

***Cladophora* spp. as a prominent global algal monitor for trace metal pollutants**

1. High concentration stresses and modes of biodeposition

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Cladophora fascicularis, a naturally occurring hardy algal species, was found to biodeposit the trace elements Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn in the order of ∞ , 258.2, 185, ∞ , ∞ , undetectable, 264.1, 32.43, 71.9 and 971.3 times, respectively, as compared to amounts of the metals prevalent in the coastal marine waters of its niche. Similarly, the relationship of the amounts biodeposited with sedimented levels demonstrated the trace elements of Cd, Co, Cr, Fe and Ni to be also greater by 1.12, 3.38, 2.0, 1.4 and 3.05 times, accordingly.

High concentration stresses and studies of biodeposition modes over an exposure period of 48 hr to the trace metals ranging between 50-500 ppms indicated the bioconcentration factors to be highest for 50 ppm stresses in most instances except for Cd, Co and Cr (100 ppm stresses): Cd; 275.68 (48 hr), Co; 208.53 (12 hr), Cr; 3.69 (48 hr), Cu; 406.27 (12 hr), Fe; 165.13 (24 hr), Mn; 2.22 (24 hr), Ni; 147.12 (48 hr), Pb; 176.53 (12 hr) and Zn; 624.64 (24 hr). The modes of biodeposition at higher concentrations generally reflected rapid biodeposition occurring during the 12-24 hr of exposure time followed subsequently by a depuration and then a gradual increase in the biodeposition processes.

Key Index Words: Biodeposit; bioindicator; *Cladophora*; high concentration stress; trace metal pollutants.

Evaluation of the pollution of the aquatic environment by trace elements employing both fauna (LORD 1974, PHILLIPS 1976, SCHULZ-BLADES 1975, SIVALINGAM 1979, SIVALINGAM and BHASKARAN 1978, SIVALINGAM *et al.* 1979, TAYLOR and BRIGHT 1973) and flora (BLACK and MITCHELL 1952, BRYAN 1969, GUTKNECHT 1965, SIVALINGAM 1978 & 1979, STOKES *et al.* 1973, THROWER and EUSTACE 1973) has been well demonstrated. Regarding marine fauna as a bio-monitor of pollutants, GOLDBERG (1965) had suggested that the "Mussel-Watch Programme" be carried out on a global basis. This, at the present moment, is drawing

much attention.

Cladophora or Atagib (Algoquian) (FJERDINGSTAD 1965), a common component of freshwater and marine environments (WHITTON 1970), has been classified as a water scavenger of certain radionuclides and a bioaccumulator of heavy metals (NEIL 1975) from the view point of aquatic flora bio-indicators. This approach has been verified further by the studies of STEVEN (1978), TAFT and KISHLER (1973) and KEENEY *et al.* (1976). It has also been postulated recently (KEENEY *et al.* 1976) that *C. glomerata* can act as an aquatic floral bioindicator of trace metals with a reasonably constant bio-

concentration factor (=BF) ranging from 1.0×10^3 to 49×10^3 for Zn, Cd, Pb and Cu. Studies by TAFT and KISHLER (1973) and FUNK (1973) have also positively indicated that the BF for Cu in *Cladophora* from Western Lake Erie (1×10^3) and Upper Spokane River (2.5×10^3) to be remarkably stable. These phenomena have been related to the high cationic exchange capacity of the cell wall imparted by high levels of pectin which might act as the factor for sorbing exotic metals (STEVEN 1978).

In view of both the fairly large tolerance of *Cladophora* spp. in general for high levels of biodeposited trace elements from the low concentrations existing in the medium and on the genus' global distribution, the local species in Malaysia, *Cladophora fascicularis* (MERT.) KÜTZING, is used in this study as an algal bioindicator for trace metals pollution in the Malaysian region, following the studies done on *Cladophora glomerata* by WHITTON (1970).

The authors have initiated three types of experiment using *Cladophora fascicularis* as the experimental material: a) high concentration stresses (50–500 ppms) of trace metals and their modes of biodeposition, b) low concentration stresses (2–10 ppms), their biodeposition and depuration (=biodischarge), and c) the complexing effects of trace metals. The goal is to comprehend the effects of trace metals on this algal species, keeping in mind its possible use as a global bioindicator of marine pollution. This paper presents only the first of the experiments. The other two sections will follow subsequently.

Materials and Methods

Cladophora fascicularis (MERT.) KÜTZING was harvested during low tides off the rocky shores of Batu Ferringhi, Penang Island (Fig. 1) between July–December, 1978. The harvested algae were brought back immediately to the laboratory in a plastic container. Prior to experimentation, the algal fronds were carefully cleaned of epiphytes and con-

taminants and washed thoroughly 3 times in membrane-filtered seawater. Adequate amounts of these thalli were cultured over a period of 48 hr in separate 1 liter Erlenmeyer flasks containing membrane-filtered seawater together with the relevant trace metals at high concentrations of 50, 100, 200, 300 and 500 ppms each, as salts of $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{K}_2\text{Cr}_2\text{O}_7$, CuCl , FeCl_3 , $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{Pb}(\text{NO}_3)_2$ and ZnCl_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 the incubation light source.

To follow the rate of biodeposition, sampling was done at fixed time intervals of 3, 6, 12, 24 and 48 hr. The sampled fronds were initially thoroughly washed 3 times in 1 liter of membrane-filtered seawater followed by washing with distilled water of the same volume prior to drying at 100°C for 48 hr over an air oven.

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

In addition, the trace metal content of the medium and sediments of the habitat of *C. fascicularis* was also analyzed for comparative purposes

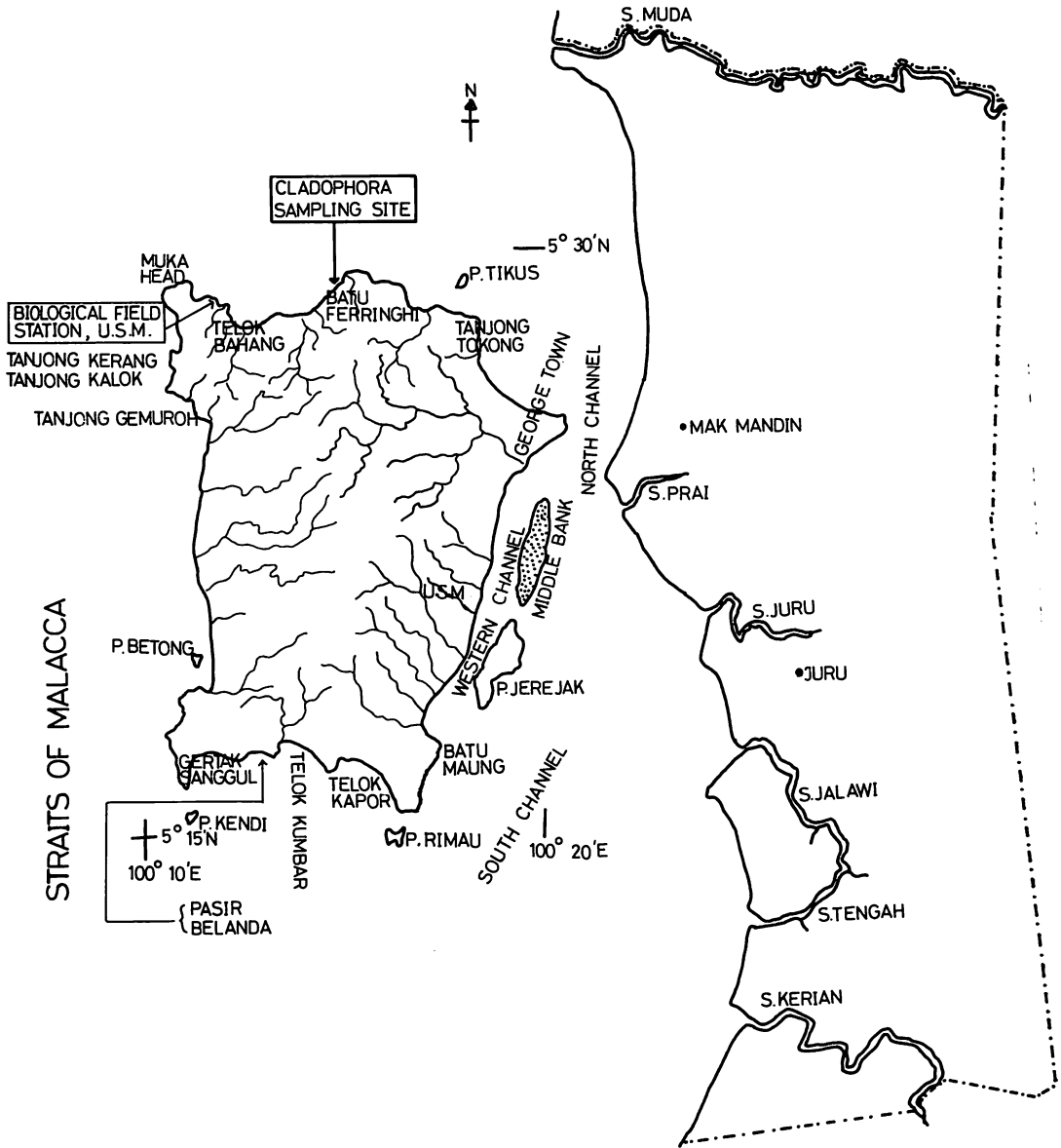


Fig. 1. Map illustrating the sampling site of *Cladophora fascicularis* (MERT.) KÜTZING on the Island of Penang.

Results

Table 1 indicates the results of the trace metals contents of *Cladophora fascicularis*, and of the water and sediment samples in its niche and oceanic waters. It is evident that the trace metal content in these coastal waters is comparatively high relative to

values for oceanic waters. However, they merely reflect the characteristics of normal coastal waters, and not of a highly polluted area. In contrast, the levels of trace metals in sediments are relatively high, in particular Fe, suggesting a high binding capacity by the precipitated particles possibly of organic origin (algae/humic acid) or by clay particles brought down by the river "Kuala Muda".

Table 1. Trace metal content of water samples, sediments, and *Cladophora fascicularis* (MERT.) KÜTZING obtained from the sampling site.

Samples	Cd	Co	Trace metals ($\mu\text{g. gm}^{-1}$)				Mn	Ni	Pb	Zn
			Cr	Cu	Fe	Hg				
<i>Cladophora fascicularis</i>	9.25	33.56	33.3	7.4	4,735.5	BDL	92.45	67.12	12.95	38.85
H ₂ O sample from sampling site(A)	BDL ⁺	0.13	0.18	BDL	BDL	BDL	0.35	2.07	0.18	0.04
Oceanic waters(B)	0.00011	0.00027	0.0005	0.003	0.01	BDL	0.002	0.0054	0.00004	0.01
(A)/(B)	BDL	481.48	360	BDL	BDL	—	175	383.33	6000	4
Sediments from sampling site										
1 cm depth	25	12	17.5	9	17,800	BDL	195	22.5	33.5	89.25
2 cm depth	BDL	7.75	23.25	4.5	18,325	BDL	150.5	30	32.25	51
3 cm depth	BDL	10	9	3.0	17,050	BDL	96.25	13.25	28.25	42

BDL⁺=Below detectable level.

Based on the foregoing (Table 1), it appears that from the fairly low levels of trace metals in the medium of its natural habitat, *Cladophora fascicularis* is capable of bioaccumulating Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn by ∞ , 58.2, 185, ∞ , ∞ , —, 264.1, 32.43, 71.9 and 971.3 times, respectively. On the contrary, in comparison with levels found in sediments the relative ratios are smaller, i.e., Cd; 1.12, Co; 3.38, Cr; 2.0, Cu; 1.4, Fe; 0.267, Hg; —, Mn; 0.628, Ni; 3.05, Pb; 0.413 and Zn; 0.64 times, respectively. This fact is quite conspicuous and facilitates further investigations with regard to the leaching effects of trace metals from sediments on a long-term basis.

Fig. 2 and Table 2 indicate the modes of biodeposition of the trace metals and their BF values during exposure. Generally, the trend of biodeposition appears initially as a sharp uptake during the first 12-24 hr of incubation. This uptake was then followed by either an increasing or decreasing trend thereafter for all the trace metals.

In the 50 ppm stresses, identical trends were observed for Cd, Co and Cr with a peak at 12 hrs followed by a decrease and then an increase in biodeposition thereafter. The uptake peaks of Cu, Fe, Mn and Pb at the 24 hr, on the other hand, is followed by a gradual decrease in uptake. However, Ni

and Zn were absorbed with each gradual increase and showed practically no peak in biodeposition at any time.

At 100 ppm stresses Cd, Co, Cr, Cu and Mn manifest a similar sharp uptake at 12 hrs followed by a decrease and gradual increase. The corresponding peaks for Fe and Pb were observed at 24 and 6 hr incubations, respectively, subsequently followed by a gradual decrease. The trends for Ni and Zn are different with only a gradual increase at any time.

Exposure to 200 ppm stresses indicate that Co, Cr, Fe, Mn and Ni have similar modes of biodeposition, i.e., a spiked uptake at 12 hrs followed by a decrease and gradual increase. The trends were similar for Pb and Zn except that no subsequent peaks were observed. In contrast, Cu and Ni have a plateau between 12-24 hr followed by a rapid increase in biodeposition. The initial rapid uptake of Cd continued to decrease thereafter.

Similarly, for the 300 ppm stresses the uptakes of Cd, Co, Cr, Cu, Fe, Mn and Ni showed the initial peak at the 12 hr incubation followed by a decrease and gradual increase. That of Pb reached a maximum nearly at the same incubation period, but it displayed a gradual decrease continuously thereafter. In contrast, Zn uptake increased

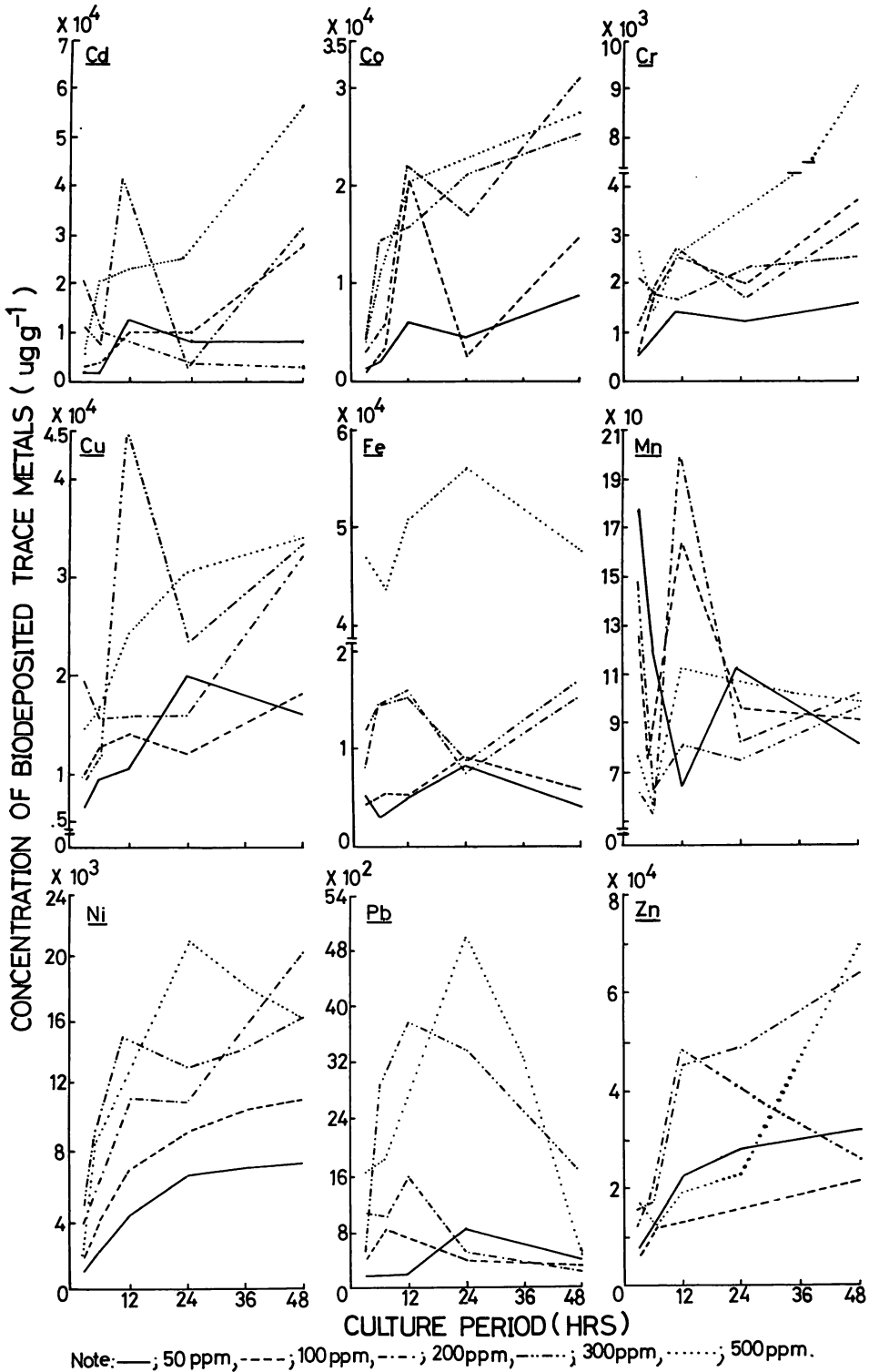


Fig. 2. Modes of bioaccumulation of trace metals in the Chlorophyta, *Cladophora fascicularis* (MERT.) KÜTZING, under various high concentration stresses.

Table 2. Bioconcentration factors of the various bioaccumulated trace metals by *Cladophora fascicularis* (MERT.) KÜRTZING under various high concentration stresses with incubation time.

Concentration of Heavy Metals (ppm)	Incubation Time (hrs)	Bioconcentration Factors ⁺								
		Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
50	3	32.90	24.88	1.02	127.39	100.93	3.55	24.03	41.04	163.62
	6	25.96	44.80	1.67	190.48	52.53	2.45	59.29	44.75	263.79
	12	<u>234.62</u>	<u>128.85</u>	2.78	<u>406.27</u>	108.5	1.31	96.91	44.96	446.65
	24	155.28	86.78	2.40	<u>216.87</u>	<u>165.13</u>	2.22	132.51	<u>176.53</u>	558.83
	36	159.31	98.12	2.81	275.18	145.19	1.87	141.32	83.12	598.21
	48	<u>165.69</u>	<u>167.80</u>	3.15	<u>318.07</u>	81.72	1.63	<u>147.17</u>	77.97	<u>624.64</u>
100	3	29.69	1.67	0.84	96.27	48.14	1.27	19.75	39.58	57.50
	6	34.60	31.57	1.74	128.04	58.42	0.77	40.67	90.74	116.18
	12	98.57	<u>208.53</u>	2.56	<u>136.72</u>	53.47	<u>1.64</u>	67.90	78.61	134.45
	24	98.51	27.19	2.01	124.37	<u>94.81</u>	0.96	90.39	41.28	159.47
	36	152.31	76.31	2.43	135.21	68.21	0.93	99.21	40.13	183.21
	48	<u>276.68</u>	<u>141.68</u>	3.88	<u>183.69</u>	60.90	0.91	<u>110.79</u>	36.73	<u>206.08</u>
200	3	<u>106.58</u>	13.24	0.56	<u>97.78</u>	41.18	0.31	20.10	56.13	62.28
	6	51.10	29.13	0.99	79.36	73.86	0.26	30.84	55.28	103.45
	12	41.75	<u>112.15</u>	1.37	79.16	<u>80.02</u>	<u>0.99</u>	<u>55.39</u>	<u>79.11</u>	<u>245.06</u>
	24	19.26	84.54	0.92	80.83	39.19	0.41	54.50	27.82	199.66
	36	17.31	131.21	1.35	88.21	70.31	0.49	71.31	14.31	121.31
	48	16.67	<u>155.56</u>	1.60	<u>100.56</u>	<u>81.85</u>	0.54	<u>101.09</u>	13.99	131.48
300	3	35.51	14.38	0.69	32.91	39.88	0.50	7.31	18.49	55.48
	6	23.43	47.92	0.61	39.54	49.32	0.21	30.59	102.18	63.34
	12	<u>139.12</u>	52.69	0.56	<u>146.75</u>	<u>51.32</u>	<u>0.27</u>	<u>49.75</u>	<u>133.83</u>	155.11
	24	<u>112.72</u>	71.63	0.77	78.03	29.30	0.25	43.82	130.69	164.54
	36	155.83	81.51	0.80	83.21	43.21	0.29	44.91	98.31	184.21
	48	<u>123.98</u>	<u>84.86</u>	0.85	<u>109.80</u>	<u>55.15</u>	<u>0.32</u>	<u>53.83</u>	47.78	<u>212.23</u>
500	3	10.86	7.37	0.54	29.00	94.47	0.15	9.23	33.14	39.09
	6	40.49	22.96	0.29	33.39	87.32	0.11	17.65	35.04	25.16
	12	45.02	41.84	0.47	48.66	101.78	<u>0.22</u>	22.47	57.62	36.95
	24	49.77	45.29	0.70	60.98	<u>111.57</u>	0.21	<u>42.69</u>	<u>97.35</u>	46.79
	36	61.31	47.31	0.98	64.21	104.31	0.20	35.31	52.31	111.31
	48	<u>131.98</u>	<u>54.50</u>	1.82	<u>67.97</u>	95.56	0.20	32.0	9.33	<u>138.16</u>

NOTE —————; indicates the highest concentration factor at the particular concentration and incubation time.

.....; indicates a peak of high concentration factor but lower than the highest concentration factor at the particular concentration and incubation time.

+; derived by the division of bioaccumulated trace metals with the relevant concentration in the medium.

gradually during the incubation.

For the highest 500 ppm concentration stresses Cd, Co, Cr, Cu and Zn only manifested a gradual uptake all the time, although slight variations in their lag period were noticeable. Nevertheless, Fe, Ni and Pb each showed maximum peaks at 24 hr incubation and their uptake decreased gradually thereafter. In the case of Mn, however, the maximum peak was found at 12 hrs incubation.

All the biodeposition patterns in Fig. 2 were listed precisely in terms of the bioconcentration factors in Table 2, in order to comprehend the algal biodeposition. The general patterns of biodepositional trends during incubation are shown graphically in Table 3.

From these Tables it is obvious that the BF's for Cd, Co and Cr are maximal at 100 ppm stresses (Cd; 275.68 (48 hr), Co; 208.53 (12 hr), Cr; 3.69 (48 hr)) while for the other trace metals at 50 ppm stresses (Cu; 406.27 (12 hr), Fe; 165.13 (24 hr), Mn; 2.22 (24 hr), Ni; 147.12 (48 hr), Pb; 176.53 (12 hr) and Zn; 624.64 (24 hr)).

Discussion

Biodeposition of heavy metals in *Cladophora fracta* was shown to be dependent on

the concentration of the elements in the media (GILEVA 1964), but little work has been done on the mechanisms of biodeposition of these elements. Similarly, BRYAN (1969) indicated a linear uptake relationship of Zn, Cu and Pb by *Laminaria digitata* depending on their concentration in the media. In this connection, SUTCHIFFE (1962) has indicated the uptake of cations by cells to commonly occur at two stages — an initial rapid, passive uptake and a slower uptake dependent on its mechanism. Further, another possible mechanism of trace metal uptake was suggested by HAGERHALL (1973) as one occurring via an irreversible accumulation over a membrane system.

It is obvious from the results of the experiments that under high concentration stresses between 50-500 ppms the modes of biodeposition vary according to the type of trace metal. This fact suggests that the pattern of biodeposition of trace metals is not so simple as explained by the various authors mentioned above with regard to low concentration stresses.

The biodeposition and depuration of trace metals in *Cladophora fascicularis* appears to be greatly dependent on potential variations within the membrane created by the trace metals and their toxicity to the enzyme systems of the algae leading to regulatory

Table 3. General patterns of biodeposition of the trace metals at the various stress concentrations.

Stress concentration. (ppm)	Patterns						
50					Cd, Co & Cr.	Cu, Fe, Mn, & Pb.	Ni & Zn.
100				Pb.	Cd, Co, Cr, Cu & Mn.	Fe.	Ni & Zn.
200	Pb & Zn.		Cu & Ni.		Co, Cr, Fe, Mn & Ni.		Cd.
300	Pb.				Cd, Co, Cr, Cu, Fe, Mn & Ni.		Zn.
500	Mn.	Fe, Ni, Pb.					Cd, Co, Cr, Cu & Zn.

+ : indicates the maximum hr of biodeposition.
 ++ : indicates the plateau hr during biodeposition.
 +++ : incubation time from 3 hr (=A) to 48 hr (=B).

mechanisms. In most cases, the trace metals appear to have a rapid peaked uptake between 12-24 hrs of exposure up to 300 ppm stress, with a different tendency for 500 ppm stress — either a gradual continuous uptake (Cd, Co, Cr, Cu and Zn) or a maximum peaked uptake between 12-24 hrs for the remaining trace metals. Patterns other than a maximum between the 12-24 hrs of incubation are also observable in the 50-300 ppm stress groups depending both on the specific trace metal and its concentration (Table 3).

Based on the BF values of the high concentration stress experiments it is clear that most of the trace metals have their highest values in the 50 ppm lots excluding those for Cd, Co and Cr. The rate of their uptake at high concentration stresses is as follows: Zn > Cu > Cd > Co > Pb > Fe > Ni > Cr > Mn. On comparison with concentrations in naturally occurring *Cladophora fascicularis* in low complexed concentrations of trace metals in the natural medium, the patterns are fairly similar: Cd, Cu and Fe > / < Zn > Mn > Cr > Pb > Co > Ni > Hg. The difference in placement of the highest category could be attributed to the very low concentrations of Cd, Cu and Fe (=BDL) in the natural medium. Further, the fluctuations of Mn, Fe, Co and Pb could also be related to the existence of high concentrations of these trace metals in the medium and their influence on the various physiological and biochemical processes. The tendency of greatly biodepositing Zn, Cu, Cd, Co, Pb, Fe and Ni seem to be quite relevant to the statements of KEENEY *et al.* (1976), TAFT and KISHER (1973) and FUNK (1973) for Zn, Cu and Pb.

Another intriguing point to be mentioned here is the initial high values of absorption observed for the stress experiments during the 3 hr incubation period: Cu and Cd at 200 ppm, Mn at all concentrations, Cd at 300 ppm, Cr at 300 and 500 ppms, Fe at 50 ppm and Zn at 500 ppm (Fig. 2). To comprehend these mechanisms further studies are necessary at shorter time intervals between 0-3 hr at high concentrations.

Considering the high concentration stresses of trace metals in *Cladophora fascicularis* and observations of no physical damage to the alga, it appears that the algal species is a suitable biomonitor of trace metals in the local aquatic environment and these results may possibly be expanded to other related species on a global basis as a "*Cladophora* Monitoring Programme".

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P. M. シバリング・R. イスマイル: 緑藻 *Cladophora* spp. による微量金属汚染の生物モニター 1. 高濃度圧と生物濃縮のモード

緑藻シオグサ属の一種, *Cladophora fascicularis* の体内の微量金属濃度は, 本種が生息する海水中のそれと比較すると検出されなかった Hg を除いて Cd, Cu, Fe では ∞ , Cd で 258.2 倍, Cr で 185 倍, Mn で 264.1 倍, Ni で 32.43 倍, Pb で 71.9 倍, Zn で 971.3 倍もの濃度で濃縮されており, また沈澱物中のそれと比較しても Cd で 1.12 倍, Co で 3.38 倍, Cr で 2.0 倍, Fe で 1.4 倍, Ni で 3 倍もの濃度で濃縮されていることが判明した。

培地中に与えられた 50~500 ppm の高濃度の微量金属濃度 (高濃度圧) 下での 48 時間の暴露実験では, 本種による生物濃縮は Cd, Co, Cr の微量金属類を除いて 50 ppm で最も高く, Cu で 406.27 倍 (暴露後 12 hr), Ni で 147.12 倍 (48 hr 後), Fe で 165.13 倍 (24 hr 後), Mn で 2.22 倍 (24 hr 後), Zn で 624.64 倍 (24 hr 後) の値を示し, また Cd, Co, Cr は 100 ppm の圧力下で高く, その濃度はそれぞれ 275.68 倍 (48 hr 後), 208.53 倍 (12 hr 後), 3.69 倍 (48 hr 後) であった。高濃度圧下での生物濃縮のモードは一般に暴露後 12~24 時間の間で迅速な濃縮を行い, その後放出過程がみられ, 再び濃縮過程がおこるといったパターンを示した。