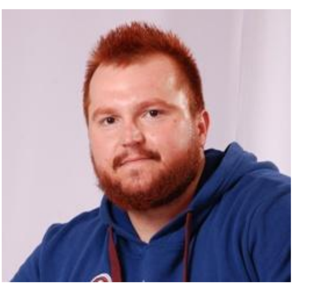
One microbial bioassay for a method of cyanobacterial and algal removal – microalgae causing biodeterioration



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Introduction

Stone is the oldest entity of Earth, it creates ecosystem which includes also environmental factors (light, nutritions, climate, pollution). The factors of weathering never affect separatly but together in a lot of antagonistic or synergic interactions. The significant deterioration of monuments has been begun since yaers 1870-1880, this phenomenon has not been possible to stop since 1950 when the defects of buildings have gone over the level of carrying-capacity.

Intensity of colonisation of organisms, biotransport and biodeterioration are directly proportionated to the poriness of stone, this phenomenon confirmed Vendellsaz et al. (1996) and Laiglesia et al. (1994) in Spain. Limestone, dolomite, sandstone, bricks and cement materials are the most colonized materials by microorganisms (Hueck van der Plas, 1968).Color changes of stone surface include biological aspects, the black color represents colonisations of micromycetes or of cyanobacteria, the green, orange, brown or yellow growths represent derivates of green chlorofyles of algae, red or pink biofilms represents the chemoautotrofic bacteria (Urzi et al., 1992). Biofilms induce changes of mineral and chemical composition of stone, microorganisms change its stability, permeability, density and color (van der Molen et al., 1980). Usually, the changes of the surface are very good visible, the presence of some species can be also endolitic, which are not visible by eye, there are species, mainly cyanobacteria, specialised to the rifts of substrata or to cavernities and to pores of material (Golubic et al., 1981; Asencio & Aboal, 2000).

Actual state of the protection of the monuments stones is different in Europe. Research of the photosynthetic lithobionts on monuments is in exces of importance to understood the stucture of the characteristic microbial associations considering the biodecay effects. It is one part of the doctoral thesis "Cyanobacteria and algae as agents of biodeterioration of stone substrata". This part of thesis titled "El Estuco's Experiments" was accomplished in Laboratory of Algology (Department of Botany, Murcia University, Spain).

Material & Methods

Samples were taken from the 16 subaerial places, include monuments (12: Real Monasterio de Encar-nacion de Monjas Clarisas, Castillo de los Vélez, Monasterio de San Francisco, Iglesia del Santo Cristo, Iglesia del Salvador, Iglesia Nuestra Señora de la Asuncini, Colegiata de San Patricio, Palacio de Guevara, Palacio Episcopal, Catedral de Murcia, Medina Siyasa, Castillo de Monteagudo, see Fig. 1), calcareous rocks (3 – La Puerta) and a new building (Faculdad de Biologia). The representative substrata hosting the algal biofilm were limestone, sandstone and in one case concrete. The samples were samplied and collected during the spring and summer seasons of years 1996 and 2003. The part of scrapied field material was aseptically spread into test tubes and over surface of Petri dishes containing medium BG11 (Rippka et al. 1979), $BG11_0$ (Rippka et al. 1988) and BBM (Smith & Bold 1967), which was liqiud or agarized. The test tubes and Petri dishes were incubated by constant condition t = 20ºC, humidity 64.2 %, irradiation 75 $\mu E\ m^2\ s^{-1}$, light 3000 lx and in 8/16 dark-light regime in laboratory of the S.A.C.E. (Servicio de Apoyo a las Ciencias Experimentales, Murcia University). Observations of samples were done by stereomicroscope OLYMPUS SZH and microscope OLYMPUS BH2. From the all cultivated samples were isolated 58 unialgal strains, which are maintaing in LAUM collection (Laboratory of Algology, Murcia University, Spain). Colected samles preserving dry and the photographic documentation of species are stored in Laboratory of Algology, Murcia University (Spain). The powder mixture (el estuco) is used traditionally for coating buildings in Mediterranean regions, mainly in Spain. There are a lot of kind of procedures how to prepare coating for buildings, usually is used mixture with water 1:1, 1:2 or 1:3 ratio. This coating is the natural powder, prepared from calcareous material or rocks. The exact composition of this material was not known. The powder diffraction analysis of this material was made in Laboratorio de Geologia (Dpto. de Química Agrícola, Geología y Edafología, Facultad de Química) by María Teresa Fernández Tapia. The analysis showed that it is mineral $CaRuO_4.2H_2O$ - Calcium Ruthenium Oxide Hydrate. For grow inhibition test it was finally used 4 successfully grown algal strains, which were incubated in constant conditions in the laboratory of the S.A.C.E. (Servicio de Apoyo a las Ciencias Experimentales, Murcia University). In general, the BBM medium (Smith & Bold, 1966), either liquid or agarized was used. Four strains FAB1/02 (*Diadesmis cf. contenta), FAB1/03A* (*Xanthonema sp.*, Fig. 5), *FAB2/01A (Klebsormidium flaccidum), BES5/03A (Chlorella kessleri) are* maintained in a LAUM culture collection (Laboratory of Algology, Murcia University, Spain).

Results

The 47 epilithic and chasmoendolithic algae were identified: 22 cyanophytes/ cyanobacteria (48%), 4 heterokontophytes (9%) and 20 chlorophytes (43%). 47 species: *Pseudocapsa dubia*, *Cyanobacterium cedrorum*, *Synechocystis* sp. 1, *Synechocystis* sp. 2, *Aphanocapsa muscicola*, *Chroococcidiopsis kashaii*, *Hyella balani*, *Pseudanabaena* sp., *Leptolyngbya* sp. 1, *Leptolyngbya* sp. 2, *Leptolyngbya* sp. 3, *Leptolyngbya nostocorum Leptolyngbya* sp. 5, *Leptolyngbya* sp. 6, *Leptolyngbya* sp. 7, *Schizothrix friesii* , *Phormidium autumnale*, *Microcoleus vaginatus*, *Scytonema julianum*, *Tolypothrix byssoidea*, *Calothrix fusca var. crassa*, *Nostoc sphaericum* (Fig. 4), *Botrydiopsis* sp., *Heterothrix (Xanthonema)* sp., *Heteropedia* cf. *simplex*, *Hantzschia amphioxys*, *Diadesmis* cf. *contenta*, *Nautococcus terrestris*, *Tetracystis sarcinalis*, *Trebouxia arboricola*, *Myrmecia* cf. *globosa*, *Apatococcus lobatus*, *Chlorosarcinopsis* sp. 1, *Chlorosarcinopsis* cf. *Arenicola* (Fig. 6), *Ecdysichlamys obliqua*, *Oocystis asymetrica*, *Muriella terrestris*, *Chlorella vulgaris*, *Chlorella miniata*, *Chlorella kessleri*, *Scenedesmus obtusiusculus*, *Klebsormidium nitens*, *Klebsormidium flaccidum*, *Klebsormidium crenulatum*, *Stichococcus allas*, *Stichococcus bacillaris*, *Stichococcus minutus.* The above data confirm that coccoid species outnumber filamentous species (totally represent 55 % of all investigated algae). The inoculated algae have not grown on the all agar plates contained "el estuco"powder this material Calcium Ruthenium Oxide Hydrate totally inhibited and eradicated growths of algae in all case of used concentrations (Fig. 3).

Main Aims

- to examine the taxonomic and autecological traits of subaerial mikroalgae occuring in urban habitats in the Region of Murcia
- to reassume in research of subaerial algae in apllied way, i.e. to design a growth inhibition test on subaerial microalgae and to test a microalgae causing actively biodeterioration which potentially damage the building stone. A type of growth inhibitor was used white powder called in Spain "el estuco".

Fig. 1: Sampling site - Castillo de Monteagudo (author: Jose Pedro Marin Murcia)

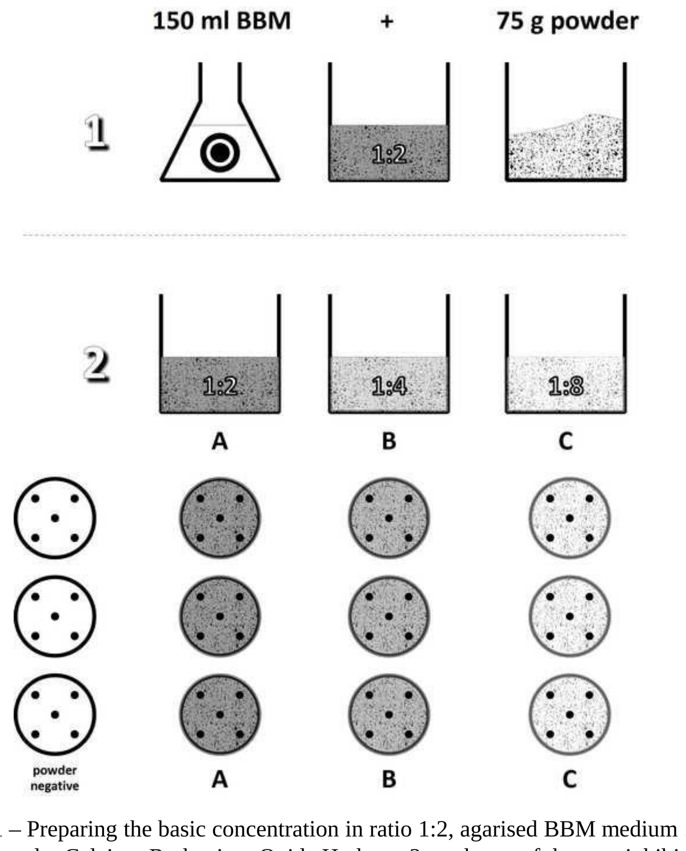

Fig. 2: 1 – Preparing the basic concentration in ratio 1:2, agarised BBM medium : sterile powder Calcium Ruthenium Oxide Hydrate; 2 – scheme of the new inhibition test, Petri dishes with diameter 3.5 cm and 5 inoculation per agar plate, first row of Petri dishes is powder negative, the next rows with powder in mixture with different ratio. (author: Bohuslav Uher)

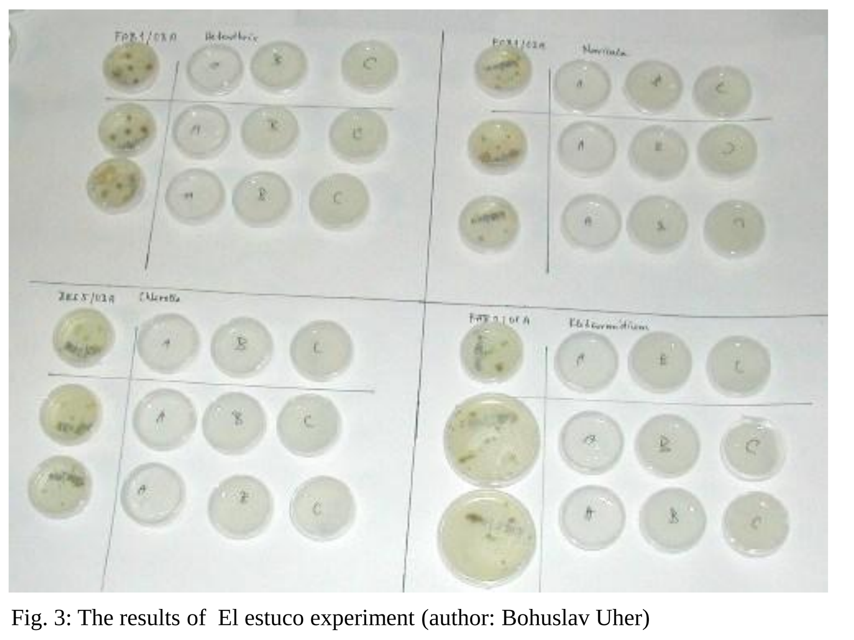

Fig. 3: The results of El estuco experiment (author: Bohuslav Uher)

Fig. 4: The subaerial cyanobacterium *Nostoc sphaericum* (author: Bohuslav Uher)

Discussion

The qualitative composition of species is different in several places of Europe, the results of them shown clearly that the habitats with a high water potencial, ensures that the cells remain aquatic for short periods when the habitat is dry, hosting mainly green algae. It is typical for temperate humid areas (Schlichting, 1975; Rifón-Lastra & Noguerol-Seoane, 2001; Godyová et al., 2003; Darienko & Hoffmann, 2003; Rindi & Guiry, 2003), with very short exposition of extrem osmotic stress on the cell. Others artificial stone habitats exposed to extrem osmotic stress very long time such as 4 months and more, are hosting predominantly by epi- or endolithic cyanobacteria, which are adapted to extrem dessication and teperature conditions. Firstly it is clear, that the important influence on the composition of subaerial phycoflora has mainly the moisture as the most important environmental parameter in lithic environment, which confirms also Grilli Ciaola et al. (1987), Potts (1994), Nienow (1996). Secondarilly the type of lithic substrata has less or non-significant influence on composition of lithic phycoflora in the case of our localities. The epi- and (chasmo)endolithic algae from subaerial monument-like environment, show a dominance of prokaryotes microorganisms, as occurs in hot regions, in caves, and others extrem environments.

Specific laboratory tests of biodeterioration were investigated by laboratories at the universities in Messina and Oldenburg (Anagnostidis et al., 1992). The selection of the most suitable method depended on the type and the stage of organisms which were deteriorating monuments. Sometimes a good effect was obtained by the combination of many methods which were successfully applied by Krumbein et al. (1992) on the 50 architectonic monuments. Dupuy et al. (1976), Pietrini et al. (1985) and Tiano (1979) investigated the effect of algicides and its reversibility effects. The biotest with antibiotics were investigated by Lefěvre et al. (1964) and the effects of UV-rays were investigated by Molen et al. (1980). The new technique designed and successfully used in laboratory required considerable modification when used on others photosynthetic organisms. This methodology is a pioneer test for culture-testing of algae required both technical and biological expertise, furthermore one needed to be able to improvise. It was developed the taxonomical and afterwards experimental program, including the compilation of bibliographical data on some strains. In addition, this collection has active interests in developing miniaturized algal bioassays, algal biosensors and development of cultivation tests. This experiment has considerable expertise in algal culture and the spectrum of cultivation units from colonies on agar plates. In addition, work on subaerial algae caused biodeterioration has highlighted their practical potencial, e.g. use in the new growth inhibition test.

Acknowledgements.

I am grateful to Prof. Dra. Marina Aboal Sanjurjo for her invitation to work in her lab and for providing the research work at the University of Murcia in Spain for the successful realization of experiments conducted these experiments confirmed the inhibition effect of "Estuco" material for the development and growing of microalgae, obtained results that are able to be applied in the control of biodeterioration and are currently in the acceptation process of patent of invention. Special thanks are expressed to Lcd. Jose Pedro Marín Murcia, Dra. Antonia Dolores Asencio Martinez, Juana María García Reverte, Lcda. Encarna Zafra Pastor and Lcda. Elena Ruíz Valero for their cooperation in the lab (Laboratorio de Algologia and S.A.C.E.) I further would like to thank Dra. María Teresa Fernández Tapia (Laboratory of Geology) for her research cooperation in the "Estuco" project.

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Fig. 5: The subaerial heterokonthophyte alga *Xanthonema)*sp. (author: Bohuslav Uher)

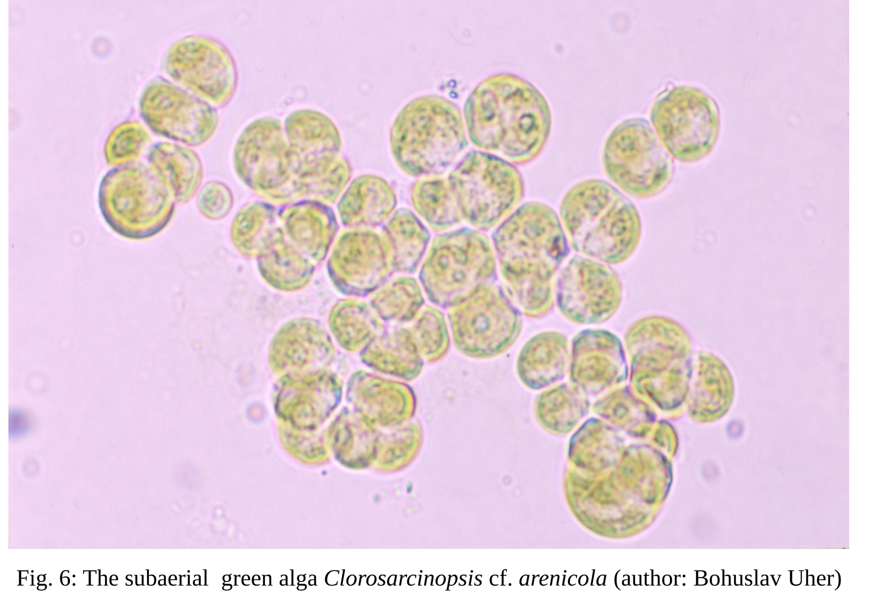

Fig. 6: The subaerial green alga *Clorosarcinopsis* cf. *arenicola* (author: Bohuslav Uher)