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Perturbations of Population Growth in a Microcosm by Industrial Metal Plating and Ice Cream Mills Wastewater and Landfill Leachate

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Environmental contaminants and pollutants are being introduced widely into the environment. Therefore, there have been several attempts to assess the biological impact of agents such as surfactants, herbicides, heavy metals, irradiation, landfill leachate and industrial wastewaters (Pratt and Barreiro, 1998; Fuma et al., 2000, 2001), some of which accumulate in organisms through the food chain (Zelikoff et al., 1995). However, the impacts of these hazards on the ecosystem are not clearly known.

As a guideline to assess the impacts of these chemicals on organisms, the Organization for Economic Cooperation and Development (OECD) suggested that 50% lethal concentrations (LC_{50}), 50% effective concentrations (EC_{50}) and mutagenicity should be determined with mice and rats (OECD, 1984). However, with the use of single species the ecotoxicological tests would not be representative of the impacts of these chemicals on the ecosystem (Abbott, 1967). Therefore, the use of more complex systems such as microcosm which include the producer, consumer and decomposer would be valuable to assess the impacts of these hazards on the natural environment. Abbott (1966) suggested the use of multispecies-microcosm systems for toxicity testing and such systems have been developed (Hill and Wiegert, 1980; Niederlehner et al., 1990; Abrams and Roth, 1994). Matthew et al. (1996) suggested the establishment of a standard protocol for assessing the impacts of these hazards using a test microcosm system, which takes advantage of the known species for composing a test microcosm system with high reproducibility and stability (Abbott, 1966; Kassen et al., 2000; Wui et al., *unpublished data*). Actually, such a microcosm system is stable and can be perturbed by the introduction of hazardous chemicals to the system. Therefore, perturbing such a microcosm system with hazards would likely indicate a biomarker for the perturbation of the ecosystem in the natural environment.

In this report, we examined the effects of industrial wastewaters (metal plating mill and ice cream mill wastewaters) and landfill leachate on the population growth and dynamics in a microcosm which included a producer *Chlorella vulgaris*, a consumer *Cyclidium glaucoma* and a decomposer *Pseudomonas putida*. We discuss the use and potential of this system for evaluating the effects of environmental pollutants.

MATERIALS AND METHODS

To assess the effects of industrial wastewaters from metal plating and ice cream

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Table 1. Composition of Taub's basal medium

Stock solution	Composition	Amount (g/L)
A	$MgSO_4 \cdot 7H_2O$	24.65
B	KH_2PO_4	13.6
	NaOH	2.8
C	$CaCl_2 \cdot 2H_2O$	14.7
D	NaCl	5.84
E	$FeSO_4 \cdot 7H_2O$	24.9
	Na_2EDTA	13.6
F	H_3BO_3	1.854
	$ZnSO_4 \cdot 7H_2O$	0.287
	$MnCl_2 \cdot 4H_2O$	1.98
	$NaMoO_4 \cdot 2H_2O$	0.024
	$CuSO_4 \cdot 5H_2O$	0.0499
	$Co(NO_3)_2 \cdot 6H_2O$	0.291

mills and landfill leachate on the population growth and dynamics of a microcosm which included a producer *Ch. vulgaris*, a consumer *C. glaucoma* and a decomposer *P. putida,* we set up the microcosm system in 300 mL flasks.

The cultures were static at 25 °C with a 12 hr light cycle (2,800 lux) and were in 200 mL of Taub's basal medium (pH 7.0; 1 mL A, 1 mL B, 20 mL C, 30 mL D, 0.125 mL E, 0.5 mL F) (Table 1) (Taub and Dollar, 1964).

The microcosm system reached stability two weeks after seeding. The average numbers of cells in the microcosm system which were highly reproducible were 7.2×10^6 /mL *Ch. vulgaris*, 1.75×10^3 /mL *C. glaucoma* and 4.5×10^5 /mL *P. putida*. After sterilization by filtration through 0.22 μm pore membranes, various amounts of metal plating mill wastewaters, ice cream mill wastewaters or landfill leachate were added to the stable microcosm systems.

To determine the numbers of cells in the microcosm systems, 2-3 mL samples were sonicated for 10 sec and then diluted with PY medium (5 g polypeptone, 3 g yeast extracts, 4 g NaCl per liter). *Ch. vulgaris* and *C. glaucoma* were counted with a hemocytometer after suitable dilutions. *P. putida* was counted as colony-forming units on PY medium.

Cells were counted 2, 4 and 7 days after each pollutant was added. The cell numbers are expressed as the % of growth ([number in experimental group/number in control]) x 100).

RESULTS AND DISCUSSION

Two weeks after seeding, the static flask culture microcosms showed reproducible stable growth curves of *Ch. vulgaris*, *C. glaucoma* and *P. putida* (Fig. 1).

We used this microcosm system to evaluate the effects of various environmental pollutants. Microcosm were treated with different concentrations (0.5, 1 and 2.5%) of the metal plating mill wastewater. The metal plating mill wastewater contained 2,235 mg Cr, 521 mg Zn, 3 mg Cu and 43 mg CN per liter.

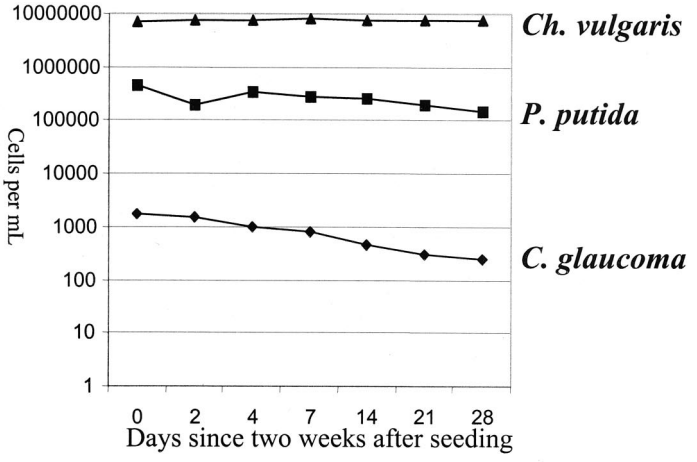

Figure 1. Growth patterns of populations in the microcosm after they reached stability (2 weeks after seeding)

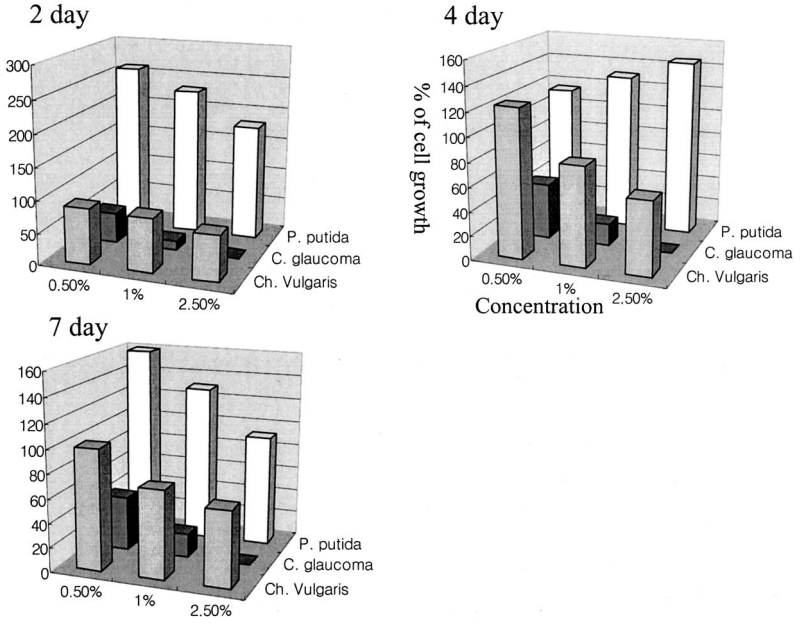

Figure 2. Changes in population growth in the microcosm after addition of metal plating mill wastewater

Quantitatively, the Cr and Zn ions were the main pollutants. When the microcosm system was treated with 0.5 to 1% of the metal plating mill wastewater, the growth of *Ch. vulgaris* (producer) was not significantly changed at day 2 but was affected at days 4 and 7 (Fig. 2).

However, the consumer *C. glaucoma* showed dramatic concentration-dependent changes after the metal plating mill wastewater treatment. On day 4, the *C. glaucoma* showed a small increase in number with increasing *Ch. vulgaris*. *P. putida* initially showed overgrowth (about 2.5 times compared to control) after the treatment in a concentration-dependent manner but had a tendency to slow down thereafter. Thus, *P. putida* was not dramatically affected by treatment with the metal plating mill wastewater, compared to *Ch. vulgaris* and *C. glaucoma* (Fig. 2). With a higher concentration (2.5%) of the metal plating mill wastewater, *C. glaucoma* was completely damaged but the other two species (*Ch. vulgaris*, *P. putida*) were not damaged significantly until 4 weeks after the treatment with this wastewater. With respect to the effects of metal on the microcosm, Shannon et al. (1986) reported that a single treatment with Cu (0.5 mg/L) affected the producer in a microcosm. However, this does not indicate anything about the effects of other metals such as Cr and Zn, which are the main chemical pollutants in this wastewater. Thus, we can not make a comparison with other microcosm systems.

The microcosm system was treated with various concentrations of the ice cream mill wastewater (0.5, 1, 2.5, 5, 10 and 25%). The ice cream mill wastewater was composed of 1,820 mg Biochemical Oxygen Demand (BOD), 1,216 mg Chemical Oxygen Demand (COD), 650 mg Suspended Substances (SS) and 420 mg NH_4-N per L, pH 6.7. Although we checked a high concentration (25%) of this wastewater, it did not significantly damage the producer *Ch. vulgaris*. Rather, it promoted the growth in a time-dependent manner playing a role as a source of nutrients (Fig. 3) because it contains lots of organics. However, the growth of the consumer *C. glaucoma* and decomposer *P. putida* showed significant changes with various concentrations of the ice cream mill wastewater with an initial increase of *P. putida* in a concentration-dependent manner as shown in Fig. 3. Therefore, it is likely that *C. glaucoma* and *P. putida* used the ice cream mill wastewater as a source of nutrients. Inamori and Takamatsu (1996) suggested that altered growth of species in a microcosm system, negative or positive, caused by pollutants would reflect the pattern affecting the ecosystem showing that those chemicals would play a role as environmental pollutants. In this case, the organics from the ice cream mill wastewater which was introduced into the microcosm system were the source of altered growth patterns. We conclude that the ice cream mill wastewater affected the population of the microcosm system by causing overgrowth of *C. glaucoma* and *P. putida* in time- and concentration-dependent manners. Thus, it would be an environmental pollutant.

Lastly, we examined the effect of landfill leachate at concentrations of 0.5, 1, 2.5, 5, 10and 25% on the microcosm system. The landfill leachate was composed of 5,700 mg BOD, 1,827 mg COD, 323 mg SS, 434 mg Total Nitrogen, 11 mg Total Phosphorus per L; pH 7.1. The landfill leachate did not clearly show inhibition of growth in the microcosm system as that of the ice cream mill wastewater. Interestingly, it promoted the growth of *P. putida* in a concentration-dependent manner (especially at concentrations of 10% to 25%) 2 days after treatment (Fig. 4). This growth pattern was similar to that with ice cream mill wastewater, while on day 2, another microorganism (*Ch. vulgaris*) clearly showed decreased growth. *C. glaucoma* showed dramatic growth on day 4 in a

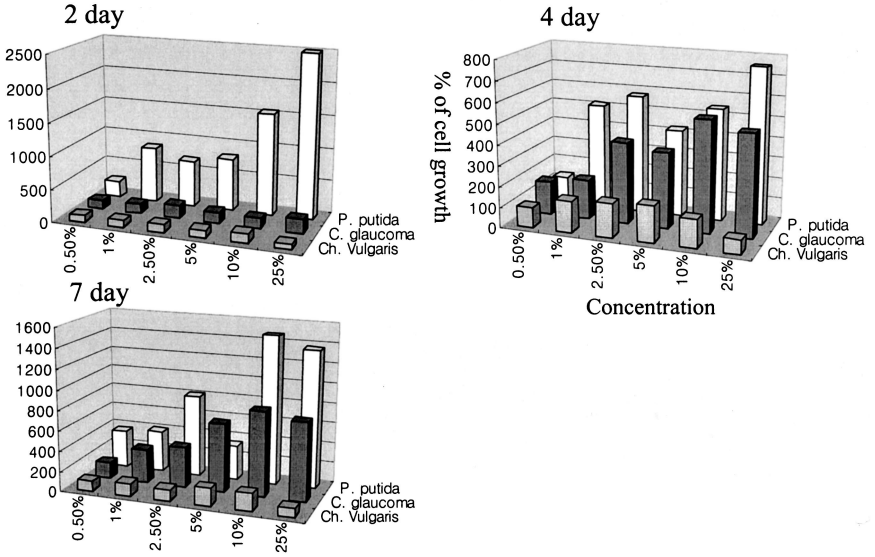

Figure 3. Changes in population growth in the microcosm after addition of ice cream mill wastewater

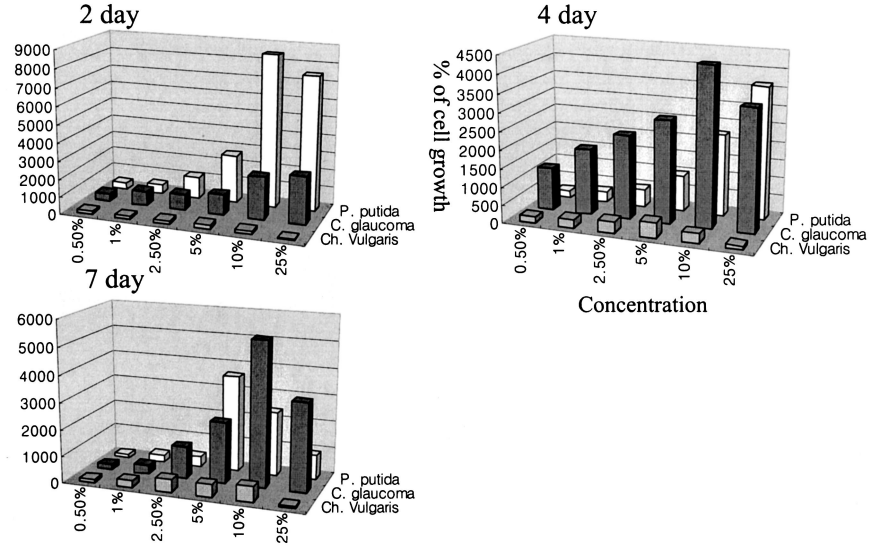

Figure 4. Changes in population growth in the microcosm after addition of landfill leachate wastewater

concentration-dependent manner, and it continued at later stages.

Overall, with abundant organics in the microcosm, the producer *Ch. vulgaris* did not show a steep increase in population mass but the other two microorganisms (*C. glaucoma* and *P. putida*) dramatically increased in concentration-dependent manners. Thus, the introduction of a rich organic environment to the microcosm did not affect the producer *Ch. vulgaris* but played a pivotal role in providing nutrients for the consumer and the decomposer, resulting in the steep growth of those populations. To assess the effect of landfill leachate, Sudo et al. (1995) set up a microcosm system which included bacteria (*Bacillus cereus*, *P. putida*, *Acinetobacter* sp., *Coryneform*), a blue green algae (*Tolypothrix* sp.) and other species (*C. glaucoma*, *Lepadella* sp., *Philodina* sp., *Aleosoma hemprichi*) and tested landfill leachate concentrations of 0.5, 1, 5, 10, 25 and 50%. Most species showed an increase in population growth except for *L.* sp. and *A. hemprichi*, indicating a perturbation of population growth in the microcosm. They also tested the effect of nitrogen source, which is one of the pollutants in landfill leachate, and got similar results. Furthermore, Sudo et al. (1995) used various concentrations of the NH_4-N solution as a single nitrogen source and they found similar on growth in the microcosm. Thus, out results with landfill leachate are similar to those of Sudo et al. (1995), although we used different testing species in the microcosm system.

In this paper, we established the potential use of a microcosm system for predicting the perturbation of the natural environment ecosystem by examining the effects on population growth of several concentrations of industrial wastewaters (metal plating and ice cream mill wastewaters) and landfill leachate. The results suggest that the microcosm could be a model system for testing the effects of pollutants on the microcosm and more diverse pollutants should be tested for establishing this system as a reliable method for checking the effects of various environmental pollutants on the ecosystem in the natural environment.

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