BIOREMEDIATION OF POLLUTED SUBSOILS FOR PROTECTION OF GROUNDWATER SUPPLIES

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INTRODUCTION

Groundwater constitutes a significant water source in most areas of the United States with approximately one-half of the residents relying on groundwater that has not been treated or disinfected and is often contaminated with organics. Sources of groundwater contamination include spills or leaks from storage tanks or pipelines, improperly constructed waste disposal sites, and applications of agricultural chemicals (1, 2). Two widely used classes of chemicals that have found their way into the subsoil and groundwater as a result of woodtreating operations include the polycyclic aromatic hydrocarbons (PAHs) and chlorinated phenols such as pentachlorophenol (PCP). These contaminants have been linked to health problems such as cancer, damage to the liver, kidney, and central nervous systems and, therefore, warrant attention. According to the Environmental Protection Agency, the PAH compounds have polluted more U.S. groundwater drinking supply by volume than has any other class of chemicals (3). Cleanup efforts have failed to keep pace with this toxic contamination, in part because many of the physical and chemical properties of groundwater, subsoils, and the aquifers remain poorly understood. In recent years a cleanup technology called bioremediation has been developed for contaminated soils and groundwater which uses microorganisms found in soil to degrade organic pollutants (4). By adding nutrients and other materials to the soil, large populations of adapted microorganisms can be developed that will rapidly degrade these organic pollutants. These techniques have been successfully used to clean up contaminated groundwater in situ (5, 6). This study was the final part of a four phase investigation to determine the conditions required to increase the population of microorganisms in the soil in order to maximize degradation of organic pollutants. Phase one determined that the addition of inorganic nutrients such as nitrogen, calcium, and phosphorus affected different soil types in different ways. Phase two determined that the addition of oxygen was best achieved with pressurized air as opposed to hydrogen peroxide. Phase three determined that the indigenous microorganisms were just as effective in degrading the PAHs and PCP as was a known PAH and PCP degrading bacteria. This paper reports the results of the final phase of this study in which effects of soil, air, and porosity on bioremediation of PAHs and PCP were evaluated.

MATERIALS AND METHODS

Phase IV Microcosom Setup

Twenty four microcosms (122 cm X 5 cm) were constructed from PVC pipe which had been sterilized in a solution of chlorox (1:1) in water and rinsed with ethanol. Closures for each of the microcosms consisted of a PVC threaded cap with a hole drilled through the top. Copper tubing was attached and sealed to the hole in the cap using a sealant. This provided inlets for the addition of air and water and outlets for the collection of the leachate. Glass wool was placed at the outlet end of each microcosm closure to prevent particulate accumulation in leachates. The microcosms were attached to specially constructed support shelves in the lab using metal clamps. Clay soil from the Wiggins treating site was screened through 0.3175 cm mesh screen and divided equally into two separate piles. One soil pile was sterilized using methyl bromide while the other pile remained unsterilized. Each soil pile was subdivided into two piles and sterilized sand was added (30% by weight) to one sterilized soil pile and one unsterilized soil pile. This gave four treatments of soil. The soil for each treatment was spiked with five PAHs (napthalene, phenanthrene, anthracene, chrysene, and benzo(a)pyrene) at a loading rate of 500 ppm each by weight and 250 ppm by weight PCP. The soil was thoroughly mixed and three replicate samples of 3500 g was removed from each treatment and added to the microcosms. A selected strain of Arthrobacter sp., isolated from soil from a wood treating site near Joplin, Missouri, was added to all the microcosms each week of the experiment. This bacteria has been isolated and extensively tested at the Mississippi Forest Products Laboratory and at field sites for its ability to degrade PAHs and PCP. Six of the microcosms within each of the four treatment groups

were further subdivided into groups of three with the addition of air and three without the addition of air. Air, pumped into the appropriate microcosms at a pressure of 0.035 kg/cm^2, was filtered and saturated with water to prevent drying of the microcosms. Sterilized deionized water (one liter) was added to each of the microcosms at days 0, 12, 24, 48, 60, 72, and 90. The leachate was collected and analyzed for PAHs and PCP by High Pressure Liquid Chromatography (HPLC).

Leachate Extraction Procedure

The leachate sample (500 mL) was transferred to a one liter separatory funnel. Internal standard solutions of 500 ppm 9,10-Diphenylanthracene and 1000 ppm 2,4,6-Tribromophenol (1 mL each) were added to the leachate sample. The leachate was acidified to pH 2 with sulfuric acid and extracted twice with 200 mL portions of methylene chloride. The combined methylene chloride extracts were concentrated to 5 mL using a roto-evaporator. Basic water (2 mL, pH 12) was added to the methylene chloride extract and the sample was mixed on a vortex mixer for two minutes followed by centrifuging for five minutes at 1500 rpm. The methylene chloride layer was removed and filtered through a 0.2µ nylon disposable filter into an autosampler vial and was ready for analysis of PAHs by HPLC. The water layer (1 mL) was diluted with 1 mL acetonitrile; acidified to pH 5 with acetic acid and filtered, as previously described, for the PAHs; and analyzed for PCP by HPLC.

HPLC Conditions

Column:	5µ Econosphere C-18 reverse phase
Mobile Phase:	80:20 Acetonitrile:water with 1% acetic acid
Detector:	254nm
Flow rate:	1.0 mL/min

RESULTS AND CONCLUSIONS

Phase 4 results indicated that the mobility of added microorganisms is more rapid through the clay soil/sand mixture than the clay soil alone as determined by bacteria colony numbers in the leachate (Figure 1). The concentration of total PAHs and PCP in the leachate of the soil/sand mixture was higher than that in the clay soil microcosms. This relationship, however, decreased below that of the clay soil after two weeks (Figures 2 and 3) and was most likely due to the higher numbers of microorganisms in the clay soil/sand mixture. In testing the differences in sterilized and nonsterilized soils on the mobility of added microorganisms through the clay soil/sand mixture, there appeared to be no observable differences in the microbial counts of these soils (Figure 4). This indicates that the indigenous microoganisms were as efficient in moving through the soil as those microorganisms which were added to the soils. Data from the study to determine the effect of additional air on the microbial count was inconclusive. The microbial counts of leachate from soils both with and without additional air remained essentially unchanged and below approximately one million for the duration of the sampling period (Figure 5). Apparently the microbes were tightly bound to the soil particles or conditions were not favorable for microbial growth.

ACKNOWLEDGEMENT

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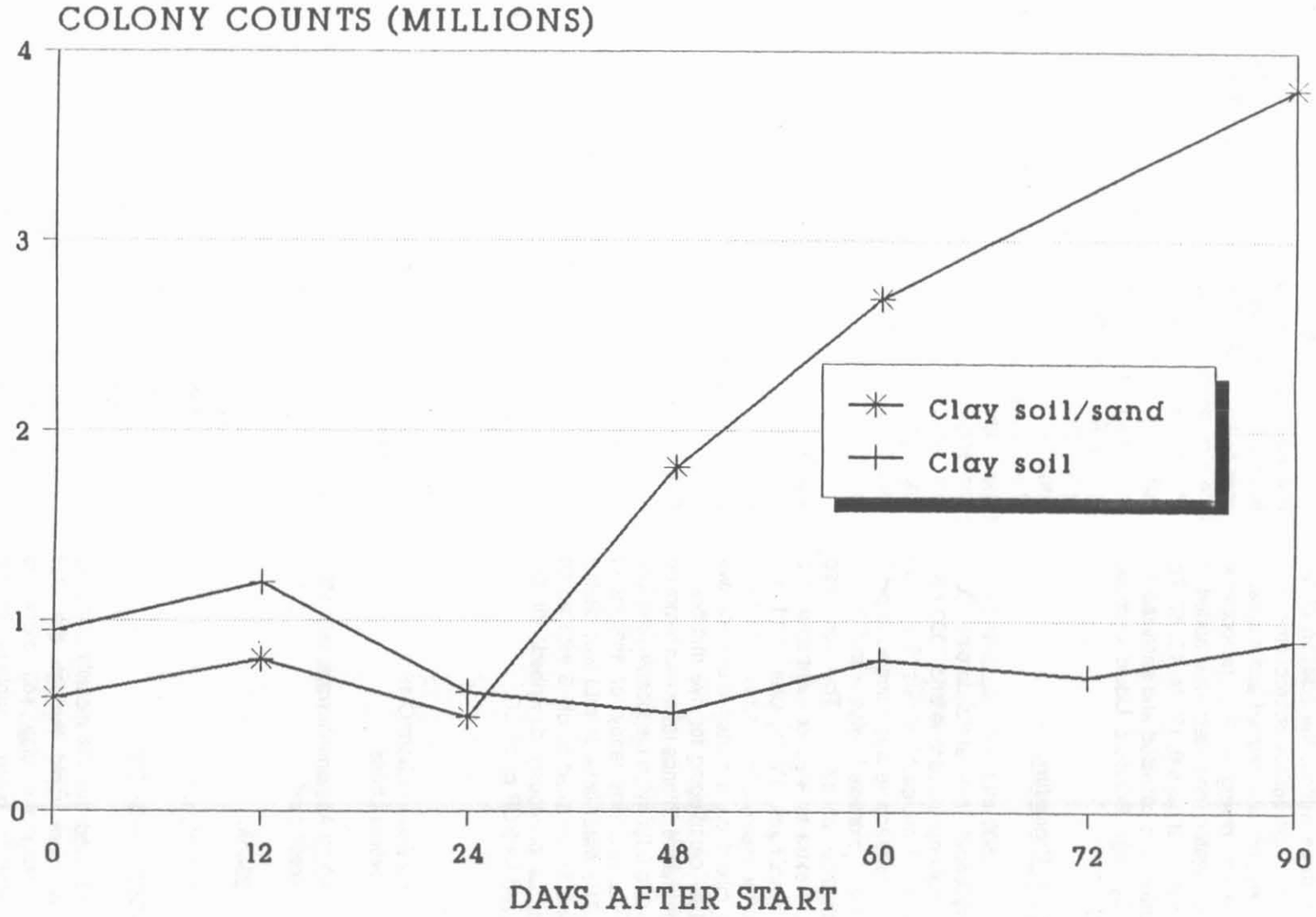

FIG. 1 MICROBIAL COUNTS IN LEACHATE

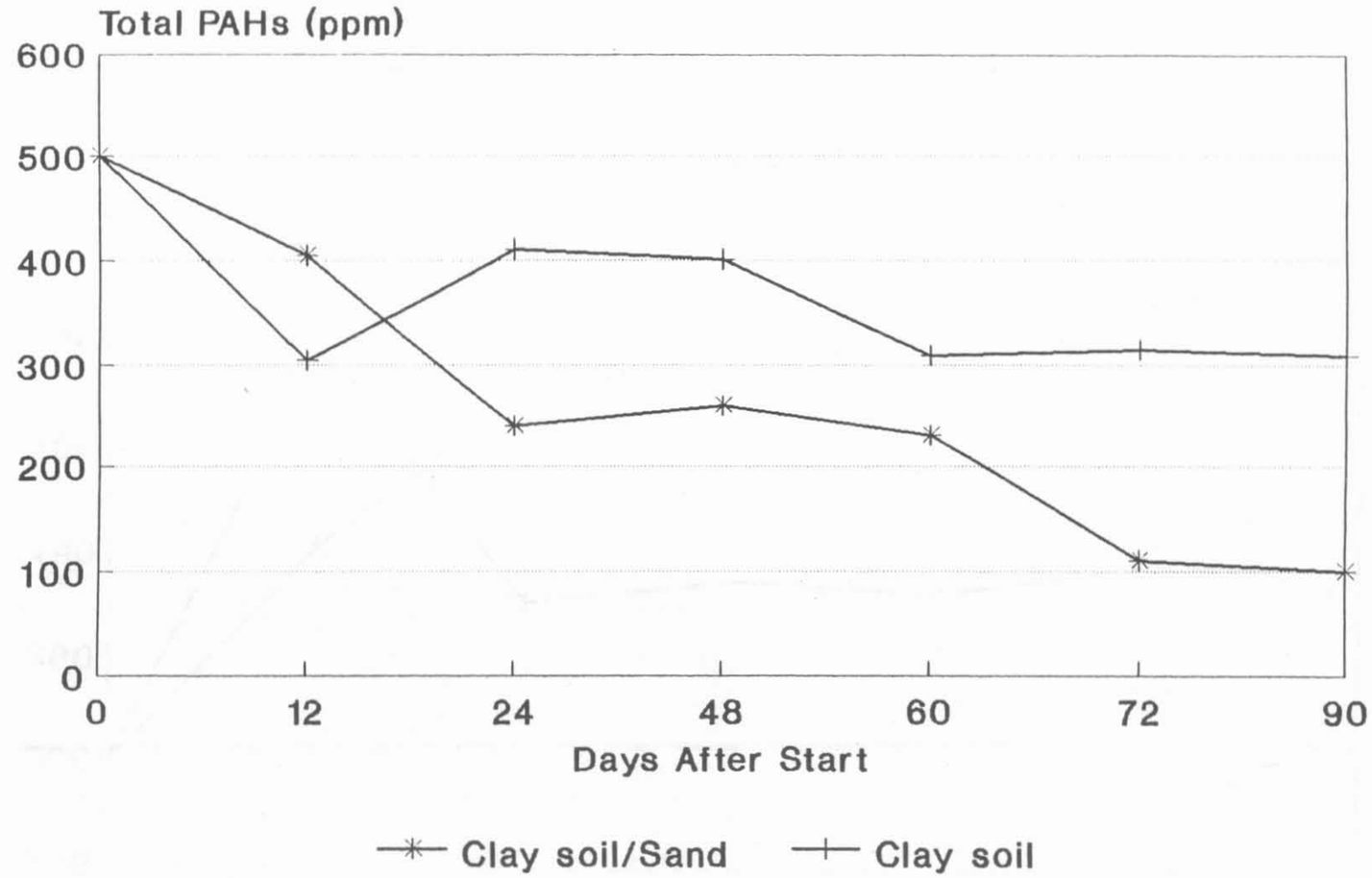

FIG. 2 TOTAL PAHs IN LEACHATE

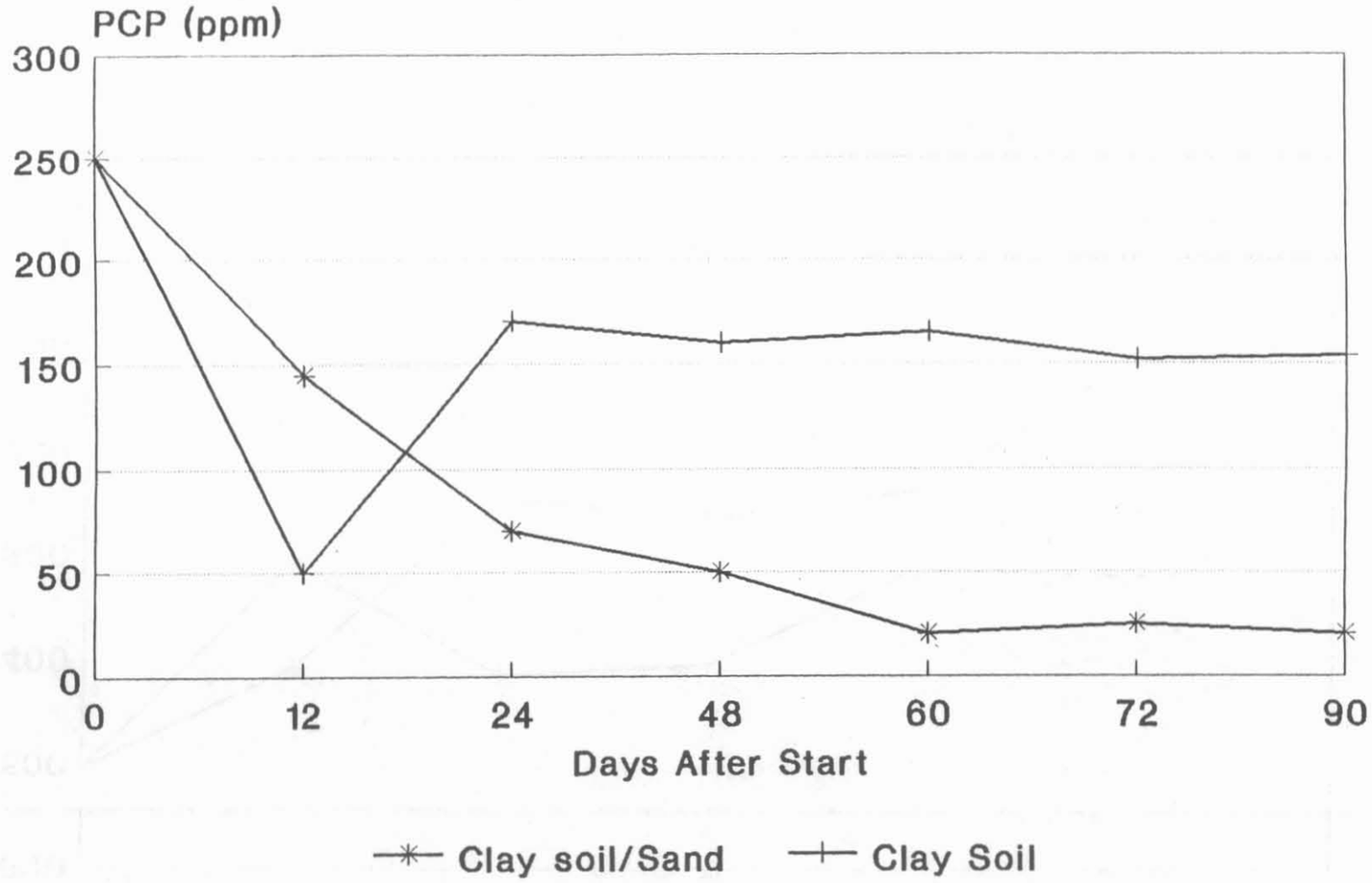

FIG. 3 PCP IN LEACHATE

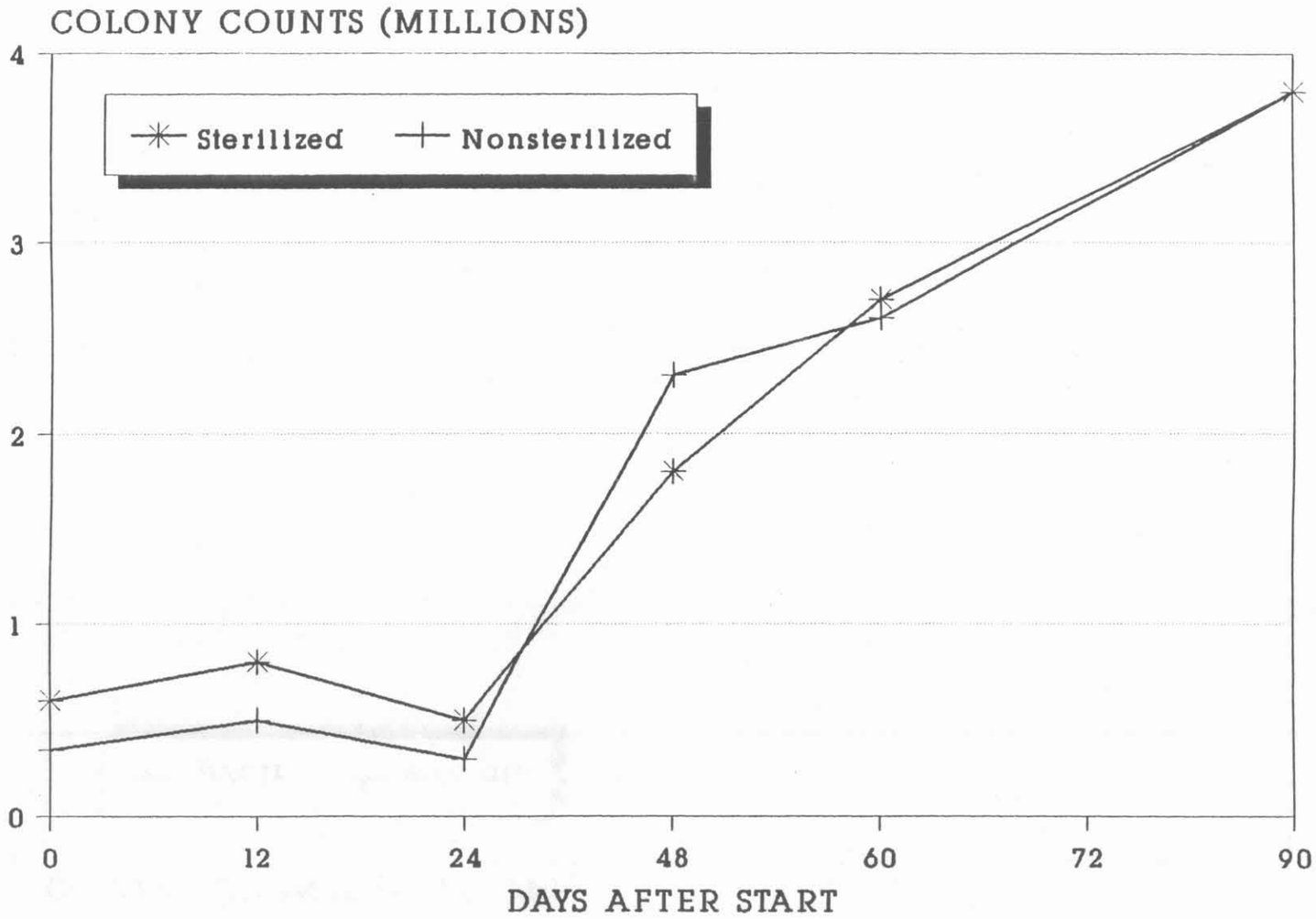

FIG. 4 MICROBIAL COUNTS IN LEACHATE

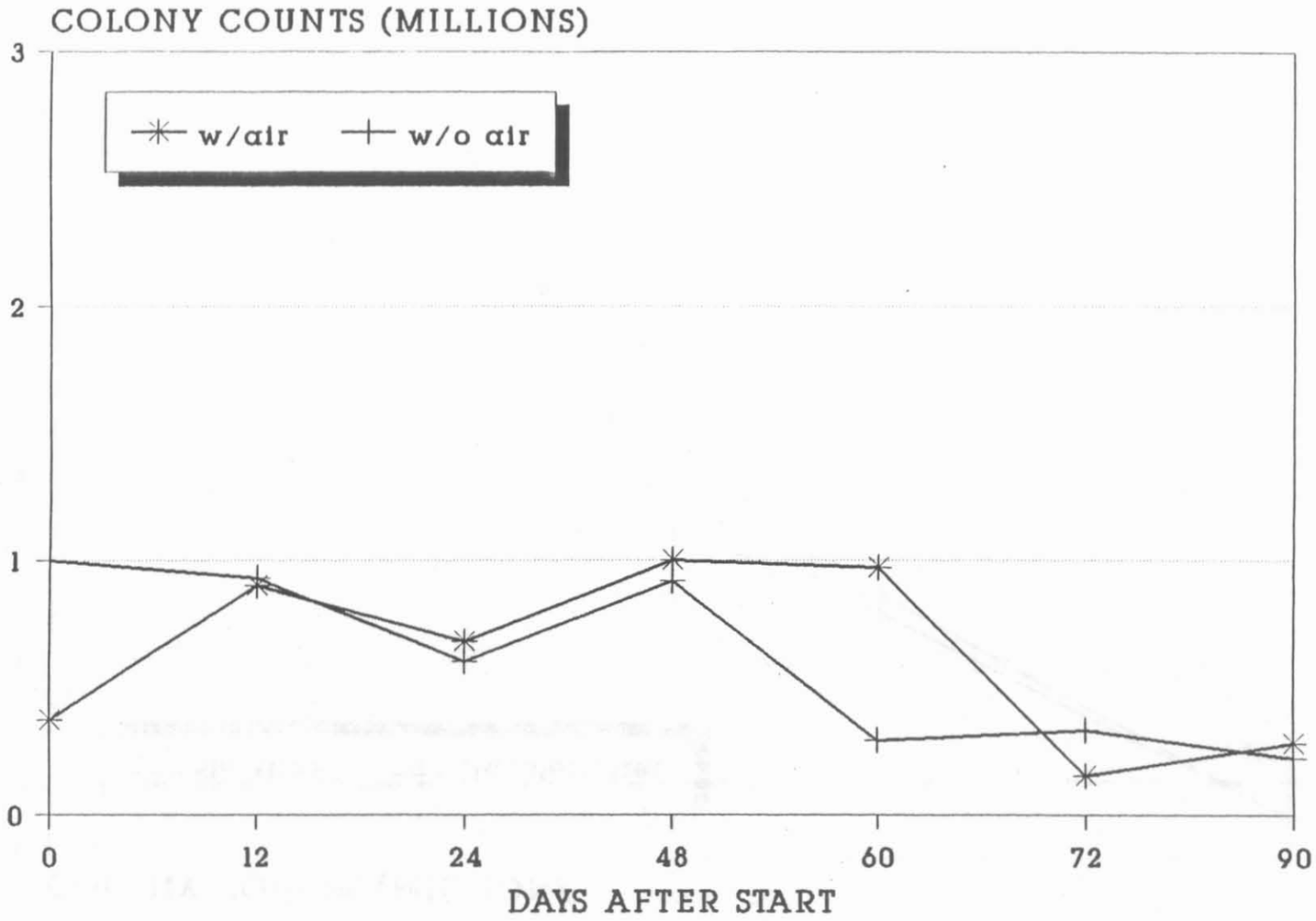

FIG. 5 MICROBILA COUNTS IN LEACHATE