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ATP-TOX SYSTEM---A NEW RAPID, SENSITIVE BACTERIAL TOXICITY SCREENING SYSTEM BASED ON THE DETERMINATION OF ATP

by

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ABSTRACT

A new toxicity screening test, based on the inhibition of bacterial growth and luciferase activity by toxicants was developed. In the ATP-TOX System, chemical toxicity was found to be time-dependent and increased with increasing exposure time up to five hours. Three organisms were evaluated in this study, E. coli K-12 PQ-37, Pseudomonas fluorescens and Salmonella typhimurium. E. coli K-12 PQ 37 was found to be the most sensitive organism. It was also shown that P. fluorescens was more sensitive to toxicants when grown in minimal medium than in nutrient broth, suggesting that nutrients may have a protective effect on the bacterium. In comparative studies using selected toxic chemicals the ATP-TOX System was found to be more sensitive than the Spirillum volutans test and comparable to the Microtox test.

Toxicant activity in sediment samples was found to be time dependent and increased with increasing exposure time in both ATP-TOX (E. coli) System and Microtox. The ATP-TOX System is complementary to the Microtox test as it also provides indications of low grade toxicant activity which is only manifested in actively growing cells over several life cycles. Thus, the ATP-TOX System appears to be an ideal screening test for sediment toxicity. The data indicate that the ATP-TOX System is sensitive, rapid, reproducible, economical an has great potential in applied studies.

SOMMAIRE

On a mis au point une nouvelle épreuve de dépistage de la toxicité. Dans l'épreuve ATP-TOX, la toxicité varie en fonction du temps et augmente donc proportionnellement à la durée du contact jusqu'à cinq heures au maximum. Dans cette étude, on a évalué trois organismes, E. coli K-12 PQ-37, Pseudomonas fluorescens et Salmonella typhimurium. Il ressort de l'étude que le micro-organisme le plus sensible est E. coli K-12 PQ-37. Par ailleurs, on a constaté que le Pseudomonas fluorescens s'est montré plus susceptible aux substances toxiques s'il se développe dans un milieu appauvri plutôt que dans un bouillon de culture. Il est permis de penser que les substances nutritives peuvent favoriser la résistance de la bactérie. À la suite d'études comparatives effectuées à l'aide de substances toxiques choisies, on a pu établir que l'épreuve ATP-TOX est plus sensible que celle basée sur le Spirillum volutans et aussi sensible que l'épreuve Microtox. L'activité des substances toxiques est fonction du temps de sorte que la toxicité augmente proportionnellement à la durée du contact avec ces substances autant pour l'épreuve Microtox que pour l'épreuve ATP-TOX (E. coli). Cependant, cette dernière complète l'épreuve Microtox dans la mesure où elle fournit aussi des indications quant aux effets des substances toxiques présentes à faible niveau, lesquels ne se font sentir dans les cellules vivantes qu'au terme de plusieurs générations. Par conséquent, l'épreuve ATP-TOX est une technique idéale de dépistage de la toxicité dans les sédiments. D'après les résultats obtenus, l'épreuve ATP-TOX paraît sensible, rapide, reproduisible et économique. Elle semble prometteuse pour les études appliquées.

EXECUTIVE SUMMARY

A new toxicity screening test, the ATP-TOX System, was developed. This test is based on both the inhibition of bacterial growth and luciferase activity by toxicants.

In comparative studies using selected toxic chemicals and sediment extracts the ATP-TOX System was found to be comparable and at times more sensitive than the Microtox test. The data indicate that the ATP-TOX System is a sensitive, economical, rapid, reproducible and easily-performed toxicity screening test. It is at least as sensitive as the Microtox test and adds an additional dimension (life cycle effects) to rapid toxicity screening test batteries.

The ATP-TOX System can also be used to study chemical inhibition of luciferase activity as well as chemical toxicity patterns during the bacterial life cycle. It can be used to test environmental samples as well as pure and mixed chemical samples. Another economical feature of this procedure is that the instrument used in this test can also be used to determine viable biomass in water, sewage or sediment sample.

The ATP-TOX System applied to environmental samples could use predetermined bacterial species or organisms (pure or naturally mixed cultures) indigenous to the environmental samples being tested. Organism resistance patterns to specific toxicants can also be studied to clarify and understand natural resistance patterns in the environment.

SOMMAIRE ADMINISTRATIF

On a mis au point une nouvelle épreuve de dépistage de la toxicité, l'épreuve ATP-TOX. Celle-ci est fondée sur le fait que les substances toxiques inhibent la croissance bactérienne ainsi que l'activité de la luciférase.

Dans les études comparatives ayant pour objet des substances toxiques et des extraits de sédiments choisis, l'épreuve ATP-TOX s'est révélée aussi sensible que l'épreuve Microtox et même parfois davantage. Les données confirment que l'épreuve de dépistage de la toxicité ATP-TOX est sensible, économique, rapide, reproduisible et facile à exécuter. Elle est au moins aussi sensible que l'épreuve Microtox et ajoute aux épreuves utilisées pour le dépistage rapide de la toxicité une autre dimension, à savoir les effets sur le cycle de vie.

On peut également se servir de l'épreuve ATP-TOX pour étudier les phénomènes d'inhibition chimique sur l'activité de la luciférase ainsi que les effets des substances chimiques toxiques sur les cycles de vie des bactéries. On peut l'utiliser pour analyser des échantillons prélevés en milieu naturel de même que des échantillons de substances chimiques purs ou mélangés. Par ailleurs, l'épreuve est économique dans la mesure où elle permet aussi de déterminer la quantité de la biomasse viable que renferme un échantillon donné d'eau, de sédiments ou d'eau d'égout.

Si on applique l'épreuve ATP-TOX à des échantillons tirés du milieu ambiant, on peut se servir de bactéries ou de micro-organismes d'espèces connues (cultures pures ou mélangées de façon naturelle) qu'on trouve à l'état naturel dans les échantillons à vérifier. À l'aide de cette épreuve, on peut également étudier la résistance des micro-organismes à des substances

toxiques particulières de manière à élucider la nature de ce mecanisme dans le milieu ambiant.

Titre : L'épreuve ATP-TOX : une nouvelle épreuve de dépistage de la toxicité à la fois rapide et sensible fondée sur la mesure de l'ATP

INTRODUCTION

Traditionally, toxicant levels in water and effluents have been estimated by bioassay proedures in which fish or macroinvertebrates were the sensing agents. Within the past ten years there has been an increasing tendency to use microbial systems for screening toxicants in water, effluents and sediment extracts (Liu and Dutka 1984). These microbial systems have a few advantages over the traditional tests. They are rapid, sensitive and economical.

Being a product of catabolic reactions, ATP (adenosine triphosphate) is found in all living cells. The fact that ATP is rapidly destroyed after cell death makes it ideal for distinguishing between live and dead cells. The basic assay of ATP consists of measuring the light emission following the reaction of firefly luciferin with ATP in the presence of luciferase and Mg^{++} (Holm-Hansen 1973).

ATP has been considered to be a good biomass indicator in fresh water (Holm-Hansen and Booth 1966), sea water (Cavari 1976) and sewage sludge (Patterson et al. 1970). In sewage treatment it has been found useful in indicating changes in operating conditions (Levin et al. 1975) as well as in measuring live biomass in effluents. This factor was also used by Brezonik and Patterson (1972) in testing the toxicant activity in activated sludge.

However, the potential of using ATP content of actively growing cells for toxicity assessments has seldom been systematically evaluated. Recent studies in this area include Kennicutt's (1980) evaluation of ATP assays in toxicity screening of water samples, Seyfried and Horgan's (1983) study on the effects of cadmium on lake bacteria by measuring ATP contents and Parker and Pribyl's (1984) study of short-term response of bacterial ATP to a few chemicals.

In this study, we report the development of a sensitive reproducible and inexpensive ATP-TOX System for use in toxicity screening of chemicals and environmental samples. The test is based on the measurement of the growth inhibition and enzyme activity.

METHODS

Toxicity Tests

(1) Microtox test:

Microtox test was performed using the luminescent bacterium Photobacterium phosphoreum and following the procedure detailed in Beckman Microtox System Operation Manual (1982) with contact time of 15 minutes (Dutka and Kwan 1981). The metabolism of the luminescent bacteria is influenced by the test toxicants and any alteration of metabolism affects the intensity of the organisms' light output. By

sensing these changes of light output in a photometer, the presence and relative concentrations of toxicants can be obtained by establishing the EC_{50} levels from graphed data. EC_{50} being, in this case, the concentration of toxicant (or dilution of unknown) causing a 50% reduction in light output from the base level.

(2) ATP-TOX System:

Reagents:

The ATP-TOX System test was performed using the following bacteria: Pseudomonas fluorescens, an environmental isolate; Escerichia coli K-12 PQ37 obtained from the SOS Chromotest procedure; and Salmonella typhimurium TA-98 used in Ames' mutagen screening test. Media and reagents used in this procedure are: Nutrient Broth (Bacto-beef extract 3 g, Bacto-peptone 5 g, and 1000 mL distilled water); Minimal Medium, was prepared from: K_2HPO_4 10.5 g, KH_2PO_4 4.5 g, $(NH_4)_2SO_4$ 1 g, sodium citrate $2H_2O$ 0.5 g, distilled water 800 mL, presterilized $MgSO_4.7H_2O$ (1%) 1 mL, Vitamin B1 (1%) 0.5 mL, glucose (20%) 10 mL and making up to volume of 1000 mL with sterilized distilled water; ATP hepes buffer, ATP releasing reagent, luciferin-luciferase and ATP standard solution which were supplied by Turner Designs (Mountain View, California, USA).

Procedures:

To determine the inhibition of toxicants on the total ATP-TOX System as measured by changes in light output of the luciferin-luciferase system after bacteria are exposed to toxicants, the following procedures were used:

(a) overnight cultures growing in nutrient broth or minimal medium were prepared;

(b) overnight cultures were diluted 100 times with nutrient broth or minimal medium;

(c) two-fold serial dilutions (1 mL) of the test chemicals or solutions were placed into 12 x 100 mm sterile tubes (negative control tubes using 1 mL distilled water were also prepared);

(d) 1 mL of diluted bacterial culture was dispensed into each tube, capped and incubated (E. coli and S. typhimurium at 37°C; P. fluorescens at 26°C) on a shaker (100 rpm) for up to five hours;

(e) light output of luciferin-luciferase system was checked at various intervals by taking 50 µL from each tube (test and control) and placing into 1.6 mL polypropylene tubes which fitted the chamber of Turner Designs Model TD-20e Luminometer;

(f) to these tubes 50 µL ATP releasing agent and 50 µL ATP hepes buffer were added, mixed well and the tube was placed into the luminometer;

(g) 50 µL of luciferin-luciferase solution were injected into the tube and the light output was read on the Luminometer screen and recorded;

(h) during every batch of tests, 50 μL of standard ATP solution ($5x10^{-8}$ g/mL) was used to monitor the enzyme activity and 50 μL of sterile distilled water was used as blank, then steps f and g were followed.

To determine the inhibitory effect of toxicants on the activity of luciferase, the following procedures were used:

(a) fifty μL of toxicant solution at specific concentrations and sterile distilled water (negative control) were added to 1.6 mL polypropylene tubes;

(b) fifty μL ATP standard solution ($5x10^{-8}$ g/mL) and 50 μL hepes buffer were quickly added to each tube, mixed well and placed into the luminometer chamber. Blank readings were taken by adding 50 μL sterile distilled water instead of ATP standard solution;

(c) 50 μL of luciferin-luciferase solution were immediately injected into the tube and the light output was read on the screen and recorded.

Results expression:

To calculate the percentage inhibition of toxicants on the total ATP-TOX System or on the luciferase activity, the following equation is applicable:

$$I(\%) = \frac{C - U}{C - B} \times 100\%$$

where I = inhibition percentage; C = reading for control; B = reading for blank; U = reading for unknown samples.

IC_{50} is defined as the concentration of toxicant causing 50% inhibition as compared to the negative control.

If the total inhibition (It) of the ATP-TOX System and the enzyme activity (Ie) inhibition by the toxicant at specific concentration are known, the toxicants inhibitory effect on bacterial growth (Ig) can be calculated by the following:

$$It = Iq + Ie$$

Chemicals tested:

Copper sulfate ($CuSO_4$), mercury chloride ($HgCl_2$), cadmium chloride ($CdCl_2.2.5H_2O$), zinc sulfate ($ZnSO_4.7H_2O$), nickle chloride ($NiCl_2.6H_2O$), phenol, p-nitrophenol, O-chlorophenol, 3,5-dichlorophenol, sodium lauryl sulfate, cetyltrimethylammonium chloride, α-naphthol, 2,4-dichlorophenoxy acetic acid, and 2,4,6-trichlorophenol. The pHs of the tested chemicals tested were 6.8.

Sample collection and treatment:

Four sediment samples were collected by Ekman dredge from Hamilton Harbour on Lake Ontario (Fig. 1). The top 10 cm of each sample was removed, placed into sterile plastic bottle, then placed in a melting ice bath and brought to the laboratory for processing.

Fifty grams of each sediment were weighed and placed into an acid washed and Milli-Q reagent grade water rinsed (five times) BOD bottle. Fifty mL of distilled water were added to the sediment, and the bottle was stoppered and vigorously shaken by hand for two minutes. The mixtures were poured into 250 mL Nalgene centrifuge bottles (acid washed and Milli-Q reagent grade water rinsed) and centrifuged for 20 minutes at 10,000 rpm in a refrigerated centrifuge. After centrifugation the supernatants were decanted into chemically clean flasks and sterilized in a microwave oven by quickly bringing the extracts to boiling three times. This precautionary procedure was carried out to eliminate possible interference by viable microorganisms to ATP level measurements.

RESULTS AND DISCUSSION

Generally speaking, the toxicity of a chemical or mix of chemicals on a biological indicator system will increase with increasing exposure time provided the toxicant dose is not excessive. This effect is seen in Figure 2, where P. fluorescens was used in the ATP-TOX System to assess the toxicity of Hg^{++}, Zn^{++}, Ni^{++}, phenol, α-naphthol and 3,5-dichlorophenol. Data in Figure 2 also show that chemical toxicity is contact time dependent and increases with increasing exposure (or incubation) time up to five hours. There is a possibility that for certain chemicals or samples a more sensitive testing could be obtained by extending the exposure time beyond five

hours. However, there are two main drawbacks with this proposal: (1) incubation time beyond five hours is difficult to fit into a traditional working day schedule and (2) complications in the testing format may arise due to accumulation of growth-death byproducts and biodegradation of organic toxicants. In Figure 2, it can be seen that there are several different patterns of time-dependent toxicity. Some chemicals produced greatly increased toxic effects with increased incubation as shown by phenol, Ni^{++}, Zn^{++} and Hg^{++}. For instance, the IC_{50} of Zn^{++} is 219 mg/L at 1.2 hour and 4.0 mg/L at five hours, a 50 times increase in toxicity by increasing the incubation period 3.8 hours. However, for 3,5-dichlorophenol, IC_{50} at 5, 3.5 and 1.2 hour is 8.0, 8.5 and 12 mg/L, respectively, lying within a very narrow concentration range. For α-naphthol, the effect of incubation on toxicant activity tended to decrease after 3.5 hours. Similar results were obtained with E. coli K-12 PQ37 when used as the test organism in the ATP-TOX System.

E. coli was found to be the most sensitive organism in the ATP-TOX System when all the test organisms were grown in nutrient broth (Figure 3). The sensitivity of E. coli to toxicants was especially noticeable when screened against low chemical concentrations. These studies also showed that Salmonella typhimurium's sensitivity pattern was close to that of E. coli and much higher than that of P. fluorescens in the ATP-TOX System. The higher sensitivity of E. coli and S. typhimurium to toxicants compared to P. fluorescens

may be due to their environmental niches. Both E. coli and S. typhimurium are usually found in human and animal guts while P. fluorescens is a typical water organism and only rarely found in mammalian enteric flora. Perhaps due to evolutionary stresses the "gut" organisms and environmental organisms have been subjected to different kinds or concentrations of chemicals and, in their adaptation over time, the physiology of the organisms have been modified to withstand various chemical stresses. Thus, it would appear that the environmental organism may be slightly more tolerant to chemical stresses than the gut organisms.

In Figure 4, a comparison of the effect of chemicals on luciferase activity and on the total ATP-TOX System is shown, using P. fluorescens as test organism. Here it can be seen that the effects of sodium lauryl sulfate (SLS) appeared to be mainly on luciferase activity and that at lower concentrations of SLS, bacterial growth seemed to be stimulated, because the total inhibition was smaller than enzyme activity inhibition ($Ig = It - Ie < 0$, means stimulation). The stimulation of the bacterial growth may be indicative of SLS degradation by P. fluorescens. For phenol, at 370 mg/L, total inhibition was 76% while enzyme activity inhibition was 10%, thus bacterial growth was inhibited 66%. Conversely, inhibitory effects of Zn^{++} and Cu^{++} on luciferase activity were minimal compared to their inhibition on bacterial growth.

Nutrient levels, of media in which the testing organisms are stressed by toxicants, are very critical. In this study, it was noted that most of the chemicals tested were more toxic to P. fluorescens when the organisms was tested and grown in minimal medium than in nutrient broth (Figure 5). Studies by Bird et al. (1985) readily confirm these observations. They reported that growth media contained a wide variety of chemical reagents including amino acids, proteins, lipids and inorganic phosphates as well as numerous metal ions. Thus growth media cannot be considered as being chemically inert whose sole function is to support microbial growth. Bird et al. found that growth media even at concentrations as low as 1% (v/v) produced significant chemical changes, converting simple copper ions into one or more unidentified copper complexes which obviously result in changes in the bioavailability and biochemical reactivity of copper. It is believed that this phenomenon is common with most chemicals or mixtures of chemicals which are screened for their toxicity. In the case of P. fluorescens, it is believed that nutrient broth has a protective effect on the organism, which may be explained by either or both of the following: physical interactions between media nutrients and toxicants creating less toxic effects or/and osmotic equilibrium changes between bacterial cells and their environment that result in a decrease in adsorption of the toxicants. Attempts to grow E. coli K-12 PQ37 in minimal medium resulted in poor growth and insufficient ATP for monitoring. When grown in nutrient broth, P. fluorescens was

found to be much less sensitive to test chemicals than E. coli; however, when grown in minimal medium, its sensitivity to the test chemicals increased and was in some cases greater than that of E. coli (Table 1).

In Table 1, a comparison of the sensitivity patterns of the ATP-TOX Systems, Microtox and Spirillum volutans procedure to 12 chemicals is shown. It can be seen that the ATP-TOX Systems were more sensitive than S. volutans test and by combining the two organism tests (P. fluorescens and E. coli K-12 PQ37) the sensitivity of the ATP-TOX System was comparable to and often greater than that of the Microtox test. The Microtox system still has a few advantages in that it is a much shorter term test, approximately 45 minutes from start to finish, it has slightly fewer manipulation procedures and it has a world wide data base. However, the ATP-TOX System can complement the Microtox data as it determines relatively longer term effects (at least eight generations) of toxicants on growing cells, while being as sensitive and less expensive to perform.

Parker and Pribyl (1984) developed a test based on equilibrium ATP changes using a variety of microorganisms, notably E. coli and N. europaea, to test for toxicity. While the data from this 20-minute test supported the use of the ATP-time response and cause-effect curves to indicate relative toxicity, Parker and Pribyl concluded that the sensitivity of the ATP responses precluded the use of ATP response without further refinement as a toxicant screening test. In Table 2,

some of the Parker and Pribyl's data, based on E. coli are shown and compared with the ATP-TOX System also using E. coli. Aside from the magnitude of the sensitivity differences (ten-fold or greater), there is surprisingly good agreement between the two tests. These results strongly support the significance of an extended growth period when using ATP tests to evaluate toxicity. Since the amount of ATP per cell remains relatively stable throughout all phases of bacterial growth, particularly during the log phase (D'Eustachio and Johnson 1968), significant toxic responses (death of cells) can be observed only at high chemical concentrations through ATP changes in short-term response studies (shorter than 30 minutes---the time needed for cell doubling), as in Parker and Pribyl's study (1984). On the other hand, with a few hours for cell growth, bacteria are more sensitive to very low chemical concentrations. Thus, within limits, the longer the incubation (or exposure) time, the more sensitive the system is to a toxicant's activity (see Figure 2).

The toxicities of the four sediment extracts as measured by the ATP-TOX Systems and the Microtox test are shown in Figure 6. While all the toxicity screening tests showed different sensitivity patterns, it can be seen that the Microtox test and ATP-TOX (E. coli) System were both more sensitive to the sediment extracts than the ATP-TOX (P. fluorescens) System. Extracts from samples 1 and 2 were more toxic in the Microtox test while extracts from samples 3 and 4 were more toxic in the ATP-TOX (E. coli) System. These findings may

be indicative that the samples contain the same or different kinds of pollutants in different ratio mixes. Pollutants in samples 1 and 2 appear to promote acute toxicity effects while those in samples 3 and 4 are slightly less toxic and may be indicative of pollutants with accumulative effects.

It can also be seen in Figure 6 (samples 2, 3 and 4) that the ATP-TOX (E. coli) System responded to lower concentrations of toxicants than the Microtox test, which suggests that the ATP-TOX System is a very sensitive system for screening samples. The increased sensitivity of the ATP-TOX System over the Microtox System can also be seen in samples 3 and 4 (Figure 7) where at concentration of 0.0625 g/mL, the Microtox System showed 0% inhibition and 4% inhibition, respectively, while the ATP-TOX (E. coli) System was inhibited 40% and 43%, respectively. These results are indicative of different toxicant activities as shown by two different types of response, inhibition of fluorescence (Microtox) and inhibition of mainly bacterial growth and perhaps fluorescence (ATP-TOX). At very high toxicant levels, cell death may occur in both systems. From these results, it would appear that the two techniques are complementary and mutually supportive and it would be advantageous to use both techniques [acute test and longer term (five hours) test] as part of a battery of screening tests.

With all four samples, an IC_{50} value for ATP-TOX (P. fluorescens) System could not be attained with the extract concentrations used, suggesting that P. fluorescens was much less sensitive to sediment

extracts than E. coli. A similar phenomenon was observed when pure chemicals were tested. These findings support the hypothesis that differences in sensitivity to toxicants may be due to the original environmental niches of the testing bacteria (i.e. mammal gut versus soil and water environments) and thus during evolution the organisms have been exposed to different types and concentrations of chemicals. This phenomenon is similar to the observed bacterial resistance to antibiotics and may be called the "immune" reaction of bacteria. Since E. coli are closely identified with mammals, especially human beings, it may be more meaningful than P. fluorescens and the results of ATP-TOX (E. coli) System may eventually be shown to be more representative of mammal-chemical interactions than that of ATP-TOX (P. fluorescens) System.

In Table 3, the effect of exposure time on toxicant activity manifestation is shown. It can be seen that in both the ATP-TOX (E. coli) System and Microtox test, the longer the exposure time of the testing cells to the samples, the smaller the amount of sediment extract required to produce an IC_{50} (or EC_{50}). When exposed for eight hours in ATP-TOX (E. coli) System, three to four times less extract solution of samples 3 and 4 was required to produce an IC_{50} compared to the routine five-hour test. Thus, exposure time is critical to the sensitivity of most toxicant screening tests. For sample 1, it can be seen that Microtox test is much more sensitive than ATP-TOX (E. coli) System, even with eight hours of exposure time. Again the data are

supportive of our belief that the two tests are complementary and should be part of any battery of microbial toxicant screening tests.

Due to the long-term effects of chemicals discharged into natural water systems, research efforts are being directed at short-term bioassays in an attempt to alert monitoring agencies as well as effluent dischargers of toxic conditions. Undoubtedly the short-term screening tests (30-45 minutes) have many advantages over the traditional long-term toxicity tests, however, the advantages of long-term tests cannot be totally ignored. They have a value in studying delayed toxicant effects, complex interractions and establishing time-dependent toxicity patterns. Compared to most long-term toxicity tests, e.g. 24-96 hours static and flow through fish tests, four day algal growth tests, protozoan colonization tests (Cairns et al. 1985) and 18 hours bacterial tests (Dutka and Kwan 1981), the ATP-TOX System is a relatively short-term toxicity screening test (five hours) with most of the benefits of those long-term tests but with increased sensitivity. From Figure 7, it can be seen that the ATP-TOX System is a very reliable test capable of providing reproducible results.

In using the ATP-TOX System, it is advisable to use a Turner Designs Luminometer and reagents, especially the releasing reagent which makes the most crucial procedure, the extraction of ATP from bacterial cells, very easy and reliable. Seyfried and Horgan (1983) and Parker and Pribyl (1984) in their studies both used the heat

extraction procedure which is comparatively more time-consuming and complicated, whereas by using Turner Designs hepes buffer and releasing reagent, one can measure ATP levels of any bacterial sample directly in less than 50 seconds.

Although only three different bacteria were evaluated as test organisms in the ATP-TOX System, it is probable that any bacterium with a short duplication time could be used. The main effect of using many different bacteria would be that each bacterium probably has its own sensitivity pattern to chemicals. Whether this sensitivity pattern to toxicants could be related to Family, Tribe, Genus or Species levels is not known. The variety of sensitivity patterns was also noted by Seyfried and Horgan (1983) in their studies to evaluate cadmium toxicity by ATP measurements in six bacterial species. It is also believed that eucaryotic microorganisms are promising candidates as test organisms of the ATP-TOX System.

CONCLUSIONS

The ATP-TOX System is a sensitive, reproducible and easily performed toxicity screening test. It is at least as sensitive as the Microtox test and adds an additional dimension (life cycle effects) to rapid toxicity screening test batteries. The ATP-TOX System can also be used to study chemical inhibition of luciferase activity as well as chemical toxicity patterns during the bacterial life cycle. It can be

used to test environmental samples as well as pure and mixed chemical samples. The ATP-TOX System applied to environmental samples could use predetermined bacterial species or organisms (pure or naturally mixed cultures) indigenous to the environmental samples being tested. Organism resistance patterns to specific toxicants can also be studied to clarify and understand natural resistance patterns in the environment.

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Table 1 Sensitivity of three toxicity screening procedures to various chemicals.

Chemicals	Concentration in mg/L to give typical endpoint						
	Microtox EC_{50} (15 mins)		ATP-TOX IC_{50} (5 hours) *P. fluorescens*		ATP-TOX IC_{50} (5 hours) *E. coli* K12		*S. volutans* (120 mins)*
Hg^{++}	0.05		0.02	a	0.07		0.20
Zn^{++}	5.50		4.00	a	6.80		11.60
Cu^{++}	2.30	a	8.10		11.90		10.00
Ni^{++}	22.50		4.33	a	10.40		20.00
Cd^{++}	18.80		2.35		1.36	a	---
a-Naphthol	6.25	a	6.30		42.50		10.00
Sodium Lauryl Sulfate	1.80	a	65.80		10.00		43.00
3,5-dichlorophenol	3.95		7.81		2.49	a	5.00
Phenol	31.00	a	218.00		448.00		300.00
p-Nitrophenol	9.40		5.00	a	35.00		---
Cetyltrimethl-ammonium chloride	1.50		0.59	a	---		1.45
2,4-dichlorphenoxy-acetic Acid	31.25		20.30		12.50	a	95.00

a most sensitive;

* Data for *S. volutans* from Dutka and Kwan (1981).

Table 2 **Comparison of Parker and Pribyl's test and ATP-TOX System using E. coli on four phenol compounds.**

Chemicals	IC_{50} (mg/L)	
	Parker and Pribyl's Test* (20 minutes)	ATP-TOX System (5 hours)
Phenol	4600	448
o-Chlorophenol	>1000	275
p-Nitrophenol	460	35
2,4,6-Trichlorophenol	360	29

* Results calculated from Parker and Pribyl (1984).

Table 3 Effect of exposure time on toxicity of sediment samples.

Sample Number	ATP-TOX (*E. coli*) IC_{50} (g/mL*)		Microtox EC_{50} (g/mL)		
	5 h	8 h	5 min	10 min	15 min
1	0.330	0.271	0.215	0.195	0.176
2	0.372	0.063	0.176	0.171	0.161
3	0.200	<0.063	0.479	0.425	0.400
4	0.280	<0.063	0.500	0.465	0.460

* Sample concentration corresponds to the wet weight of sediment per unit volume from which chemicals were extracted.

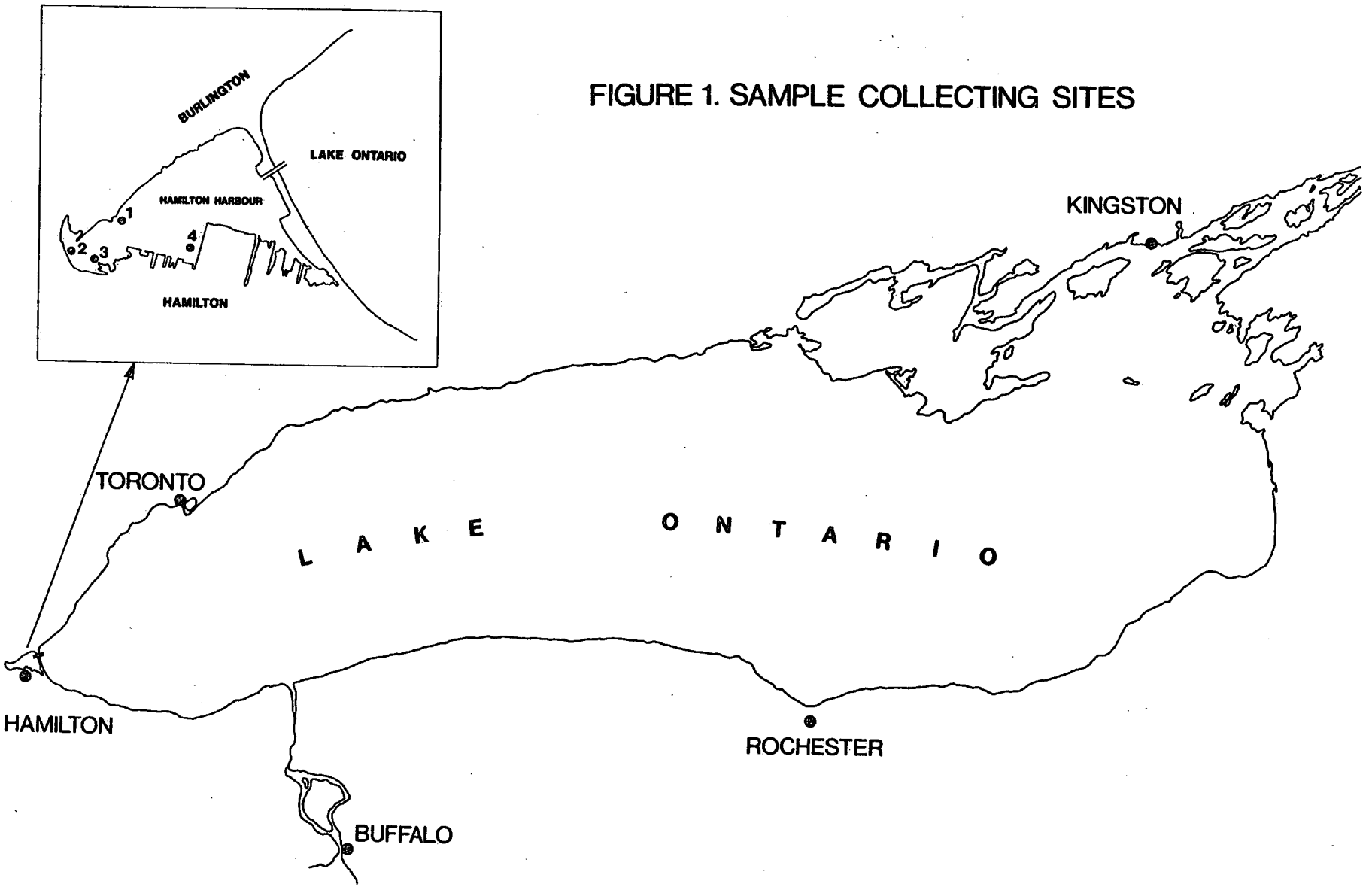

FIGURE 1. SAMPLE COLLECTING SITES

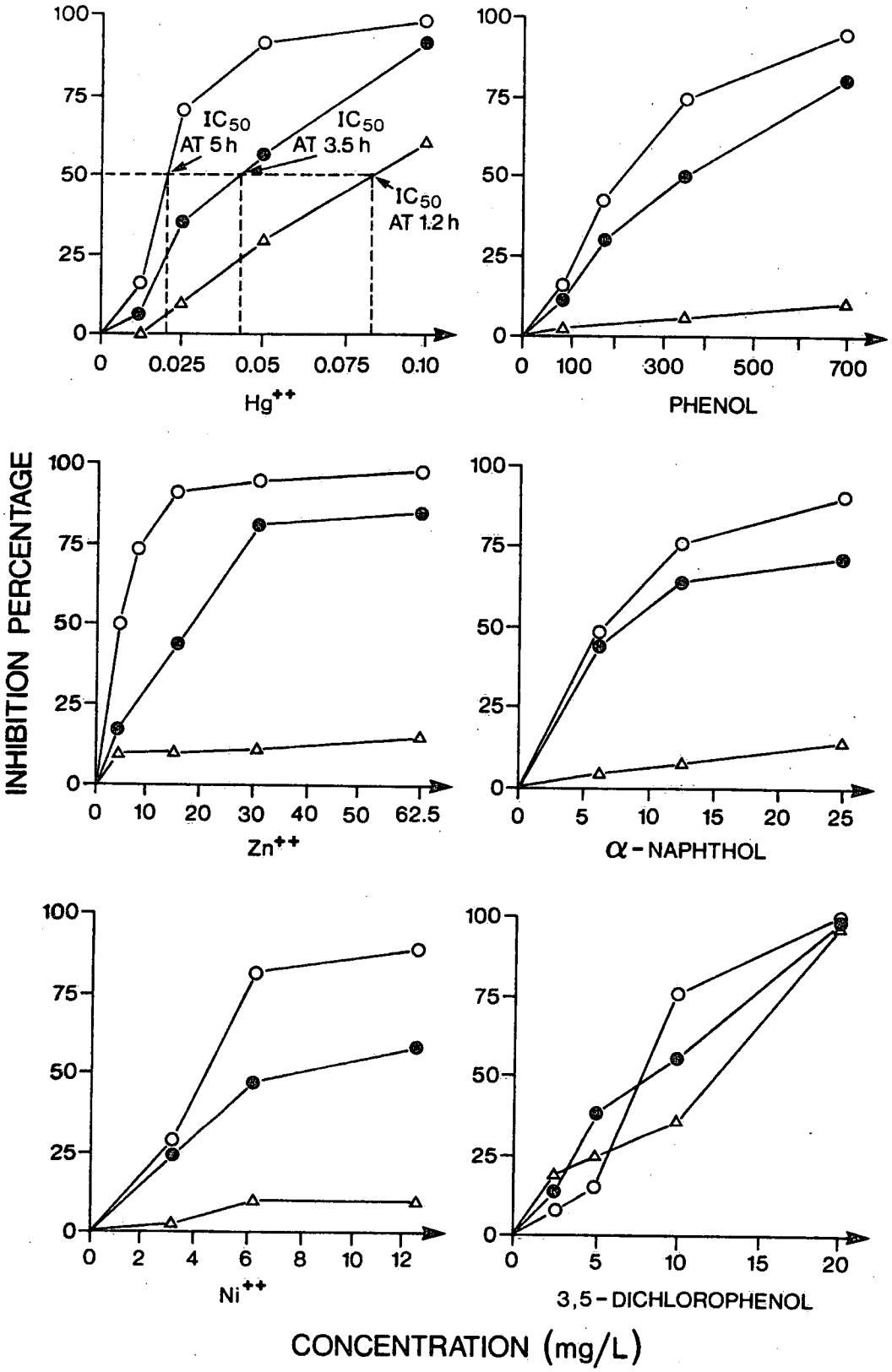

FIGURE 2. EFFECT OF INCREASING EXPOSURE PERIOD ON THE TOXICITY OF CHEMICALS VIA THE ATP-TOX SYSTEM (using P. fluorescens).

△——△ 1.2 h EXPOSURE

●——● 3.5 h EXPOSURE

○——○ 5.0 h EXPOSURE

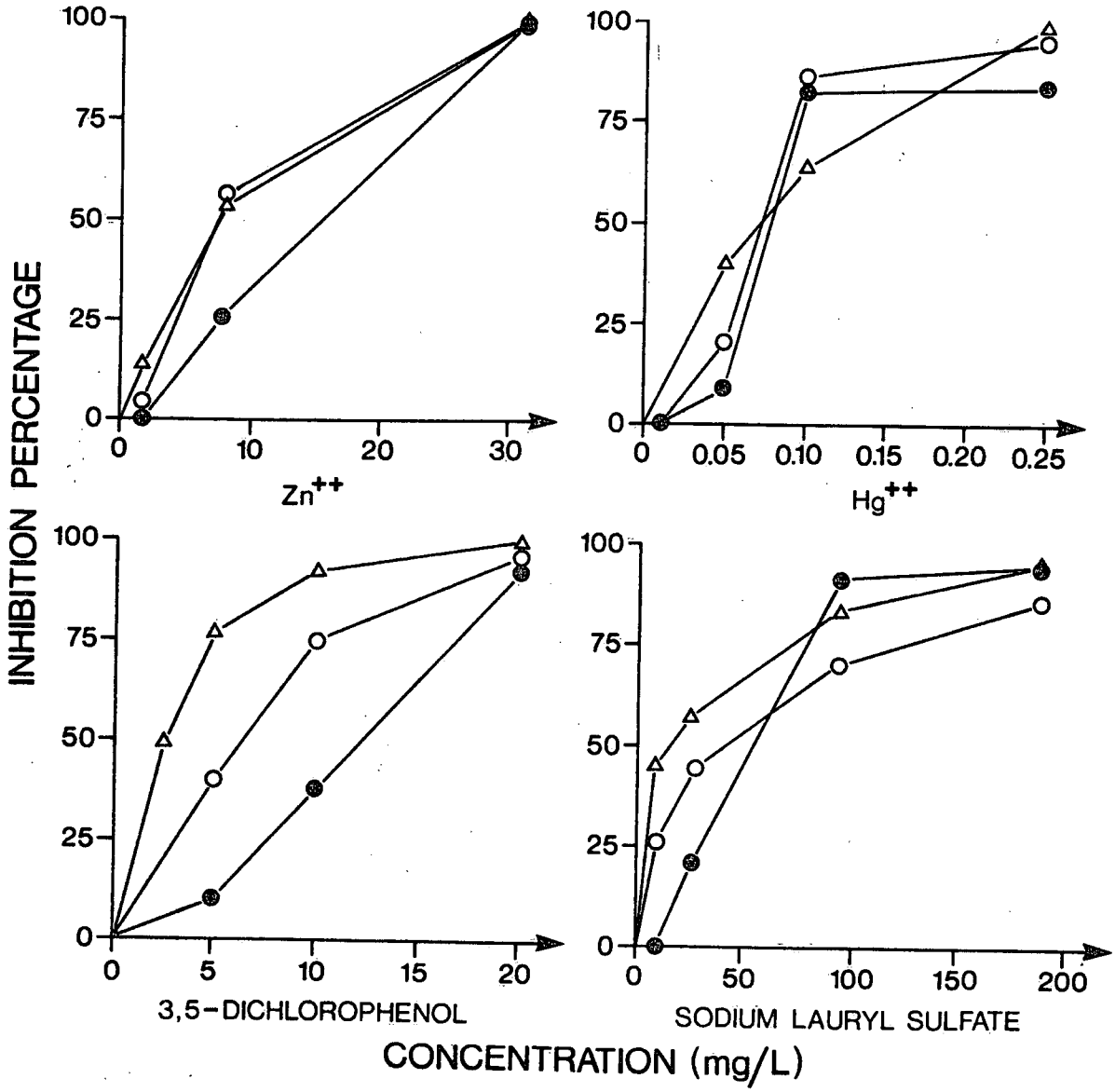

FIGURE 3. EVALUATION OF BACTERIAL SENSITIVITY IN THE ATP-TOX SYSTEM (Based on 5 h exposure period, in nutrient broth).
PSEUDOMONAS FLUORESCENS : ●———●;
SALMONELLA TYPHIMURIUM TA-98 : ○———○;
ESCHERICHIA COLI K-12 PQ 37 : △———△.

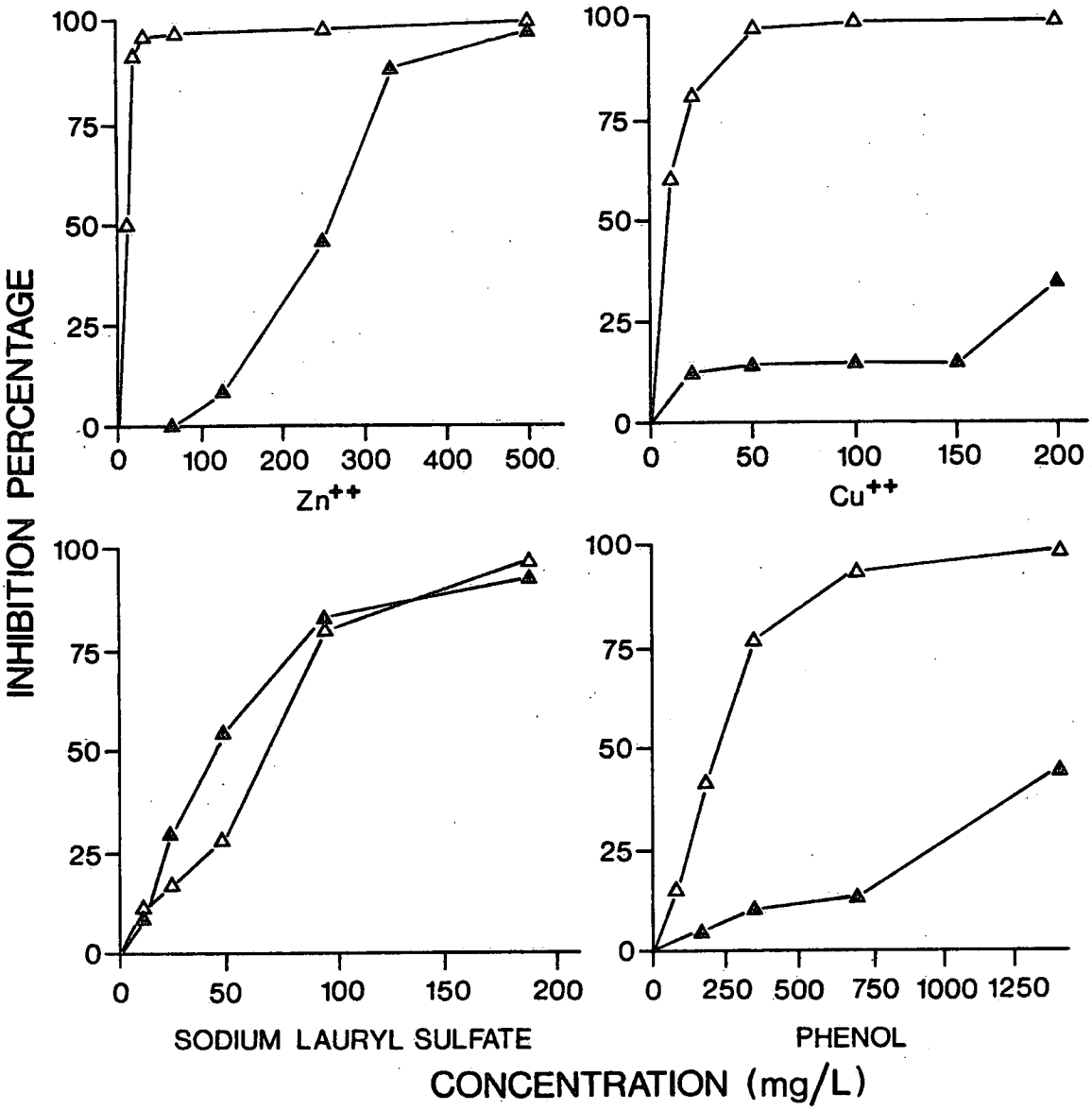

FIGURE 4. INHIBITION OF CHEMICALS ON THE ACTIVITY OF LUCIFERASE (▲——▲) AS COMPARED TO THEIR EFFECT ON THE ATP-TOX SYSTEM (P. fluorescens, 5 h exposure, △——△). P. FLUORESCENS WAS GROWN IN MINIMAL MEDIUM

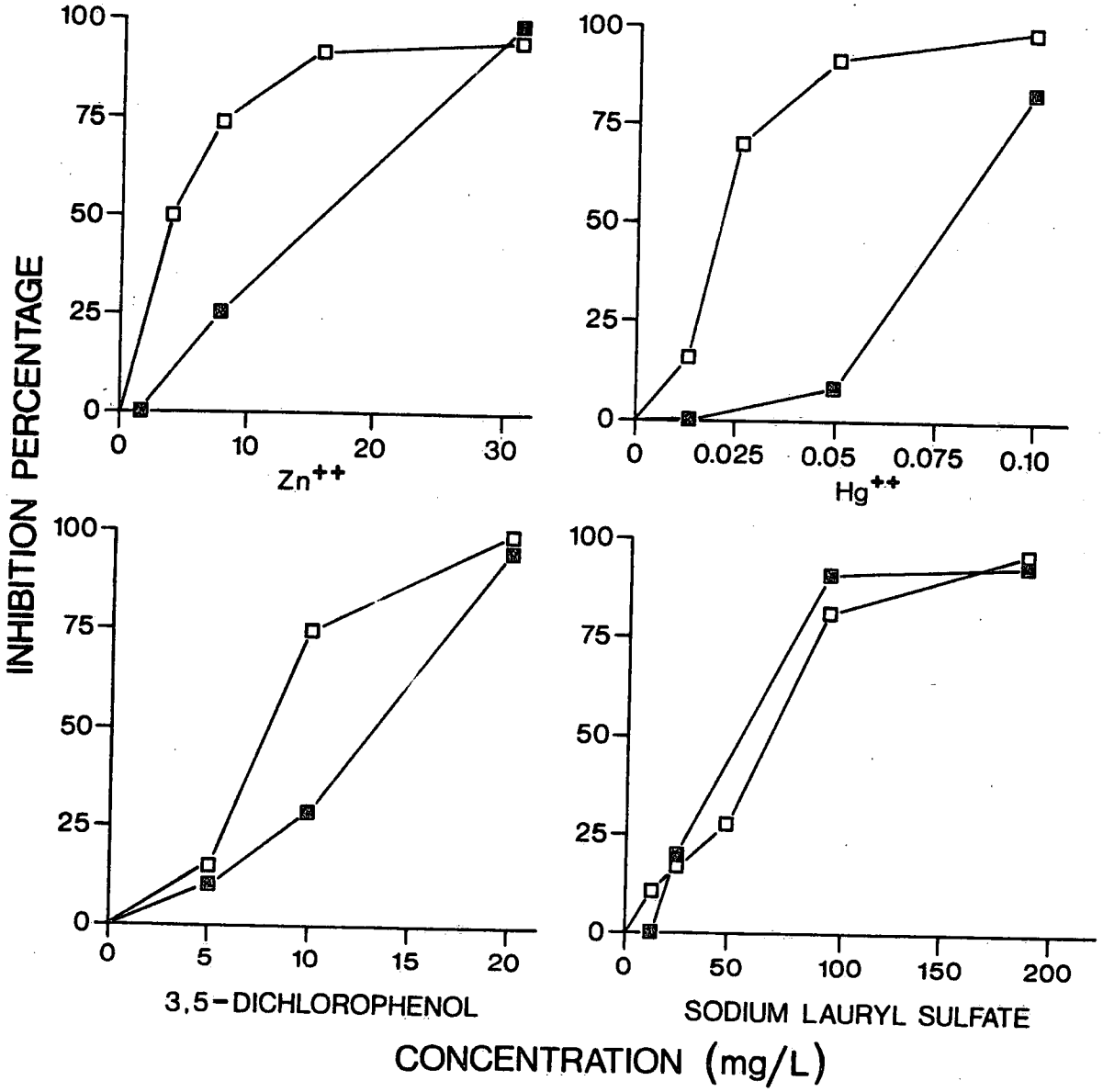

FIGURE 5. INFLUENCE OF CULTURE MEDIA ON THE SENSITIVITY OF THE ATP-TOX SYSTEM (P. fluorescens, 5 h exposure) P. FLUORESCENS WAS GROWN EITHER IN MINIMAL MEDIUM (□——□) OR IN NUTRIENT BROTH (■——■)

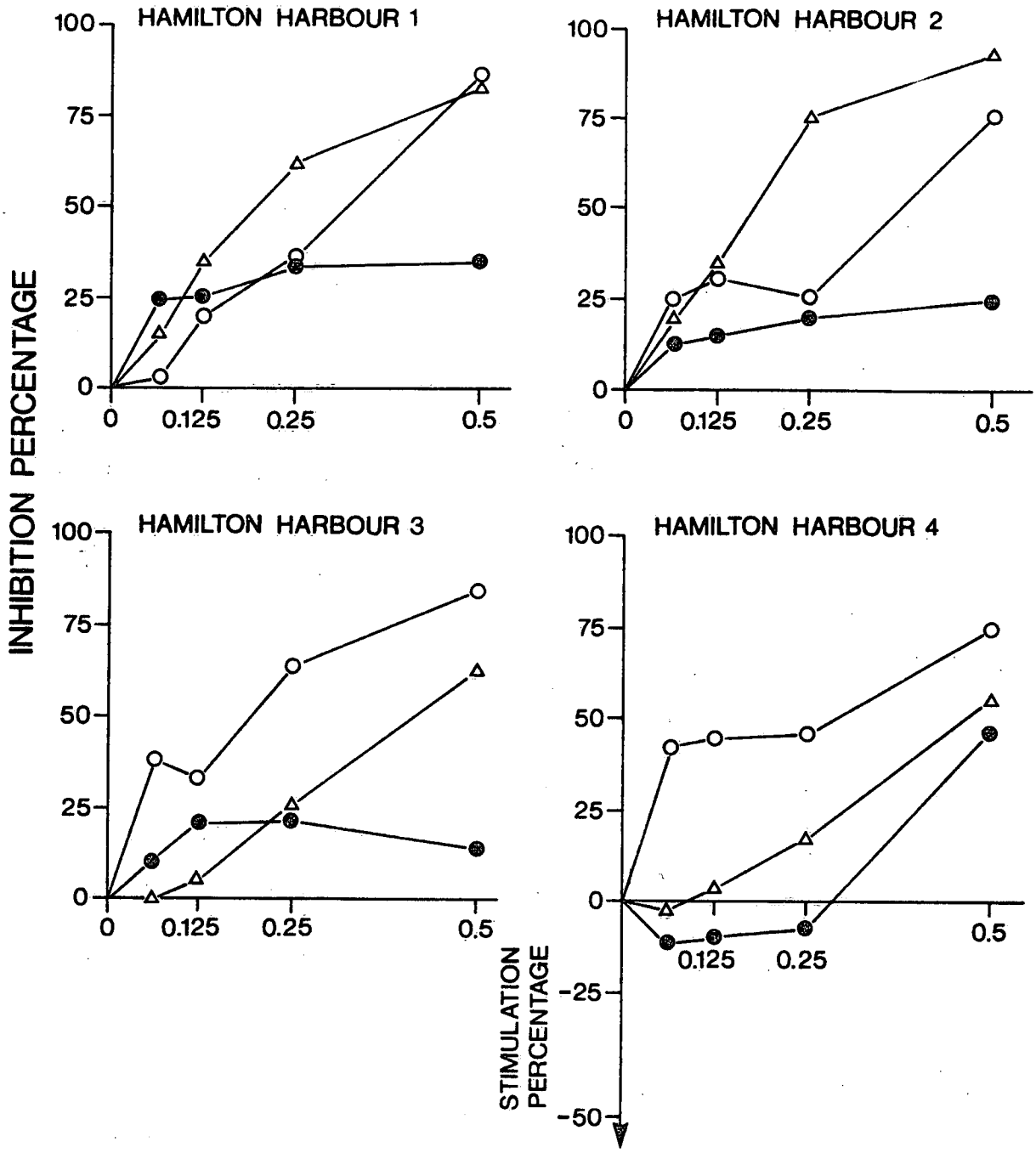

FIGURE 6. TOXICITY OF FOUR HAMILTON HARBOUR SEDIMENT EXTRACTS AS MEASURED BY MICROTOX (△——△) AND ATP-TOX SYSTEMS (E. coli K-12 PQ 37, ○——○; P. fluorescens ●——●).

* Sample concentration corresponds to the wet weight of sediment per unit volume from which chemicals were extracted

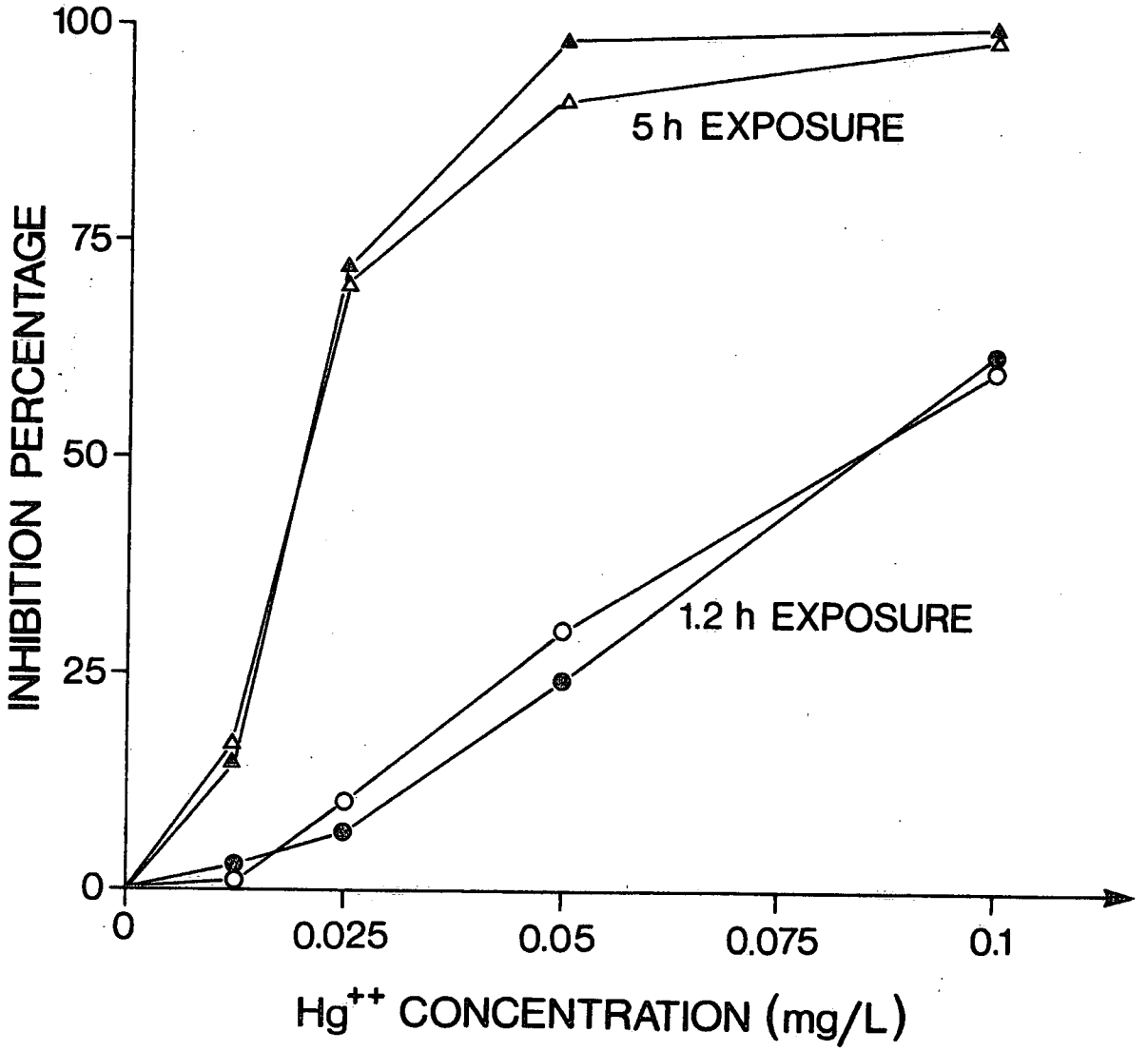

FIGURE 7. REPRODUCIBILITY OF THE ATP-TOX SYSTEM (P. fluorescens). TWO INDEPENDENT TRIALS, 3 DAYS APART ARE SHOWN. P. FLUORESCENS WAS GROWN IN MINIMAL MEDIUM