2 mm sieve.

For the description of the euglenoids obtained from the soil samples, morphological and physiological characteristics were investigated, such as: colour, presence of hematochromes, size and number of flagella, number and chloroplast shape, number and type of pyrenoids, mobility and metabolic movement (change in cell shape). These features were identified under an Olympus (CX 41) microscope coupled with a Sony (5.1 megapixels) digital camera.

The genera and species determination were performed using the key proposed by Cassie (1983) and with recent studies on morphospecies such as Alves-da-Silva & Tamanaha (2008), Alves-da-Silva & Bicudo (2002); Rosowski (2003).

Liquid samples containing living vegetative cells were collected from the water surface (Figure 2b), using wide-mouth bottle and analyzed under a microscope using the same methodology of the soil samples.

Cell dimensions were determined by the AutoCAD 2004 softwere from images obtained under the microscope.

Culture in solid medium

Red euglenoids were cultivated in Bold solid medium for microalgae, as described in Starr (1968) for microalgae. The medium was solidified with 10 gL^{-1} of agar, sterilized by autoclaving for 20 minutes at 120 °C and 1.0 atm pressure and poured into Petri dishes by volume of approximately 25 ml.

The solution containing soil and the resting spores was prepared to the concentration of 1:10 (1 gram of soil to 10 ml of distilled water) from which aliquots of 0.5 ml were seeded into Petri dishes containing 25 ml of sterile medium. Liquid samples containing vegetative cells collected during the bloom were inoculated in the same manner and volume. Subsequently, the inoculated plates were transferred to an environmental chamber (Cienlab), with photoperiod of 12 hours light and 12 hours dark, temperature of 23 ± 2 °C and fluorescent light intensity of 35 µmol $m^{-2}s^{-1}$, where they remained incubated for two weeks.

Culture in liquid medium

Bold medium (Starr,1968) without agar was used for liquid cultures, and inoculation was performed in test tubes containing 10 ml of nutrient medium and 0.5 ml of the liquid sample collected in blooms. The tubes were incubated at the same conditions used for the cultures in solid medium.

The isolation of cells grown in solid media was performed by the method described in Rippka (1979), in which cells previously identified under the microscope are transferred to new plates containing the same culture medium, using sterile platinum loops. The plates were incubated under the same conditions as the initial culture.

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Blooms of red euglena monitored by chemical and physical parameters

Chemical and physical variables describing the water environment from where the organisms were collected (pH, water temperature, solar radiation, air temperature, wind speed, percentage of clouds and bloom intensity) were monitored weekly, always at 10 A.M. beginning in the first week after the flooding of the experimental blocks.

Water pH and temperature were measured in the field using a pH meter (Digimed) and a mercury thermometer. Data relating to atmospheric temperature, solar radiation, and wind speed were obtained from the meteorological station of the Federal University of Santa Maria. The percentage of clouds and intensity of blooms were determined using the following criteria:

Cloud percentage:

No clouds: 0%

Less than five small clouds: 2%

Between five and ten small clouds: 5%

Between ten and twenty small clouds: 10%

Over twenty clouds: cloudy

Bloom intensity:

Very weak: small patches in the soil around the experimental blocks.

Weak: small patches in the soil around the experimental blocks and water surface.

Moderate: occasional patches water surface

Intense: more than 50% of water surface covered by red euglenoid cells.

The development of euglenoid blooms and evolution of blooming were determined by "in situ" observation and microscopic analyzes.

Results

Microscopic analysis of red *Euglena* developed both in culture medium and in natural water revealed the presence of numerous finger-shaped chloroplasts deeply immersed in the cytoplasm extending from the pyrenoid to the cell surface, forming ribbons. The pellicle is thin and with many grooved mucosysts.

Body shape varies from cylindrical to fusiform with a round apex. Two flagella were identified, one as long as the cell body and the other with approximately half of the lenght of the cell. Hematochromegranules are present across the cell surface under conditions of high luminosity or restricted to the central region of the cell if reduced light conditions (Fig.3 first and 3b, respectively).

The dimensions obtained by the measurements of the cultured cells at different stages of the cell cycle varied between 72.59 and 148.64 µm in length and from 23.96 to 53.38 µm in width (Fig. 4 and Table 1). The coefficients of variation obtained for both sets of measurements of length and width cell showed an average dispersion of the measures in relation to the standard deviation concluding, thus, homogeneity in the samples and the experimental conditions.

The red euglenas collected and cultured in this study showed a wide range of form variation during metabolic movements. The cellular forms varied between cylindrical, spindle, and spherical among others as shown in Figure 5.

The reproductive behavior of red euglenoids in culture conditions, after approximately two weeks, revealed a predominance of multiple binary fission from encysted cells. There was a sequence of cell development that

began with the breakup of the mucilaginous envelope that protects the cell, releasing numerous new cells, of green color and small size. These cells rapidly increase in size evolving to a palmelloid stage (spherical), which was immediately followed by vegetative (adult)stage when the cells were mostly green and, depending on light intensity, showed a central region with concentration of red pigment (Figure 6 a, 4b, 4c, 4d).

The blooms of red euglena emerged about a week after flooding of the experimental blocks, a period when about 50% of the nitrogen required by the rice crop has been applied. 50% of the dose wass added to the culture at the beginning of tillering. The remaining 50% wass applied on the panicle differentiation stage, for short cycle cultivars, (135 days). A few days after flooding and first nitrogen application (50% of the dose) red euglenoid blooms covered most of the free water surface considered intense blooms (Table 2).

Discussion

The results of morphological and physiological analyses indicated that the species responsible for red euglenoid blooms in flooded rice fields is *Euglena sanguinea* Ehr. Very similar descriptions of this species were given by Alves-da-Silva *et al.*(2008), Cassie (1983) and Kim *et al.*(1998).

Euglenophyceae and other pigmented and non pigmented species do not display sexual reproduction, but only vegetative division by longitudinal binary fission of parental cells that divide into two sister cells (Kim, 1998). However, in unfavorable environmental conditions, the cells develop a mucilaginous layer that surrounds the entire encysted cell. The encystment of vegetative cells is stimulated by the lack of nutrients, oxygen, water stress or temperature (Philipose 1982). When environmental conditions are again favorable, the encysted cell undergoes multiple binary fission and forms many clonal cells (16-32) which are released into the medium after the breakup of the mucilaginous envelope.

In the present study, in both solid and liquid media, only a few cells progressed to adult stage. A possible explanation for this result is the depletion of nutrients in the culture medium, particularly sources of nitrogen. This situation is not observed in the field, where this nutrient is introduced more than once.

Nitrogen is essential for the synthesis of amino acids required for the assembly of proteins and hence formation of biomass. Thus, it is very likely that the nitrogen load imposed on the rice fields is the stimulus not only for the full development of red euglenoids, but also for the formation of blooms. Furthermore, there may be other nitrogen sources such as irrigation water originated from fish farming ponds and rain water that carries nutrients from the surrounding wetlands.

Thus, it was observed that a combination of environmental factors including: pH between 6.0 and 7.2, temperatures (air and water) above 23 ° C, wind speed lower than 4.0 m / s, high solar irradiation, and the availability of high levels of nitrogen are key factors in the onset of euglenoid red blooms in flooded rice fields.

Such blooms in flooded rice fields have been observed and reported frequently in recent decades. However, references on the impact that these phenomena may cause to these environments are not found in the literature. We have observed in the field, and also compiled information from observations made by rice growers (unpublished information), that the continued occurrence of red blooms may interfere with the development of rice plants by blocking the light and preventing it from reaching the submerged parts of the plants Also, euglenoid cells in below the surface adhere to the plant leaves when it is in full development, causing negative impacts on the photosynthetic process and thus interfering with plant growth.

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CONCLUSIONS

The methodology used in this study allowed the identification of only one morphotype of red Euglena in both cells present in environmental samples as in cells grown in culture.

The appearance of blooms of red euglenas monitored in this study is due to the joint action of mainly three of the monitored factors, which are pH, atmospheric temperature and solar radiation.

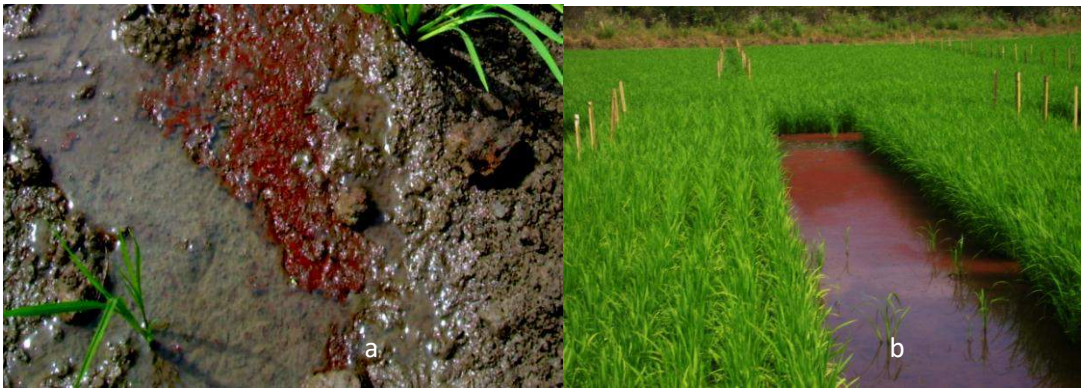

Figure 2: Resting spores in soil, sedimented after drainage of the experimental block (a) and vegetative cells on the water surface, in bloom condition (b) observed in the experimental blocks of rice of the Plant Science Department of the Federal University of Santa Maria in December 2010.

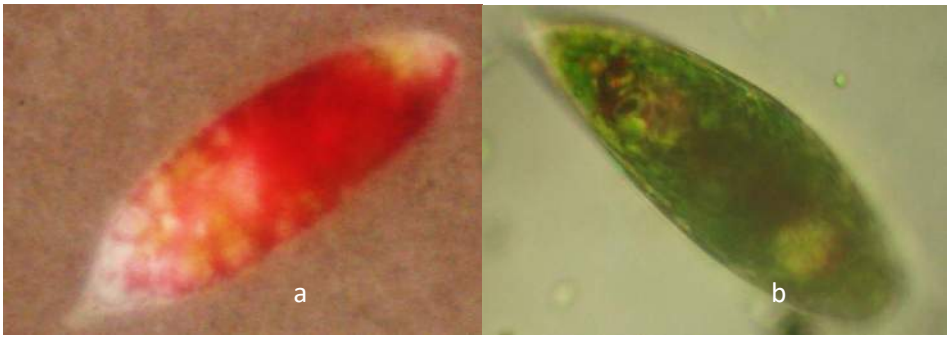

Figure 3: Adult cells of red euglenas of environmental samples with the red cell surface covered by hematochromegranules under conditions of stress (a) and the hematochrome granules concentrated only in the center of the cell, under natural conditions (b).

Figure 4: Adult red euglena cell with scale of measurement made with the aid of AutoCAD 2004 *software*.

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Table 1 Results of measurements of microscopic images of the thirty red euglenas cells spindle stage in environmental samples.

Parameter	Cell measurements														
Cell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cell Length (µm)	140.00	120.00	80.29	99.57	72.59	99.06	85.98	106.43	105.22	134.83	118.54	106.52	147.60	148.64	128.44
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	152.19	123.15	100.55	117.31	133.49	136.90	121.91	135.68	151.99	142.17	138.99	113.92	157.46	131.97	139.13
	Average			Variance			Standadrd deviation			Coefficient of variation					
	121.82			409.23			20,23			16,61% ≤ 20%					
Cell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Cell width (µm)	38.00	40.00	38.28	35.52	31.64	23.96	26.40	40.66	38.78	50.11	53.44	55.41	48.28	46.61	44.78
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
	46.22	40.64	45.22	49.55	49.39	53.38	38.64	49.88	58.55	46.24	49.79	45.08	52.60	46.77	48.27
	Average			Variance			Standadrd deviation			Coefficient of variation					
	44.40			63.83			7.99			17.99 % ≤ 20%					

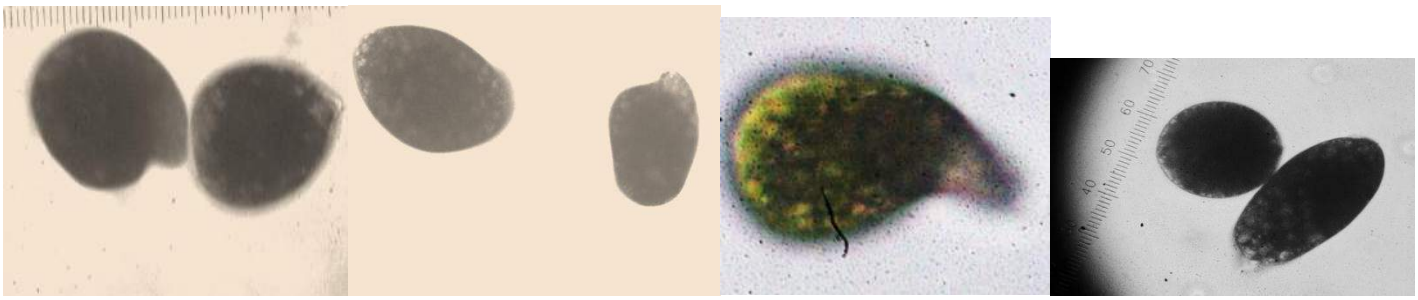

Figure 5: Cellular forms observed inred euglena cells collected in the rice paddy field observed microscopically (400x)

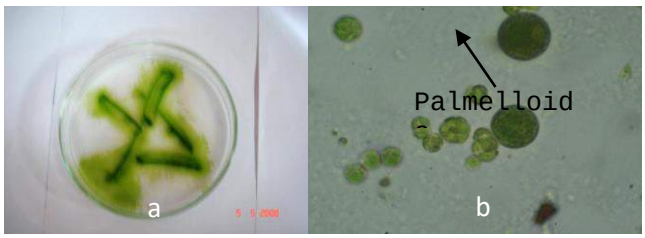

Figure 6: Evolution of the development of red *Euglena* cells in culture conditions.
Growth in culture medium after two weeks incubation (a) Gradual increase in cell size to the palmelloid stage (b)

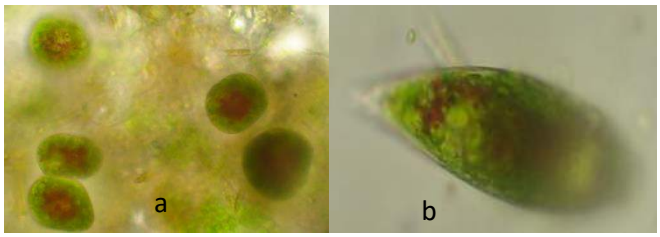

Figure 7: Evolution of the development of a red Euglena cell culture conditions.
Cells palmeloid stage, presenting hematochromes (a) and adult cell (b).

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Table 2: Physical and chemical variables describing the environment and experimental blocks where red euglenoid blooms were registered from December 2010 to February 2011, Santa Maria, RS.

	Clouds	pH	Water. temp.	air temp.	Rad.	Windspeed.	Bloom
	%	Un.	oC	oC	KJm2	m/s	
1st. week after flooding							
Q1				10:00 A.M.			
P1	0	6.5	20.9	26.0	1260	6.5	Absent
P2	0	6.3	20.9	26.5	1260	6.5	Absent
P3	0	6.4	20.9	26.0	1260	6.5	Absent
Q2							
P1	0	8.5	20.9	35.0	1260	6.5	Absent
P2	0	7.0	20.9	30.0	1260	6.5	Absent
P3	0	6.9	20.9	30.0	1260	6.5	Absent
2nd. week after flooding							
Q1				10:00 A.M.			
P1	10	6.3	23.9	25.0	959	3.8	Absent
P2	10	6.7	23.9	26.0	959	3.8	weak
P3	10	7.0	23.9	26.0	959	3.8	Absent
Q2							
P1	5	6.8	23.9	30.0	959	3.8	Intense
P2	5	7.3	23.9	25.5	959	3.8	Moderate
P3	5	7.1	23.9	25.0	959	3.8	Intese
3rd. week after flooding							
Q1				10:00 A.M.			
P1	0	6.6	17.7	23.0	1204	5.1	Absent
P2	0	6.5	17.7	28.0	1204	5.1	Absent
P3	0	6.4	17.7	24.4	1204	5.1	Absent
Q2							
P1	2	9.1	17.7	30.0	1204	5.1	Very week
P2	2	6.0	17.7	31.0	1204	5.1	Moderate
P3	2	6.0	17.7	28.8	1204	5.1	Absent

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REFERENCES

Alves-da-Silva, S.M.; Tamanaha, M. da Silva. Ocorrência de Euglenophyceae pigmentadas na rizipiscicultura na região do Vale do Itajaí. **Acta Bot. Bras**. 22(1): 145-163, 2008.

Alves-da-Silva, S.M. e Bicudo, C.E.M. Contribution to the knowledge of genus Lepocinclis (Euglenophyceae) of the state of Rio Grande do Sul, Southern Brazil. **Algological Studies** , 106: 77-97, 2002.

AGENCIA NACIONAL DE ÁGUAS - Conservação de Água e Preservação Ambiental nas Lavouras de Arroz do Rio Grande do Sul: Produção Mais Limpa, Brasília: ANA, 2009.

Araújo, C. B.; Siva, T. L.; Lucio-Filho, M.Q.L.; Ssntos, M.E. & Sá-Oliveira, J. C. 2007. **Ocorrência de Euglenophytas Pigmentadas na Lagoa dos Índios, Macapá-Ap, Brasil.** In*:* SHIRATA, M.T. & NEVES, P.S. Estudo de Euglenophyta pigmentadas em dois. lagos na área de proação/PUCPR. Município de Tijucas do Sul,Paraná, Brasil. Amapá, 2007.

Cassie, V. 1983. A guide to algae in oxidation ponds in the Auckland district. **TANE** 29, 1993. 119-132.

EMBRAPA. Centro Nacional de Pesquisa de Solos (Rio de Janeiro, RJ). Sistema brasileiro de classificação dos solos. 2. ed. Rio de Janeiro: Embrapa Solos, 2006. 306p.

EMBRAPA, 2009.Disponível em:

http://www.cnpaf.embrapa.br/apps/socioeconomia/docs/arroz/produtores_arroz.htmAcesso em: maio 2011.

Grützmacher, D. D. *et al.* Monitoramento de agrotóxicos em dois mananciais hídricos no sul do Brasil. Departamento de Fitossanidade/UFPel, 2008.

Kim, T. J.; Boo, M.S.; Zarkrys, B. Floristic and Taxonomic Accounts of the Genus *Euglena* (Euglenophyceae) from Korean Fresh Waters. **Algae**, V. 13(2):173-197, 1998.

Philipose, M. T. Contributions to our knowledge of Indian algae III. Euglenineae Part 1. The genus *Euglena* Ehrenberg. **Proceedings, Plant Sciences** (Indian Academy of Sciences) , 91:551–599, 1982.

Rosowski, J.R. 2003. **Photosynthetic Euglenoids**. In: Freshwater Algae of North Amerca, Ecology and Classification. (Wehr, J.D. & Sheath, R.G. Eds), pp. 383-422. San Diego: Academic Press, 2003.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. & Stanier, R. Y. Generic assignments, strain stories and properties of pure cultures of Cyanobacteria. **J. Gen. Microbiol**, 111:1–61, 1979.

Roger, P.A.; Heong, K.L. & Teng, P.S. **Biodiversity and sustainability of wetland rice production**: role and potential of microorganisms and invertebrates. International Rice Research Irzst itu t e , PO Box 933, Manila, Philippines 117-136, 1991.

SOSBAI - SOCIEDADE SUL-BRASILEIRA DE ARROZ IRRIGADO - Arroz Irrigado: Recomendações Técnicas da Pesquisa para o Sul do Brasil. Porto Alegre, 2010.

Starr, R.C. The culture collection of algae at the University of Texas at Austin. J. Phycol. 14 (supplement): 47-100. In: STARR, R.C. The culture collection of algae at the University of Texas atAustin. **J. Phycol**. 1978. 14 (supplement): 47-100, 1978.

Von Sperling, M. 1996. Introdução à qualidade das águas e ao tratamento de esgotos. DESA-UFMG, 1996.

Vasconcellos, Noeli Júlia Schüssler de. Cianobactérias Heterocitadas e Euglenas Vermelhas em Lavoura de Arroz Irrigado por Inundação em Planossolo Háplico, Santa Maria, Rs.2010. 94 f. Tese (Doutorado em Ciências do Solo), Programa de Pós-Graduação em Ciência do Solo, Universidade Federal de Santa Maria, Santa Maria, 2010.

Zimba, P. V.; Moeller, P. D.; Beauchesne, K.; Lane, H. E; Trieme, R. E. Identification of euglenophycin – a toxin found in certain euglenoids. **Toxicon** 55: 100-104, 2010.