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Investigation of Three Wastewaters Entering İzmit Bay (Turkey) by Means of Batch and Chemostat Culture Algal Bioassays

O. S. Okay,[a]* L. Egesel,[a] V. Tüfekçi,[a] E. Morkoç[a] and A. Gaines[b]

[a]Energy Systems and Environmental Research Institute, Marmara Research Center, TÜBİTAK, PO Box 21, 41470, Gebze, Kocaeli, Turkey
[b]Marine Sciences Institute, Middle East Technical University, PO Box 28, 33731, Erdemli, İçel, Turkey

ABSTRACT

The bay of İzmit is the focus of much of Turkey's industrial development and three of the most important of the wastes discharged into its surface waters have been studied as a necessary step in minimising their environmental impact. Gas chromatography/mass spectrometry of the extracted wastes revealed the presence of organic xenobiotics listed as priority pollutants. Nevertheless, measurements of cell populations, chlorophyll fluorescence and the consumption of nutrients demonstrated that most of the wastes stimulated the growth of both batch and chemostat cultures of Phaeodactylum tricornutum *(Bohlin). Experiments showed the growth of the cultures to be phosphate limited and it appears that the major effect of the wastes was due to their excessive loading of phosphate.*

INTRODUCTION

İzmit Bay, one of the most important enclosed seas in Turkey, situated not far from the Bosphorus and the metropolis of Istanbul, has been strongly affected by increases in the surrounding population and by industrialization (Tuğrul *et al.*, 1986).

As a part of a continuing effort to maintain and improve its marine environment, the seasonal oceanography of the Bay was monitored systematically between 1984 and 1988 (Tuğrul *et al.*, 1989) and 1994–1996 (Morkoç *et al.*, 1996). Eight major discharges of wastewaters into the Bay have been identified and characterised. The eight discharges are sources of both toxicity and inorganic nutrients. The toxicity has been quantified by short-term ^{14}C algal bioassays and the measured changes of toxicity with dilution of the wastes have permitted the toxicity to be treated as a simple chemical property. From the toxic

*To whom correspondence should be addressed.

intensity of each discharge, the toxicity field of İzmit Bay was simulated for four seasonal oceanographic conditions. The eight discharges entering the Bay have been ranked in terms of toxicity; the shallower, inner eastern part of the Bay receives nearly 70% of the toxic pollutant. The simulations of pollutant dispersion in the Bay waters revealed that uptake by phytoplankton is still the dominant mechanism whereby toxicity in the Bay is reduced (Okay *et al.*, 1996). Simultaneously, the marine circulation has been simulated to see the seasonal distribution of chemicals throughout the Bay (Morkoç *et al.*, 1996).

The seasonal distribution of the total suspended solids (TSS) was also simulated since the TSS is subject to precise legal regulation. The simulation showed there to be times when the TSS levels exceeded the legal limits in certain regions of the Bay (Legoviç *et al.*, 1997). The overwhelming majority of the TSS in the Bay is phytoplankton and calculation and simulation based on the observed behaviour of the Bay shows the TSS would remain within legal limits were the nutrient contents of the discharges to be halved (Morkoç *et al.*, 1996) Total phosphorus levels in the eastern part of the Bay were found to be approximately twice those at the open boundary with the Marmara Sea (Legoviç *et al.*, 1995).

The simulations revealed the importance of three particular wastewaters for the quality of the Bay waters. These were two complex wastewaters and the wastewaters from the pulp and paper industries (PPW) which combine with a domestic discharge (DW) before entering the bay. One of the complex wastewaters (CW1) is a mixture of wastes from over 50 factories including drug, metal and fermentation industries. It is discharged into the eastern section of the Bay which is the most polluted, industrialized and shallowest. The wastewaters from the pulp and paper industries were also discharged into this section of the Bay. The second complex wastewater (CW2) collects the wastewaters from 20 factories and is discharged to the western part of the Bay.

Both the short-term ^{14}C algal toxicity bioassays and the simulation studies showed the necessity for further investigations. Therefore batch and chemostat bioassays have now been performed in order to determine the long term and steady state responses to the three wastewaters and to determine the nutrient limiting growth.

MATERIALS AND METHODS

Cultures of *Phaeodactylum tricornutum* Bohlin (a diatom) grown in modified f/2 medium were utilized in bioassay studies. Batch bioassays were performed as described in the US EPA procedure, *Selenastrum capricornutum* Printz Algal Assay Bottle Test (Miller *et al.*, 1978). The dilution water was the filtered and autoclaved surface waters of the Marmara Sea with a salinity of 22–28 psu. For batch bioassays, a 50% dilution series was prepared and the results were compared with those from control samples having the same dilution prepared with distilled water instead of wastewater. The batch bioassays were designed so as to see the type of effect, i.e. stimulative or toxic, *Phaeodactylum* cells were inoculated in the wastewater + seawater mixtures without and with addition of f/2 medium (Guillard and Ryther, 1962).

In chemostat bioassays, wastewaters were diluted with 50% of seawater. CW1 was enriched with 1.120 mg l^{-1} PO_4-P. The chemostat was run without addition of PO_4-P in the case of PPW + DW mixtures.

Phaeodactylum cells were counted under a microscope and the fluorescence of their chlorophyll-a was also measured. In chemostat experiments the concentrations of nitrate

and phosphate in the outflow were analyzed. Flow rates and inflow nitrate and phosphate concentrations were controlled daily.

Erlenmayer flasks (1000 ml) and a chemostat culture chamber (500 ml) were used. A light intensity of 3500 lux and a room temperature of $22 \pm 1°C$ were held constant throughout the experiments.

One liter samples of industrial wastewaters were extracted with (15% diethyl-ether + 85% hexane) and with CCl_4 and the extracts were analysed by GC–MS for priority pollutants.

RESULTS AND DISCUSSION

Table 1 shows the flow rates and analysis of the wastewaters and Fig. 1(a) and (b) shows the results of batch bioassay experiments with CW1, CW2 and PPW. These wastewaters were diluted 50% with seawater. In Fig. 1(a) the wastewater and control samples had been enriched with nutrients in order to observe clearly the toxic effect of the wastes. Figure 1(b), showing the results of batch bioassays of the same wastewaters in an unenriched media, demonstrates the stimulative effect of the wastes. Two wastewaters, CW1 and PP, were found to be stimulative and CW2 was strongly toxic to algal growth. The toxic effect of CW2 probably resulted from the high phenol content.

Batch experiments revealed the necessity for the continuation of impact studies to determine the steady state response of algal cultures. CW1 and PPW (+DW combining with PPW) were studied in a chemostat culture to identify which nutrient limited algal growth. Although analysis shows the concentration of nutrients in wastewaters, this is not always correlated with the stimulative effect, nor is the concentration of toxic substances always directly related to the inhibitive effect (Walsh and Merill, 1984).

TABLE 1

(a) Characteristics of Wastewaters. (b) Metal Analysis Results of Wastewaters. (c) Priority Pollutants Found in Wastewaters

(a) Wastewater type	*NH_4-N* *(mg l^{-1})*	*NO_3-N* *(mg l^{-1})*	*PO_4-P* *(mg l^{-1})*	*pH*	*TOC* *(mg l^{-1})*	*Phenol* *(mg l^{-1})*	*AOX* *(mg l^{-1})*
Complex no. 1 (CW1)	10.5	7.1	0.86	8.91	13.3	< 0.4	0.056
Complex no. 2 (CW2)	8.6	3.5	0.01	9.01	17.2	30	0.21
Pulp and paper (PPW)	0.44	2.52	2.4	7.51	6.3	< 0.4	0.06
Domestic (DW)	—	1.58	0.01	6.35	25	—	—

(b) Wastewater type	*Fe* *(mg l^{-1})*	*Mn* *(mg l^{-1})*	*Zn* *(mg l^{-1})*	*Cu* *(mg l^{-1})*	*Pb,Cd,Co,As,Sb,Be,Cr,Ni* *(mg l^{-1})*
Complex no. 1	0.99	0.23	0.22	0.01	< 0.01
Complex no. 2	0.33	0.07	0.14	0.01	< 0.01
Pulp and paper	0.08	0.01	0.01	0.01	< 0.01

(c) Wastewater type	
Complex no. 1	Phenol, phenanthrene, naphthalene, indene, benzene, toluene
Complex no. 2	Toluene, naphthalene, indene, benzene, phenol, anthracene, dibutylphthalate
Pulp and paper	Toluene, indene, benzene, dibutylphthalate, naphthalene

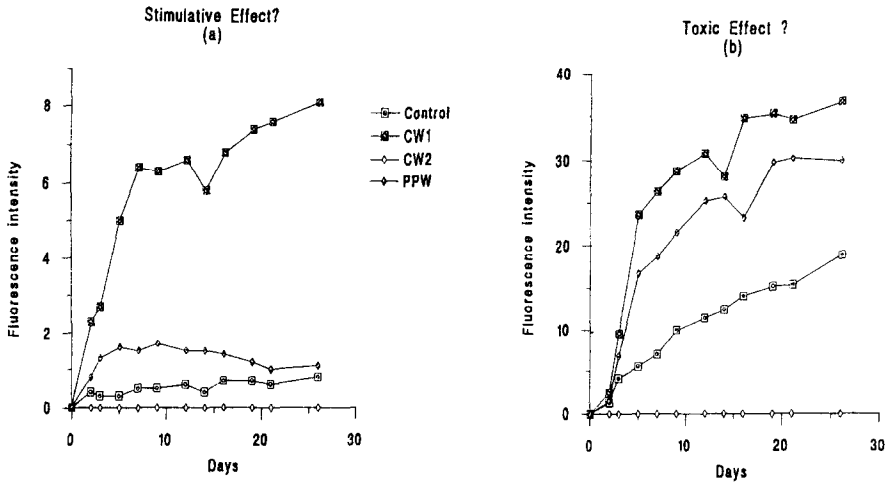

Fig. 1. The results of batch bioassays.

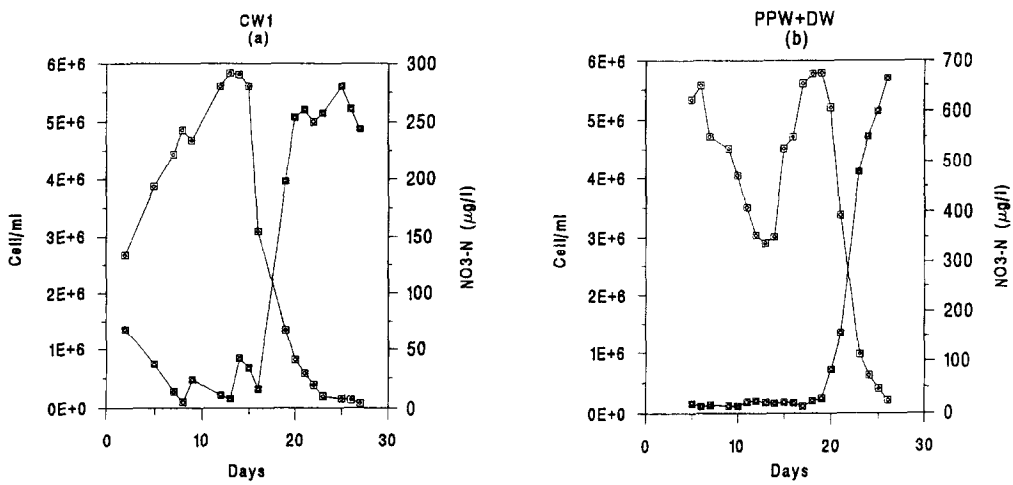

Fig. 2. The results of chemostat bioassays.

Figure 2(a) shows the temporal changes of cell concentration and outflow NO_3–N concentration in CW1 chemostat experiments. The culture was first inoculated batchwise in a 50% seawater + wastewater mixture with addition of phosphate. When the culture reached a reasonable cell concentration it was fed at a dilution rate of 0.4 day^{-1}. After 12–13 days the cell concentration in the culture chamber reached a steady state and PO_4–P was removed from the feeding medium. Figure 2(a) shows that as a result, the cell concentration in the chemostat chamber decreased to a very low level and, as expected, NO_3–N was observed in the outflow.

Figure 2(b) shows the analysis of chemostat cultures in the presence of (PPW + DW) + seawater mixtures. The ratios of the wastewater mixtures (PPW:DW) were chosen

according to the observed seasonal changes in flow rates. The wastewater mixture with a ratio of 3:1 reached a steady state after 12–13 days and the ratio was then changed to 3:4. DW was removed from the feeding medium after 18–19 days when the culture had reached the second steady state and the cell concentration decreased rapidly. From the figure it is clear that when it is mixed with PPW, DW causes a stimulative effect due to its PO_4–P content.

CONCLUSION

According to the batch bioassays:

(a) CW2 is strongly toxic to algal growth and the toxic substances in this wastewater should be removed before the waste is discharged.
(b) CW1 and PPW have stimulative effects on algal growth, the effect being more pronounced with CW1.

According to the chemostat bioassays: the limiting nutrient for CW1 and DW is PO_4–P. Because these wastewaters contain sufficient of other nutrients for algal growth, they are potential sources of eutrophication. PPW contains both nitrate and phosphate and mixing of this wastewater with DW causes a strong stimulative effect especially with a 3:4 mixing ratio. This prompts eutrophication of the Bay waters.

The previous simulations showed that the majority of TP originating from the land-based sources of the Bay proper ends up in the lower layer of the Bay and is a potential source of eutrophication especially in the shallow parts of the Bay (Legoviç *et al.*, 1995) Bioassay studies performed in the Bay waters revealed that the phytoplankton growth was limited by silicate, phosphorus and nitrogen in decreasing order of importance (Morkoç *et al.*, 1988). Thus, the only solution to the eutrophication problem in the Bay is to remove both nitrogen and phosphorus from these wastewaters.

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