Effects of L-methionine-DL-Sulphoximine(MSO) and 3-(3,4-dichlorophenyl)-N-N-dimethylurea(DCMU) on Physiological Activity of Cyanobacteria *Nostoc species* Isolated from Lichen *Peltigeracanina*

Jabbar F- Al-maadhidi
Department of Biology, Madenat Al-elem University College.
Baghdad, Iraq.
E-mail: dral_maadhidi@yahoo.com

Abstract
Lichen is a Symbiotic system consist of nitrogen fixing Cyanobacteria or and algae and fungus. Cyanobacteria can fix nitrogen in a peculiar differentiated cells called heterocyst under aerobic conditions, those heterocyst are the loci of nitrogenase activity. The organic nitrogen and carbohydrates produced by cyanobacteria utilized by fungi and the fungus supplying the cyanobacteria and algae by inorganic metals coming from dissolved rocks by acids produced by fungi. Culture of *Nostocspp* treated with MOS excreted, the newly fixed nitrogen in form of ammonia into liquid media, which is proportions with the concentration of the analog and detected after 6 h of treatment. Acetylene reduction technique (nitrogenase activity) was not affected by analog (MSO) treatment. The growth of Cyanobacteria *Nostocspp* was slightly inhibited starting after 6 h of treatment. The rates of carbon fixation were highly enhanced after treatment leading to increase the number of (PGBS) in the Cyanobacteria cells. The combination treatment of *Nostocspp* culture by MSO and DCMU showed the following: carbon and nitrogen fixation are dependent of each other, slight inhibitions in culture growth, 50% inhibition in ammonia release, complete inhibition of carbon fixation and disappeared of extra PGB. Light intensities and carbon fixation are dependent even in the presence of MSO. The cultivation of *Nostocspp* culture under $Ar/O_2/CO_2$ in presence of MSO gives reverse relationship between the cellular incorporated carbon and time. Electron micrograph showed an increase in PGBs of the lichen *P. conina* treated with MSO through the first hour of treatment and then disappeared after 24 h.

Key words: MSO, DCMU, Nitrogen fixation, Lichen.

تأثير كل من MSO, DCMU على الفعاليات الفسلجيه لخلايا السيانوبكتريا *Nostoc* spp. المعزولة من الاشن *Peltigeraconina*

جبار فرحان المعاضيدي
كلية مدينة العلم الجامعة / قسم علوم الحياة

الملخص

اظهرت المزارع الخلوية للنوع *Nostoc spp.* المعاملة بمادة MSO قدرتها على افراز الناتروجين ألمثبت حديثاً الى الوسط الزرعي السائل بعد ستة ساعات من المعامله على شكل امونيا وان الكمية المفروزة تناسب طردياً مع تركيز مادة MSO. لم تتاثر فعالية انزيم النتروجينتز بمعاملة الـ MSO, في حين كان هناك تثبيط بسيط في النمو مما ادى الى زيادة في اعداد جسيمات PGBS في السايتوبلازم للخلايا. ان تداخل معاملة الخلايا للنوع *Nostoc spp.* بمادة MSO وبـ DCMU اوضحت الاتي : ان عملية تثبيت الكاربـــــون والنتروجين معتمدان بعضهما على البعض , تثبيط بسيط في نمو الخلايا, تثبيط 50% من امونيا المفروزة خارج الخلية , تثبيطكاملتثبيت الكاربون واختفاء جسيمات PGB الزائدة , شدة الاضاءة وتثبيت الكربون يعتمدان على بعضها حتى في حالة معاملة الخلايا بمادة MSO , ان تنمية المزارع الخلوية للنوع *Nostoc spp.* المعاملة بمادة MSO تحت خليط مـن CO_2 / O_2/ Ar ادى الى علاقة عكسية بين الكاربون الخلوي التركيبي والزمن المعتمد في تنمية الخلايا , كما تبين دراســـة المجهر الالكتروني لعينات لآسنات من النوع *P. canina* المعاملة بمادة MSO ازدياد عدد جسيمات PGBS خلال الساعة الاولى من المعاملة واختفائها كلياً بد 24 ساعة من المعامـــــلة .

Introduction

Lichen is a Symbiotic system consist of nitrogen fixing Cyanobacteria or and algae and fungus [1,2]. Cyanobacteria can fix nitrogen in a peculiar differentiated cells called heterocyst under aerobic conditions, those heterocyst are the loci of nitrogenase activity [3]. The organic nitrogen and carbohydratesproduced by cyanobacteria utilized by fungi, and the fungus supplying the cyanobacteria and algae by inorganic metals coming from dissolved rocks by acids produced by fungi [4]. In Legume nodules, the first product of N_2 fixation in bacteroids is NH_3. The NH_3 is then exported to the host plant cytosol where it is further metabolized to amino acids and amides [5]. Glutamine Synthase (GS) plays a key role in nitrogen metabolism, thus the fine regulation of this enzyme in *Prochloroccus*, which is especially important in the oligotrophic oceans where this marine *Cyanobacterium thrives* [6]. Metabolism of c14 glutamate showed that in white light glutamine was the main labeled product and in the dark label was principally in compounds closely associated with tri carboxylic acid cycle metabolism [7]. Also, glutamine synthase (GS) rapidly converts blood – borne ammonia into glutamine which in high concentration may cause mitochondrial dysfunction and osmotic brain edema [8], and it has been proposed that elevated glutamine levels during hyper ammonia lead to astrocyte swelling and cerebral edema

using MSO as inhibitors for nitrogen metabolism [9].

The symbiotic relation in lichen components (algae, cyanobacteria and fungus) sharing the nitrogen and carbon fixed which affecting the physiological activity of all organisms in lichens. Nitrogen metabolism as a function of GS can inhibited by glutamine analog MSO, and carbon fixation (photo system II) can inhibited by DCMU [3,10-13].

In this study MSO and DCMU were used to explain the relationship between nitrogen fixation and carbon fixation in lichen (lichen system), the flow of fixed nitrogen, and the inhibition in growth of *Nostocspp.*isolated from lichens *peltigeraconina.*

Materials and methods

Organisms and culture conditions

The alga *Nostocspp* isolated from the lichen peltigeracanina at the Department of microbiology. Sciences University of Dundee, UK was grown in the N-free medium [14] under continuous culture conditions. The light intensity was 3000 lux and the temperature 26 °C. Aliquots of alga were withdrawn regularly for experimental purposes, all experiments mentioned below were conducted at 3000 lux, 26 °C and with gentle shaking.

Effects of MSO and DCMU on some algal physiological processes

Effects of MSO on the relation between alga and fungus within symbiotic systems in lichens was studied in experiments carried out in conical flasks of 200ml capacity containing 50ml algal suspension (batch culture) treated with freshly prepared 0.5, 1.0, and 2 µm of MSO. Algal suspension without MSO served as control. Samples were regularly withdrawn during the period of the experiment (24 h) and assayed for (a) ammonia released to the medium (15), (b) rates of 14 CO_2 for 30 min being fixed intracellular and released to the medium (16),(c) algal growth chlorophyll a (17), and (d) nitrogenase activity (18). Algal samples were fixed at the beginning and at the end of the experiment for examination by electron microscopy. Effects of the combination between MSO and DCMU on the alga were examined. the experiments was carried out in 200 ml conical flasks containing 50ml of algal suspension treated with 1 µm of MSO or 10 µm of DCMU or a combination of both chemicals. Untreated algal suspension served as control. Samples were assayed as in the previous experiment.

Effects of light intensities and MSO on the algal rates of 14 CO_2fixation

Algal suspension treated with 1µm of MSO for 24 was exposed to different light intensities of 100, 300, 500, 1000, 3000, lux and dark respectively in order to study the rates of carbon fixation. The alga was exposed for 30 min. to radioactive sodium bicarbonate (5 µci/ml) at the various intensities. Organic 14 CO_2 fixed cellular and released to the medium was calculated (16).

Effect of MSO on the incorporation of carbon under Ar/O_2/CO_2

The experiment was carried out in 200 ml conical flasks containing 50 ml aliquots of *Nostoc spp.* The alga was treated with 0.5 and 1.0 µm of MSO for 48hr. under a gas mixture of Ar/CO_2 (77.96/22.00/0.4, v/v) with continuous light. Two ml from each treatment were withdrawn and assayed regularly every 12 h for 14 CO_2 fixation after exposure for 30 min to the radioactive sodium bicarbonate organic fixed cellular and released to the medium was again estimated.

Effect of MSO on lichens

A lichen of 1cm in diameter was saturated with a solution of 1.0 µm of MSO for 24 samples were fixed at the beginning and at the end of the experiment for electron microscopy study.

Chemicals

MSO, L-methionine-DL-sulphoximine, sigma ltd., London. DCMU 3-(3,4-dichlorophynile-1,1-dymethel-urea,sigma ltd., London.

Results and discussions

The results presented in this study demonstrate the effects of MSO and DCMU on some physiological activities of the alga *Nostocspp* isolated from the lichen *p. canina*. As shows in figure 1 the reduction of acetylene was more or less unaffected by addition of virus concentration of MSO, while the amount of ammonia released was proportional to the increase in the concentration of the analogue. Only low quantities of ammonia were detected in the untreated algal culture.Ammonia was detected in the medium after six hours of treatment with different concentration of the analogue. These results indicate that newly fixed ammonia is only partly incorporated in to amino acid synthesis in presence of MSO. This is likely due to a partial blocking of the ammoniating pathway in this case the GS – GOGAT system was inhibited, by MSO as shown earlier by (1,3,19) for the blue – green alga *A. cylindrica*, and(20) for Azotobacter. The effect of treatment with MSO on algal growth was shown in Fig.2.The treatment leads to a slight inhibition in the algal growth, starting after six hours as compared to the control. The inhibition in growth may be caused by nitrogen starvation which due to the partial inhibition in the protein synthesis (21). Stewart and rowell(1975)(3) reported a 10% inhibition in vitro in the activity of ammonia assimilating enzymes GS, GOGAT after treatment of the alga *A. cylindrica* with MSO (1µm). Furthermore, electron micrograph of the algal samples treated with MSO for 24hr show a high increases in the number of polyglucoside bodies (PGB), which are considered to be storage bodies of carbon in prokaryotes .Again this increase could be due to inhibition in the ammonia incorporation activity, with a decrease in the protein synthesis leading to a decreases in the carbon consumed by the cells.

Experiment results studying the relation between carbon and nitrogen fixationare confirmed they are dependent (Table 1). Treatment of the alga with 10µM and 1µM of MSOled to induce in the ammonia release to the medium by about 5 and 10 times respectively higher than the control. However, the ammonia release was found inhibited 50% when the alga was treated with DCMU and MSO together. The result showed that inhibitor blocking the electron transport between Ps 11 and Ps 1, and MSO simultaneously. Furthermore, treatment of the alga with the same concentration of MSO increased the capacity for fixing carbon about three times compared to the control , while it was completely inhibited by DCMU alone, and with DCMU and MSO together (Table 1). The organic carbon released to the medium was less than the control in all treatments, which indicates that there are no effects from MSO or DCMU on the permeability of the algal cell walls (Figure 3). Algal growth was slightly inhibited in all treatments,a greater or complete inhibition in carbon fixation however was happen with DCMU alone (Table.1), this result is agree with that fined by Singh (2011) (12). Electron micrograph show disappearance of the PGB from the algal cells treated with DCMU or DCMU and MSO (Figure 4). The rapid inhibition in carbon fixation of the alga treated with DCMU due to inhibit ATP supply for carbon and nitrogen fixation [22].

Effects of different light intensities 0n the rates of carbon fixation of the alga show thenormal relation between the intensity of

light and photosynthetic activity, i.e. the rates of carbon fixation was enhanced gradually with increase in light intensity (Figure 3). Treatment of the alga with 0.5 µM and 1.0 µM of MSO stimulated the carbon fixation rate 5 and 10 times, respectively, compared with the untreated alga. These results confirm the effects of MSO on carbon fixation demonstrated in the previous parts of this study. As is also seen in figure 3 the organic carbon released show no significant differences between treated and untreated alga at the different light intensities used.

Cultivating the alga with and without 1.0 µM MSO under Ar/O_2 / CO_2 in absence of N2 gave a reverse relationship between the cellular incorporated carbon and time (Figure 5). Under the incubation condition, less carbon was accumulated when the algae was treated with MSO. The carbon released, however, increased with maximum carbon released being 13% of the total counts after 48 h (Figure 5), for comparative purpose, the lichen *Peltigeracanina*, harboring heterocyst cyanobacteria *Nostocspp* in cephalodia, was treated with MSO. Electron micrograph showed an increase in the PGB over the first hour of treatment, while these disappeared at the end of the experiment (24 h) (Figure 6). The increase in PGB noted over the first hour is probably due to the presence of MSO, leading to increase in the rates of carbon fixation as shown above for free-living algae. However the disappearance of the bodies might be caused by the fungus of the cephalodia consuming MSO at high rates over the experimental time and as a consequence the rates of carbon fixated by the alga will regress to the normal condition that is why with few PGB.

As a conclusion, the acting mechanism system in lichen is not completely similar to the ammonia assimilating inhibitor (MSO), but this is one of theexplanation. Also, there are another factors coming from the physiological intact between the algae and fungus, and the explanation of that is the accumulation of polyglucoside bodies as shown in the electron microscopy study and finally there is a direct physiological relation between Carbon and Nitrogen fixation.

Table.1 Effect of combination between 1 µM of MSO and 10 µM of DCMU on ammonia released, growth and carbon fixation in *Nostocspp*(intercellular and extracellular).

Treatments	$nmNH_3$	Chloro.a µg/ml	CPM of $^{14}CO_2$ fixed (µg chloro.a)$^{-1}$ (ml filtrate)$^{-1}$	
			Intracellular (µgchloro.a)	Extracellular (ml filtrate)
Control	17.50	7.35	1711.00	449.00
1 µM MSO	163.00	7.29	4811.00	314.00
10 µM DCMU	82.00	8.15	3.00	31.00
MSO and DCMU	70.00	6.47	15.00	4.00

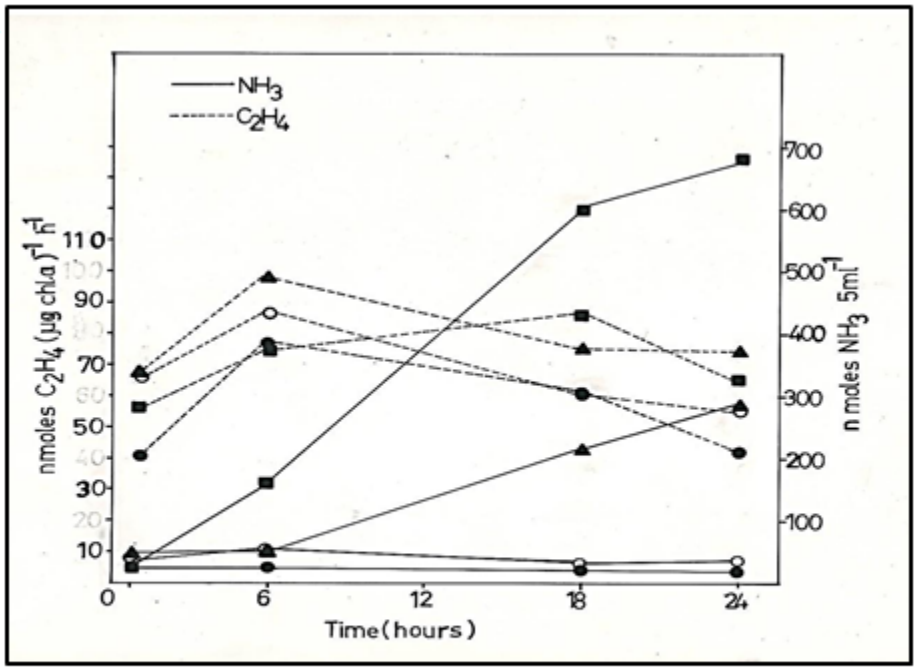

Figure 1. Effect of MSO (0.5µM, 1.0 µM and 2.0 µM) on the nitrogenase activity (C_2H_2) reduction and ammonia released, 0.5 µM (●), 1.0 µM (▲), 2.0µM (■), controls (○).

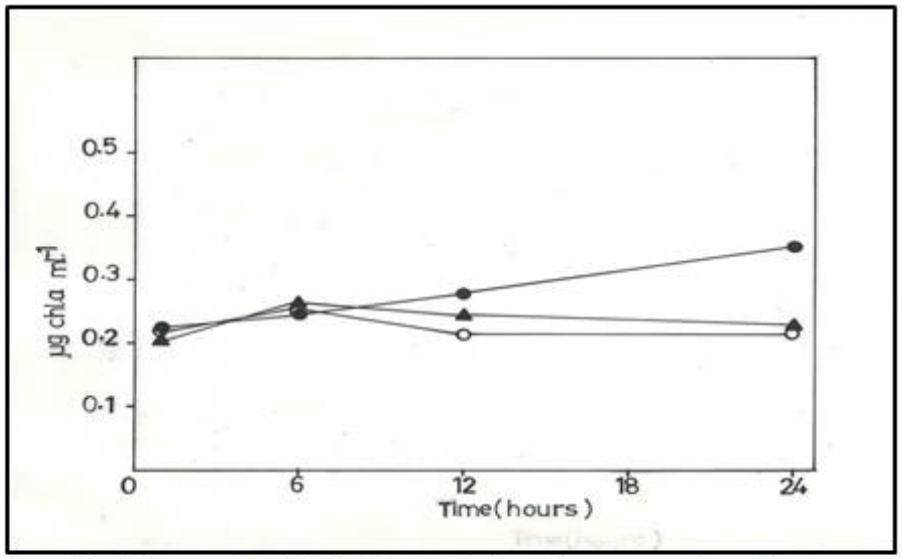

Figure 2. Effect of MSO (0.5µM, 1.0 µM) on growth of *Nostoc*spp. controls (●), 0.5 µM (▲), 1.0µM (○).

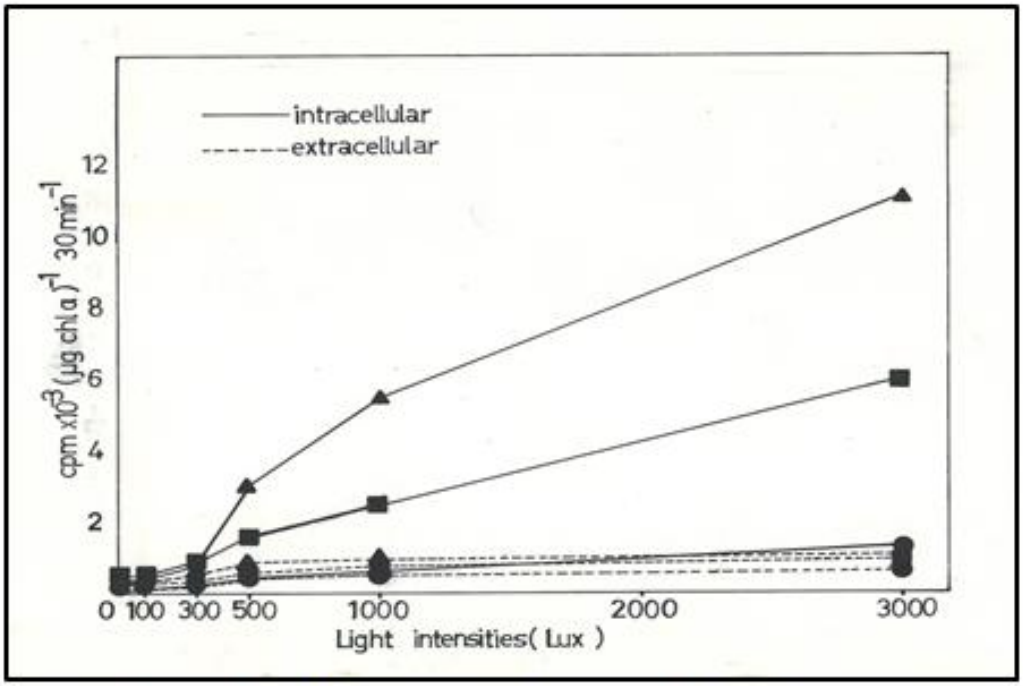

Figure 3. Effect of Light intensities on the rate of carbon fixation of *Nostoc spp.* treated with 0.5 µM, 1.0 µM of MSO.controls (●), 0.5 µM (■), 1.0µM (▲).

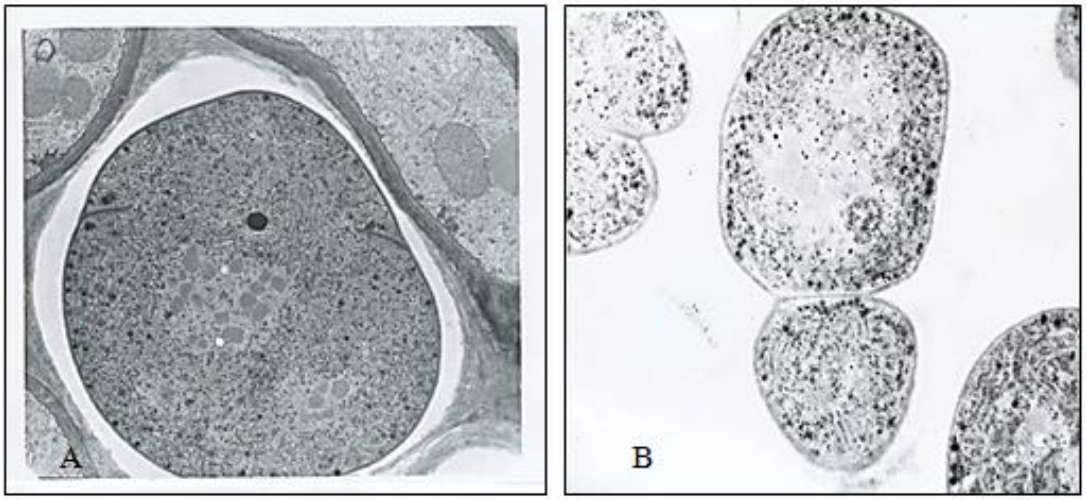

Figure 4. Effects of MSO (0.5µM) on the PGES of the algae *Nostoc spp.* Electron microscopy 10000 x. A, zero time; B, 24 h.

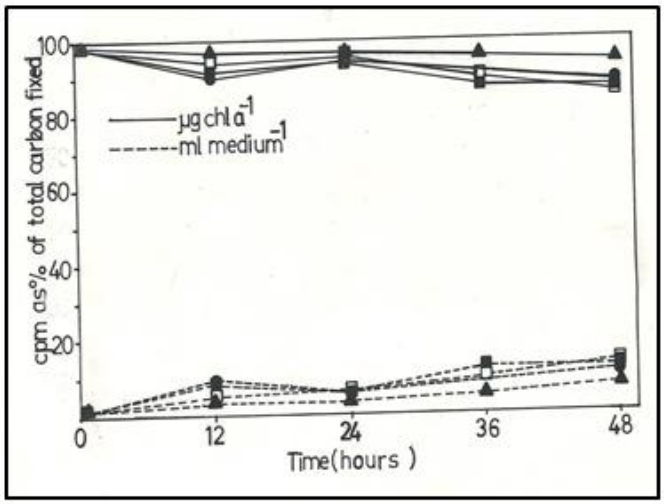

Figure 5. Incorporation of $^{14}CO_2$ by the algae *Nostoc spp.* treated with MSO (0.5 µM, 1.0 µM) cultivation under $Ar/O_2/CO_2$. Control under air (□), control under $Ar/O_2/CO_2$ (●), 0.5µM under$Ar/O_2/CO_2$(▲),1.0µM under$Ar/O_2/CO_2$(■).

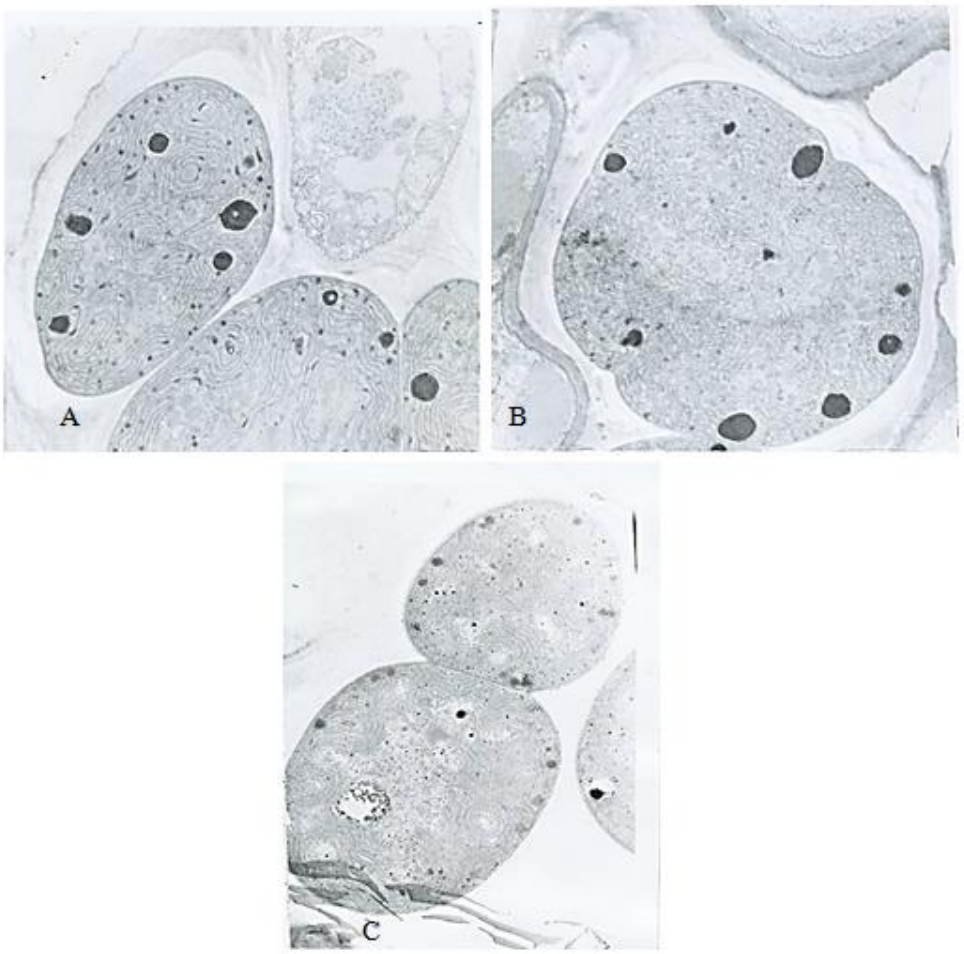

Figure 6.Effects of MSO and DCMU on the PGE numbers of the phycobionta*Nostoc spp.* in the Lichen *P. conina*. *Electron microscopy 10000x.* A, zero time 1.0 µM (MSO); B, 24 h, Controls C, 0.5 µM MSO+DCMU 24 h.

References

1- Rai, AN, Rowell, P. and Stewart, W. D. P. (1980). NH^{+}_{4} assimilation and nitrogenase regulation in the Lichen *Peltigeraaphthosa*Willd . New phytol. 85:545-555.

2- American Psychological Association (APA). Lichen.(n.d.) the American heritage Science Dictionary. Retrieved October 11, 2015, from Dictionary. Com Website http : /dictionary. Reference. Cam/browse/Lichen.

3- Stewart, W.D.P. and Rowell, P. (1975). Effects of L – methionine – DL- Sulphoximine on the assimilation of newly fixed NH_3, acetylene reduction and heterocyst production in *Anabaena cylindrica.* Biochemical and Biophysical Research Communications. Vol:65,No,3:846-856.

4- Chen, J. Blume, H – P, Beyer, L. (2000). Weathering of rocks induced by Lichen Colonization – a review. Catena, 39:121 – 146 .

5- Ohyama, T. and Kumazawa, K (1980). Nitrogen assimilation in Soybean nodules. II. N_2 assimilation in bacteroidand cytosol fraction of soybean. Soil Sci. Plant Nutr., 26:205 -213.

6- Gomez – Baena, G, Dominguez – Martin, M.A., Donaldson, R. P, Gracia – Fernandaz, J.M and Diez, J.(2015). Glutamine Synthetase sensitivity to oxidative modification during nutrient starvation in *Prochlorococcusmarinus*pcc 9511. Plos one 13:10 (8): eo1 35322. Epub, Aug 13.

7- Jordan, B. R. and Givan, C. V. (1979). Effects of Light and inhibitors on glutamate metabolism in leaf discs of *Viciafaba*., Plant Physiol., 64:1043 -1047.

8- Fries, A.W,Dadsetan, S., Keiding, S., Bak, L. K.,Schousboe, A, w(aagepetersen, H.S., Simonsen, M.,Ott, P., Vilstrup,H. and Sorensen, M. (2014). Effect of glutaminsynthetase inhibition on brain and inter organ ammonia metabolism in bile duct Ligated rats. J .cerebral Blood flow and Metabolism, 34:460 – 466.

9- Dadsetan, S., Kukolj, E., Bak, L.K. , Sorensen, M., Ott, P., Vistrup, H., Schousboe, A., Keiding, S. and waagepetersen, H.S.(2013).Brain alanine formation as an ammonia – Scavenging Pathway during hyperammonemia : Effects of glutamine synthetase inhibition in rats and astrocyte – neuron co – cultures . J. cerebral Blood Flow and metabolism, 33:1235–1241.

10- Stewart, W.D.P. and Rodgers, G.A. (1977).The Cyanophyte-hepaticsymbiosisII.nitrogen fixation and the interchange of nitrogen and carbon. newphytol., 78:459- 471.

11- Godd, G.A. and Cossar, J.D. (1978) the site of inhibition of photosystem II by 3-(3,4-dicholorophenyl)-N-N-dimethylurea in thylakoieds of the Cyanobacterium*Anabaena cylindrical*. Biochemical and Biophysical Research Communications Vol. 83,No.1,342 -346.

12- Singh,S.,D. Atta,P. and Tirkey, A. (2011) response of multiple herbicide resistant strain of diazotrophiccyanobacterium, *Anabaenavariabilis*, exposed to atrazine and DCMU. Indian J. Experimental Biology, Vol. 49: 298-303.

13- Rowsthoren,S.,Minchin,E.R.,Summerfield,R.J.,Cookson,C, Coombs, J. (1980). Carbon and Nitrogen metabolism in legume root nodules. Phytochemistry, 19:341-355.
14- Allen, M.B., Arnon, D.J. (1955) Studies on nitrogen fixing blue-green algae. I- growth and nitrogen fixation by *Anabaena cylindrical Lemm*. Plant Physiol. 30:366-372.
15- Salorzano, L. (1969). Determination of ammonia in natural water by the phenylhypochloritemethod. Limnology and Occanography, 14:799.
16- Oleary, M.H. (1988). Carbon isotopes in photosynthesis. Bioscience, 38(5):328-336.
17- Mackiney,G. (1941). Absorption of light by chlorophyll solution. J,Biol,Chem.,140:315.
18- Stewart,W.D.P., Fitzgerald,G.P., Burris,R.H. (1968). Acetylene reduction by nitrogen fixing blue-green algae. Arch Microbiol 62:336-348.
19- Rigano, VDIM, Vona, V., Fuggi, A, Rigano,C. (1981) effect of L - methionine–DL-Sulphoximine, a specific inhibitor of glutamine synthetase on ammonium and nitrate metabolism in the unicellular alga *Cylindium caldarium*. Physiol. Plant. 54:47–51.
20- Gordon,J.K., Brill,W.J. (1974). Depression of nitrogenous synthesis in the presence of excess of NH_4Biochem,Biophys Res. Commun., 59:967-971.
21- Ownby, J.D., Shannahan, M., Hood, E. (1979). Protein synthesis and degradation in *Anabaena*during Nitrogen Starvation. J. General microbiol., 110:255-261.
22- Rathnam, C.K.M., Zlinskas, B. (1977). Reversal of 3–(3,4–Dichlorophenyl) –1,1–Dimethylurea inhibition of Carbon dioxide fixation in spinach chloroplast and photoplast by dicarboxylic acid. Plant Physiology. Vol, 50, No. L: 51-53.