



## EFFECT OF COPPER ON THE ULTRASTRUCTURE OF MARINE PHYTOPLANKTON

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### Abstract

Electron microscopy technique was used to study the copper induced ultrastructural changes in the blue green alga, *Synechocystis salina*. Wislouch and golden yellow flagellate *Isochrysis galbana* Parke. The blue green alga exhibited significant deterioration in the cell's internal organisation. Presence of membrane whorls, disorganisation of cell envelop. Photosynthetic lamelleae at certain regions of the cell, reduction in cell size, increase in number of polyhedral bodies and absence of lipid inclusion was observed. Drastic change was not noticed in the golden yellow flagellate except for the presence of enlarged vacuoles and more number of osmophilic bodies. Toxicity and detoxification mechanisms are discussed and the utilization of electron microscopic analysis is established in the studies of metal toxicity at the cellular level

### Introduction

One of the most commonly observed effects of heavy metal poisoning is a change in cell size or morphology. This has been observed in a wide variety of organisms including representatives from the Chlorophyceae (Rosko and Rachlin, 1977) Chrysophyceae (Davies, 1974.) and Bacillariophyceae.

The application of electron microscopic morphometry to evaluate and quantify intracellular effects of heavy metals on phytoplankton became evident only after the publications of Weibel and Bolender (1973) and Sickgoat and Stoermer (1979). An understanding of the internal anatomy of cells exposed to heavy metals would provide a first step towards documenting the physiological effects of heavy metal pollution at the cellular level.

Except for the study on morphological aberrations in diatom cells (Thomas *et al* (1982-1984) Rai *et al* (1990) to date, there is no information on metal toxicity on the ultrastructural changes in marine phytoplankton used as live feed for marine bivalve larvae

The purpose of the present study is to examine the copper induced intracellular structural changes in *Synechocystis salina*, Wislouch and *Isochrysis galbana* Parke using transmission electron microscope.

### Materials and Methods

The two unicellular algae, *Synechocystis salina* Wislouch (blue green alga) and *Isochrysis galbana* Parke (golden yellow flagellate) were grown in Miquel's medium under a light intensity of  $34.6.1 \times 10^{15}$  quanta  $\text{cm}^2 \times \text{sec}^{-1}$  light /dark cycle of 10:14hrs. Temperature ranging from 24°C -30°C and a salinity of 15-20ppt for *S. salina* and 30-35 ppt for *I. galbana*. Cultures were obtained from CMFRI, Cochin laboratory collection. Cultures from the exponential phase were used for the toxicity tests. Batch cultures of *S. salina* and *I. galbana* were exposed to  $0.200. \mu\text{gml}^{-1}$  of copper and control cells to metal free distilled water. Cells were harvested, washed, fixed, embedded in spurr resin, sectioned and examined by transmission electron microscopy using a Carl Zeiss Electron microscope. Negative staining technique was adopted to study the fine structure of *I. galbana*. A drop of culture suspension was applied to formvar coated carbon grid stained with 0.5% ammonium molybdate, dried and observed in TEM.

**Literature cited**

- Anon 1978 A report on the survey of marine algal resources of Tamil Nadu 1971-1976  
CSMCRI pp 1-25
- Anon 1984 A report on the survey of marine algal resources of Andhra Pradesh 1979-1982  
CSMCRI pp 1-8
- Anon 1989 A report on the seaweed resources off the Tuticorin - Tiruchendur coast, Tamil Nadu CMFRI & CSMCRI pp. 1-19
- Anon 1990 A report on the seaweed resources off Tamil Nadu coast, India II. Sector - Alantalai to Manapad and Vembar to Nallatanni Tivu. CSMCRI & CMFRI pp. 1-30
- Anon 1993 A report on the seaweed resources off Tamil Nadu coast, India, III. Sector - Valinokkam - Keelakarai and Manapad - Kanyakumari 1989-1990 CMFRI & CSMCRI pp. 1-23
- Anon 1993 b A report on the seaweed resources off Tamil Nadu coast, India, IV Sector - Keelakarai - Rameswaram Island (Dhanushkodi) 1990-1991 CSMCRI & CMFRI pp. 1-20.
- Chauhan V.D. and V. Krishnamurthy 1968 An estimate of algin bearing seaweeds in the Gulf of Kutch  
Curr. Sci. 37: 648
- Chauhan, V.D. and O.P. Mairh 1978 Report on the survey of marine algae resources of Saurashtra coast.  
India Salt Res. Ind. 14 (2) 21-41
- Chennubhotla, V.S.K., B.S. Ramachandrudu, P. Kaladharan and S.K. Dharmaraja 1988 Seaweed resources of Kerala coast Aquatic Biology 7:69-74
- Dhargalkar, V.K. 1981 Studies on marine algae of the Goa coast Ph.D. thesis, Bombay University pp 1-186.
- Kaliaperumal N. 1993 Seaweed culture In Handbook on Aquafarming "Seaweed, Sea urchin and Sea cucumber, MPEDA, Cochin pp 1-22
- Kaliaperumal, N., V.S.K. Chennubhotla and S. Kalimuthu 1987 Seaweed resources of India CMFRI Bulletin 41:51-54
- Kaliaperumal, N., P. Kaladharan and S. Kalimuthu 1989 Seaweed and Seagrass resources. CMFRI Bulletin 43:162-175
- Kalimuthu, S. and N. Kaliaperumal, 1996 Commercial exploitation of seaweeds in India and need for their large scale cultivation Proc. Natl. Symp. on Aquaculture for 2000 AD Palani Paramount Publications, Palani pp 215-219
- Kalimuthu, S., N. Kaliaperumal J.R. Ramalingam and V.S.K. Chennubhotla 1990 Present status of seaweed exploitation and seaweed industry in India. Mar. Fish. Infor. Serv. T&E Ser. 103:7-8
- Kalimuthu, S., N. Kaliaperumal and J.R. Ramalingam 1995 Distribution of algae and seagrasses in the estuaries and backwaters of Tamil Nadu and Pondicherry. Seaweed Res. Utiln 17 (1&2): 79-86
- Mitra, G. 1946 Development of Chilka Lake, Cuttack
- Silas, E.G., and S. Kalimuthu 1987 Commercial exploitation of Seaweeds in India CMFRI Bulletin 41:55-59
- Silas, E.G. V.S.K. Chennubhotla and N. Kaliaperumal 1986 Seaweed resources, products and utilisation Seaweed Res. Utiln 9(1&2) : 11-24
- Untawale A.G., V.K. Dhargalkar, V.V. Agadi and T.G. Jagtap 1979 Marine algal resources of Maharashtra coast Tech. Report Natl. Inst. of Oceanography pp1-48
- Untawale, A.G., V.V. Agadi 1981 Distribution of marine algae in the littoral zone of Karnataka coast.

Table 5: Occurrence of agar, carrageenan and algin yielding seaweeds in other parts of Indian coast

Name of the seaweed	Place of occurrence
<b>Agarophytes</b>	
<i>Gelidiella acerosa</i>	Okha, Dwarka, Porbandar, Diu, Veraval, Lakshadweep and Andaman-Nicobar
<i>Gracilaria edulis</i>	Lakshadweep and Andaman-Nicobar
<i>G. crassa</i>	Andaman-nicobar
<i>G.corticata var corticata</i>	Dwaraka.Bombay.Karwar. Goa,Tikkoti Quilon.Varkala. Vizhinjam. Visakhapatnam.and Andaman-Nicobar
<i>G.foliifera</i>	Gopnath.Okha,Bombay Tikkoti, and Andaman-nicobar
<i>G.verrucosa</i>	Okha,Bombay,Goa,Chilka and Andaman-Nicobar
<b>Alginophytes</b>	
<i>Sargassum wightii</i>	Bombay,Goa,Alleppey.,Vizhinjam.and Andaman -Nicobar
<i>S.tenerrimum</i>	Gulf of Kutch,Okha,Dwaraka, Bombay, Goa,Karwar,Visakhapatnam and Andaman-Nicobar
<i>S. myriocystum</i>	Andaman-Nicobar
<i>S.ilicifolium</i>	Bombay,Goa,Karwar,Visakhapatnam,and Andaman -Nicobar
<i>S. cinereum var. berberifolia</i>	Gulf of Kutch, Bombay,Goa, Karwar,and Vishinjam.
<i>S. johnstonii</i>	Okha
<i>S.vulgare</i>	Dwaraka,Okha and Visakhapatnam
<i>S.duplicatum</i>	Lakshadweep and Andaman -Nicobar
<i>Turbinaria conoides</i>	Lakshadweep, and Nadaman -Nicobar
<i>T.ornata</i>	Dwarka, Lakshadweep and Andaman -Nicobar
<i>T.decurrens</i>	Andaman -Nicobar
<i>Cystoseira trinodis</i>	Okha,and Andaman -Nicobar
<i>Hormophysa triquetra</i>	Okha and Andaman-Nicobar
<b>Carrageenophytes</b>	
<i>Hypnea musciformis</i>	Gopnath,Okha,Dwarka, Bombay,Goa, Karwar,Visakhapatnam, Lakshadweep and Andaman-Nicobar
<i>H.valentiae.</i>	Bombay,Tikkoti, Vizhinjam and Lakshadweep

Table 4: Time-table for commercial harvest of economically important seaweeds from Tamil Nadu Coast

Name of the seaweed	Period of Occurrence	Suitable period for harvest
<b>Agarophytes</b>		
<i>Gelidiella acerosa</i>	Throughout the year	January to March July to September
<i>Gracilaria edulis</i>	--do--	January to March & August to September
<i>G. crassa</i>	--do--	--do--
<i>G. foliifera</i>	--do--	--do--
<i>G. corticata</i> var <i>corticata</i>	--do--	June to August November to December
<i>G. verrucosa</i>	March to November	May to August
<b>Alginophytes</b>		
<i>Sargassum wightii</i>	Through out the year	October to December
<i>S. myriocystum</i>	--do--	May to August
<i>S. ilicifolium</i>	--do--	July to September
<i>Turbinaria conoides</i>	--do--	October to December
<i>T. ornata</i>	--do--	--do--
<i>T. decurrens</i>	--do--	December -January
<b>Carrageenophytes</b>		
<i>Hypnea musciformis</i>	--do--	December to March.
<i>H. valentiae</i>	--do--	January to March

The commercial exploitation of seaweeds is concentrated for several years only along south Tamil Nadu coast. The natural resources of algin yielding seaweeds *Sargassum* and *Turbinaria* in Tamil Nadu coast are adequate. At present only about 50% of the standing crop is harvested and there is no paucity for these plants. The agar yielding seaweeds *Gelidiella acerosa*, *Gracilaria edulis*, *G. crassa*, *G. corticata* var. *coricata*, *G. foliifera* and *G. verrucosa*; algin yielding seaweeds *Sargassum* spp, *Turbinaria* spp, *Cystoserira trinodis* and *Hormophysa triquetra* and carrageenan yielding seaweeds *Hypnea musciformis* and *H. valentiae* growing in harvestable quantities in other parts of Indian coast, Lakshadweep and Andaman- Nicobar Islands (Table -5) may be exploited during their maximum growth periods to meet the raw material requirements of Indian seaweed industries and to conserve the seaweed beds of Tamil Nadu coast. This will provide additional employment to the people living in the coastal areas and to earn more foreign exchange to the country by promoting the export of seaweeds in semi processed form and value added seaweed products namely agar, sodium alginate and carrageenan.

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All these seaweeds are harvested since 1966 from the natural seaweed beds occurring in south Tamil Nadu coast from Rameswaram to Kanyakumari (Kalimuthu *et al* 1990 and Kalimuthu and Kaliaperumal 1996). Data were collected monthly from 1978 to 1995 on the quantity of seaweeds harvested from this area and landed at different centres. During the period 1978 to 1995, the quantity of agar yielding seaweeds *Gelidiella acerosa*, *Gracilaria edulis*, *G. crassa* and *G. foliifera* exploited in a year varied from 248 to 1289 tons (dry wt), algin yielding seaweeds *Sargassum* spp and *Turbinaria* spp from 651 to 5537 tons (dry wt) and all the above seaweeds from 1173 to 6420 tons (dry wt) depending on the availability of seaweeds in the natural beds and raw material requirements from the seaweed industries (Table 2). The total quantity of seaweeds landed in 1994 was 3571 tons (dry wt) consisting of 2867 tons of *Sargassum* spp, 256 tons of *Turbinaria* spp, 261 tons of *Gelidiella acerosa* and 187 tons of *Gracilaria edulis*. During the year 1995 out of 3006 tons (dry wt) of total seaweeds landed, *Sargassum* spp contributed 2249 tons, *Turbinaria* spp 307 tons, *Gelidiella acerosa* 232 tons, *Gracilaria edulis* 108 tons and *G. foliifera* 110 tons.

Many agar and algin manufacturing industries are coming up every year in India. As the demand for raw material of agar yielding plants is more and their natural resources are less in Tamil Nadu, the agarophytes *Gelidiella acerosa* and *Gracilaria edulis* are being over exploited. Because of extensive and unrestricted commercial harvest of these seaweeds throughout the year, there is depletion in the stock of these red algae in the seaweed beds of Tamil Nadu. So it is necessary to conserve the natural stock of these two agar yielding plants by adopting rational way of commercial exploitation.

Based on the studies made on the growth, fruiting behaviour, effect of repeated harvesting on the growth and phycocolloid contents of agar, algin and carrageenan yielding seaweeds of Tamil Nadu, a time - table for commercial harvest of these seaweeds is given in Tabel 4. In order to conserve the natural stock of economically important seaweeds of Tamil Nadu coast and also to get consistant crop every year, the seaweed collections have to follow this time - table. This will ensure the regeneration and regrowth of seaweeds by vegetative and reproductive growth to harvestable size plants in the next harvesting season by means of giving sufficient interval between one harvest and the other. A single harvest in a year is recommended for all species. However, the harvesting may be done twice in a year during the periods indicated in Table 4 for *Gelidiella acerosa* and *Gracilaria* spp in areas with rich growth of these algae.

## Discussion

The resources of *Gracilaria corticata* var. *corticata* available in exploitable quantity in the east and west coast of India remains unexploited at present. It may be harvested and used along with *Gelidiella acerosa* and other *Gracilaria* spp for agar production. Similarly *Gracilaria verrucosa* occurring abundantly in Chilka Lake, Pulicat Lake and in the estuaries and backwaters of Tamil Nadu, Pondicherry and other maritime states may be exploited and used for agar manufacture. The carrageenan yielding red algae *Hypnea musciformis* and *H. valentiae* occur abundantly in Gulf of Mannar islands, near shore areas, estuaries and backwaters in different localities of Tamil Nadu and other parts of Indian coast including Lakshadweep and Andaman - Nicobar Islands. This potential resource could be utilised for the production of carrageenan in India. Some precautionary measures have to be taken while collecting the seaweeds. The regeneration of algae takes place as long as basal remnants of plants are intact with the substratum. Hence harvest has to be made by pruning the plants leaving the basal part instead of removing the whole plants. The other seaweeds growing in the harvesting area should not be removed to avoid the damage to the seaweed ecosystem.

Table 3. Quantity of seaweeds landed (dry wt in tons) at different landing centres of Tamil Nadu during 1994 and 1995

	Sargassum spp		Turbinaria spp		Gelidiella acerosa		Gracilaria edulis		Gracilaria foliifera	
	1994	1995	1994	1995	1994	1995	1994	1995	1994	1995
Rameswaram	--	10	--	12	34	21	5	9	--	--
	85	65	--	5	49	52	24	13	--	--
Mandapam	40	87	--	30	--	--	44	40	--	110
	285	343	5	15	--	--	114	43	--	--
Seeniappa Darga	380	513	--	--	--	--	--	--	--	--
Periapatnam	800	320	55	--	--	--	--	--	--	--
Kilakikarai	960	765	156	220	102	79	--	3	--	--
	217	145	40	25	53	61	--	--	--	--
Valinokkam	75	1	--	--	--	--	--	--	--	--
	25	--	--	--	--	--	--	--	--	--
Tharavaikulam	--	--	--	--	23	19	--	--	--	--
	2867	2249	256	307	261	232	187	108	--	110

Table 2. Seaweed landings (dry weight in tons) from Tamil Nadu during 1978 to 1995

Agar yielding seaweeds		Algin yielding seaweeds				Total
<i>Gelidiella acerosa</i>	<i>Gracilaria edulis</i>	<i>Gracilaria crassa</i>	<i>Gracilaria foliifera</i>	<i>Sargassum spp</i>	<i>Turbinaria spp</i>	
288	395	--	--	3636	1021	5340
541	342	--	--	4256	1281	6420
247	213	--	--	3090	438	3988
131	117	--	--	2522	222	2992
102	225	--	--	3176	704	4207
293	291	85	--	2070	375	3114
210	320	96	--	780	235	1641
189	269	45	--	2096	385	2984
261	233	28	--	491	160	1173
217	317	34	--	868	250	1686
366	330	15	--	2605	523	3839
370	400	2	--	3106	459	4337
307	982	--	--	2867	224	4380
274	318	--	3	5000	160	5755
312	399	--	50	2921	122	3804
261	187	--	--	2867	256	3571
232	105	--	110	2249	307	3006



*Drupa margaritica*, *Janthira* sp., *Pyrene* sp., *Trochus* sp., *Turbo* sp., *Cronia* sp., *Chiton* ., and *Acanthychitona* sp. and Echinoderms like *Ophiocnemics* sp. are found sheltered in the sediments retained by the holdfast of macro algae.

### Discussion

The present study coincides with the earlier studies (Varma, 1960; Mahadevan and Nagappan Nair, 1967, Srinivasan, 1969 & 1973; Kaliaperumal and Pandian, 1984; and Nair *et al* 1986) made from the east coast mainly in the floral list. The study showed the occurrence of a rather rich diversity in flora which could be attributed to the less polluted environment this coast is having when compared to the other nearby areas. The inter-relationship between the fauna associated with algal community needs further study. Such a study will contribute much to the conservation of the fauna and judicious exploitation of the flora for human use.

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### Literature Cited

- Chapman, V.J. 1955 Aspects of the fauna and flora of the Azores VI. The density of animal life in the coralline alga zone *Ann. Mag. Nat. Hist.* 512:801-805
- Colman, J 1940 On the fauna inhabiting intertidal seaweeds *J. mar. biol. Ass. U.K.* 24: 129-183
- Mohan Joseph, M. 1978 Ecological studies on the fauna associated with economic seaweeds of South India 1. Species composition, feeding habits and inter-relationships *Seaweed Res. Utiln* 3:1-25.
- Kaliaperumal, N. and C. Pandian 1984 Marine algal flora from some localities of South Tamil Nadu Coast *J. mar. biol. Ass. India* 26:159-164
- Mahadevan, S. and K. Nagappan Nayar 1967 Under water ecological observations in the Gulf of Mannar of Tuticorin *J. mar. biol. Ass. India* 9(1): 147-163
- Nair, N.B., V. Sobha, R. Chandran, M. Rathi Ammal, P.I. Miranda, S. Maya and H. Surya Narayanan 1986 Algal resources of Kerala coast II Update list of Indian Marine algae *Aquatic Biol* 6:25-52
- Sarma, A.L.N. 1974 Phytal fauna of *Caulerpa taxifolia* and *C. racemosa* of Visakapatnam coast Indian *J. mar. Sci.* 3:155-164
- Sarma, A.L.N. and P.N. Ganapati 1972 Faunal associations of algae in the intertidal region of Visakapatnam *Proc. Indian nat. Sci. Acad. Part B* 38:380-396
- Srinivasan, K.S. 1969 *Phycologia Indica* Bot. Sur. of India, Calcutta Vol.I: 1-52
- Srinivasan, K.S. 1973 *Phycologia Indica* Bot. Sur. of India, Calcutta Vol.II: 1-50
- Strickland, J.D.H. and T.R. Parsons 1972 *A practical hand book of sea water analysis* Bull. 167 Fisheries Research Board of Canada, Ottawa
- Varma, R.P. 1960 Flora of the Pearl beds of Tuticorin *J. mar. biol. Ass. India* 2: 221-225