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Effects of Heavy Metals on the Growth and Survivability of vegetative cells and the Formation of Autospore mother cells in Chlorella variegata and Chlorella vulgaris

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Abstract

The present study was aimed to find out that to what extent the growth, and/or survivability and reproduction of *Chlorella variegata* and *Chlorella vulgaris* were affected in presence of different heavy metals (zinc, copper , manganese , nickel , cadmium, lead and mercury). Both species of *Chlorella* were easily available from local habitats, and grew and reproduce well in cultures. Both species were grown in BG 11 medium (Stanier et al., 1971). Their cultures were maintained in controlled culture condition at 22±1·C temperature and 2 K lux light intensity for 16 hours a day . In order to maintain them in actively growing condition, subculturing was done after every 10-15 days of inoculation

Keywords- *C.var. Chlorella variegata* *C.vul. Chlorella vulgaris*

INTRODUCTION

For algal growth, some heavy metals are essential, since they act as co-factor or as an activator for different enzymes (Van Assche and Clijsters, 1990), e.g, copper is an essential component of plastocyanin (Katoh et al., 1961), photosystem (Bishop,1964) and enzyme amine oxidase (Palenic and Morel , 1991); the zinc act as a prosthetic group for enzymes alkaline phosphatase carbonic anhydrase and lactic dehydrogenase (Price,1962; Coleman, 1984; Cembella et al.,1984), was required to maintain mRNA activity (Altman et al., 1968) and ribosomes integrity (Rai et al., 1981)

Growth of *C.vul.* was stimulated by 0.5 ppm of nickel and 0.1 ppm of cadmium, while that in *Haematococcus capensis* by 0.1 ppm of both nickel and cadmium (Hutchinson and Stokes, 1975).

However, heavy metals proved toxic when present in excess amounts.

OBJECTIVE

The present study was undertaken to see the effects of different heavy metals such as zinc, copper, manganese, nickel , cadmium, lead and mercury on the survivability of vegetative cells and the formation of autospore mother cells in *C.var.* and *C.vul.*

METHODOLOGY

The graded amounts of different heavy metals *viz.*, zinc, copper, manganese, nickel, cadmium, lead and mercury were mixed, separately with standard BG 11 medium prior to autoclaving , so as to prepare the solutions of desired concentrations of 1,10, 100 and 1000 ppm. In each case , pH of the medium was adjusted to 7.5.

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In order to observe the effects of different heavy metals on the growth of alga, the survivability of vegetative cells,and the formation of autospore mother cells, the seven-day-old actively growing vegetative cells of both spp. of *Chlorella* were used as a source of inoculum. Controls were maintained in standard BG 11 medium. All inoculated culture tubes were placed in the culture chamber and examined periodically upto 45 days of inoculation . The growth in alga was determined as usual.

The percentage of vegetative cells (living cells), autospores mother cells (enlarged cells having autospores) and dead cells (empty, hyaline cells) were determined by counting their numbers out of about 2000-3000 cells from each of three replicates.

RESULTS AND DISCUSSION

Copper, manganese , nickel, cadmium, lead and mercury , all , at 1 to 1000 ppm progressively inhibited the survivability of vegetative cells in both species of *Chlorella* used. (Table I)The vegetative cells of *C.var*. were more sensitive to manganese , nickel , cadmium, lead and mercury than of *C.vul*. However , the vegetative cells of *C.vul* were more sensitive to copper than of *C.var*. while those of both species were equally sensitive to 100 and 1000 ppm of zinc. *C.var*. can not tolerate 1000 ppm of mercury and lead even by 5 day but the same of nickel and manganese at least by 5 day and the same of cadmium , copper and zinc at least by 10, 15 and 30 day , respectively .(Table I) *C.vul*. can not to lerate 1000 ppm of mercury even by 5 day , the same of lead and copper at least by 5day, the same of manganese, nickel and cadmium by at least 15 day, and the same of zinc at least by 30 day. At 100 ppm of mercury vegetative cells of *C. var*. died quick without differentiation into any autospore mother cell . But vegetative cells of both *Chlorella* species do differentiate into autospore mother cells in most of the metals treatments given, where they were surviving. Autospore mother cells of both species seems to be slightly more tolerant to most of the metals than vegetative cells.

TABLE I: *C. VARIEGATA* AND *C. VULGARIS* PERCENTAGE SURVIVAL OF VEGETATIVE CELLS (VS) AND AUTOSPORE MOTHER CELLS (AM) REST DEAD CELLS, IF ANY WITH RESPECT TO CULTURE AGE IN HEAVY METALS

HEAVY METAL, PPM		CULTURE AGE, DAY	*C. VARIEGATA*		*C. VULGARIS*	
			VS	AM	VS	AM
ZINC	1	15	84	6	82	7
		30	81	7	79	8
		45	79	8	76	9
	10	15	83	6	81	7
		30	82	6	79	8
		45	79	8	77	8.5
	100	15	43	4	38	3.5
		30	23	3	23	3
		45	0	0	0	0
	1000	10	53	3	50	3
		15	21	2	21	0
		30	10	0	11	0
		45	0	0	0	0
COPPER	1	15	80	8	72	9
		30	76	9	69	6
		45	68	6	57	5
	10	15	72	11	68	10
		30	43	10	0	0
		45	38	5	-	-
	100	15	54	10	43	5
		30	28	11	0	0
		45	0	0	-	-

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HEAVY METAL, PPM		CULTURE AGE, DAY	C. VARIEGATA		C. VULGARIS	
			VS	AM	VS	AM
MANGANESE	1	15	73	8	75	12
		30	43	10	72	12
		45	18	12	37	14
	10	10	79	7	79	9
		15	67	10	73	12
		30	0	0	64	13
		45	-	-	0	0
	100	10	63	10	65	12
		15	52	12	58	13
		30	0	0	51	13
		45	-	-	0	0
NICKEL	1	15	56	11	62	11
		30	39	12	49	13
		45	23	13	46	12
	10	10	33	9	47	10
		15	16	9	26	10
		30	0	0	11	12
		45	-	-	0	0
	100	10	26	8	36	12
		15	14	8	17	12
		30	0	0	0	0

Contd...

HEAVY METAL, PPM		CULTURE AGE, DAY	C. VARIEGATA		C. VULGARIS	
			VS	AM	VS	AM
CADMIUM	1	15	59	8	72	9
		30	34	10	65	11
		45	0	0	53	12
	10	15	44	9	57	10
		30	0	0	47	12
		45	-	-	0	0
	100	10	29	9	54	10
		15	0	0	42	11
		30	-	-	32	12
		45	-	-	0	0
LEAD	1	15	57	10	58	11
		30	24	11	37	12
		45	0	0	0	0
	10	10	59	10	62	11
		15	46	12	50	12
		30	0	0	0	0
	100	10	52	10	55	12
		15	0	0	46	10
		30	-	-	0	0
MERCURY	1	10	40	11	56	10
		15	13	6	30	5
		30	0	0	0	0
	10	5	30	14	69	9
		10	0	0	43	7
		15	-	-	0	0
	100	5	24	0	58	9
		10	0	0	0	0

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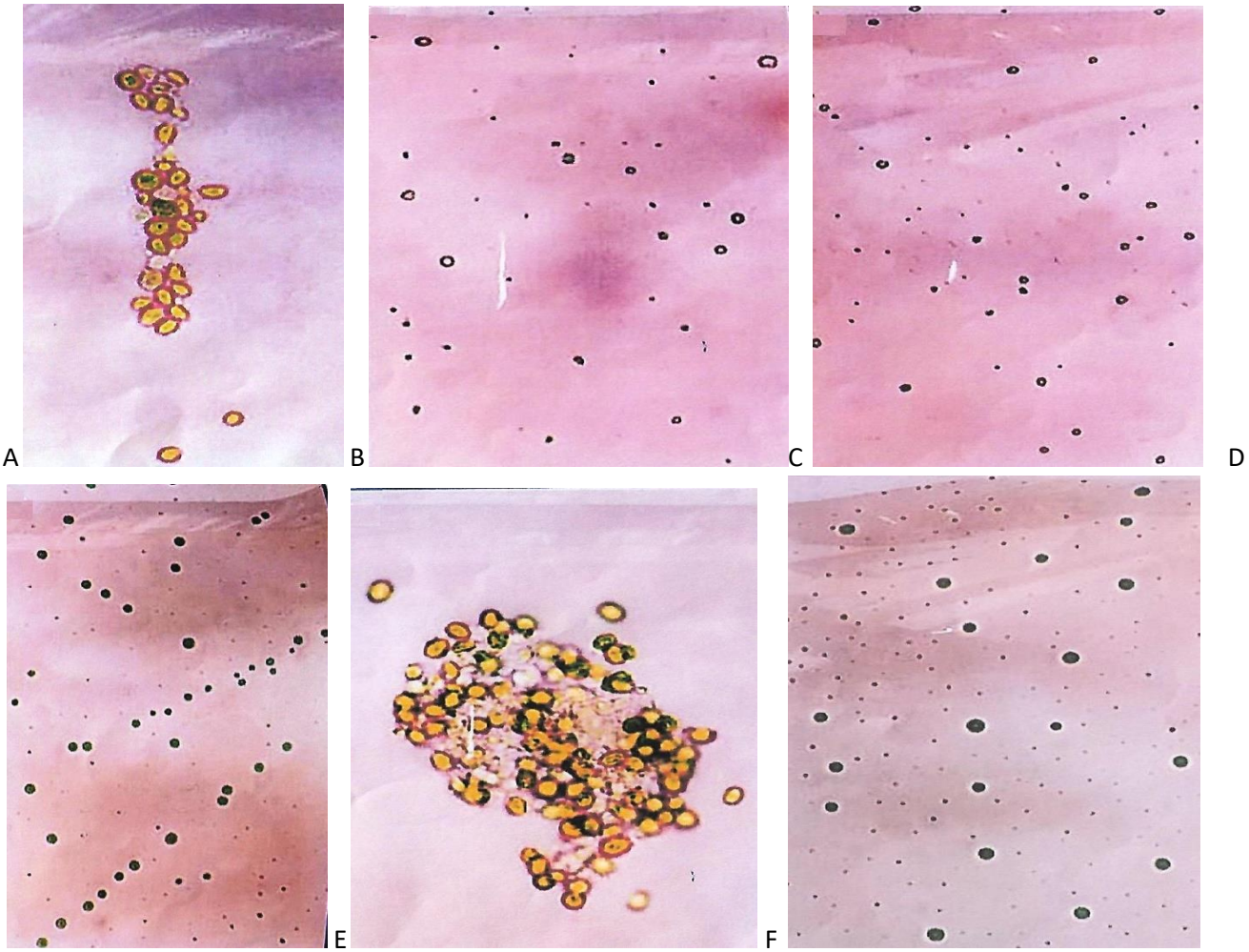

Fig.1. A: *C. vulgaris* at 10 ppm of zinc on 45 days, showing discolouration of cells.
B: C. *variegata* at 100 ppm of manganese on 30 days. cells are very much shrinked.
C*: C. variegata* at 100 ppm of nickel on 30 days. The cells have shrink and collapse.
D: *C. vulgaris* at 100 ppm of cadmium on 45 days. All cells have shrink very much.
E: *C. vulgaris* at 10 ppm of lead on 15 days. cells shows shrinkage of chloroplast and discolouration.
F: *C. vulgaris* at 100 ppm of mercury on 10 days. All cells have shrink very much.

In the present study , mercury and lead were most toxic metal to both species of *Chlorella* while zinc the least . The toxicity of nickel , cadmium, manganese and copper varied between two species of *Chlorella*.However , green algae *Stigeoclonium tenue* and *Cladophora glomerata* showed equal tolerance to copper, lead and zinc (McLean,1974).

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CONCLUSION –

1. Irrespective of a slight growth of alga at 1 and 10 ppm zinc, the alga maintained the different life cycle stages constant upto 45 days.
2. Zinc at 100 and 1000 ppm; copper and manganese at 10-1000 ppm and nickel, cadmium, lead and mercury, at 1-1000 ppm inhibited growth and decreased vegetative survival, progressive in both species of *Chlorella* used.
3. Small sized, less pigmented, *C.var.* vegetative cells were more sensitive to metals manganese, nickel, cadmium, lead and mercury than comparatively large sized, more pigmented *C.vul.* cells. However both species were similarly sensitive to zinc. But *C.vul* . was more sensitive to copper than *C.var* (Fig. 1.).
4. Presence of autospore mother cells till the survival of vegetatative cells in most of the metals treatment indicate that the vegetative cells do differentiate into autospore mother cells even in presence of metals.
5. An increased ratio of autospore mother cells to vegetative cells in most of the metals treatments than in controls indicate that autospore mother cells were slightly more resistant to most of the metals than vegetative cells.
6. Mercury and lead were most toxic metals while zinc the least to both species of *Chlorella* used.

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