Bull. Environm. Contam. Toxicol. 25, 75-78 (1980)

# Effects of Di-*n*-butyl Phthalate and Salinity on the Growth of the Diatom *Skeletonema costatum*

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The toxicity level of di-n-butyl phthalate (DBP) to the diatom Skeletonema costatum was determined by subjecting culture portions of this organism grown at five salinities to various concentrations of DBP. The toxicity levels of seven phthalate compounds to Gymnodinium breve were reported and of those compounds tested, DBP was shown to be the most toxic (WILSON et al. 1978). High quantities of phthalate compounds have been produced and dispersed in the environment (CEKIS 1976, GIAM 1975), but little is known of their effects on phytoplankton and other organisms in both estuarine and marine waters (ATLAS 1979).

## MATERIAL AND METHODS

Stock cultures of S. costatum were established in artificial sea water media (NH-15) (GATES & WILSON 1960) and incubated in a 24°C growth chamber with 1000 foot-candles of continuous incident light supplied by 40 watt cool-white fluorescent lights. Media with salinity levels of 14, 22, 27, & 36 o/oo were prepared by adjusting the concentrations of the major salts. Cultures were transferred into each salinity level with subsequent retransfers to precondition S. costatum to each salinity.

Various concentrations of DBP were prepared at each salinity. A 0.2 mL portion of the DBP compound was added to 200 mL of NH-15 media at each salinity. The DBP and NH-15 media were manually shaken for 5 min, allowed to settle, centrifuged, and filtered to remove undissolved phthalate. The aqueous saturated solution was used in the assays. Portions of this saturated solution were frozen and later analyzed (GIAM 1975) to determine the concentrations of DBP at each salinity (TABLE 1).

TABLE 1. Concentrations (µg/µL) of Di-n-butyl Phthalate in Fortified Water Samples. SD = Standard Deviation for 4 Replicates

| Salinity (o/oo) | Concentration (µg/µL ± SD) |
|---|---|
| 14 | 1.0 ± 0.3 |
| 22 | 1.4 ± 0.3 |
| 27 | 1.5 ± 0.3 |
| 36 | 1.2 ± 0.3 |

0007-4861/80/0025-0075 $01.00

Dilutions of each DBP and NH-15 media were made with NH-15 at the corresponding salinity to obtain seven different "percent saturated solutions" (TABLE 2). Five mL portions of these dilutions were pipetted into 3 replicate 16 x 125 mm Pyrex brand, disposable test tubes with a polypropylene cap. To these dilutions were added 5 mL of S. costatum culture in logarithmic growth phase at each salinity, and the final "percent saturated solution" test conditions resulted (TABLE 2). Controls consisted of 5.0 mL of the saturated solution (undiluted) and 5.0 mL of NH-15 at each salinity level as well as "no add" controls consisting of 5.0 mL of culture and 5.0 mL of NH-15 at each salinity set up in triplicate.

TABLE 2. Stock Dilutions of Saturated Solutions

| Milliliters of Saturated Solutions | Milliliters of NH-15 | Resulting Percent Saturated Solution | With Cultures Final Percent Saturated Solution |
|---|---|---|---|
| 25 | 0 | 100 | 50 |
| 10 | 15 | 40 | 20 |
| 5 | 20 | 20 | 10 |
| 2.5 | 22.5 | 10 | 5 |
| 0.5 | 24.5 | 2 | 1 |
| 0.25 | 24.75 | 1 | 0.5 |
| 0.05 | 24.95 | 0.2 | 0.1 |

These test portions were incubated as described above. Relative chlorophyll a measurements were made with a Turner Model III Fluorometer initially and at 24 hour intervals thereafter for 4 days. Cultures were fixed by adding Lugol's preservative. Initial and final cell counts were made with a Coulter Counter. The average daily division rate (K) was calculated from: $K = \ln(\frac{C_t}{C_o})\ (\frac{1}{t \ln 2})$ where $C_t$ and $C_o$ are cell concentrations at time t and o respectively, with $C_t$ representing the mean terminal population for the 3 replicates (SMAYDA 1969). Standard regression analyses were conducted on untransformed data. The data were fitted to a quadratic model in each case. A Student's t test was performed on paired data in columns and rows (TABLE 3). A two way analysis of variance (Model One) was also performed.

## RESULTS AND DISCUSSION

Skeletonema costatum grew in all combinations of salinity and DBP concentrations (TABLE 3, FIG., 1). A two way analysis of variance showed that both the concentration of DBP, the salinity, and the interaction of these factors had a significant effect on the growth rate of the diatom ($P < 0.05$). A t test showed that cells grown in

media at 14 o/oo salinity were significantly different ($P < 0.05$) from cells grown at 27 and 36 o/oo salinity, but not from cells grown at 22 o/oo salinity. The mean growth rates of cells in media at all salinities with a 50% concentration of DBP were significantly different ($P < 0.05$) from those grown at all other salinity-DBP combinations and controls.

TABLE 3. Growth Rates (K) of Skeletonema costatum at Various Concentrations of DBP and Salinity

| Concentration of DBP (%) | Salinity (o/oo) 14 | 22 | 27 | 36 |
|---|---|---|---|---|
| 50 | 0.34 | 0.50 | 1.24 | 1.23 |
| 20 | 0.90 | 1.02 | 1.33 | 1.25 |
| 10 | 1.00 | 1.11 | 1.30 | 1.23 |
| 5 | 0.90 | 1.26 | 1.28 | 1.25 |
| 1 | 1.07 | 1.26 | 1.25 | 1.21 |
| 0.5 | 1.06 | 1.28 | 1.20 | 1.22 |
| 0.1 | 1.08 | 1.24 | 1.26 | 1.30 |
| Control | 1.00 | 1.16 | 1.24 | 1.18 |

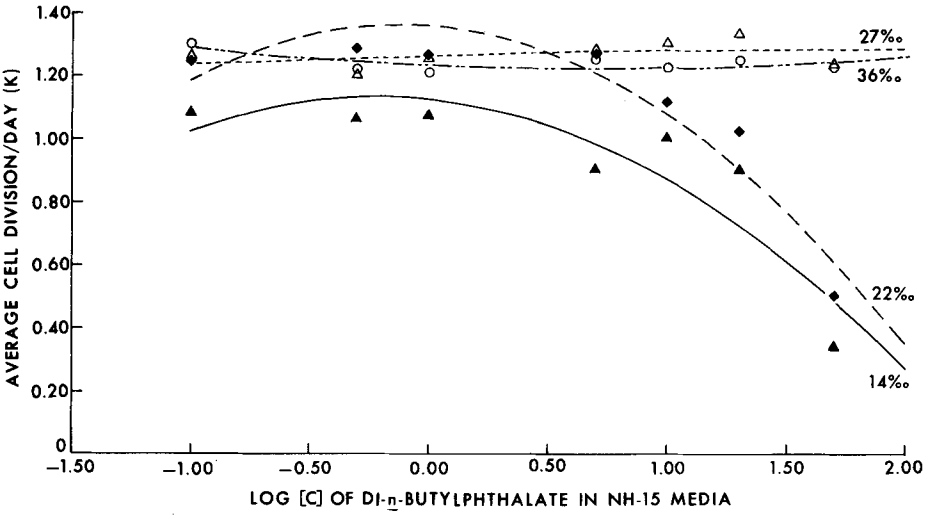


FIGURE 1. Influence of di-n-butyl phthalate on the average cell divisions/day (K) of the diatom, Skeletonema costatum, grown at 4 different salinities. 14 o/oo (▲), 22 o/oo (◆), 27 o/oo (△), 36 o/oo (○)

It appears that DBP is more toxic to Skeletonema in media of a lower salinity and that increasing salinity overrides the effect of the concentration of DBP on the growth of Skeletonema. Highest growth rates for the diatom occurred in media of 27 o/oo salinity which is near its optimal range. Thus, the toxicity of DBP to S. costatum appears to be less in media of high salinity (that approaching normal sea water) and to be reduced in media of lower salinity (that approximately equivalent to the waters found in bays and estuaries). However, estuarine isolates of several species of phytoplankton have been shown to be more tolerant of pollutants, perhaps because these organisms have evolved mechanisms that can deal with widely fluctuating environmental changes (FISHER 1977). This isolate of S. costatum has been in culture for several years and may not respond as a more recent isolate of the species from the estuaries might under similar test conditions.

Acknowledgements. Financial support of the National Science Foundation, International Decade of Oceanographic Exploration (NSF-IDOE) is gratefully acknowledged. The assistance of B. Crowder, D. Patlan, and W. B. Wilson is gratefully acknowledged.

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