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Cladophora as a prominent global algal monitor for trace metal pollutants (2) Long-term low concentration stresses, its biodeposition and depuration

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Long-term, i. e. 30 days, low concentration biodeposition studies of trace metals in *Cladophora fascicularis* indicated their maximal bioaccumulation factors having a sequential order of 2 ppm stress lots>5 ppm stress lots>10 ppm stress lots for Cd, Co, Cr, Cu, Fe, Mn and Ni-though their maximal bioaccumulation periods vary: Cd; 600.00 (22 days), Co; 425.00 (30 days), Cr; 78.00 (24 hr), Cu; 1037.05 (22 days), Fe; 1105.00 (6 days), Mn; 23.00 (24 hr) and Ni; 790.00 (22 days). In the case of the trace metals Pb and Zn this maximal is observed in the 5 ppm lots: Pb; 620.00 (26 days) and Zn; 790.00 (30 days). This information confirms the previous study at high concentration stresses whereby the tendency of this algal species to bioaccumulate trace metals at lower concentrations is verified.

Continuous subsequent long-term, i. e. 30 days, studies on their depuration following those of bioaccumulations indicated linear decreasing trends in biodischarge of the trace metals at all concentrations with maximal depuration rates (%) as follows: Cd; 47.1 (2 ppm), Co; 81.9 (10 ppm), Cr; 95.4 (5 ppm), Cu; 48.4 (5 ppm), Fe; 51.6 (10 ppm), Mn; 78.6 (10 ppm), Ni; 61.4 (10 ppm), Pb; 87.0 (2 ppm) and Zn; 88.6 (5 ppm).

The modes of biodeposition under low concentration stresses as compared to those at high concentration stresses, i. e. 50-500 ppms, are much diversified demonstrating a substantially different biochemical and physiological interaction within the algal thallus. Continued exposure for a further 30 days complicates the issue.

Key Index Words: *algal monitor*; Cladophora; *pollutants*; *trace metals*.

In a previous manuscript (1981) the authors have demonstrated the feasibility of employing *Cladophora* spp. on a global basis in the form of a "*Cladophora* Monitoring Programme" for trace metal contaminants due to its capacity to bioaccumulate fairly large amounts from a very low concentration of medium and even the comparability of certain biodeposited elements to those existing in sediments within its natural habitat. Related high concentration experimental stress studies over a period of 48 hours to trace metals between 50-500 ppms indicated Cu, Fe, Mn, Ni, Pb and Zn as having their highest bioaccumulation factors at the lowest concentration such as 50 ppm while Cd, Co and Cr were at 100 ppm. Similarly, their modes of biodeposition generally reflected rapid biodeposition between 12-24 hours followed by a depuration and then a gradual increase in the biodeposition processes. Based on this information and the tolerability of *Cladpohora fascicularis* to high concentrations of trace metals without any physical damage, it was postulated that the biodeposition and depuration processes of trace metals in this alga are greatly dependent on the possible physiological conditions prevalent within the algal membrane and that they may be related to metabolic regulatory mechanisms.

As an extension of the forementioned studies, and as the second stage the authors have endeavoured further to clarify the unknown bioaccumulation mechanisms of trace metals in *Cladophora fascicularis* experimentally at low concentration stress exposures between 2-10 ppms over a period of 30 days followed consequently by a depuration period of the same duration. Here in this report, the results are presented as a further support to the proposal of the "*Cladophora* Monitoring Programme".

Materials and Methods

Cladophora fascicularis (MERT.) KÜTZING was harvested during low tides off the rocky shores of Batu Ferringhi, Penang Island between July-December, 1978 (Sivalingam and Rodziah Ismail, 1981). The algae harvested were brought immediately to the laboratory in a plastic container. Prior to experimentation, the algal fronds were carefully cleaned of epiphytes and contaminants and washed thoroughly 3 times in membrane-filtered sea water. Adequate amounts of these thalli were cultured over a period of one month in separate 1 liter Elenmeyer flasks containing membrane-filtered sea water together with the relevant trace metals at low concentrations of 2, 5 and 10 ppms each, supplemented with salts such as $CdCl_2$. $2^1/_2H_2O$, $CoCl_2$. $6H_2O$, $K_2Cr_2O_7$, CuCl, $FeCl_3$, $MnCl_2$. $4H_2O$, $Pb(NO_3)_2$ and $ZuCl_2$, respectively. All culture flasks were incubated in a "Nikko Tron" growth chamber with a 12 hr light-dark periodicity and at a constant temperature of 20°C. Normally, triplicate experiments at each concentration of the trace metals were carried out. Actinic light with an intensity of 13,500 lux was used as light source.

The bioaccumulation modes of trace metals was followed by sampling adequate amounts of the thalli from the culture media at fixed time intervals of 3, 6, 12 and 48 hr and 6, 10, 14, 18, 22, 26 and 30 days. Suitable nutrient conditions and constant levels of the trace metals in the media were maintained by replacing the media regularly every five days with the appropriate concentration of the heavy metals. After 30 days of experimentation on biodeposition, the vessels were emptied of their media containing heavy metals and filled with fresh membrane-filtered sea water to initiate depuration (=biodischarge) experiments. The modes of depuration were followed by harvesting the fronds at time intervals identical to the bioaccumulation experiments. In this experiment too, the media was constantly replaced with fresh membrane-filtered sea water every five days. The sampled fronds were initially thoroughly washed 3 times in membrane-filtered sea water of 1 liter each and subsequently with triple distilled water of the same volume prior to drying at 100°C for 48 hr over an air oven.

The dried algal fronds were then pulverized separately using a pestle and mortar. A given amount of this algal powder was predigested overnight in 100 ml Kjeldahl flasks containing 10ml of a mixture solution of nitric : perchloric acid (2 : 1). The samples were further digested the following day under low heating followed by vigorous boiling over an electrothermel heater until white fumes evolved. The digest on cooling was diluted with distilled water and filtered through Whatman No. 1 filter paper. The filterate was then made-up to 100 ml with distilled water for analysis using a Varian Techtron (AA 120) Atomic Absorption Spectrophotometer.

The obtained bioaccumulated values were calculated as dry weight gm^{-1} algae with the aid of standard curves. For precision, the background levels of trace metals content in *Cladophora fascicularis* from its natural habitat were subtracted from the values (SIVALINGAM and R. ISMAIL 1981) They were then plotted as modes of bioaccumulation/depuration (=biodischarge) with incubation times. These bioaccumulation/

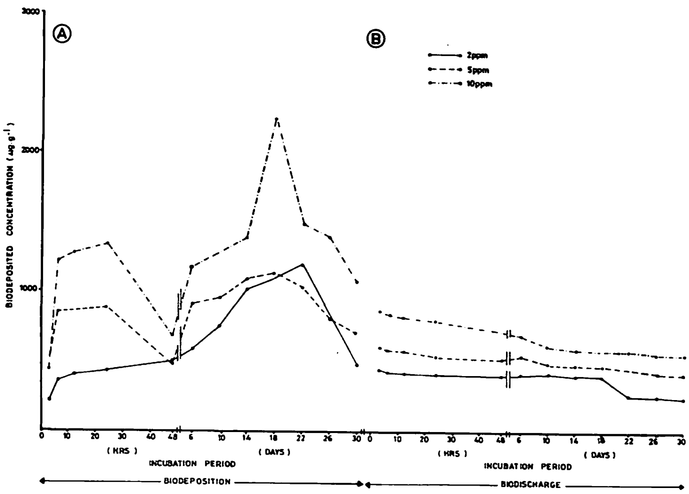

Fig. 1. Ⓐ Modes of bioconcentration of the trace metal Cd by *C. fascicularis* when subjected to three different low concentration stresses for an incubation period of one month.

Fig. 1. Ⓑ Modes of biodischarge of the trace metal Cd by *C. fascicularis* after been subjected to three different low concentration stresses for an incubation period of one month.

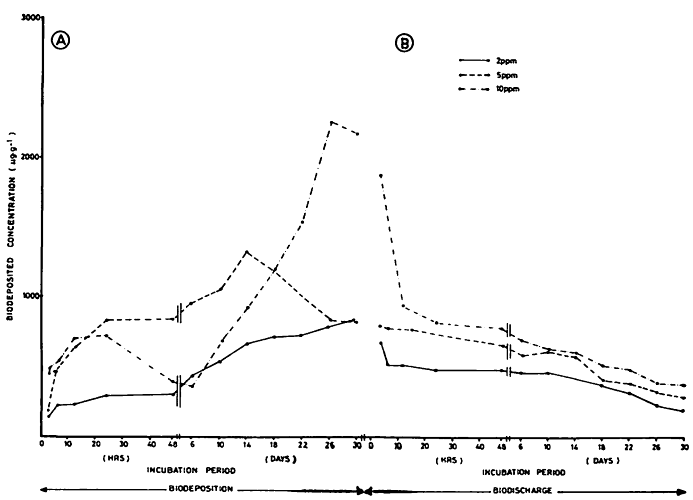

Fig. 2. Ⓐ Modes of bioconcentration of the trace metal Co by *C. fascicularis* when subjected to three different low concentration stresses for an incubation period of one month.

Fig. 2. Ⓑ Modes of biodischarge of the trace metal Co by *C. fascicularis* after been subjected to three different low concentration stresses for an incubation period of one month.

depuration values were then calculated for their bioaccumulation/depuration factors (= BF or DF) at each monitoring period by division with the concentration of the relevant trace metal in the culture media. Further, the depuration rates at each concentration of the trace metals were computed by the division of the 30th day bioaccumulation factors of the relevant trace metals with the corresponding 30th day depuration factors.

Finally, based on the modes of biodeposition, a general pattern of biodeposition series was compiled for the periods between 3-48 hours and 48 hours-30 days of exposure in order to envisage the possible biochemical and physiological mechanisms involved.

Results

Figs. 1-9 demonstrate the various modes of biodeposition and depuration of the nine trace metals at low concentration stresses over experimental exposure periods of 30 days each. It is evident that the modes of biodeposition between the initial 3-48 hrs as compared to those between 6-30 days are fairly different for most trace metals.

In contrast the depuration modes are relatively similar for all trace metals-reflec-

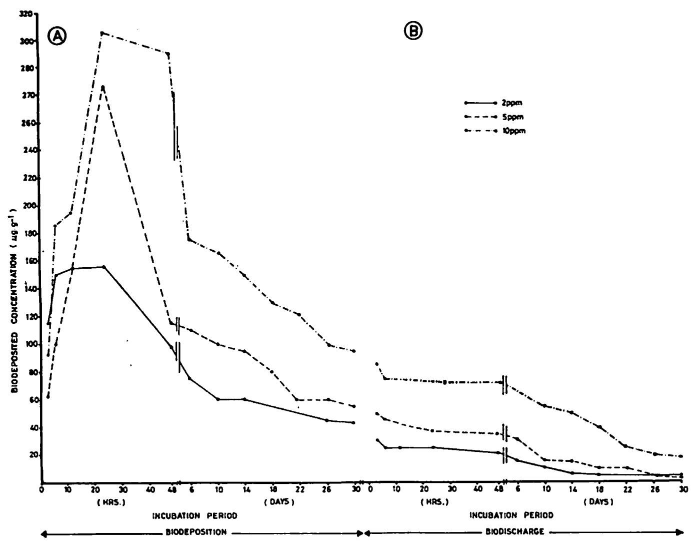

Fig. 3. Ⓐ Modes of bioconcentration of the trace metal Cr by *C. fascicularis* when subjected to three different low concentration stresses for an incubation period of one month.

Fig. 3. Ⓑ Modes of biodischarge of the trace metal Cr by *C. fascicularis* after been subjected to three different low concentration stresses for an incubation period of one month.

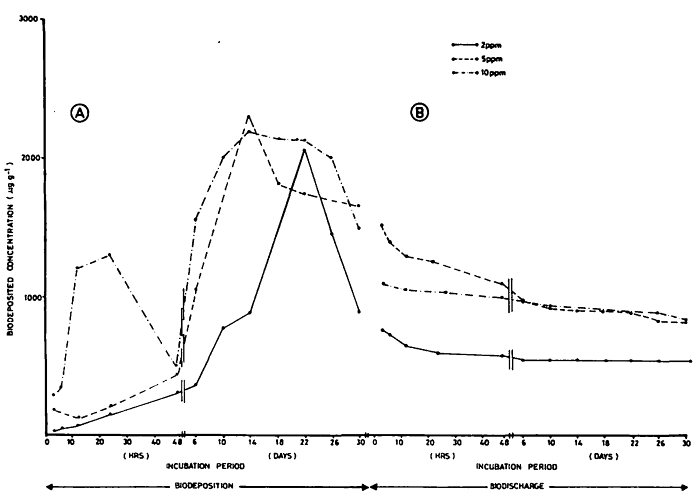

Fig. 4. Ⓐ Modes of bioconcentration of the trace metal Cu by *C. fascicularis* when subjected to three different low concentration stresses for an incubation period of one month.

Fig. 4. Ⓑ Modes of biodischarge of the trace metal Cu by *C. fascicularis* after been subjected to three different low concentration stresses for an incubation period of one month.

ting only a continuous linear decreasing trend in the biodeposited amounts of the 30 day bioaccumulation experiments. These modes of depuration for the 3 concentration stresses generally follow the sequence of 2 ppm > 5 ppm > 10 ppm except for Mn and Ni which manifest the following categories- 10 ppm > 5 ppm > 2 ppm and 10 ppm > 2 ppm > 5 ppm, respectively.

The modes of biodeposition during the first 48 hr incubation period manifest grossly a) a continuousincrease in biodeposition at all times, b) a sharp increase at the 6th/12th hours followed by a depuration and then a continuous increase, c) a sharp increase until the 6th/12th/24th hours followed by a gradual increase thereafter, d) a sharp increase until the 24th hour followed by a continuous depuration and e) a sharp increase until the 6th hour followed by a steady-state until the 24th hour ending in continuous depuration. With regard to the modes thereafter until the 30 day period, they are quite different from that of the forementioned 48 hr depuration and can be categorized as follows, a) either a

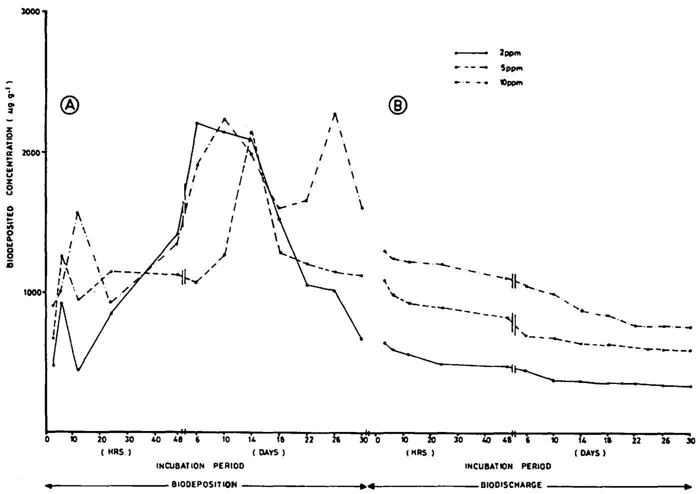

Fig. 5. Ⓐ Modes of bioconcentration of the trace metal Fe by *C. fascicularis* when subjected to three different low concentration stresses for an incubation period of one month.

Fig. 5. Ⓑ Modes of biodischarge of the trace metal Fe by *C. fascicularis* after been subjected to three different low concentration period of one month.

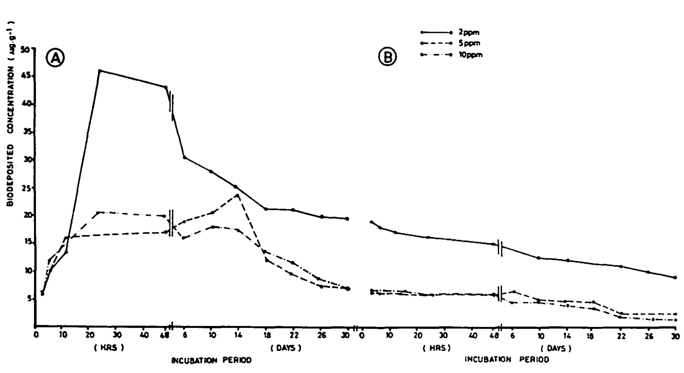

Fig. 6. Ⓐ Modes of bioconcentration of the trace metal Mn by *C. fascicularis* when subjected to three different low concentration stresses for an incubation period of one month.

Fig. 6. Ⓑ Modes of biodischarge of the trace metal Mn by *C. fascicularis* after been subjected to three different low concentration stresses for an incubation period of one month.

gradual increase/continuous depuration at all times, b) sharp increases until 10/14/18 /22/26 days followed by continuous depuration, c) a sharp increase until the 6th day followed by a steady-state period uptil 14/22 days and then a continuous depuration and d) an increase at the 6th day followed by depuration and another increase at the 22th day culminating in continuous depuration thereafter. Owing to the complications involved in these modes of biodeposition they are simplified as overall general patterns as indicated in Table 1 for clarity and better apprehendment of the mechanisms involved.

Furthermore, it may be pointed out from the above data that the highest rates of depuration for the 2 ppm experimental lots are observable for Cd and Pb, Cr, Cu and Zn for the the 5 ppm lots and Co, Fe, Mn and Ni for the 10 ppm lots, respectively. Based on these highest depuration rate values the discharge of the biodeposited trace metals could be categorized as Cr>Zn>Pb >Co>Mn>Ni>Fe>Cu>Cd. However, at each experimental trace metal concentration the categorization falls as follows: 2 ppm; Cr>Pb>Co>Mn>Fe>Zn+Cd>Ni>Cu, 5 ppm; Cr>Zn>Mn>Co>Pb>Cu>Fe>Cd> Ni and 10 ppm; Co>Cr>Mn>Pb>Zn>Ni >Fe>Cd>Cu, indicating also some complicated mechanisms involved in the depuration processes of trace metals in algae.

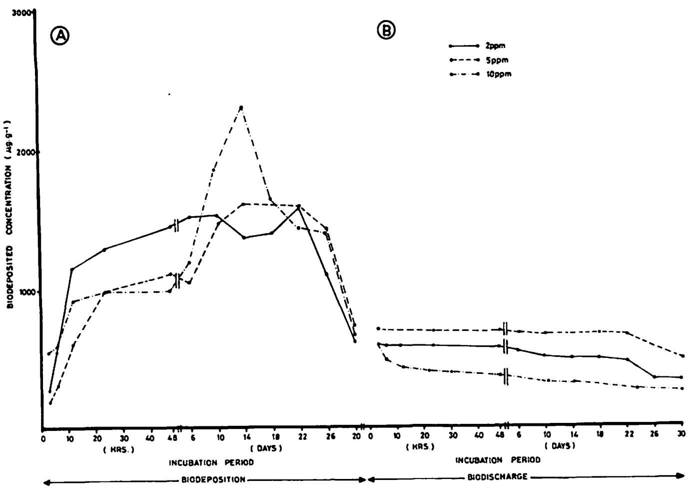

Fig. 7. Ⓐ Modes of bioconcentration of the trace metal Ni by *C. fascicularis* when subjected to three different low concentration stresses for an incubation period of one month.

Fig. 7. Ⓑ Modes of biodischarge of the trace metal Ni by *C. fascicularis* after been subjected to three different low concentration stresses for an incubation period of one month.

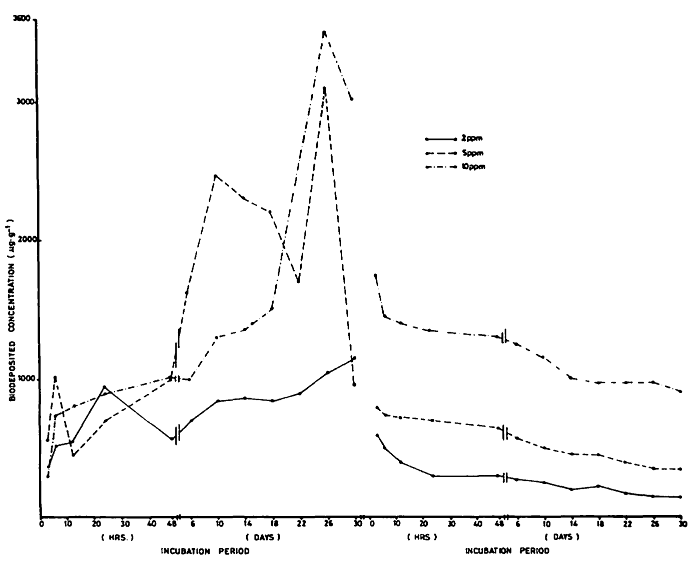

Fig. 8. Ⓐ Modes of bioconcentration of the trace metal Pb by *C. fascicularis* when subjected to three different low concentration stresses for an incubation period of one month.

Fig. 8. Ⓑ Modes of biodischarge of the trace metal Pb by *C. fascicularis* after been subjected to three different low concentration stresses for an incubation period of one month.

Discussion

To back up our previous study (1981), it is again evident in this report that the bioaccumulation factors (=BF's) are maximal at the lowest exposure concentration of 2 ppm for most trace metals excluding that of Pb and Zn, which occur at the 5 ppm stress lot. Nevertheless, the trace metals biodeposited as maximal BF's in the high concentration stress studies (1981) occurring at 50 ppm stress are Cu, Fe, Mn, Ni, Pb and Zn while Cd, Co and Cr, at 100 ppm. Obviously, this supports the concept that at different concentration exposures to trace metals their biological, biochemical and physiological interactions are in the least comparable. These phenomena can also be verified by comparison of the categorization of these maximal BF's, i.e. Cr>Zn>Pb>Co>Mn>Ni>Fe>Cu>Cd for the present study with that of the 50 ppm stress lots; Zn>Cu>Cd>Co>Pb>Fe>Ni>Cr>Mn and that of thalli harvested from the natural habitat; Cd, Cu and Fe>/<Zn>Mn>Cr>

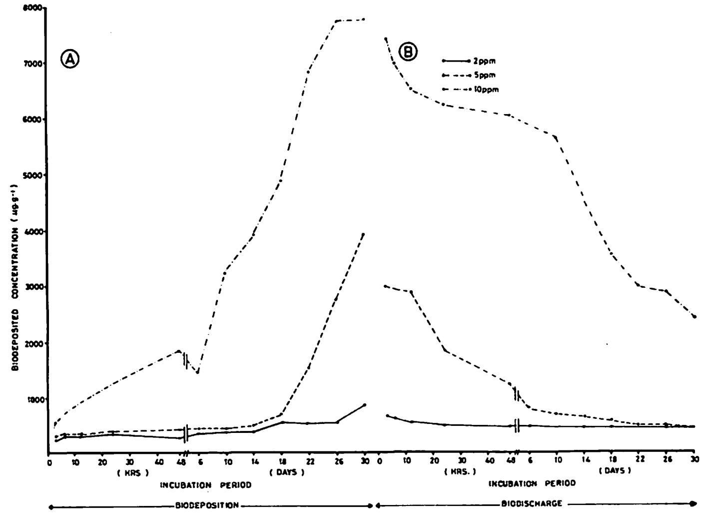

Fig. 9. Ⓐ Modes of bioconcentration of the trace metal Zn by *C. fascicularis* when subjected to three different low concentration stresses for an incubation period of one month.

Fig. 9. Ⓑ Modes of biodischarge of the trace metal Zn by *C. fascicularis* after been subjected to three different low concentration stresses for an incubation period of one month.

Table 1. General patterns in biodeposition of trace metals at low concentration stresses between incubation periods of a) 3-48 hrs and b) 48hrs-30 days in *Cladophora fascicularis* (MERT.) KÜTZING.

Stress Concentrations	Patterns (between 3~48 hrs) A+++ ↗ B++++	A ↗ 6+ ↗ B	A ↗ 12+ ↗ B	A ↗ 24+ ↗ B	A ↗ 24+ ↘ B	A ↗ 6+ → 24+ ↘ B	A ↗ 6+ ↘ ↗ B	A ↗ 12+ ↘ ↗ B
2	Cu & Zn	Cd & Co	Ni	Mn	Pb	Cr	Fe	
5	Cu & Zn	Co	Ni		Cr	Cd & Mn	Fe & Pb	
10	Zn	Pb.	Ni	Cr		Cd, Co, Cu & Mn		Fe

Stress Concentrations	Patterns (between 48 hrs~30days) B ↗ C	B ↘ C	B ↗ 10++ ↘ C	B ↗ 14++ ↘ C	B ↗ 18++ ↘ C	B ↗ 22++ ↘ C	B ↗ 26++ ↘ C	B ↗ 6++ → 14++ ↘ C	B ↗ 6++ → 22++ ↘ C	B ↗ 6++ ↘ ↗ 22++ ↘ C+++++
2	Co, Pb & Zn	Cr & Mn				Cd, Cu & Ni		Fe		
5	Zn	Cr		Co, Cu & Mn					Cd & Ni	Pb
10	Zn	Cr	Fe	Mn & Ni	Cd		Co & Pb		Cu	Fe

Note. + ; hrs, + + ; days, A+++ ; 3 hrs, B++++ ; 48 hrs and C+++++ ; 30 days.

Pb>Co>Ni>Hg, although they seem to have some correlation.

The modes of biodeposition between 3-48 hrs and that for 48 hrs-30 days again are not identical for the various trace metals during the low concentration stresses-2, 5 and 10 ppms, as compared to the high concentration stresses—50-500 ppms. In fact, due to the low concentration of the trace metals in the media and depending on their toxicities the algal thalli appear to definitely demonstrate an effective control mechanism. Hence, this does not agree with the concepts of a) BRYAN (1969) of a linear uptake relationship of Zn, Cu and Pb by *Laminaria digitata* depending on its concentration in the media, b) SUTCHIFFE (1962) of cation uptake by cells at two stages-an initial rapid, passive uptake and a slower uptake dependent on its mechanism and c) HAGERHALL (1973) as one occurring via an irreversible accumulation over a membrane system, supporting our proposal of complexed biological, biochemical and physiological mechanisms across the algal membrane.

The depuration rate data also demonstrates that regulatory mechanisms exist in the algal thallus for all the trace metals, though not at identical levels. Further, such mechanisms seem to vary from algal species to species on comparison to that reported for the Malaysian sea lettuce, *Ulva reticulata* FORSSKAL, by SIVALINGAM and ZAKARIA (1979). This statement is true also for the bioaccumulation factors at similar low concentration stresses. Notwithstanding the foregoing, due to major regional confinement, great dependence on seasonality variations in existence and susceptibility to quick thallus damage of the *Ulva* spp. this species of *Cladophora* is more suitable for being employed as an algal biomonitor on a global basis.

Finally, the authors are of the opinion that the present study together with the previous one (1981) on high concentration stresses greatly support their proposal of the "*Cladophora* Monitoring Programme" and that this proposal will be further substantiated by our following investigation of complexing effects.

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P.M. シバリンガム・R. イスメイル：緑藻 *Cladophora* による微量金属汚染の生物モニター(2)
長期にわたる低濃度圧下での生物濃縮と放出,

緑藻シオグサ属の一種 *Cladophora fascicularis* を用いて，長期間(30日間)，微量金属の低濃度圧下で暴露実験を行った結果，体内に蓄積される金属濃度が最も高い値になるのは，Cd, Co, Cr, Cu, Fe, Mn, Ni, では体外濃度圧が 2 ppm の時で，5，10 ppm と濃度圧が高くなるにつれて， 体内濃度は低い値になる。 しかし，その最高濃度に達する期間はそれぞれ異なっており，海水中の金属濃度と比較すると，Cd で 600倍 (22日後)，Co で 425倍 (30日後) Cr で78倍 (24日時間後)，Cu で1037倍 (22日後)，Fe で1105倍 (6日後)，Mn で23倍 (24時間後)，Ni・790倍 (22日後)，である。Pb, Zn では体外濃度圧が 5 ppm の時に最大で Pb で620倍 (26日後)，Zn で790倍 (30日後) であった。 本報告と前報で得られた結果から，本種では，微量金属の体外濃度圧が高い時よりも低い時では，生物濃縮が早めにおきていることが確かめられた。

生物濃縮後の微長金属の放出過程を長期間 (30日間) 連続して調べた結果， すべての体外金属濃度圧下で，体内の金属濃度には直線的温少傾向がみられた。最大の放出率(%)をみると，Cd では 47.1% (2 ppm の濃度圧下) Co では 81.9% (10 ppm)，Cr では 95.4% (5 ppm)， Cu では 48.4% (5 ppm)．Fe では 51.6% (10 ppm)，Mn では 78.6% (10 ppm)，Ni では 61.4% (10 ppm)，Pb では 87.0% (2 ppm)，Zn では 88.6% (5 ppm) であった。

低濃度圧下での生物濃縮のモードは高濃後圧下 (50～500 ppms) でのそれとはちがい， 大変多様であり，藻体内の生化学的， 生理学的相互関係は本質的に異なっている。 30日間の連続暴露実験はこの問題をさらに複雑にしている。