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Toxicities of trace metals on *Chlorella vulgaris* isolated from palm oil mill sludge

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Studies on the influences of ten trace metals, viz. Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn, at different concentrations, i. e., 0.5, 1, 1.5, 2.5, 5, 10, 15, 25, 50, 100, 200 and 300 ppms, on the proliferation of *Chlorella vulgaris* isolated from scum of palm oil mill sludge indicated that at most instances the productivity maxima occur after a culture period of 15 days as compared to the control which is 10 days. A definite pattern of inhibition by concentration was only observable for the trace metals of Cd, Cr and Cu while the other elements reflected independent effects.

It was found that the toxicities of the trace metals examined on the proliferation of *Chlorella vulgaris* fall into the following category: Cu>Cr>Cd=Zn>Co>Fe=Pb>Ni>Hg>Mn.

Key Index Words: Chlorella; *palm oil mill sludge*; *toxicity*; *trace metals.*

The production of palm oil is of prime national interest from the viewpoint of economics as a foreign exchange earner in the Malaysian context. Concomitantly, it is also the major pollutant of our aquatic environment culminating in at least 42 rivers been grossly polluted while 16 others fairly. Numerous attempts have been made to eliminate this organic pollution problem by local scientists (COLLIER and CHICK 1977, DALZELL 1977, MUTHURAJAH and DEVENDRAN 1975, RAJAGOPALAN and SIVALINGAM 1975, SEOW 1977, WEBB *et al.* 1975). However, these approaches are yet to meet the legislation promulgated by the Ministry of Environment, Science and Technology, Malaysia (MAHESWARAN 1978).

In this connection, SIVALINGAM *et al.* (1979) and SIVALINGAM (1980) have demonstrated that through the propagation of *Chlorella vulgaris* isolated from the scum of palm oil mill sludge the B. O. D. load could be lowered from 1,080 ppm to 40 ppm. It is envisaged that with the availability of this new information and other presently investigated technology the overcoming of this agro-based industrial pollution problem is just round the corner.

Knowledge on this strain of *Chlorella vulgaris* which has the capability to sustain under such noxious conditions is still lacking. Hence, the author has attempted to verify toxicity effects of various trace metals on its proliferation at different concentration levels with time. The results of this investigation are presented here.

Materials and Methods

Isolated *Chlorella vulgaris* (Fig. 1) cells from palm oil mill sludge (SIVALINGAM *et al.* 1979, SIVALINGAM 1980) were used in all the experiments that are to follow. Experiments on the effects of the various trace metals were performed in the most suitable dilution by 4 times of fermented palm oil mill sludge. The various trace metals used in the experiments were salts

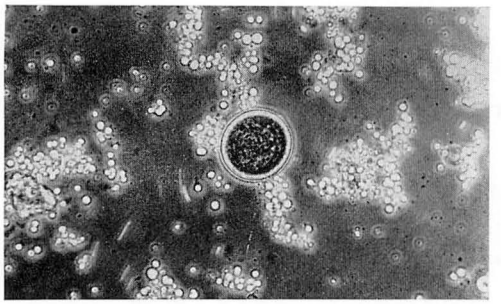

Fig. 1. Isolated *Chlorella vulgaris* cells from the scum of palm oil mill sludge (200 × magnification).

of the following: $CdCl_2\frac{1}{2}H_2O$, $CoCl_26H_2O$, CuCl, $K_2Cr_2O_7$, $FeCl_3$, $HgCl_2$, $MnCl_24H_2O$, $NiCl_2H_2O$, $Pb(NO_3)_2$ and $ZnCl_2$, at concentrations of 0.5, 1, 1.5, 2.5, 5, 10, 15, 25, 50, 100, 200 and 300 ppms, respectively. As blank the culture medium did not contain any of the trace metals. Each amendment of the trace metals concentration were performed in triplicates and their mean propagation of innoculated *Chlorella vulgaris* cells were followed periodically every five days for a period of 25 days. The number of cells for each innoculation was 3.8×10^3/m*l* of the culture medium. All the above-mentioned culture experiments were performed in a "Nikko Tron" at 17,000 lux actinic light intensity with a day-light period of 12 hours and chamber temperature of 28°C.

Results

Tables 1–10 demonstrate the various toxicity effects of the trace metals examined. It is evident that almost all the concentrations of the trace metals have a maximum productivity period on the 15th day of culture as compared to the reference experiment of 0 trace metal treatment which is only 10 days. These productivity peaks are greater than the control in the following order: Cr, 1.26, 1.167 and 1.07 times for the 0.5, 1.0 and 1.5 ppm concentrations, Co; 1.118, 1.118, 1.216, 1.07 and 1.677 times for the 1.5, 5, 10, 25 and 200 ppm concentrations, Cr; 1.26 and 1.67 times for the 0.5 and 1.0 ppm concentrations, Fe; 1.216, 1.216, 1.507, 1.751, 1.217, 1.216 and 1.119 for the 0.5, 2.5, 10, 15, 50, 100 and 200 ppm concentrations, Hg; 1.459, 1.41, 1.459, 1.459, 1.07, 1.119, 1.119, 1.119 and

Table 1. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of cadmium with time

Conc. (ppm)	Cell No. ($\times 10^3$)/m*l* with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	8.42	7.37	3.913	3.911
0.5	3.8	5.897	5.16	10.647	4.586	2.62
1.0	3.8	2.621	3.302	9.828	5.897	3.276
1.5	3.8	5.897	2.889	9.009	5.241	3.931
2.5	3.8	3.931	3.715	4.504	3.276	2.62
5	3.8	3.275	4.128	5.733	3.276	2.62
10	3.8	2.621	4.128	6.961	4.586	1.966
15	3.8	3.931	3.096	8.190	3.276	3.276
25	3.8	3.275	3.715	7.371	3.276	2.62
50	3.8	6.552	3.302	6.142	3.276	3.931
100	3.8	1.310	4.128	6.142	3.276	1.31
200	3.8	3.275	3.096	4.504	3.276	2.62
300	3.8	2.621	4.128	6.142	3.276	1.966

—— : productivity maximum

Table 2. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of cobalt with time

Conc. (ppm)	Cell No. ($\times 10^3$)/m*l* with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	8.42	7.37	3.931	3.911
0.5	3.8	1.310	4.953	8.190	4.586	2.62
1.0	3.8	0.655	4.54	7.371	5.897	1.966
1.5	3.8	0.655	5.366	9.418	1.310	2.62
2.5	3.8	0.655	4.334	6.125	3.276	1.31
5	3.8	0.012	5.16	9.418	4.581	1.966
10	3.8	0.655	3.096	10.237	5.897	1.966
15	3.8	1.310	5.779	7.371	3.936	2.62
25	3.8	0.015	5.16	9.009	6.552	3.276
50	3.8	0.013	4.128	7.780	6.552	2.62
100	3.8	0.011	3.302	6.142	6.552	2.62
200	3.8	0.045	5.779	9.828	3.276	1.966
300	3.8	0.032	3.096	2.866	2.621	1.31

—— : productivity maximum

Table 3. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of chromium with time

Conc. (ppm)	Cell No. ($\times 10^3$)/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	8.42	7.37	3.931	3.911
0.5	3.8	5.241	5.159	10.237	9.172	3.276
1.0	3.8	3.931	6.191	9.828	7.862	3.931
1.5	3.8	3.275	5.572	7.780	7.207	3.931
2.5	3.8	3.275	5.159	7.780	6.552	3.931
5	3.8	3.275	2.064	4.095	6.552	2.62
10	3.8	2.621	1.445	2.457	2.621	1.31
15	3.8	2.621	1.0319	2.860	2.621	1.31
25	3.8	1.966	1.445	1.638	3.276	1.31
50	3.8	1.966	1.0319	2.457	2.621	1.31
100	3.8	2.097	1.238	1.228	2.621	0.655
200	3.8	2.293	1.857	2.047	2.621	1.21
300	3.8	2.293	1.657	1.638	1.966	1.31

— · —: productivity maximum

Table 4. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of copper with time

Conc. (ppm)	Cell No. ($\times 10^3$)/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	8.42	7.37	3.931	3.911
0.5	3.8	3.931	5.572	15.560	6.552	3.931
1.0	3.8	1.966	4.747	6.901	5.241	3.931
1.5	3.8	0.378	4.128	7.371	5.897	3.276
2.5	3.8	0.021	2.064	4.095	5.241	1.21
5	3.8	0.022	2.064	4.095	3.936	1.966
10	3.8	0.025	1.238	3.685	3.936	1.966
15	3.8	0.023	1.0319	4.095	3.276	1.966
25	3.8	0.021	1.0319	5.323	1.966	2.62
50	3.8	0.027	1.0319	4.914	1.966	2.62
100	3.8	0.024	1.0319	5.323	1.966	0.655
200	3.8	0.020	1.238	3.685	3.276	0.655
300	3.8	0.019	1.651	2.647	5.897	0.542

——: productivity maximum

Table 5. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of iron with time

Conc. (ppm)	Cell No. ($\times 10^3$)/mℓ with incubation time (Days)					
	0	5	10	15	20	25
0	3.8	3.735	8.42	7.37	3.931	3.911
0.5	3.8	2.621	6.191	10.237	3.276	3.931
1.0	3.8	2.621	5.159	7.780	3.276	3.931
1.5	3.8	0.655	6.191	8.190	5.241	2.62
2.5	3.8	0.655	5.366	10.237	3.931	2.62
5	3.8	1.310	4.411	8.190	5.897	3.276
10	3.8	0.561	6.312	12.694	4.586	3.276
15	3.8	3.275	7.842	14.741	7.862	5.897
25	3.8	2.621	7.223	7.371	5.897	5.241
50	3.8	3.931	7.223	10.237	7.862	5.897
100	3.8	3.276	6.604	10.237	9.828	6.552
200	3.8	2.621	5.572	9.418	6.552	6.552
300	3.8	3.256	6.191	8.190	5.897	5.897

– – –: productivity maximum

Table 6. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of mercury with time

Conc. (ppm)	Cell No. ($\times 10^3$)/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	8.42	7.37	3.931	3.911
0.5	3.8	2.621	5.159	12.286	7.207	3.276
1.0	3.8	3.931	4.128	11.875	4.586	3.931
1.5	3.8	5.241	3.508	12.286	6.552	3.931
2.5	3.8	3.931	4.953	12.286	5.897	3.931
5	3.8	0.655	2.063	9.009	4.581	2.62
10	3.8	0.655	3.090	9.418	3.276	3.276
15	3.8	0.321	2.063	6.142	2.621	1.966
25	3.8	0.378	2.063	8.190	1.966	1.21
50	3.8	0.378	1.0318	9.418	3.276	2.62
100	3.8	0.983	1.0318	9.418	2.621	1.966
200	3.8	0.421	0.826	8.190	3.936	1.966
300	3.8	2.621	2.063	9.148	3.276	1.21

——: productivity maximum

1.119 times for 0.5, 1.0, 1.5, 2.5, 5.0, 10, 50, 100 and 300 ppm cencentrations, Mn ; 1.702, 1.605, 1.605, 1.799, 1.508, 1.264, 1.653, 1.167, 1.167, 1.216, 1.362 and 1.459 times for the 0.5, 1.0, 1.5, 2.5, 5, 10, 15, 25, 50, 100, 200 and 300 ppm concentrations, Ni ; 1.07, 1.264, 1.654, 1.09, 1.216, 1.313, 1.313 and 1.012 for the 0.5, 1.0, 2.5, 5, 10, 15, 25 and 100

Table 7. Proliferation of *Cholrella vulgaris* on exposure to different concentrations of manganese with time

Conc. (ppm)	Cell No. ($\times 10^3$)/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	8.42	7.37	3.931	3.911
0.5	3.8	5.241	7.223	14.33	7.207	3.931
1.0	3.8	3.931	6.398	13.512	5.241	3.276
1.5	3.8	2.621	6.191	13.512	5.897	3.276
2.5	3.8	2.621	6.811	15.151	5.897	3.276
5	3.8	3.276	7.223	12.694	5.897	3.276
10	3.8	2.621	7.223	10.647	6.552	4.586
15	3.8	2.621	7.223	13.922	6.552	6.531
25	3.8	1.966	6.191	9.828	6.552	10.483
50	3.8	2.621	6.191	9.828	5.241	1.966
100	3.8	2.621	7.223	10.237	9.172	6.522
200	3.8	1.310	6.604	11.467	7.862	1.966
300	3.8	0.655	6.604	12.285	7.207	2.62

——: productivity maximum

Table 8. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of nickel with time

Conc. (ppm)	Cell No. ($\times 10^3$)/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	8.42	7.37	3.931	3.911
0.5	3.8	6.552	5.159	9.009	3.936	3.276
1.0	3.8	6.552	4.540	10.647	3.276	2.62
1.5	3.8	6.552	4.540	8.190	4.586	2.62
2.5	3.8	4.586	4.953	13.922	4.586	3.931
5	3.8	6.552	5.779	9.009	3.276	3.276
10	3.8	3.275	4.747	10.237	3.936	2.62
15	3.8	3.275	4.747	11.056	4.581	3.276
25	3.8	3.275	4.128	11.056	3.276	2.62
50	3.8	1.310	2.683	4.504	3.276	2.62
100	3.8	3.275	2.477	3.685	8.517	1.21
200	3.8	1.310	2.064	3.276	1.966	1.21
300	3.8	2.621	1.857	2.866	2.621	1.966

——: productivity maximum

Table 9. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of lead with time

Conc. (ppm)	Cell No. ($\times 10^3$)/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	8.42	7.37	3.931	3.911
0.5	3.8	3.275	6.191	14.133	3.276	3.261
1.0	3.8	3.275	5.159	14.131	3.276	3.258
1.5	3.8	3.275	5.159	14.132	5.241	4.013
2.5	3.8	3.275	4.128	9.009	3.931	2.911
5	3.8	2.621	4.128	6.961	5.897	3.623
10	3.8	1.966	3.715	7.371	4.586	2.431
15	3.8	1.310	5.159	9.009	7.862	5.821
25	3.8	1.310	4.128	7.371	5.897	3.897
50	3.8	1.966	7.842	9.148	7.862	3.213
100	3.8	0.655	5.159	14.133	9.828	6.714
200	3.8	0.655	5.366	16.379	6.552	2.381
300	3.8	2.621	4.334	18.427	5.897	3.438

——: productivity maximum

Table 10. Proliferation of *Chlorella vulgaris* on exposure to different concentrations of zinc with time

Conc. (ppm)	Cell No. ($\times 10^3$)/mℓ with incubation period (Days)					
	0	5	10	15	20	25
0	3.8	3.735	8.42	7.37	3.931	3.911
0.5	3.8	3.276	6.604	10.237	5.897	5.241
1.0	3.8	3.276	5.779	6.961	8.517	2.62
1.5	3.8	3.931	6.191	12.285	6.552	3.276
2.5	3.8	2.621	3.715	8.190	7.207	1.966
5	3.8	1.966	2.064	12.694	3.276	1.31
10	3.8	1.310	2.064	2.047	5.241	1.21
15	3.8	1.310	2.477	4.095	3.276	0.655
25	3.8	1.310	1.857	18.427	3.276	0.655
50	3.8	0.655	1.445	4.504	3.276	1.21
100	3.8	0.655	2.064	5.733	3.936	0.655
200	3.8	3.276	2.064	4.095	3.276	0.655
300	3.8	2.621	2.064	2.866	3.276	0.665

——: productivity maximum

(20-day culture period) ppm concentrations, Pb; 1.679, 1.678, 1.679, 1.07, 1.07, 1.679, 1.945 and 2.189 times for the 0.5, 1, 1.5, 2.5, 15, 100, 200 and 300 ppm concentrations and Zn; 1.216, 1.012, 1.459, 1.508 and 2.188 times for the 0.5, 1 (20-day culture period), 1.5, 5 and 25 ppm concentrations.

Hence it is evident that at certain con-

centrations of the different trace metals they act as a stimulant instead of a toxicant as compared to the contents though the maximum productivity is delayed by 5 days.

Discussion

This study obviously indicates that the toxicities of the various trace metals are such that the peak of maximum productivity is shifted by 5 days as a delayed phenomenon. Also, it is noticeable that most of the trace metals indicate some sort of an inhibitory action at the early stages of culturing since normally there is a drop in cell numbers as compared to the initial inoculation.

The increase in cell numbers at the 15-day cultures of the trace metal experiments as compared to the maximum of the controls at 10-day cultures is of great interest. This could be attribute to the fact that the metals could be acting as a "kink" in its life cycle. The thought of probably excreted products by *Chlorella vulgaris* capable in complexing with metals and rendering the metals less toxic as suggested by OVERNELL (1976) should also be considered. Further, this algal species could also be less sensitive to toxic metals as compared to *Chlorella pyrenoidosa* as reported by WONG *et al.* (1979), SCHROLL (1978) and for other algae by SPARLING (1968).

Finally, the author is of the opinion that this strain of *Chlorella vulgaris* isolated from the scums of palm oil mill sludge appears to be fairly tolerant to high concentrations of trace metals contaminations and that studies should be performed to verify whether the organism bioaccumulates them or there in reality exists a mechanism of complexing by excreted extracellular substances to curb the toxicity effects of the trace metals.

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P. M. シバリンガム：ヤシ油工場汚泥から分離された *Chlorella vulgaris* に及ぼす微量金属の影響

ヤシ油工場汚泥表面から分離された *Chlorella vulgaris* の生長に及ぼす10種類の微量金属の影響を研究した。使用した金属はカドミウム，コバルト，クロム，銅，鉄，水銀，マンガン，ニッケル，鉛，亜鉛で，それぞれ 0.5, 1, 1.5, 2.5, 5, 10, 15, 25, 50, 100, 200, 300 ppm の濃度であった。

Chlorella rulgaris の生長に及ぼす微量金属の毒性は次のような順序となる：Cu>Cr>Cd=Zn>Co>Fe=Pb>Ni>Hg>Mn. (School of Biological Sciences, Universiti Sains Malaysia, Minden, Pulau Pinang, Malaysia)