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Most of southern California's municipal wastewaters are discharged directly into the ocean via deep water outfalls. Seven treatment facilities discharge a total of approximately 1200 million gallons of effluent per day, over 90% of the total municipal discharge into the Southern California Bight. Biological monitoring of these effluents has been limited to tests with freshwater organisms (primarily acute survival tests with fish). There is little recent data available on the relative toxicity of these effluents to sensitive marine organisms. A survey of the relative toxicity of wastewater effluent from these seven southern California sewage treatment plants to marine organisms was conducted in 1982, 1987, and 1988. Effluent toxicity to sea urchin embryos was measured for most samples. The toxicity of selected effluent samples collected in 1988 and 1987 was also evaluated using Microtox and sea urchin sperm tests. The results of these studies are summarized below, providing insight into effects of differences in effluent composition and species sensitivity on toxicity measurements.

METHODS

Effluent samples from the following treatment plants were tested: JWPCP (Los Angeles County), Hyperion (City of Los Angeles), CSDOC (Orange County), Pt. Loma (City of San Diego), City of Oxnard, ENCINA (City of Carlsbad), and SERRA (Orange County). The samples used in this project were 24-h composites of final effluent collected by the dischargers. The physical and chemical characteristics of the 1987 composites were measured by the dischargers as part of their routine monitoring efforts. Additional effluent composition data used in this report are annual averages of discharger monitoring data.

All toxicity tests were conducted at 15°C. Two different types of sea urchin toxicity tests were conducted, using either gametes or embryos of the purple sea urchin, *Strongylocentrotus purpuratus*. Sea urchin sperm tests were conducted on all 1987 and 1988 samples according to the methods of Dinnel et al.[1] Sperm were exposed to seawater dilutions of effluent for 1 h. Unfertilized eggs were then added to the samples and given 20 min for fertilization to occur. Egg samples were examined with a microscope to determine fertilization success.

Sea urchin embryo tests involved the determination of developmental abnormalities and mortality following 48 h of exposure to effluent dilutions. Embryo condition was assessed by two methods, microscopic examination and echinochrome pigment measurement. The percentage of embryos attaining a normal prism stage of development was determined with the microscope. The amount of echinochrome pigment produced by the embryos was determined by ethanol extraction according to the methods of Bay et al.[2] Production of this pigment is correlated with degree of embryo development; toxic effects are indicated by a reduction in the amount of pigment present. Microscopic examination of embryos was used to assess toxicity in all 1987 samples and in selected 1988 samples. Echinochrome measurement was used to assess toxic effects in all 1982 and 1987 samples. Selected 1988 samples were also evaluated with the echinochrome test.

A Microtox assay was conducted on all 1987 and selected 1988 effluent samples using the methods of Bulich.[3] Luminescent bacteria were exposed to effluent dilutions for 30 min. Toxicity was indicated as a reduction in luminescence following exposure.

Toxicity test results have been expressed as the percentage change relative to the dilution water control in order to facilitate the comparison of results from different experiments and test methods. EC50 values for the sperm test results were calculated using probit analysis.

RESULTS AND DISCUSSION

Substantial differences in effluent sensitivity were observed between the various test methods used. The sperm test was by far the most sensitive to all of the effluent types. Statistically significant reductions in fertilization percentage were often found at effluent concentrations below 1% during the 1987 sampling. Representative results are shown in Figure 1 for the Pt. Loma (1987) and JWPCP (1988) effluents.

The Microtox test was the next most sensitive method. Reductions in bacterial luminescence at effluent concentrations below 4% were usually greater than effects seen with the sea urchin embryo test. The Microtox test was also the least variable of the methods used; reductions in luminescence of less than 10% were often statistically significant. Bacterial luminescence did not change as rapidly with increasing effluent concentration as did the measures of sea urchin embryo development, however. As a result, the relative sensitivity of effluent toxicity estimations from Microtox results was dependent upon the method of data analysis. If toxicity was expressed as a no observable effect concentration (NOEC), Microtox results were more sensitive than the 48-h embryo test. Description of toxicity in terms of the EC50 value results in a decrease of the relative sensitivity of the Microtox results.

The State of California is currently developing effluent toxicity test methods using other marine species, such as mysids, abalone, and giant kelp. Tests of JWPCP effluent toxicity using these species were conducted by the Marine Bioassay Project in 1988.[4] These data permit a comparison of the relative sensitivities of these test methods to a similar effluent type. Responses of these species in 48- to 96-h effluent exposures are shown in Figure 2.

It must be recognized that these data represent results from several effluent samples, none of which were the same as that used with the sea urchin tests. Variations in effluent toxicity between sampling times were observed and undoubtedly had a significant effect on the magnitude of the responses shown here. Consequently, these data can only give an approximate indication of the relative sensitivities of these additional test methods. The

48-h abalone embryo development test appeared to be more sensitive to JWPCP effluent than the 96-h mysid survival or 48-h kelp zoospore germination and gametophyte growth tests. All of these methods also appeared to be considerably less sensitive than the sea urchin sperm test.

The effluent from the seven treatment plants studied in this project had very different toxicities. EC50 values for the sea urchin sperm test ranged from 0.09% (JWPCP) to 25% effluent (SERRA) for the 1987 samples (Table 1). The chemical composition of these effluent samples is shown in Table 2. The toxicity of the samples appeared to be more strongly influenced by the size of the treatment plant than to the concentrations of individual contaminants. Effluent from large plants (flow greater than 100 mgd) was approximately an order of magnitude more toxic than the smaller plants. Among the large plants, there was no simple relationship between degree of treatment or chemical concentration and toxicity. JWPCP effluent was more toxic than Hyperion or Pt. Loma, even though this effluent received a greater proportion of secondary treatment and had similar concentrations of trace metals and chlorinated hydrocarbons. Suspended solids content of the effluent appeared to be related to variations in sperm toxicity among the smaller treatment plants. The greatest toxicity was found at ENCINA, which had an effluent solids content more than double that of similar sized plants.

The 1987 sperm test results indicated an unexpectedly high level of toxicity for effluent from all plants except SERRA and Oxnard. The 1988 sperm test results indicated substantially less toxicity in effluent samples from all plants. JWPCP and Hyperion effluent samples were the most toxic in 1988, with EC50's of 0.27 and 17.9%, respectively. Toxicity was too low to calculate EC50 values for the remaining effluent types; CSDOC was the most toxic effluent within this group, having a sperm test NOEC of 2%.

The sea urchin echinochrome test results can be used to document effluent toxicity changes with time. Examination of these data indicates that substantial reductions in effluent toxicity at the largest treatment plants have occurred since 1982 (Figure 3). Improvements in sewage treatment and source control practices appear to have been responsible for these changes. Dramatic reductions in the emissions of most measured contaminants by these treatment plants have occurred in the last decade.[5] The general pattern of these contaminant reductions is usually correlated with suspended solids changes, which are shown in Figure 4a. Suspended solids concentrations at these plants declined by 25 to 58% between 1982 and 1987. Reductions in effluent toxicity appeared to be continuing in 1988, based on sea urchin sperm toxicity results (data not shown).

The temporal changes in effluent toxicity identified using sea urchin test data can be compared with discharger-supplied toxicity data based on acute survival of freshwater fish. A similar pattern of toxicity reduction is evident only for the JWPCP plant data (Figure 4b). Fish toxicity reported for the other plants is similar between the 1982 and 1987 samplings. Changes in toxicity at these plants could not be detected with fish because of the low sensitivity of this test; fish survival in 100% effluent was already high in 1982.

SUMMARY

The results of this study provide an indication of the toxicity of wastewater effluents from southern California to local marine species. It is clear that

marine tests are orders of magnitude more sensitive than acute tests with freshwater fish. Trends in toxicity identified with sea urchin tests often did not correspond with fish bioassay results, illustrating the irrelevance of such acute tests for protecting the marine environment.

Considerable variation in effluent sensitivity was also found between various marine toxicity test methods. In general, the greatest sensitivity was found with methods using the shortest exposure times (sea urchin sperm test and Microtox). The high sensitivity of the sperm and Microtox tests is probably due to their short exposure times, rather than to substantial differences in sensitivity to specific contaminants. These tests detect toxicity before the concentrations of labile toxic components in the diluted effluent have an opportunity to decrease greatly through volatilization, degradation, or adsorption processes. The sea urchin sperm and Microtox tests therefore appear to be the best choices for the accurate assessment of toxicity in a relatively unstable sample such as wastewater effluent.

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TABLE 1. Toxicity of 1987 effluent samples to sea urchin sperm. Samples were collected during April and May 1987.

Effluent	EC50 and 95% confidence limits (% effluent)
JWPCP	0.09 (0.07 - 0.12)
Hyperion	0.48 (0.39 - 0.60)
CSDOC	0.32 (0.25 - 0.41)
Pt. Loma	0.51 (0.44 - 0.60)
Oxnard	5.64 (4.19 - 7.58)
ENCINA	2.32 (1.92 - 2.81)
SERRA	25.0 (11.4 - 55.0)

TABLE 2. Characteristics of 1987 effluent samples tested for toxicity. All units are mg/L unless otherwise noted.

Constituent	JWPCP	Hyperion	CSDOC	Pt. Loma	Oxnard	ENCINA	SERRA
Flow (mgd)	386	369	270	182	20	19[a]	15[b]
% Secondary	50	25	60	0	100	30	100
Susp. Solids	75	56	52	73	23	58[a]	20[b]
BOD	106	111	64	132	26	65[a]	11[b]
Ammonia-N	37.5	15.6	NA	23.4	4.9	19.5	7.2
Arsenic	0.007	0.007	NA	0.004	<0.005	<0.005	<0.005
Cadmium	0.001	0.012	0.002	<0.005	<0.01	0.007	<0.001
Chromium	0.061	0.03	0.017	<0.02	<0.01	<0.005	<0.05
Copper	0.044	0.067	0.060	0.05	0.052	0.022	0.04
Lead	0.046	0.03	0.02	<0.05	<0.07	0.001	0.14
Mercury	0.0001	0.0002	NA	0.0005	<0.001	<0.0002	<0.001
Nickel	0.055	0.06	0.03	0.02	0.082	0.038	<0.04
Silver	0.008	0.012	0.011	<0.01	<0.02	<0.001	<0.01
Zinc	0.11	0.32	0.07	0.062	0.058	0.062	0.14
Cyanide	0.03	0.026	NA	0.005	0.011	0.01	0.10
Tot. Phenol	1.8	0.048	NA	0.007	<0.02	0.001	<0.003
Tot. DDT(ug/L)	0.07	<0.02	NA	ND	<0.05	ND	NA
Tot. PCB(ug/L)	ND	<0.1	<0.5	ND	<0.15	ND	NA
Tot. PAH(ug/L)[c]	<0.014	0.024	1.93	<0.009	<0.005	<0.005	NA
96 Hour LC50[d]	77	101	132	59[a]	>100[e]	130[f]	NA

[a]Average for the month of May.
[b]Average for the month of April.
[c]Analysis done at SCCWRP
[d]Fathead minnow bioassay, performed by the discharger.
[e]No mortality occured at any of the dilutions, so LC50 cannot be calulated.
[f]Value for different day of same week.
NA = Data not available.
ND = Not detectable, detection limit not available.

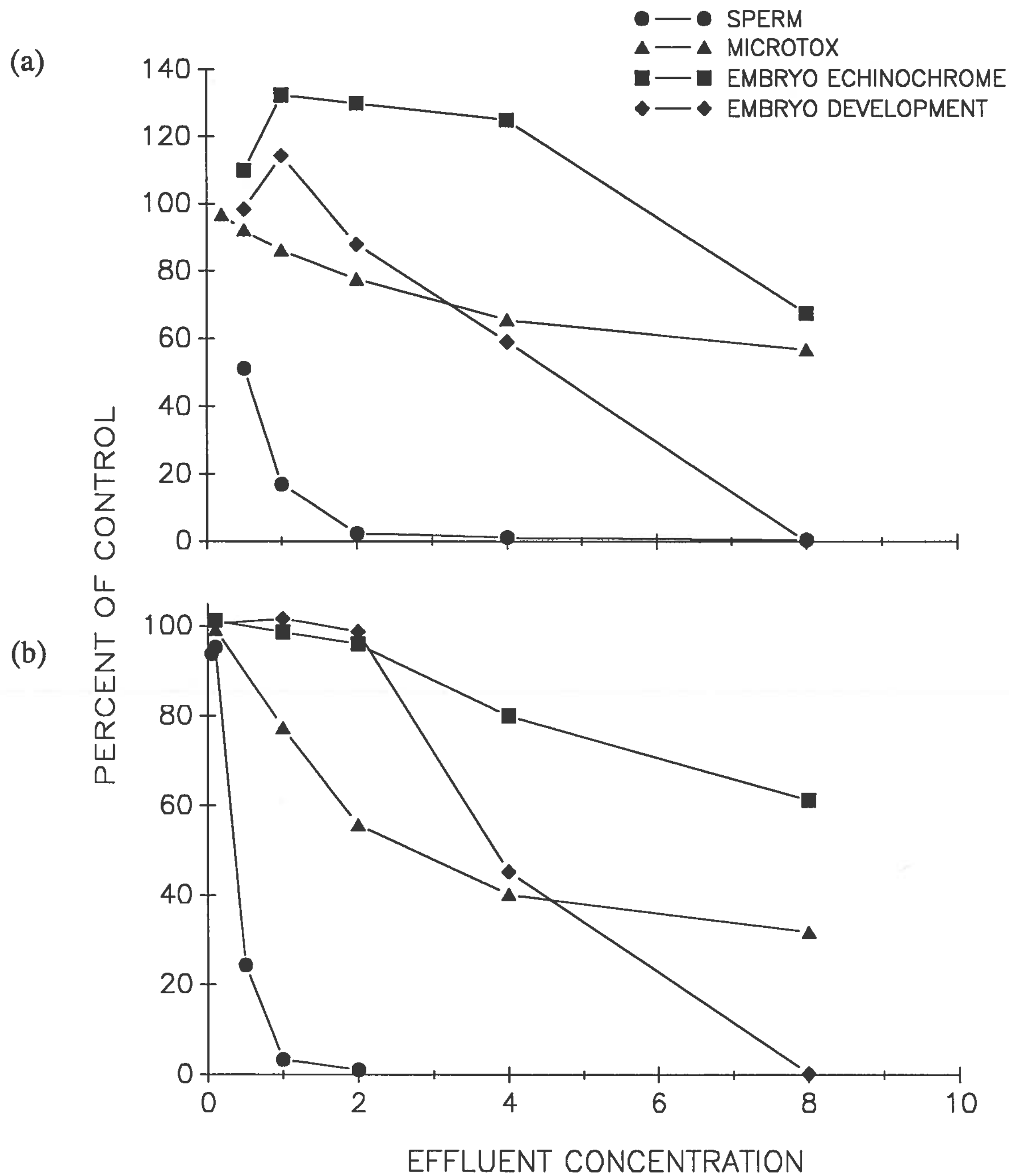

FIGURE 1. Results of Microtox and sea urchin sperm and embryo toxicity tests of wastewater effluent. (a) Test of Pt. Loma effluent sample collected in May 1987. (b) Test of JWPCP effluent sample collected in December 1988.

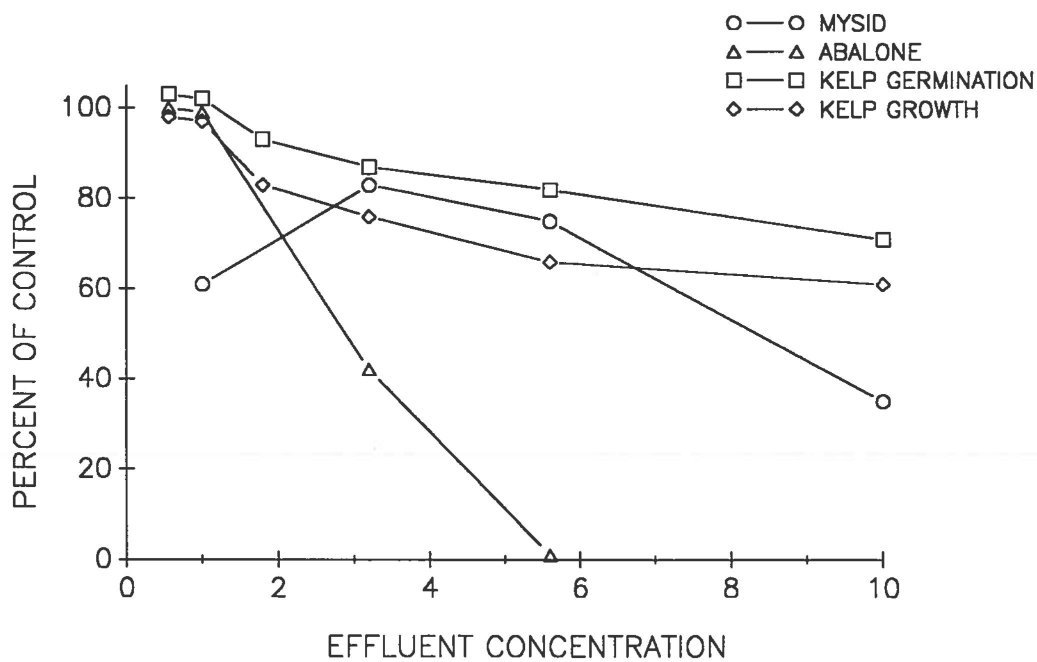

FIGURE 2. Results of mysid (*Holmesimysis costata*), abalone (*Haliotis rufescens*), and kelp (*Macrocystis pyrifera*) toxicity tests of JWPCP effluent. Different effluent samples were used for tests with each species. Data for each species are mean of results for two separate effluent samples.

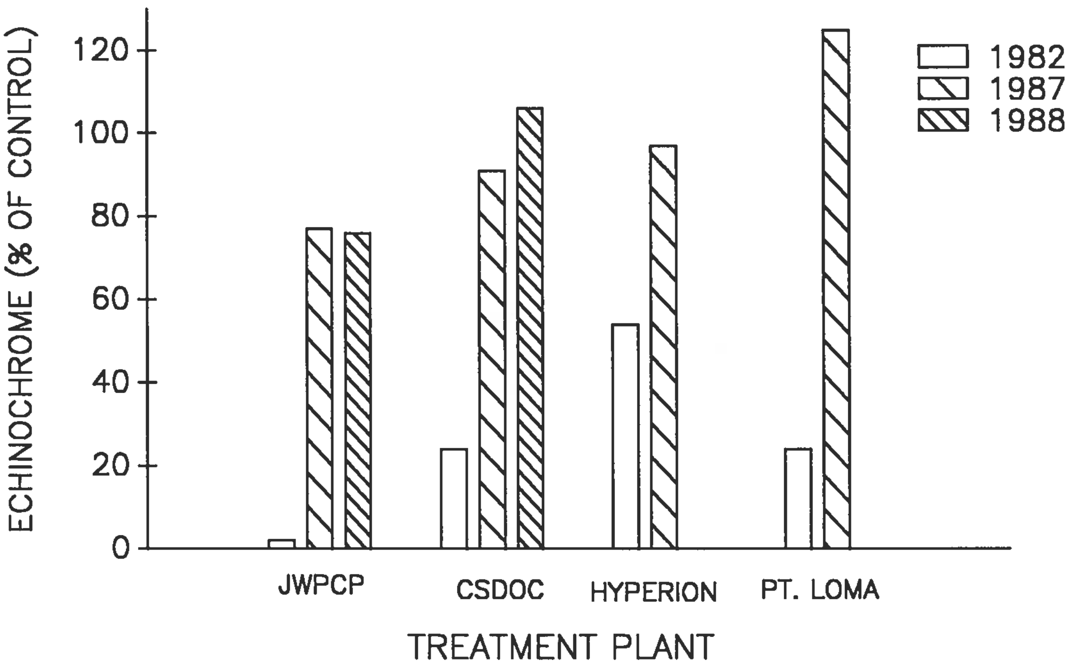

FIGURE 3. Temporal changes in effluent toxicity to sea urchin embryos (echinochrome pigment production of 48-h embryos). No 1988 toxicity measurements were made for Hyperion or Pt. Loma.

(a)

(b)

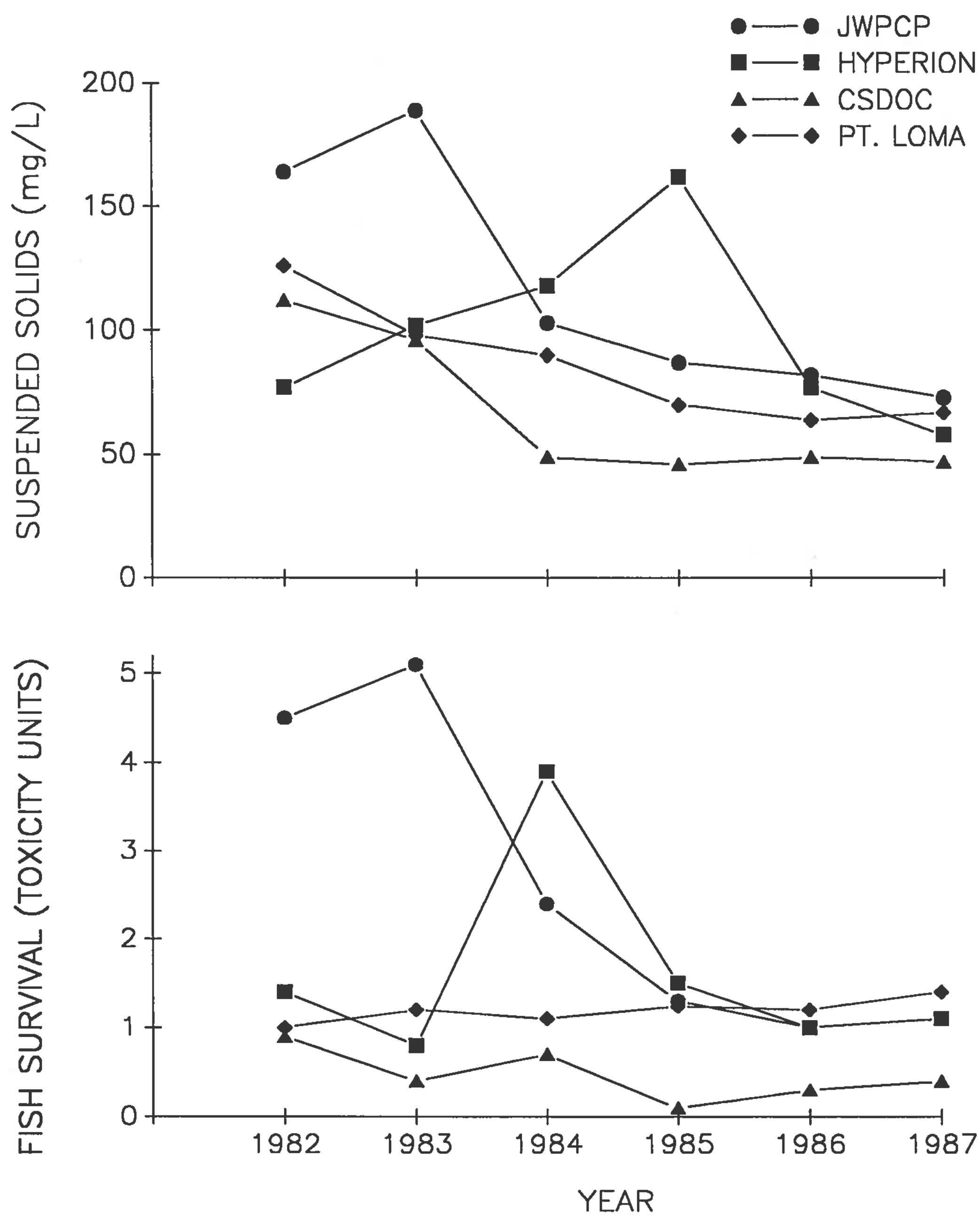

FIGURE 4. Temporal changes in effluent characteristics for southern California's largest wastewater treatment plants. (a) Suspended solids concentration (data are annual averages for each plant). (b) Annual average fish toxicity expressed as toxicity units (TU). One TU = 100/96-h LC50.