Jap. J. Phycol. 26 : 157-160. 1978

Effects of high concentration stress of trace metals on their biodeposition modes in *Ulva reticulata* FORSKAL

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SIVALINGAM, P.M. 1978. Effects of high concentration stress of trace metals on their biodeposition modes in *Ulva reticulata* FORSKAL. Jap. J. Phycol. 26 : 157-160.

Studies on the algal biodeposition of seven kinds of trace metals, viz. Cd, Cr, Co, Pb, Zn, Mn and Ni, at five different concentractions of 50, 100, 200, 300 and 500 ppm over a period of 48 hours indicated that the bioconcentration factor for the 50 and 500 ppm concentrations at 48 hours were 36.1 and 21.2 times for Cd, 18.1 and 11 times for Cr, 136.3 and 23 times for Co, 124.1 and 24 times for Pb, 48.2 and 4.4 times for Zn, 144.2 and 35.3 times for Mn and 146.4 and 10 times for Ni, respectively. Time course studies too indicated different patterns of biodeposition for each metal reflecting the possible different physiological and biochemical interactions of these trace metals in *Ulva reticulata* FORSKAL.

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Introduction

Studies by SIVALINGAM *et al.* (1978) have indicated the potentiality in the utilization of *Ulva reticulata* Forskal, which is the most abundant species found at least in Penang waters, as an animal and human feed and a soil conditioner. Based on this, the author has initiated a study to delve the possible pollution effects of trace metals on this alga when it will be exploited with time being ultimately exposed to the present rapid rate of industrial expansion. This study was first focussed on unravelling the effects and modes of biodeposition of seven sorts of trace metals within a short period of incubation at high concentration stresses, in order to examine a long term biodeposition of these trace metals at very low concentration stress in the natural medium and their possible biological discharge after exposure of the algae.

Based on the biodeposited trace metals in the thallus under different concentration stresses the bioconcentration factor at 48 hour was then calculated. This factor was obtained by dividing the amount of bioconcentrated trace metals in the thallus after 48 hour incubation at the various concentrations by the amount of trace metal concentration in each corresponding culture medium.

Methods and Materials

Experimental thalli were harvested from the shores of the Marine Depot, Penang between September 1977 and January 1978, and these were brought to the laboratory to be culled off completely of epiphytes and other contaminants. Prior to experimentation the cleaned algal fronds were conditioned in filtered seawater at 26°C under a 12-hour actinic light-dark period of 13,500 lux culture for a day. These conditioned thalli were then exposed to the various trace metals, i. e., Cd, Cr, Co, Pb, Zn, Mn and Ni, at increasing concentrations of 50, 100, 200, 300 and 500 ppm in 1 *l* Elenmeyer flasks and cultured under similar conditions. After 3, 6, 12, 24 and 48 hour exposure to these metals portions of them were picked, exhaustively washed with clean filtered seawater, then with fresh water and finally

they were dried at 104°C overnight in an air oven prior to storage in a dessicator.

Given amounts of the dried fronds were subjected to predigestion overnight in a solution of nitric-perchloric acid mixture (2 : 1) in kjeldhal flasks before heat digestion on an electrothermal heater until very little white fumes appeared. The digests were then diluted and made up to 100 ml in volumetric flasks before analysis for the trace metal contents using a Varian Techtron (AA120) Atomic Absorption Spectrophotometer.

Results

Fig. 1 shows the biodeposition patterns of various trace metals by *Ulva reticulata* Forskal over a period of 48 hours. In the case of Cd for the 50 ppm stress experiment not much of a conspicuous change is observed except for a sudden jump at the 12th hour followed by a regulating maintenance mechanism in the biodeposited concentration thereafter. The 100, 200 and 300 ppm stress experiments convey similar tendencies except for that the sudden jump in biodeposition occurs as early as the 6th hour and that after the 24th hour the 100 and 200

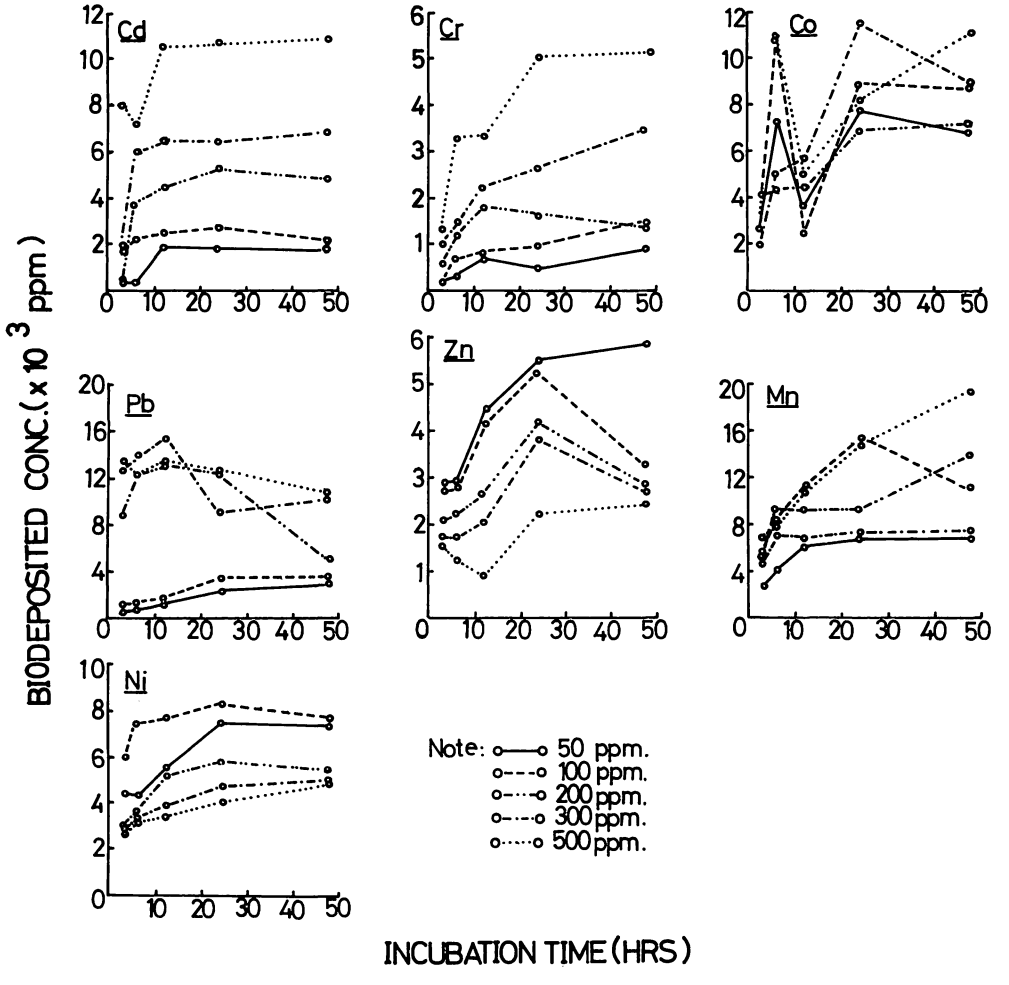

Fig. 1. Biodeposition of the various trace metals under different concentrations of culture by the alga *Ulva reticulata* Forskal.

stress lots appeared to perform some regulating discharge mechanism. The case would be identical for the 500 ppm stress experiment, in which a slight drop in concentration in the 6th hour of incubation.

Cr bioconcentration on the other hand showed gradual increment in most cases as compared to Cd. Further the biodeposited concentration was almost half that of Cd.

In relation, Co demonstrated a totally different picture. Excluding the 200 and 300 ppm stress experiments all the others manifested a sudden spike at the 6th hour incubation culture period followed consequently by a drop at the 12th hour and a renewing sharp increase by the 24th hour with some regulating mechanism thereafter. An exceptional case in this experiment is that in the 500 ppm concentration there appears to be no regulating mechanism after the 12th hour of incubation.

Among the three kinds of trace metals mentioned hitherto Pb at lower concentration stresses of 50 and 100 ppm demonstrated gradual increase at all times. For the 200, 300 and 500 ppm stress experiments there appears to be a parabolic increase between the 3rd and 12th hour incubation followed by a discharge regulating mechanism thereafter.

Zn was the reverse in its biodeposition patterns as compared to the rest. The 50 ppm stress experiment demonstrated the highest bioconcentrated level and the levels decreased in parallel with the concentration stress.

Similarly Mn showed a specific pattern of its own. The 50, 200, 300 and 500 ppm stresses demonstrated gradual increments in their biodeposition at all hours while the pattern of 100 ppm stress experiment competed with that of the 500 ppm counterpart except for some different regulating mechanisms after the 24th hour.

The trace metal Ni also reflects an interesting phenomena. The 100 ppm stress experiment bioaccumulates to the highest extent and followed by the 50, 200, 300 and 500 ppm stresses, respectively. Again here too the algal frond appeared to be having a resisting biodeposition mechanism at high concentrations as with Zn incorporation.

Table 1. Bioconcentration factor of the various trace metals taken up by *Ulva reticulata* FORSKAL at different concentration stresses after 48 hour incubation.

Concentration (ppm)	Bioconcentration factor						
	Cd	Cr	Co	Pb	Zn	Mn	Ni
50	36.1	18.1	136.3	124.1	48.2	144.2	146.4
100	19.5	12.1	82.5	32.4	31.5	112.0	73.2
200	16.3	5.9	32.5	24.3	24.3	72.5	25.8
300	20.5	10.5	27.5	30.5	7.3	25.2	14.6
500	21.2	11.0	23.0	24.0	4.4	35.3	10.0

Table 1 indicates the bioconcentration factors of various trace metals in *Ulva reticulata* FORSKAL after 48 hour incubation under different concentration stresses. It is noticeable that for the trace metals of Co, Zn, Mn and Ni there is a systematic decrease in the bioconcentration factor with higher concentrations, i. e., 50>100>200>300>500 ppm, whereas for the trace metals of Cd, Cr and Pb there is a slight increase in the 300 ppm concentration stress as compared to that of the 200 and 500 ppm ones. However, on the whole, the lower concentration stress exposures obviously showed higher concentration factors.

Discussion

It is obvious that for each trace metal there seems to be some sort of a different physiological/biochemical mechanism on their actions of biodeposition in the marine alga, *Ulva reticulata* FORSKAL. This appears to be true for most tropical marine algae (at present under investigation). The modes of biodeposition for the trace metals Cd and Cr appears to be similar except for the great difference in bioconcentrated amounts even under similar exposure to stress.

In the case of Co, unlike other trace metals, there appears to be an initial spiked intake followed by discharge and later an increased absorption culminating in a regulating mechanism. This, indirectly reflects a possible membrane type of enzymatic regula-

tion in the biodeposition of the trace metal.

Pb, on the other hand, at lower concentrations follows the normal trend of biodeposition but exhibits a totally different pattern at higher concentration exposures. At the initial hours of exposure there is rapid bioconcentration followed by discharge regulating mechanisms on longer hours of exposure.

Unlike other trace metals, Zn showed the reverse mode of biodeposition. At the 50 ppm concentration it manifested the largest amount of bioconcentration followed by the other concentrations in their highest order, throwing light into a definite rejecting mechanism to this trace metal.

Mn demonstrates similar patterns as those of Cd and Cr except for the fact that at the 100 ppm concentration it competed with the rate of bioconcentration of the 500 ppm stress experiment until the 24th hour seeming to trigger a different sort of a pattern.

As for Ni, it followed a similar mode of biodeposition as that of Zn except that the rate of bioconcentration for the 50 and 100 ppm stresses were reversed.

From the foregoing and the bioconcentration factors of the seven metals it appears that *Ulva reticulata* FORSKAL processes a definite mechanism to regulate all the examined trace metals in a pattern of their own depending on the individual trace metal itself. It is also evident that at lower concentrations the bioconcentration factors for all examined trace metals are highest than those exposed to higher concentration stresses. Hence, it can be concluded that the bioconcentration factor at lower concentrations follow the category in a nature of Ni>Mn>Co>Pb>Zn>Cd>Cr.

Acknowledgement

The author wishes to express his gratitude to the School of Biological Sciences, Universiti Sains Malaysia for the aid provided and to Miss Hajar Abdul Rani for typing this manuscript.

References

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P. M. シバリンガム：*Ulva reticulata* の生物濃縮様式に対する高濃度微量金属元素圧の影響について

アオサの一種 *Ulva reticulata* の葉状体を Cd, Cr, Do, Pb, Zn, Mn, Ni の7種の金属元素の 50, 100, 200, 300, 500 ppm の各溶液にそれぞれ 3, 6, 12, 24, 48 時間浸し，どのような割合で取り込まれ濃縮されるかをみた。濃度が高ければ高いほど取り込まれる量も多くなるが，低濃度の溶液における方がより高い割合で濃縮されること，および時間の経過につれての金属元素の取り込まれ方が，それぞれの金属によって異なることがわかった。