Bull. Korean Fish. Soc. 27(4), 373~379, 1994 韓水誌, 27(4), 373~379, 1994

Effects of Suspended Solid and Cadmium on the Shallow-sea Foodweb Ecosystem

1. Reduction of Growth Rate and Biomass Yield of Coastal Diatom Clones by Cadmium

Wonho YIH, Jae Sam YANG, Soo-Gun JO* and Ee-Yung CHUNG*

Department of Oceanography, Kunsan National University, Kunsan 573-360, KOREA

**Department of Marine Development, Kunsan National University, Kunsan 573-360, KOREA*

Final biomass yields(cells/*ml*) and growth rates(divisions/*day*) of 4 clones of marine diatoms isolated from the Korean coastal waters were measured in media with 6 different levels of added cadmium concentrations. A neritic diatom, GS-12(*Chaetoceros* sp.), showed no growth at 0.1*ppm* cadmium, and its IC_{50} for final biomass yield and growth rate was 0.03 and 0.02*ppm*, respectively. Two clones isolated from tidal pool, NC-37 and NC-29, showed enhanced tolerance to cadmium toxicity. Extremely high tolerance to cadmium addition was found in J-21 from a eutrophicated bay, with its high IC_{50} for biomass yield(1.07*ppm*) and growth rate(1.92*ppm*). Present results implied a habitat related pattern of coastal diatom clones in the cadmium tolerances. Except GS-12, the other three diatom clones are considered to be highly tolerant to cadmium stresses.

Introduction

Toxic metals in polluted seawater or oceanic deep water can even inhibit marine phytoplankton growth at their natural concentrations(Brand, 1987; Brand *et al.*, 1986; Bruland, 1983; Patin, 1982). Coastal areas of the Yellow Sea and the Southern Sea of Korea are liable to receive a high level of toxic metal mixtures from the atmospheric and inland environments. Among the toxic metals, cadmium is a trace element in seawater with its acute toxicity to marine phytoplankters even at 100*nM* (Hollibaugh *et al.*, 1980). The goals of the present study were to measure the growth rates and final biomass yields of several marine diatom clones from shallow waters of Korea under various cadmium concentrations, and to quantitatively estimate the relative tolerances of the test clones to cadmium toxicity.

Materials and Methods

The four diatom cultures for the present study (Table 1) were established by single cell isolation before enrichment(Guillard, 1973) in Marine Phytoplankton Laboratory of Kunsan National University. Basal seawater for the culture media was collected from the surface layer of 'Station 14'(36° 10.8′ N, 126° 09.2′ E) near Sochongdo, the Yellow Sea in July 1992(Yih, 1993). The seawater and individual nutrient stock solutions were all tyndal-

This paper is in part funded by a '93 grant for the research institutes of universities from Korea Research Foundation to Coastal Research Center, Kunsan National University.

Table 1. The origin of the clonal cultures

clone	species	location	year	isolator
GS-12	*Chaetoceros* sp.	Yellow Sea, off Kunsan, Korea	1992	W. Yih
NC-37	*Skeletonema costatum*	Tidal pool, Kunsan coast	1993	W. Yih
NC-29	*Thalassiosira* sp.	Tidal pool, Kunsan coast	1993	W. Yih
J-21	*Chaetoceros* sp.	Jinhae Bay, Southern Sea, Korea	1992	W. Yih

Table 2. Cadmium concentrations added to the culture media

medium	M1	M2	M3	M4	M5	M6
Cd(ppm)	0.0	10^{-3}	10^{-2}	10^{-1}	1.0	10

lized separately in teflon bottles. The 32‰ salinity seawater was enriched with 10^{-4} M $NaNO_3$, 10^{-5} M NH_4Cl, 10^{-5} M NaH_2PO_4, 10^{-4} M Na_2SiO_3, 10^{-5} M EDTA, 10^{-6} M Fe-EDTA, 10^{-7} M $ZnSO_4$, 10^{-7} M $MnSO_4$, 10^{-9} M $CuSO_4$, 10^{-8} M $CoSO_4$, 10^{-9} M biotin, 10^{-7} M thiamine, and 10^{-8} M vitamin B_{12}(Brand, 1990). To the enriched seawater, cadmium stock solution was added to make six different kinds of culture medium(Table 2). For the experiments, 20 ×150*mm* glass culture tubes with autoclavable caps (Sigma, USA) were used after a cleaning procedure. The procedure includes cleaning with detergent and repeated rinses and soaks in dilute HCl and distilled water, and final rinsing with the tyndallized seawater. The experimental culture was incubated at 20.5~22.5℃, and a light intensity of 6500 lux was maintained with a 14 : 10 L : D cycle. Each new batch culture was established sequentially by transferring an inoculum from the corresponding batch culture at its peak abundance. To monitor the growth and final biomass yield in different culture media, *in vivo* optical density at 665*nm* was measured at the same time each day with a UV/VIS spectrophotometer. Microscopic cell counting with a hemocytometer or a Palmer-Maloney slide was done for each batch culture at its peak optical density. Thus, final biomass yields are presented here as cell abundances(cells/*ml*). The growth rate(divisions/*day*) was determined from the rate of optical density increase adopting linear regression methods to the logarithmically transformed data.

Results

The final biomass yields determined for different nutrient regimes are illustrated in Fig. 1. GS-12 showed no increase of cell concentration in medium with 0.1*ppm* cadmium addition. Furthermore, the yield decreased inversely as the concentration of added cadmium increased from 0 to 0.01*ppm* only in GS-12. By contrast, in the other three clones cell growth was blocked in medium with 10 *ppm* cadmium addition. The addition of 0.1~1.0 *ppm* cadmium resulted in retarded biomass yields of the other three clones than GS-12. Relative inhibition of the biomass yield at 1.0*ppm* cadmium addition was weaker in J-21 than in NC-37 or NC-29 (Fig. 1).

Results of growth rate experiments are given in Fig. 2. Mean maximal growth rate under the present culture condition was highest in NC-29(0.95 divisions/*day* at 0.001*ppm* addition). In NC-29 0.01 *ppm* cadmium addition led to higher growth rate (0.95) than in the control medium(0.83). Except NC-29, all the clones exhibited a very narrow range in their mean maximal growth rates(0.72~0.79 divisions/day). The trends of cadmium toxicity to growth rates were quite similar to those of final biomass yields(Figs. 1, 2).

Discussion

Dissolved cadmium concentrations in the surface

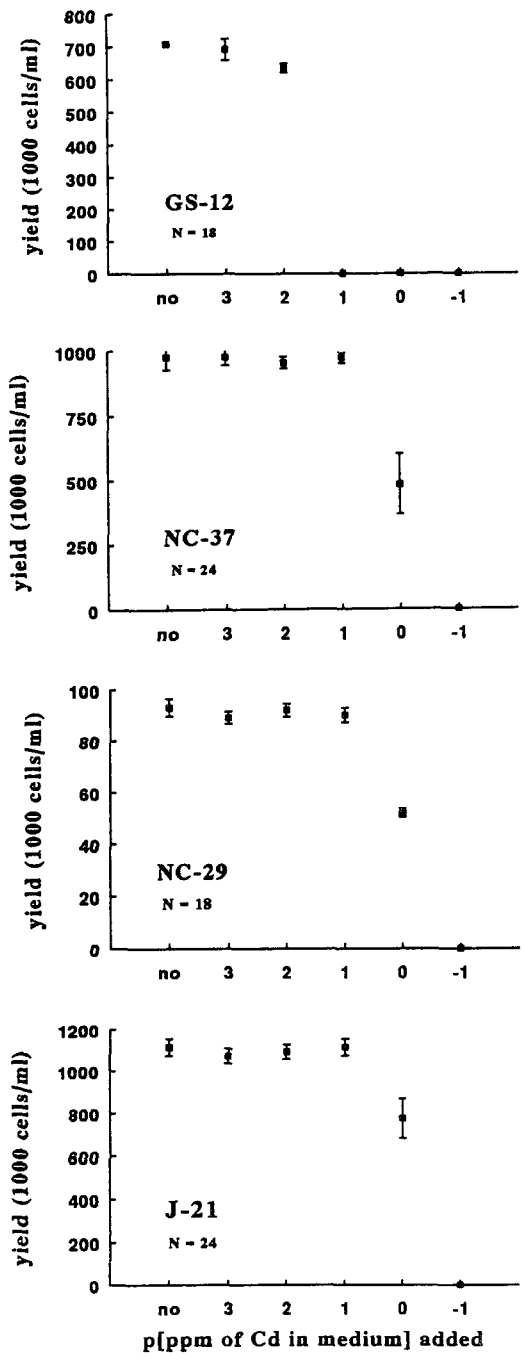

Fig. 1. Final biomass yields of the diatom clonal cultures(GS-12: *Chaetoceros* sp., NC-37: *Skeletonema costatum*, NC-29: *Thalassiosira* sp., and J-21: *Chaetoceros* sp.) in media with various concentrations of cadmium. The mean and SEM(standard error of mean) of replicate analyses are shown. N is the total number of the replicates.

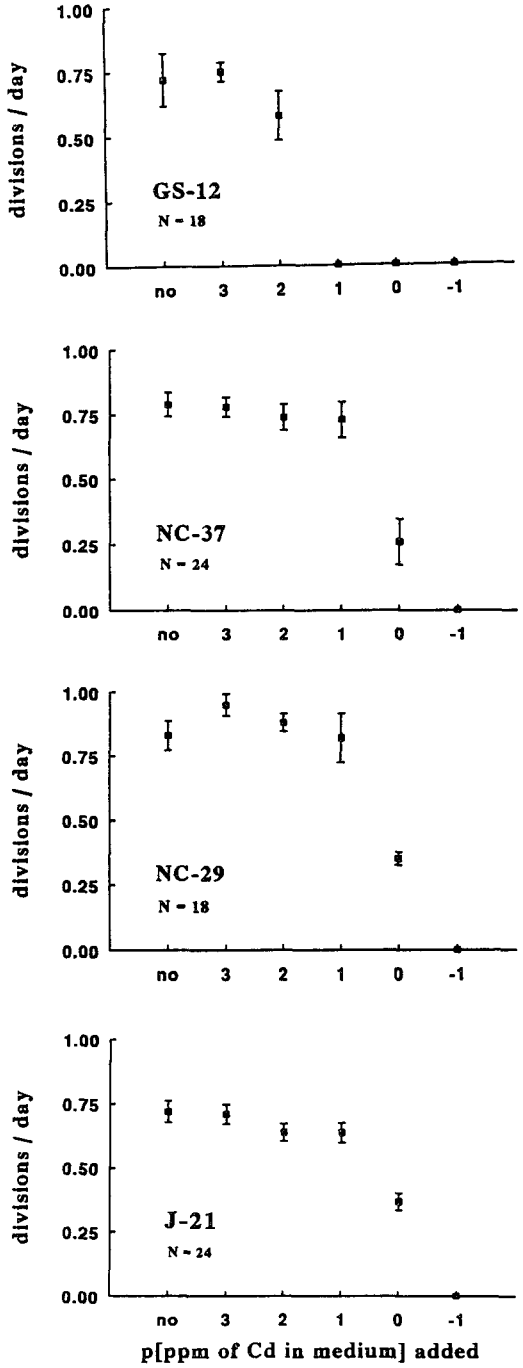

Fig. 2. Mean daily growth rates of the diatom clonal cultures(GS-12: *Chaetoceros* sp., NC-37: *Skeletonema costatum*, NC-29: *Thalassiosira* sp., and J-21: *Chaetoceros* sp.) in media with various concentrations of cadmium. The mean and SEM(standard error of mean) of replicate analyses are shown. N is the total number of the replicates.

water of the Yellow Sea were reported to range from 6.9 to 77.0*ng/l*(KORDI, 1993) except one abnormally high value(134*ng/l* at 'Station B5' in April, 1992). The cadmium concentrations reported are far greater than those in the surface water of the Sargasso Sea(0.23*ng/l*; Bruland and Franks, 1983), the Indian Ocean(0.84*ng/l*; Saager *et al.*, 19 92) and the upwelling area of the equatorial eastern Pacific(9.0*ng/l*; Boyle and Huested, 1983). Reported mean concentrations of dissolved cadmium in the surface water of the Yellow Sea(32*ng/l* or 3. 2×10^{-5}*ppm*) is well outside the inhibiting range to all the tested clones(Figs. 1, 2).

A trend of the calculated IC_{50}(concentration that causes 50% inhibition) of cadmium in this study is illustrated in Fig. 3. The IC_{50} of cadmium for final biomass yield is about 2 times higher than that for growth rate in all the tested diatom clones(Fig. 3). The IC_{50} for final biomass yield of the diatom clones isolated from the Yellow Sea ranged from 0.03(GS-12) upto 1.27 *ppm*(NC-29), and IC_{50} for growth rate from 0.02 to 0.74*ppm*(Table 3). The IC_{50} of the diatom clone from Jinhae Bay(J-21) was almost 2 times greater than that of NC-37(Fig. 3), reflecting the enhanced cadmium tolerance of J-21. The orderly increase of IC_{50} from the neritic clone (GS-12), tidal pool clones(NC-29 and NC-37), and up to the clone with its origin of eutrophicated bay implies a habitat related pattern in the cadmium tolerances among the coastal diatom clones. GS-12 is a diatom clone sensitive to cadmium toxicity in comparison with other neritic or oceanic diatoms, coastal natural populations, and Mediterranean or Red Sea diatom strains(Table 3). The other three diatom clones(NC-37, NC-29 and J-21), however, showed a extremely strong cadmium tolerance (Table 3).

The atmospheric and inland environments surrounding the Yellow Sea are predicted to change rapidly in a few decades, and then dissolved cadmium can inhibit marine diatom growth in the coastal Yellow Sea. Cadmium input through air and riverine systems into the culture medium was estimated to be very important for the cadmium accumulation in mass cultured unicellular algal cells (Payer *et al.*, 1976). Bioconcentration of cadmium through the basic marine foodweb ecosystem resulted in 2~3 times higher cadmium concentrations in commercial fish from the Caspian Sea than that

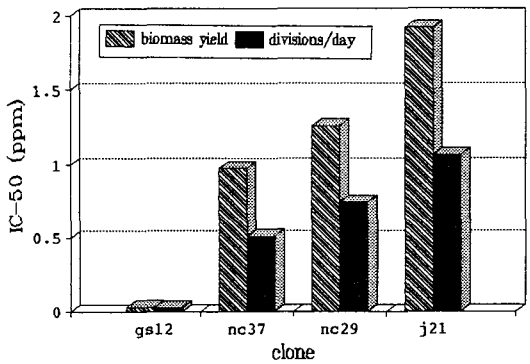

Fig. 3. IC_{50}(concentration that causes 50% inhibition) for the final biomass yield(hatched bar) and growth rate(black bar) of the diatom clonal cultures(GS-12: *Chaetoceros* sp., NC-37: *Skeletonema costatum*, NC-29: *Thalassiosira* sp., and J-21: *Chaetoceros* sp.).

Table 3. IC_{50}(in ppm) of cadmium concentrations for diatom growth rate and finalbiomass yield

origin of diatoms	IC_{50}, GR[a]	IC_{50}, FBY[b]	references
neritic water (GS-12)	0.024	0.028	present study
tidal pool(NC-37)	0.507	0.975	present study
tidal pool(NC-29)	0.740	1.265	present study
eutrophicated bay(J-21)	1.065	1.920	present study
neritic waters(8 clones)	0.008~0.140		Brand et al., 1986
oceanic waters(3 clones)	0.067~0.090		Brand et al., 1986
natural populations(*Skeletonema*>50%)		0.12	Hollibaugh et al., 1980
Mediterranean & Red Sea strains		0.1~1.0	Patin, 1982

[a]GR means growth rate. [b]FBY means final biomass yield.

from other oceanic or marine waters(Patin, 1982). The accumulation and behavior of toxic trace metals across the cell membrane of a marine unicellular algae(Lee and Fisher, 1992; Karez *et al.*, 1990; Price and Morel, 1990; Payer *et al.*, 1976) would be another topic for the next ecotoxicological studies on the foodweb ecosystem of the Yellow Sea.

Acknowledgements

Part of this research was supported by a '93 grant for the research institutes of national universities from KRF(Korea Research Foundation) to W. Yih through the Coastal Research Center of Kunsan National University. Sincere contributions to the Marine Phytoplankton Laboratory of Kunsan National University by those senior students in 19 93, G.Y. Han, H.N. Jung, S.Y. Yih, G.A. Sung and E.S. Kim, are gratefully acknowledged. The final version of this paper considerably benefited from the helpful comments and suggestions of the anonymous reviewers.

References

Boyle, E.A. and S. Huested. 1983. Aspects of the surface distribution of copper, nickel, cadmium and lead in the North Atlantic and North Pacific. *In* Trace metals in sea water, C.S. Wong, E. Boyle, K.W. Bruland, J.D. Burton and E.D. Goldberg(eds), Plenum Press. New York. 379~394.

Brand, L.E. 1990. The isolation and culture of microalgae for biotechnological applications. *In* Isolation of biotechnological organisms from nature, D.P. Labeda(ed.). McGraw Hill Inc. 81~115.

Brand, L.E. 1987. Nutrition and culture of autotrophic ultraplankton and picoplankton. *In* Photosynthetic picoplankton, T. Platt & W.K.W.Li (eds), Can. Bull. Fish. Aquat. Sci., 214, 205~223.

Brand, L.E., W.G. Sunda and R.R.L. Guillard. 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. J. Exp. Mar. Biol. Ecol., 96, 225~250.

Bruland, K.W. 1983. Trace elements in sea water. *In* Chemical Oceanography, J.P. Reiley & R. Chester(eds), vol. 8, 157~220. Academic Press.

Bruland, K.W. and R.P. Franks. 1983. Mn, Ni, Zn and Cd in the western North Atlantic. *In*, Trace metals in sea water, C.S. Wong, E. Boyle, K.W. Bruland, J.D. Burton and E.D. Goldberg(eds), Plenum Press. New York. 395~414.

Guillard, R.R.L. 1973. Methods for microflagellates and nanoplankton. *In* Handbook of phycological methods, J.R. Stein(ed), Cambridge University Press. New York, 69~85.

Hollibaugh, J.T., D.L.R. Seibert and W.H. Thomas. 1980. A comparison of acute toxicities of ten heavy metals to phytoplankton from Saanich Inlet, B.C., Canada. Estuar. Coast. Mar. Sci., 10, 93~105.

Karez, C.S., D. Allemand, G.D. Renzis, M. Gnassia-Barelli, M. Romeo and S. Puiseux-Dao. 1990. Ca-Cd interaction in the prymnesiophyte *Cricosphaera elongata*. Plant, Cell and Env., 13, 483~487.

KORDI(Korea Ocean Research and Development Institute). 1993. Coastal ocean flux study in the mouth of the Yellow Sea. BSPN 00203-614-4. 321 p.

Lee, B-G and N.S. Fisher. 1992. Degradation and elemental release rates from phytoplankton debris and their geochemical implications. Limnol. Oceanogr., 37, 1345~1360.

Patin, S.A. 1982. Chapter 6. Effect of pollutants on cultured unicellular marine algae. *In* S. A. Patin, Pollution and the biological resources of the ocean. Butterworth Scientific. London, 80~109.

Payer, H.D., K.H. Runkel, P. Schramel, E. Stengel, A. Bhumiratana and C.J. Soeder. 1976. Environmental influences on the accumulation of lead, cadmium, mercury, antimony, arsenic, selenium, bromine and tin in unicellular algae cultivated in Thailand and in Germany. Chemosphere, 6, 413~418.

Price, N.M. and F.M.M. Morel. 1990. Cadmium and

cobalt substitution for zinc in a marine diatom. Nature, 344, 658~660.

Saager, P.M., H.J.W. De Baar and R.J. Howland. 1992. Cd, Zn, Ni and Cu in the Indian Ocean. Deep-Sea Res., 39, 1, 9~35.

Yih, W. 1993. The phytoplankton clones from the coastal Yellow Sea.(I) GSNU 0001-0028. Marine Development Res., Kunsan National Univ., 5, 33~45.

Received March 19, 1994
Accepted July 2, 1994

1. Reduction of Growth Rate and Biomass Yield of Coastal Diatom Clones by Cadmium

천해역 먹이망 생태계에 대한 무기부유입자와 카드뮴의 영향

I. 연안역 규조류 단종배양체의 성장률과 생체량증가에 대한 카드뮴의 저해효과

이원호 · 양재삼 · 조수근* · 정의영*

군산대학교 자연과학대학 해양학과 · *군산대학교 자연과학대학 해양개발학과

한국 연안역에서 분리한 4종의 규조류 단종배양체를 대상으로 하여, 카드뮴 농도가 다른 6종류의 배양액에서 최종생체량과 성장률을 측정하였다. *Chaetoceros*속의 천해역 규조인 GS-12는 0.1 *ppm*의 카드뮴 농도에서 전혀 성장하지 못하였으며, 최종생체량과 성장률에 대한 IC_{50}은 각각 0.03 및 0.02 *ppm*이었다. 조수못에서 분리한 배양체인 NC-37과 NC-29는 카드뮴독성에 대해 내성이 보다 큰 편이었다. 부영양화한 만내에서 분리한 J-21은 극히 높은 카드뮴 내성을 나타내었는데, 최종생체량과 성장률에 대한 IC_{50}이 각각 1.07 및 1.92 *ppm*에 달하였다. 본 연구 결과는 연안역 규조류 단종배양체의 카드뮴 내성이 서식환경에 따라 차이가 있음을 암시한다. GS-12를 제외한 나머지 3종의 규조류 단종배양체들은 카드뮴압박에 대한 내성이 매우 강하다고 평가하였다.