

REMOVAL OF TRACE METALS FROM MARINE CULTURE MEDIA

ABSTRACT

The sodium form of purified Chelex-100 can be used to remove trace metals from artificial and natural seawater without altering the major cation-anion composition or contributing organic toxicants or chelators. Specific contaminant trace metals, often present in artificial seawater at toxic levels, are reduced to levels that permit the growth of sensitive marine phytoplankton and the development of certain invertebrates from the egg through early larval stages. The concentrations of essential trace metals are reduced below the nutritional requirements of selected species of marine phytoplankton.

To perform meaningful experiments in trace metal requirements and metal toxicity, it is essential to prepare a reproducible and precisely defined chemical environment. Artificial seawater prepared from the purest chemicals available often contains enough contaminant trace metals to satisfy the nutritional requirements of most organisms. High concentrations of copper in artificial seawater make it unsuitable as a calibration standard for seawater measurements of copper (Strickland and Parsons 1968). We have measured concentrations of copper of 2×10^{-7} M in freshly prepared artificial seawater, enough to inhibit the growth of certain marine phytoplankton. To resolve these problems, we developed a technique that removes trace metals from artificial and natural seawater without altering the major cation or anion composition or contributing organic toxicants or chelators.

Riley and Taylor (1968) reported that seawater that had been passed through columns of the hydrogen form of Chelex-100 to remove trace metals and then enriched with chelated trace metals failed to support the growth of even hardy species of phytoplankton. Presumably it contained toxic materials dissolved from the resin. We have found, however, that artificial and natural seawater passed through the sodium form of carefully purified Chelex-100, when properly enriched, supported the growth of several sensitive species of marine phytoplankton. Artificial seawater

treated with Chelex-100 also supported the egg to larval development of the American oyster *Crassostrea virginica* (Zaroogian, Pesch, and Morrison 1969), the brine shrimp *Artemia salina*, and the calanoid copepod *Acartia tonsa* (J. Gonzalez, personal communication).

PROCEDURES

The chelating resin, Chelex-100 (Bio-Rad), is a purified form of Dowex A-1. A 50-100-mesh particle size was used. The resin was purified further by heating at 60°C in 10 N NaOH for 24 hr (Bio-Rad Laboratories 1964), followed by a rinse with distilled deionized water (DDW), a wash with anhydrous methanol, and 10 alternate 1 N HCl and 1 N NaOH washes interspersed with DDW. A final treatment with 1 N NaOH yielded the sodium form of the resin which was rinsed with DDW to remove excess NaOH before storage.

A 1.4-cm ID $\times$ 20-cm borosilicate glass column was filled within 2 cm of the top with the purified sodium Chelex-100. The resin in the column was given a final 1 N acid-base wash and DDW rinse just before use. Each medium was passed through the column at a flow rate of about 5 ml/min. To equilibrate the resin and reduce any cation-anion alteration of the media, we discarded the first 10 bed volumes of effluent before medium for culturing was collected.

The resin efficiency for removing trace metals from seawater was determined with radioisotopes at concentrations between 1 and 5 μ Ci/liter, depending on the counting efficiency for the isotope used. After the sample had run through the column, the resin was rinsed free of salts with DDW and trace metals were eluted with either 2 N HCl or HNO₃. Radioactivity remaining in the effluent was measured by liquid scintillation counting using a Triton X-100 toluene scintillation fluid.

Medium for the phytoplankton cultures was collected from the column into linear

TABLE 1. Cadmium-115m not retained by sodium or hydrogen Chelex-100

Aliquot No.	^{115m} Cd in column effluent			
	Hydrogen form		Sodium form	
	(cpm/ml)	(%)	(cpm/ml)	(%)
Initial	1,760	—	1,760	—
1	1,022	58.0	4	0.2
2	672	38.2	0	0.0
3	279	15.8	0	0.0
4	134	7.6	1	0.1
5	71	4.0	0	0.0
Total loss in 500 ml		24.75		0.01

polyethylene containers and adjusted to pH 8.0 using Ultrex HCl (J. T. Baker Co.). The medium was filter-sterilized through a 0.22- μ membrane supported on an all-Teflon 142-mm filter holder with a positive pressure of 0.14 kg/cm². The membrane was prewashed with 2 liters of 2 N HCl followed by 4 liters of particle free, low organic, high resistivity (18 megohm at 20C) water to remove contaminant trace metals.

Cultures of 50 ml were maintained at 20C, 2,500 lux in 125-ml erlenmeyer flasks with foam plugs. All glassware was acid washed in 6 N HCl followed by 10 running rinses with high resistivity water, heated to 400C, and silicone coated (General Electric SC-87, 2.5% SC-87 in cyclohexane). Trace metal solutions were prepared from Specpure trace metals (Johnson Matthey Chem., Ltd.), and Ultrex HCl and HNO₃. Cells were counted with an electronic particle counter (Coulter, Model B, with MCV computer).

RESULTS AND DISCUSSION

The hydrogen and sodium forms of Chelex-100 were compared for the efficiency of trace metal removal. One liter of natural seawater containing 1 μ Ci ^{115m}Cd was divided into 2 equal portions. One was passed through a column of the hydrogen form of Chelex-100 and the other through the sodium form. Both columns were run at a flow rate of 5 ml/min, 100-ml aliquots were collected, and replicate 1-ml samples

counted. The results (Table 1) show better than 99% uptake of ^{115m}Cd on the sodium form, whereas 24.75% of the radioactivity passed through the hydrogen form. More than 500 ml of water were necessary to equilibrate the hydrogen form to the sodium form efficiency. The effluent from the sodium form was pH 8.0, whereas the effluent from the hydrogen form was pH 2.0. The processors of Chelex-100 state that if the resin is left in the hydrogen form for more than a few hours it tends to lose chelation capacity (Bio-Rad Laboratories 1964).

Using the sodium form of Chelex-100, we could remove from both artificial and natural seawater better than 99% of ⁶⁵Zn, ^{115m}Cd, ⁵⁴Mn, and ⁶⁴Cu; 95% of ²¹⁰Pb; 92% of ⁶³Ni and ⁵⁹Fe; but only 32.6% of ^{110m}Ag. These radioisotope removal efficiencies are similar to those reported by Goya and Lai (1967). However, there is some disagreement with the efficiencies stated by Riley and Taylor (1968), especially the 100% value reported for silver.

One milliliter of wet sodium Chelex-100 could remove 99% of ⁶⁵Zn from 2–2.5 liters of seawater passed through a 0.4-cm ID $\times$ 9-cm column at 5 ml/min. However, no matter what volume of resin was used, only 85 to 90% of the above isotopes were recovered from the columns by elution with up to 200 ml of 2 N HNO₃ or HCl. In our experiments, the first 25 ml of either acid yielded less than 70% isotope recovery rather than 100% as indicated by Riley and Taylor (1968).

Artificial seawater from which trace metals were removed by purified sodium Chelex-100 supported the growth of several species of phytoplankton from our culture collection when suitable trace metal enrichments were made. There was no indication that toxic products from the resin were present. Even sensitive dinoflagellates such as *Gymnodinium breve*, *Gymnodinium splendens*, and *Gonyaulax tamarensis* were unaffected.

Figure 1 summarizes the growth response of *Cyclotella nana* in medium from which trace metals were removed by puri-

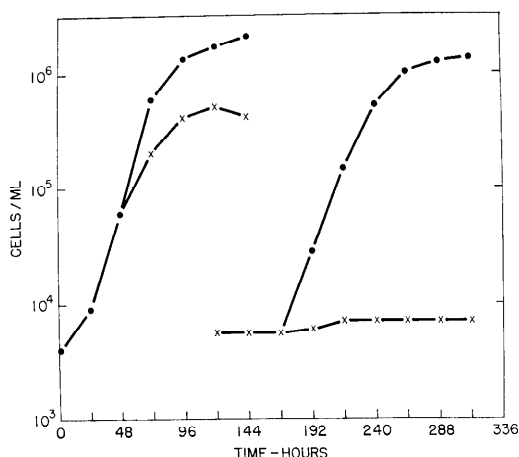


FIG. 1. Growth of *Cyclotella nana* in artificial seawater free of trace metals (X—X) and with complete trace metals (●—●).

fied sodium Chelex-100. On the first transfer into medium treated with Chelex-100, both growth rate and maximum population density were lower than in the same medium to which trace metals had been added. Growth of this control was, incidentally, identical to that observed for the untreated medium. The culture deficient in trace metals was used as inoculum for a second experiment. Eight flasks of the deficient medium were inoculated to give an initial cell density of 5.5×10^3 cells/ml. None of the flasks showed growth after 48 hr. At this time, four were enriched with trace metals. Resumption of growth in these cultures was immediate and the resultant growth rate and maximum cell density were similar to the controls. Cultures not receiving trace metal enrichments failed to grow. Similar growth responses were obtained with *Skeletonema costatum*, *Olisthodiscus luteus*, and *Monochrysis lutheri*.

Finally, to determine whether organic material from the resin was contributing chelating properties to the medium, we made bioassays for copper toxicity on both Chelex-100 treated and untreated medium

to which trace metals had been added. Bioassays with *C. nana* gave growth rates in treated and untreated artificial seawater identical for any given copper concentration. Since there was no decrease in copper toxicity using the medium treated with Chelex-100—the expected response for a chelator—the resin did not appear to contribute any chelating materials in excess of those present in the untreated medium. Nutritional studies on manganese and iron have provided further evidence that treated medium is essentially free of chelating organics.

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