Polychlorinated Biphenyl Inhibition of Marine Phytoplankton Photosynthesis in the Northern Adriatic Sea

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Polychlorinated biphenyls (PCBs) are widespread contaminants of the marine ecosystem. Residues of these chlorinated hydrocarbons have been detected in samples from a variety of trophic levels (RISEBROUGH et al., 1968; JENSEN et al., 1969; PEAKALL AND LINCER 1970). The kinetics of the various processes involved in biological accumulation of PCBs have not been adequately defined, however, as the mechanisms of environmental input are diverse and complex.

Marine phytoplankton comprise the base of complex oceanic food webs. Photosynthetic conversion of carbon from the inorganic to the organic form is the principal mechanism whereby energy enters the ecosystem and is made available to the various biotic components. Detrimental effects induced by PCBs at that resource base with resultant decreased phytoplankton production or altered community structure could markedly disrupt an entire marine system.

Previous studies have indicated that PCBs affect the growth kinetics of monospecific phytoplankton cultures (KEIL et al., 1971; FISHER et al., 1973; FISHER AND WURSTER 1973). In addition, PCBs induced changes in the species composition of mixed algal cultures (MOSSER et al., 1972; FISHER et al., 1974). The effects of polychlorinated biphenyls on phytoplankton photosynthesis have been described for several species in axenic culture (FISHER 1975). The present communication describes an evaluation of PCB-induced photosynthetic inhibition of natural phytoplankton assemblages from the northern Adriatic Sea.

STUDY AREA

The northern Adriatic Sea displays many of the characteristics of an estuary due to the shallow nature of an extended coastal shelf and the dominating influence of the Po River on oceanographic processes. Relatively high nutrient levels in this region contrast with lower concentrations typical of the generally oligotrophic Mediterranean Sea (BULJAN 1963; FAGANELLI 1961; FRANCO 1970). Phytoplankton primary production is substantial and provides the food base for commercially important sardine (Sardina pilchardus) and anchovy (Engraulis encrasicholus) fisheries (MAJOR 1970; KVEDER et al., 1971; REVELANTE AND GILMARTIN 1975).

In addition to providing a nutrient source for autotrophic planktonic forms, the waters of the Po River carry industrial and domestic wastes into this shallow marine basin (BARLETTA AND MARCHETTI 1971; GENERAL FISHERIES COUNCIL FOR THE MEDITERRANEAN 1971; FAO 1974). Previous studies indicate that the northern Adriatic may be considered a heavily stressed region with regard to several pollutant classes including heavy metals (SELLI et al., 1972; GILMARTIN AND REVELANTE 1975) and chlorinated hydrocarbons

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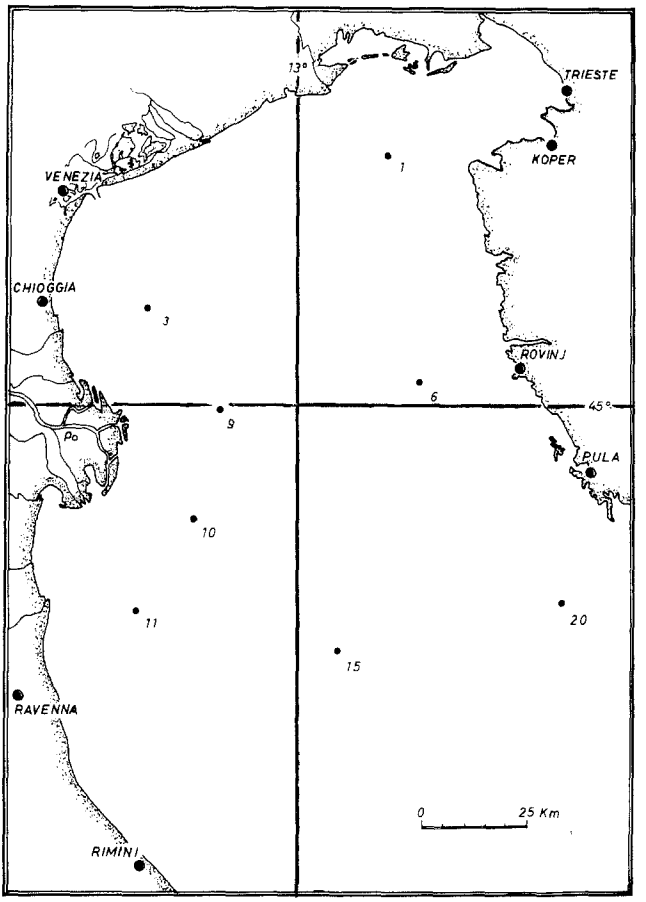

Figure 1. The northern Adriatic Sea showing sample sites for photosynthetic inhibition studies.

(CRISETIG et al., 1973; VIVIANI et al., 1969; 1973a; 1973b; REVELANTE AND GILMARTIN 1975). The location of the Po River and other sources of contaminant input with respect to circulation patterns and prevalent meteorological conditions renders the northern Adriatic Sea an ideal system for the analysis of pollutant impact on regional biological processes.

MATERIALS AND METHODS

Hydrographic and biological parameters measured in the continued study of primary production in the N. Adriatic were used in the selection of eight representative stations (see Fig. 1). Monthly cruises were conducted aboard RV Vila Velebita from October through December 1973 and from May through July 1975.

Standard oceanographic data were collected at each station; the methods employed have been described (GILMARTIN et al., 1972; 1973; 1974; 1975.).

Seawater samples were collected from each station with 6-liter capacity Van Dorn bottles from 0.5 meters depth to avoid sampling the surface layer. Individual 250 ml aliquots were immediately placed in light and dark incubation bottles with duplicate light bottles. PCB (Aroclor 1254) in 10 µl ethanol was added to yield the desired concentration in each bottle. The range of concentrations tested was 1.0 to 100.0 µg l^{-1} (parts per billion) with individual samples receiving 1.0, 10.0, 50.0 or 100.0 µg l^{-1} Aroclor 1254. Controls consisted of a sample run without addition and one run with the carrier solvent, i. e. 10 µl ethanol, only. The addition of ethanol did not significantly alter photosynthetic carbon incorporation.

Approximately 4 µCi ^{14}C-sodium bicarbonate was added to each bottle and the samples were incubated for six hours on shipboard in modified Doty incubators (DOTY 1955). Following incubation bottle contents were filtered onto membrane filters (HA Millipore 0.45 µm pore size, 25 mm diameter). The filters were stored in a dessicator prior to counting with the Nuclear Chicago Model 115 Geiger-Müller Counter. The means of duplicate light bottle counts were used for all calculations unless the individual measurements differed by greater than ten percent. Values which did not meet that criterion were discarded.

RESULTS AND DISCUSSION

Polychlorinated biphenyls reduced phytoplankton photosynthesis when administered in doses as low as 10.0 µg l^{-1} (ppB). Table 1 shows values for photosynthetic inhibition obtained on six separate occasions over a two-year interval. Phytoplankton communities from different temporal regimes exhibited distinct photosynthetic responses to polychlorinated biphenyl addition. Analysis of variance revealed significant contributions to the variance by inter-station differences ($P < .05$).

The data for individual stations and sample periods were also treated as independent measurements. Analysis of variance indicated no significant contribution to the variance by differences among individual measurements. Overall means for photosynthetic reduction were calculated and are included in Table 3 with an estimate for ED_{50} determined by the graphical method of LITCHFIELD AND WILCOXIN (1949).

The results included in Table 2 and the corresponding analysis of variance indicate differences among stations as an important factor governing the magnitude of PCB-induced photosynthetic inhibition. Various factors which could account for such response differences were considered and an empirical separation of the stations into two groups was attempted. The parameters examined were primarily hydrographic and biological. Photosynthetic pigment concentrations, primary production, nutrient levels, salinity and temperature, seston content and geographical position with regard to suspected sources of contaminant introduction were

TABLE 1

SAMPLE PERIOD	N	CONCENTRATION AROCLOR 1254 ($\mu g\ l^{-1}$) 1.0	10.0	50.0	100.0
OCT. 1973	6	85.4±6.4	85.3±7.6	--	25.2±0.9**
NOV. 1973	8	125.7±18.6	89.2±7.9	66.8±13.8*	64.9±15.7
DEC. 1973	4	85.6±7.1	62.6±11.3*	46.8±9.2*	39.8±6.6**
MAY 1975	8	103.2±5.2	96.9±6.6	52.1±7.0**	41.4±8.2**
JUNE 1975	7	98.8±1.9	87.5±7.4	49.3±3.3**	39.3±3.4**
JULY 1975	8	105.4±1.5**	78.2±4.2**	53.7±5.0**	45.8±5.3**

Table 1. Mean photosynthesis with standard errors expressed as percent of control for six sample periods at the four PCB concentrations examined. Student-t tests were performed for all values to determine the significance of differences from control rates (*$P<.05$; **$P<.01$).

among the factors used in classification of the stations.

Stations 1, 3, 9, 10 and 11 comprise Group I and stations 6, 15, and 20 constitute Group II. Group I stations are characterized by relatively high nutrient levels, dense plankton standing stocks, substantial freshwater influence and proximity to sources of industrial and domestic wastes. Group II stations are consistently poorer in nutrient content, photosynthetic pigment concentrations, primary production and suspended particulate material than the constituents of Group I. In addition, the stations that comprise Group II are further from the Po River and industrial centers such as the Gulf of Trieste and the Venice lagoons than are their Group I counterparts (see Figure 1).

The photosynthetic inhibition means for both groups are included in Table 3. Analysis of variance did not indicate a significant contribution to response magnitude variations by between-group differences. A Student-t test, however, revealed a significant ($P<.05$) difference in response between Groups I and II at the 100.0 $\mu g\ l^{-1}$ dose. The ED_{50}s were estimated from Group I and II means and the values are included in Table 3. These values were not significantly different.

Product moment correlation coefficients were calculated for photosynthetic inhibition data and several biological indicators including chlorophyll *a*, phaeophytin *a*, seston, nitrate and phosphate concentrations. The only significant correlation ($P<.05$) found was between chlorophyll *a* concentration and photosynthetic response at the 100.0 $\mu g\ l^{-1}$ dose level. This relationship between standing stock, as estimated from pigment content, and the magnitude of photosynthetic inhibition may derive from the polychlorinated biphenyl dose per cell.

TABLE 2

STATION	N	CONCENTRATION AROCLOR 1254 ($\mu g\ l^{-1}$) 1.0	10.0	50.0	100.0
1	5(4)	132.5±29.2	100.5±12.4	66.3±11.9	47.9±14.2*
3	4(3)	94.7±3.1	77.0±3.0**	38.4±4.0**	29.1±5.5**
6	6(5)	97.1±6.0	73.9±5.4**	49.4±2.5**	38.0±3.8**
9	6(5)	97.0±6.7	84.0±9.3	42.0±7.2**	30.3±4.6**
10	6(5)	99.9±2.6	103.0±8.6	60.0±6.3**	43.9±6.0**
11	6(5)	93.9±8.0	74.4±4.4**	45.2±4.6**	37.5±7.4**
15	4(3)	100.1±5.0	90.7±9.9	63.6±12.7*	58.6±17.0
20	4(3)	113.3±5.2	76.0±1.4**	78.9±19.9	78.4±19.5

Table 2. Mean photosynthesis with standard errors expressed as percent of control for the eight stations at the four PCB concentrations tested. Student-t tests were performed for all values to determine the significance of differences from control values (*P<.05, **P<.01). Note: Sample sizes for experiments run at 50 $\mu g\ l^{-1}$ Aroclor 1254 are indicated in parentheses.

TABLE 3

	N	CONCENTRATION AROCLOR 1254 ($\mu g\ l^{-1}$) 1.0	10.0	50.0	100.0	ED_{50}
INDEPENDENT	41(35)	103.0±4.5	85.1±3.3**	54.7±4.1**	44.0±4.2**	74.0
GROUP I (See text)	27(22)	103.0±6.5	88.1±4.4**	50.8±4.0**	38.0±3.9**	59.0
GROUP II (See text)	14(13)	102.6±3.8	79.3±4.2**	61.3±8.4**	56.7±8.7**	115.2

Table 3. Mean photosynthesis with standard errors expressed as percent of control for ungrouped and grouped data (see text). Student-t tests were performed to determine the significance of differences from control values (*P<.05; **P<.01). Note: Sample sizes for experiments run at 50 $\mu g\ l^{-1}$ Aroclor 1254 are indicated in parentheses.

The polychlorinated biphenyl concentrations which inhibited phytoplankton photosynthesis in the N. Adriatic are substantially higher than those recorded for natural marine systems. HARVEY et al. (1973; 1974) measured seawater PCB concentrations in the North Atlantic. The highest concentration detected was 150 ng l^{-1} (ppT), while the mean level was 20 ng l^{-1}. The experiments described here did not indicate photosynthetic inhibition at these levels.

The photosynthetic response differences detected in N. Adriatic phytoplankton from different locations and sample periods indicate that polychlorinated biphenyl inhibition of carbon incorporation is not a simple dose-effect phenomenon. An interpretation of photosynthetic inhibition based solely on comparisons between reported PCB concentrations in seawater and levels sufficient to yield decreased production ignores the spatial and temporal variability encountered in these experiments.

The manner in which PCBs are introduced into marine systems and partitioned among particulate components, and the form in which these contaminants are available to phytoplankton are important considerations. The concentrations of PCBs that have been measured in seawater may not accurately represent the amounts accessible to phytoplankton. The low water solubility (ZITKO 1970) and high affinity of chlorinated hydrocarbons for particulate matter makes the measurement of seawater concentrations of these compounds difficult. The amounts available to phytoplankton may be far greater than the quantities detected in seawater as polychlorinated biphenyls are removed from seawater through various biological and non-biological uptake mechanisms almost immediately upon their introduction. Concentrations in seawater, therefore, may reflect merely the transient presence of these compounds prior to their physical or biological removal. It is difficult to determine from measurements of seawater PCB concentrations alone either the levels to which phytoplankton are exposed or the form in which these compounds actually exist in natural systems.

The composition of the particulate fraction may partially govern the availability of chlorinated hydrocarbons for biological uptake, effect and transport. Chlorinated hydrocarbons exhibit a high affinity for particle surfaces and are relatively insoluble in seawater, but they are particularly soluble in lipid-containing material (JENSEN 1972). Elevated phytoplankton standing stocks and increased concentrations of suspended organic materials, for instance, could influence the manner in which lipophilic compounds are partitioned among particulate components. Qualitative and quantitative differences in particle composition realized either spatially or temporally may, therefore, affect the chlorinated hydrocarbon concentrations available to phytoplankton.

It has been observed that different phytoplankton species exhibit different tolerances for chlorinated hydrocarbons (FISHER AND WURSTER 1973; FISHER 1975). Phytoplankton communities of different composition may show physiological responses which reflect such differences in species composition. Similar doses of PCB may thereby elicit dissimilar responses from various phyto-

plankton aggregates. Seasonal and geographical differences in plankton composition could serve as one explanation for differences in the magnitude of photosynthetic inhibition among sample stations and periods.

Excessive polychlorinated biphenyl concentrations in organisms may reflect local sources of contaminant introduction (OLSSON et al., 1973). The proximity of domestic and industrial waste inputs via rivers, sewage outfalls or commercial effluent sources may result in gradients of pollutant levels various distances from such locations. Geographic differences in ambient chlorinated hydrocarbon concentrations in phytoplankton could develop within such a region. The concentration of exogenous PCB necessary to elicit phytosynthetic inhibition may be affected by such differences in background levels.

Significant temporal and geographic photosynthetic response differences to the introduction of polychlorinated biphenyls were discerned in this study of the N. Adriatic. These findings are not surprising based on the complex mechanisms involved in contaminant availability. Indeed, the effectors that have been discussed virtually insure the differences which were noted for this region. The Po River contributes large amounts of suspended particulate matter to the N. Adriatic in addition to high concentrations of nutrient salts and various pollutant forms from domestic and industrial sources. There exist substantial horizontal gradients with regard to nutrient material of biotic and abiotic origin and presumably contamination. For these reasons determinations of polychlorinated biphenyl concentrations necessary to elicit photosynthetic inhibition are difficult. Additional research is required to further elucidate the mechanisms involved in PCB uptake by phytoplankton and the effects of these compounds on photosynthesis, cell division and other physiological processes which may substantially alter natural marine systems.

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