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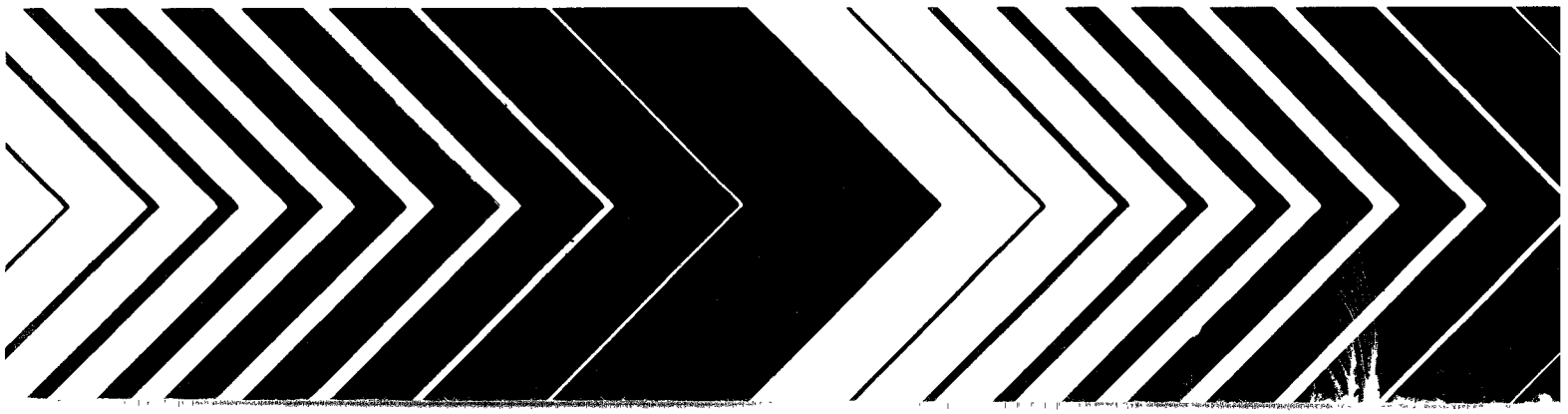
Enhanced Bioremediation Utilizing Hydrogen Peroxide as a Supplemental Source of Oxygen

A Laboratory and Field Study



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ENHANCED BIOREMEDIATION UTILIZING HYDROGEN PEROXIDE as a SUPPLEMENTAL SOURCE of OXYGEN: A LABORATORY AND FIELD STUDY

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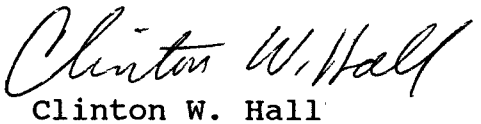
FOREWORD

EPA is charged by Congress to protect the Nation's land, air, and water systems. Under a mandate of national environmental laws focused on air and water quality, solid waste management and the control of toxic substances, pesticides, noise, and radiation, the Agency strives to formulate and implement actions which lead to a compatible balance between human activities and the ability of natural systems to support and nurture life.

The Robert S. Kerr Environmental Research Laboratory is the Agency's center of expertise for investigation of the soil and subsurface environment. Personnel at the Laboratory are responsible for management of research programs to: (a) determine the fate, transport and transformation rates of pollutants in the soil, the unsaturated and the saturated zones of the subsurface environment; (b) define the processes to be used in characterizing the soil and subsurface environment as a receptor of pollutants; (c) develop techniques for predicting the effect of pollutants on groundwater, soil, and indigenous organisms; and (d) define and demonstrate the applicability and limitations of using natural processes, indigenous to the soil and subsurface environment, for the protection of this resource.

This report describes research conducted to investigate the efficacy of utilizing hydrogen peroxide as a supplemental source of oxygen in the enhanced bioremediation of aviation gasoline contaminated aquifer material in both laboratory-scale and field-scale studies.



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ABSTRACT

Remedial actions at hazardous waste sites involving in-situ treatment are widely recognized as a preferred treatment option. Bioremediation, a treatment technology which can be implemented in-situ, utilizes available oxygen to metabolize organic contaminants. Laboratory and field scale studies were conducted to investigate the feasibility of using hydrogen peroxide as a supplemental source of oxygen for the bioremediation of an aviation gasoline fuel spill.

Field samples of aviation gasoline contaminated aquifer material collected from a spill site in Traverse City, Michigan were artificially enhanced with nutrients to promote microbiological degradation of fuel carbon in a laboratory column experiment. Oxygen provided from hydrogen peroxide decomposition was utilized biologically in the columns. However, the rapid rate of hydrogen peroxide decomposition at 100.0 mg/l resulted in the production of oxygen gas. An oxygen mass balance indicated that approximately 44.0% and 45.0% of the influent oxygen was recovered in aqueous and gaseous phases respectively. Reduced rates of oxygen consumption during this period indicated that microbial inhibition may have occurred.

A mass balance of the fuel carbon indicated that approximately 36% of the initial mass leached out in the aqueous phase, 10.0% remained, and 54.0% degraded. The ratio of oxygen consumed to aviation gasoline degraded was greater than that predicted by the ideal stoichiometric conversion. Hydrogen peroxide breakthrough in the column effluent never exceeded 11.0% of the influent concentration. The influent hydrogen peroxide concentration in triplicate columns (18 cm. in length) was 50.0, 100.0, and 200.0 mg/l.

Ground-water data from the enhanced in-situ bioremediation pilot field study indicates that hydrogen peroxide successfully increased the concentration of available oxygen downgradient. In this study, however, it was observed that there was a measurable increase of oxygen in the soil gas in the area where hydrogen peroxide was injected. This indicated that a significant fraction of hydrogen peroxide rapidly decomposed to oxygen gas and escaped into the unsaturated zone.

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ACKNOWLEDGEMENTS

The authors wish to thank Dorene Meyer and Michelle Hester of EPA at the Robert S. Kerr Environmental Research Laboratory who performed much of data entry associated with this study. We would also like to thank the Traverse City Group, Inc. staff, namely, James Russell, Bill Newman, and Robert Douglas, who provided several of the figures used in this report and much of the ground water data from the field-scale study at the U.S. Coast Guard Station in Traverse City Michigan.

SECTION 1

INTRODUCTION

The Superfund Amendments and Reauthorization Act (SARA) of 1986 directed EPA to prefer remedial actions involving treatment that would permanently and significantly reduce the volume and toxicity, or mobility of hazardous substances, pollutants, and contaminants over remedial actions not involving such treatment. Therefore, the off-site transport and disposal of hazardous substances or contaminated materials without treatment is the least favored remedial action where practicable alternative treatment technologies are available [9,20].

Bioremediation is a relatively new treatment technology that can be implemented in-situ in which microorganisms metabolize organic contaminants generally into harmless byproducts. Aerobic bioremediation has been reported to degrade a wide variety of organic contaminants such as alkylbenzenes (benzene, toluene, xylene (BTX)), polynuclear aromatic hydrocarbons, heterocyclic organic compounds, and some of the simpler chlorinated compounds [22b,7]. Recently, researchers have found that transformation of trichloroethylene by methane-oxidizing bacteria under aerobic conditions is possible [22a].

In aerobic respiration, free molecular oxygen accepts electrons from an electron donor, usually carbon, and is reduced to a lower oxidation state. An important aspect of these biochemical redox reactions is their irreversibility; therefore, dissolved oxygen is always consumed and never produced as a result of bacterial metabolism [13]. Oxygen, if not present in adequate concentration, will limit the ability of aerobic microorganisms to degrade contaminants. In one field experiment where BTX was injected in a sandy aquifer, researchers reported that an irregular persistence of BTX occurred in a near-zero dissolved oxygen environment [2]. Therefore, the rate of aerobic biotransformation, and thus, contaminant persistence, was reported to be controlled by the transport of oxygen into the BTX-contaminated water. At another site, the low level of dissolved oxygen in creosote contaminated ground water was identified as the probable factor limiting biodegradation [11a].

Due to the dissolved oxygen sink in biologically active contaminated aquifer systems, oxygen supplementation is required to maintain aerobic conditions. Several methods have been developed to increase and maintain the concentration of dissolved oxygen in the ground water.

Air sparging is a process that involves diffusing compressed air into water that is subsequently infiltrated or injected into the contamination zone. Oxygen will diffuse into the water at a rate proportional to the deficit of oxygen below maximum solubility. This method of oxygen supply is limited by the relatively low solubility of oxygen in water from air, typically 8-10 mg/l, and the rapid depletion of oxygen by bacteria [4a,11b].

Ozone, which rapidly decomposes to oxygen in aqueous solutions containing impurities, has been injected into ground water for the purpose of increasing the concentration of dissolved oxygen [1]. Due to the highly oxidative nature of ozone, this gas is also capable of oxidizing organic material. Information concerning ozone injection and subsequent subsurface microbial toxicity is limited.

Soil venting is used to recover volatile organic vapors in highly permeable formations [5]. Researchers have suggested that since air contains approximately twenty times more oxygen on a volume basis than water, and is less viscous, then soil venting could increase the available oxygen in the unsaturated zone for biological activity [22c]. Information concerning this method of ground water oxygenation is also limited.

Liquid or gaseous oxygen has been introduced into water and injected into the subsurface. This method takes advantage of the increased oxygen solubility, typically 40-45 mg/l, that can be obtained when using pure oxygen [8].

Hydrogen peroxide injection into the ground water has recently become a popular method of introducing oxygen to targeted contaminated low dissolved oxygen zones. Hydrogen peroxide decompostion reactions, ideally, yield one mole of water and one mole of oxygen (equation 1), thereby introducing pure oxygen into ground water. Hydrogen peroxide is highly soluble and potentially highly mobile, thus offering numerous operational advantages in the field. However, relatively little information concerning its utilization in a biologically active contaminated aquifer system is currently available.

Hydrogen peroxide decomposition can be characterized by the net result reaction [14]:

$$2H_2O_2 \text{-------} > 2H_2O + O_2 \qquad (1)$$

The most important aspect of the reaction is the liberation of one mole of oxygen. The reaction product, oxygen, is the basis for injecting hydrogen peroxide due to the subsequent replenishment of oxygen in the ground water. The stoichiometry of eqn. (1) indicates that 47.1% by weight of decomposed hydrogen peroxide will be pure oxygen. The two main mechanisms for hydrogen peroxide decomposition are enzymatic and non-enzymatic reactions. Enzymatic decomposition reactions are catalyzed by hydroperoxidases (catalases and peroxidases) which are found in most bacterial cells and are characterized by the following reactions:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2 \qquad (2)$$

$$H_2O_2 + XH_2 \xrightarrow{\text{peroxidase}} 2H_2O + X \qquad (3)$$

where X is a biological reductant [3]. Many scientific observations have been reported concerning catalysts; however, the greatest attention has been centered on the enzyme, catalase [14]. Catalase, found in most bacteria, is primarily responsible

for catalytically decomposing cell synthesized hydrogen peroxide, thus preventing the accumulation of hydrogen peroxide to a toxic level. Catalase is outstandingly effective in this process, being active at low hydrogen peroxide concentrations and at a rate far exceeding that of most other catalysts [14]. Furthermore, researchers have reported that the hydrogen peroxide decomposition observed in an infiltration gallery at an enhanced bioremediation pilot study was largely attributed to the catalase driven reaction [16].

One reviewer of non-enzymatic iron decomposition reactions [14] indicated the most notable are those in the presence of iron salts and the generally accepted mechanism is a series of complex chemical reactions involving hydroxyl and perhydroxyl radical intermediates and both ferric and ferrous iron as illustrated by the following reactions:

$$Fe^{+2} + H_2O_2 \text{ -----> } Fe^{+3} + OH^- + OH^{\cdot} \text{ (hydroxyl radical)} \quad (4)$$

$$OH^{\cdot} + H_2O_2 \text{ -----> } H_2O + H^+ + O_2^{\cdot -} \text{ (superoxide radical)} \quad (5)$$

$$O_2^{\cdot -} + H_2O_2 \text{ -----> } O_2 + OH^- + OH^{\cdot} \quad (6)$$

$$Fe^{+3} + H_2O_2 \text{ -----> } Fe^{+2} + 2H^+ + O_2^{\cdot -} \quad (7)$$

$$Fe^{+3} + O_2^{\cdot -} \text{ -----> } Fe^{+2} + O_2 \quad (8)$$

The overall stoichiometry for both catalase and iron decomposition of hydrogen peroxide is equivalent to that described in equation 1.

Non-enzymatic decomposition of hydrogen peroxide was investigated in laboratory studies to determine the effects of a potassium phosphate "stabilizer" and pH [3]. Hydrogen peroxide decomposition was not observed to be significantly effected by pH. However, the presence of a 0.01M solution of Potassium Phosphate (monobasic) was observed to significantly inhibit hydrogen peroxide decomposition. Phosphate inhibition of hydrogen peroxide decomposition is fortuitous since phosphate is also an important microbial nutrient.

Hydrogen peroxide is well known for its bactericidal properties. Three percent hydrogen peroxide is cytotoxic and commonly used as a general antiseptic. In a laboratory study where hydrogen peroxide provided the main source of oxygen for hydrocarbon degrading bacteria, plate counts were used as an indicator of microbiological activity [3]. These researchers reported that the maximum concentration tolerated by a mixed culture of gasoline degraders was 0.05% hydrogen peroxide. The tolerance was increased to 0.2% by incrementally raising the hydrogen peroxide concentration. Tolerance was determined to occur when the number of colony forming units in the test column were essentially the same for the control column. One important observation made during this study was that non-viable cell material catalyzed the decomposition of hydrogen peroxide as well as viable cell material. Field reports indicated that 100-500

mg/l hydrogen peroxide have been injected into contaminated aquifers [23,4b] and unpublished reports of 1000-10,000 mg/l are not uncommon.

The objectives of the laboratory study were to confirm that hydrogen peroxide can be used to supply oxygen in the bioremediation process, assess the tolerance of the system to hydrogen peroxide, and estimate the overall oxygen demand based on stoichiometric degradation of hydrocarbon. A field scale in-situ bioremediation pilot study in which hydrogen peroxide and nutrients were injected into contaminated aquifer material provided the opportunity to confirm laboratory observations in the field.

SECTION 2

CONCLUSIONS

LABORATORY STUDY

Hydrogen peroxide was shown to rapidly decompose and produce pure gaseous oxygen. Due to precautions taken to minimize non-enzymatic decomposition in this study, the data indicate that hydrogen peroxide decomposition to oxygen and water was the result of enzymatic catalysts. Oxygen provided by the hydrogen peroxide decomposition reaction was consumed in the columns. It was not possible to distinguish between abiotic and biotic oxygen demand in this study.

The injection of hydrogen peroxide at 100.0 mg/l had two observed notable effects. The rapid rate of decomposition resulted in a high rate of oxygen gas production. The rate of oxygen gas production far exceeded the demand, and although the solubility of dissolved oxygen had not been exceeded, gas bubbles appeared to have insufficient time to diffuse from the gaseous phase into the aqueous phase. Approximately 45% of the available oxygen injected into the columns was transferred into the gaseous phase. Secondly, the rate of oxygen consumption decreased indicating that bacterial inhibition may have occurred.

Mass balances of both oxygen and hydrocarbon was calculated to quantify the mass of oxygen consumed and the mass of hydrocarbon degraded. The ratio of estimated oxygen consumed to aviation gasoline degraded was found to be greater than the stoichiometric prediction.

Hydrogen peroxide, introduced into the biologically active columns at 50.0, 100.0, and 200.0 mg/l, never exceeded 11% breakthrough although the columns were only 18 cm in length.

FIELD STUDY

Injecting hydrogen peroxide into the aquifer at the pilot study area resulted in; increasing the concentration of available oxygen in downgradient wells; rapid decomposition of hydrogen peroxide; and the liberation of oxygen gas into the unsaturated zone resulting in a concentration much greater than background. The rate of hydrogen peroxide decomposition at the site was unknown but was expected to be rapid due to the concentration of oxygen gas measured in the pilot study area.

SECTION 3

RECOMMENDATIONS

RECOMMENDATIONS for PROCESS and APPLICATIONS RESEARCH

This study has focused on investigating the feasibility of utilizing hydrogen peroxide as a supplemental source of oxygen for the enhanced bioremediation of contaminated (aviation gasoline) aquifer material. Clearly, injection of hydrogen peroxide into the ground water will increase the concentration of dissolved oxygen which can be used as an electron acceptor in the bioremediation process. However, the rapid decomposition of hydrogen peroxide as observed in this study, and the subsequent liberation of the oxygen gas to the unsaturated zone will limit the effectiveness of hydrogen peroxide to supply oxygen to the saturated zone. The following recommendations are suggested to further evaluate the feasibility of utilizing hydrogen peroxide as a source of oxygen.

1. Research designed to differentiate between biotic and abiotic mechanisms of hydrogen peroxide decomposition will offer the opportunity to more completely understand the role of biological and chemical variables in this reaction. The application of this information could then be used to develop methods to control or minimize decomposition reactions. Understanding these processes may also help identify chemical and biological subsurface conditions that are not conducive to this method of oxygen supplementation.

2. The results of the laboratory column study found hydrogen peroxide at 100.0 mg/l to decrease the oxygen utilization rate. A decline in the oxygen utilization rate indicated that bacterial inhibition may have occurred. While other research has found the hydrogen peroxide microbial toxicity threshold to be much higher, these are usually based on bacterial enumeration. The oxygen utilization rate is a parameter which offers feedback on the performance of the biodegradation process. The concentration of hydrogen peroxide identified in this research which indicated bacterial inhibition had occurred has paramount importance since it is process oriented and is relatively low. Additional research is necessary both to verify this observation and to more closely examine the toxicity effect from a process perspective.

3. The feasibility of using hydrogen peroxide as a supplemental source of oxygen at a bioremediation site must consider also the economics and safety of this method compared to other candidate methods. Included in this evaluation are: the cost of the hydrogen peroxide; operation and maintenance costs of the equipment to store, mix, and deliver the hydrogen peroxide; the fraction of oxygen actually delivered to the saturated zone;

toxicity; and safety. A comprehensive feasibility evaluation is necessary to identify the technical and economic benefits of using various techniques of oxygen supplementation.

SECTION 4

MATERIALS and METHODS

LABORATORY STUDY

The contaminated aquifer material, characterized as a fine to medium grained sand, was retrieved from a thick glacial deposit aquifer in Traverse City, Michigan [18,10]. An aseptic, undisturbed aquifer sample was collected from the heart of an aviation gasoline plume using a modified hollow stem auger drilling tool [10]. Approximately 476 g. of wet, contaminated soil was placed in each column. An abiotic control column was not used in this experiment. A schematic of the laboratory apparatus is shown in Figure 1. The glass columns were approximately 18 cm long (4 cm I.D.). Glass wool was placed in the top and bottom followed by 1.5 cm of coarse sand. Soil columns were kept in a constant temperature chamber at 12 C.

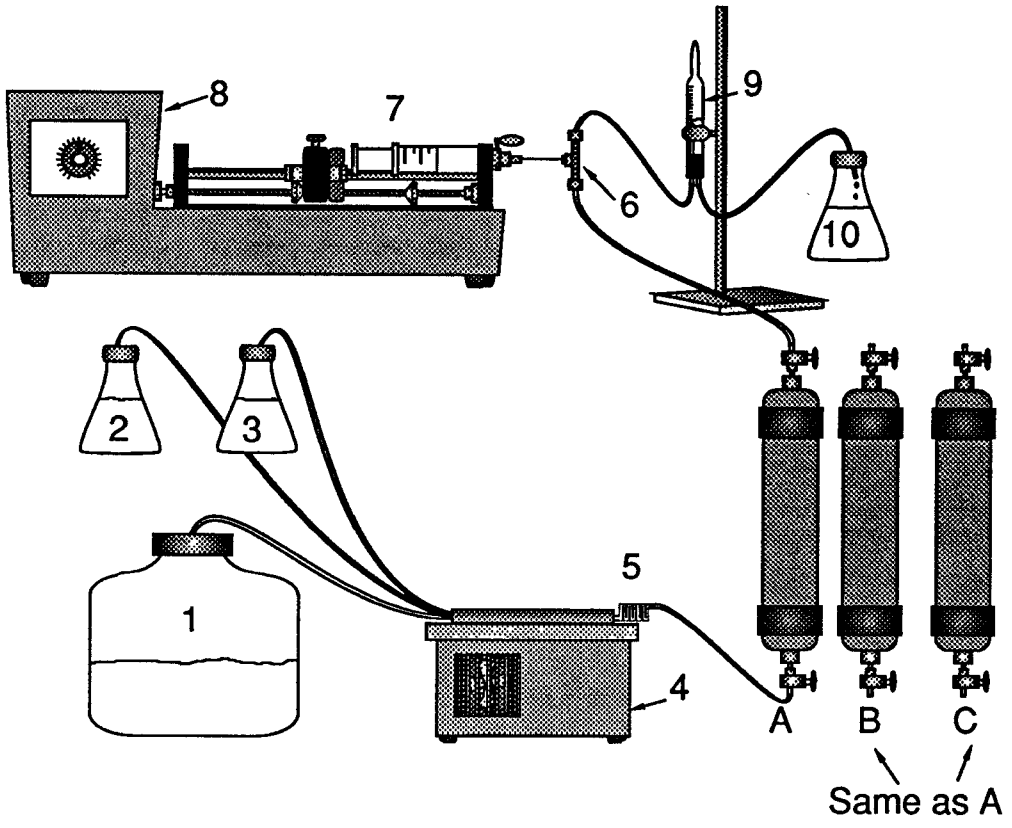


Figure 1. Schematic of soil columns. (1) feed water; (2) nutrient solution; (3) peroxide solution; (4) peristaltic pump; (5) mixing coil; (6) teflon septa sampling port; (7) glass syringe; (8) syringe pump; (9) gas trap; (10) waste collection.

A mixture of feed water, nutrients, and hydrogen peroxide was pumped (peristaltic pump) through the columns. The columns were operated in a continuous upflow mode. Feed solutions were mixed in an in-line mixing coil prior to introduction to the columns. Effluent samples from the column were retrieved in-line using a syringe pump. This enabled the retrieval of an aqueous sample in a closed system without losing volatiles and without aerating/deaerating the column effluent. An inverted centrifuge tube was installed in-line to capture and quantify the gas produced from the column.

Chemical analyses were performed in accordance with EPA methods [6]. Available oxygen (eqn. 9) from both the hydrogen

$$\text{Available oxygen} = ([DO] + 0.471\ [H2O2]) \quad (9)$$

where; [DO] = dissolved oxygen, mg/l
[H2O2] = hydrogen peroxide concentration, mg/l

peroxide and the dissolved oxygen (DO) was measured using the Winkler azide modification method (EPA Method No. 360.2). Hydrogen peroxide analysis was determined using a peroxytitanic acid colorimetric procedure. The columns were pretreated with a phosphate rich nutrient solution (128.0 mg/l Orthophosphate as P) prior to the introduction of hydrogen peroxide. This pretreatment step was a precaution taken to prevent iron decomposition of hydrogen peroxide. The influent phosphate concentration was decreased (89.2 mg/l O-P as P) after 20 hours of pretreatment when 98% breakthrough of O-P (as P) had occurred. The influent nutrient concentrations to the columns was 400.0 mg/l Ammonium Chloride, 200.0 mg/l Potassium Phosphate (monobasic), 200.0 mg/l Sodium Phosphate, and 100.0 mg/l Magnesium Sulfate. Hydrogen peroxide was introduced at 15.0 mg/l.

SECTION 5

RESULTS

LABORATORY STUDY

Due to the low oxygen demand observed during the first eight days of operation, the flow rate was reduced from 80.0 ml/hr to 45.0 ml/hr. The increased hydraulic residence time in the column resulted in greater oxygen consumption and increased the accuracy in determining the oxygen demand. The oxygen demand exerted on the influent was calculated as follows:

$$\text{Oxygen demand} = (\text{Influent [DO]} + 0.471[H_2O_2]) - \text{Effluent [DO]} \quad (10)$$

where the effluent DO concentration, as determined by the Winkler method, detects both DO and oxygen from hydrogen peroxide. Approximately two weeks was required before significant oxygen consumption was observed in all three columns, (Figures 2-4). This response was interpreted as characteristic of microbial acclimation to a new chemical or physical environment. The concentrations of nitrates and nitrites in the column effluent were consistently equivalent to background concentration (<1.0 mg/l) found in the feed water.

Hydrogen peroxide was increased from 15.0 mg/l to 30.0 mg/l after the oxygen demand exceeded approximately 80% of the available oxygen. This increase corresponded to an additional 7.1 mg/l available DO. The effluent oxygen concentration increased indicating a response to the increase in hydrogen peroxide concentration. Hydrogen peroxide was increased to 100.0 mg/l after the oxygen demand exceeded approximately 80% of the available oxygen. Initially, the oxygen demand appeared to increase greater than 50% from the previous hydrogen peroxide concentration. However, bubbles were observed in the column effluent line indicating that a loss of oxygen from the system was occurring.

During a period of fifteen days following the hydrogen peroxide injection of 100.0 mg/l, effluent DO remained constant (DO avg. = 24.6 mg/l, n = 29, st. dev. = 1.25 mg/l) in all three columns. In-line gas traps were used to capture and quantify the gas produced from the columns (Fig. 1). The average rate of gas generation during this period from columns B and C was 1.17 ml/hr. Gas chromatograph analysis of the captured gas indicated that the gas composition was approximately 65%-70% oxygen and 30%-35% nitrogen.

A mass balance was performed on the influent and effluent available oxygen in the system. The influent mass of oxygen was calculated as follows:

Fig. 2 Oxygen Response Curve, Column A

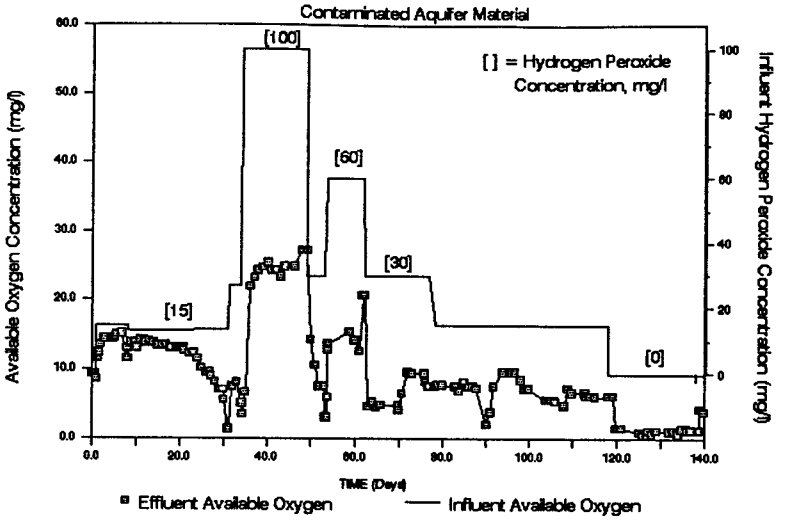


Fig. 3 Oxygen Response Curve, Column B

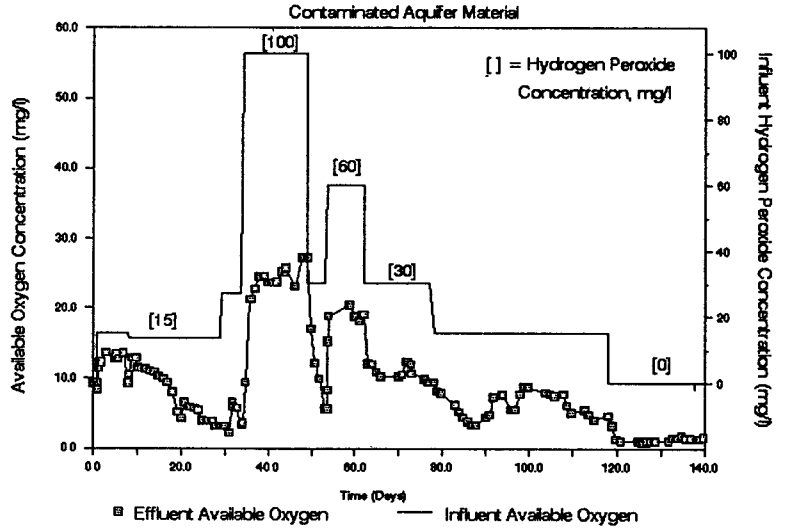
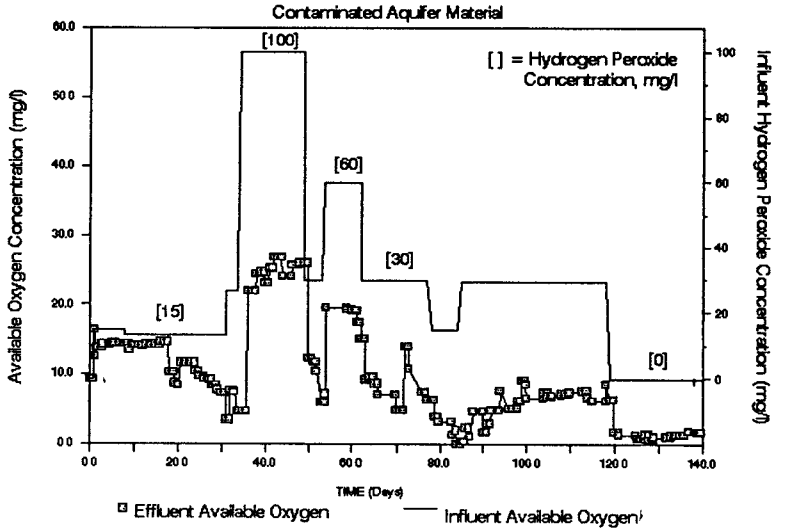


Fig. 4 Oxygen Response Curve, Column C



$$Mt,i = Q_T [DO]_i + 0.471\ Q_H [H2O2] \quad (11)$$

where; Mt,i = total influent oxygen, mg/hr
Q_T = total flow, l/hr
$[DO]_i$ = influent dissolved oxygen, avg. concentration @ 12 deg. C, 9.35 mg/L,
Q_H = Hydrogen peroxide flow, l/hr

The effluent mass of oxygen is calculated as follows:

$$Mt,e = Q_T [DO]_e + f\ O_2gas \quad (12)$$

where; Mt,e = total effluent oxygen, mg/hr
$[DO]_e$ = effluent available oxygen, mg/l includes DO + .471 [H2O2]
O_2gas = average rate of O_2gas generated, l/min
f = fraction of oxygen in mixture (.675)

Mass balance results were converted to moles/hr and are included in Table 1. The oxygen mass balance indicates that roughly 44.0% and 45.0% of the influent oxygen was recovered in the aqueous and gaseous phases respectively for a total recovery of 89%. The unrecovered oxygen was assumed to be consumed both biotically and abiotically. The cumulative total and cumulative adjusted oxygen demand curves for each column are presented in Figures 5-7. The cumulative adjusted oxygen demand curve is the difference between the cumulative total oxygen demand and the oxygen lost from

Table 1 - Column Influent and Effluent Flux of Available Oxygen(1,2) in Both Aqueous and Gaseous Phases

	COLUMNS		
	A	B	C
INFLUENT			
aqueous	7.78E-5	7.78E-5	7.78E-5
EFFLUENT			
aqueous	3.41E-5 (43.8%)	3.4E-5 (43.7%)	3.42E-5 (44.0%)
gaseous	3.5E-5(3) (45.0%)	3.71E-5 (47.7%)	3.29E-5 (42.3%)

(1) Flux rate, moles oxygen/hr.
(2) Values in parentheses indicate percent effluent of total influent flux.
(3) Average of columns B and C.

Figure 5. Cumulative Oxygen Demand, Column A

Total and Adjusted Oxygen Demand

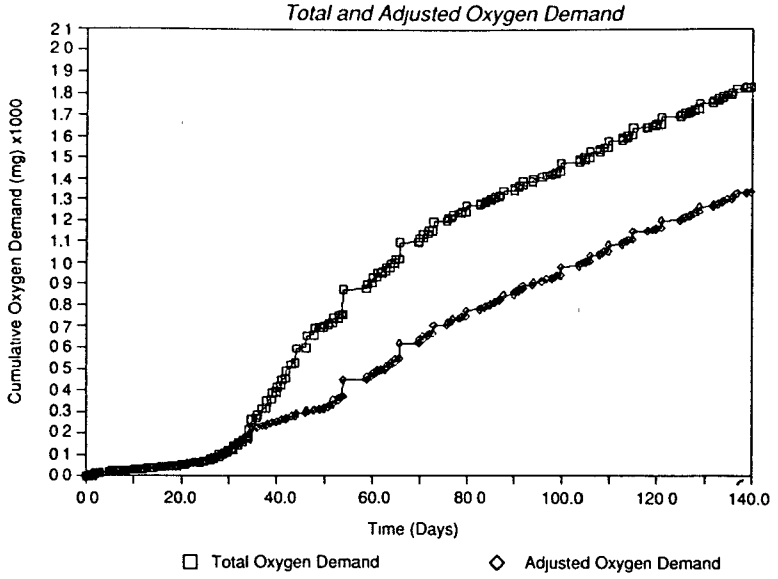


Figure 6. Cumulative Oxygen Demand, Column B

Total and Adjusted Oxygen Demand

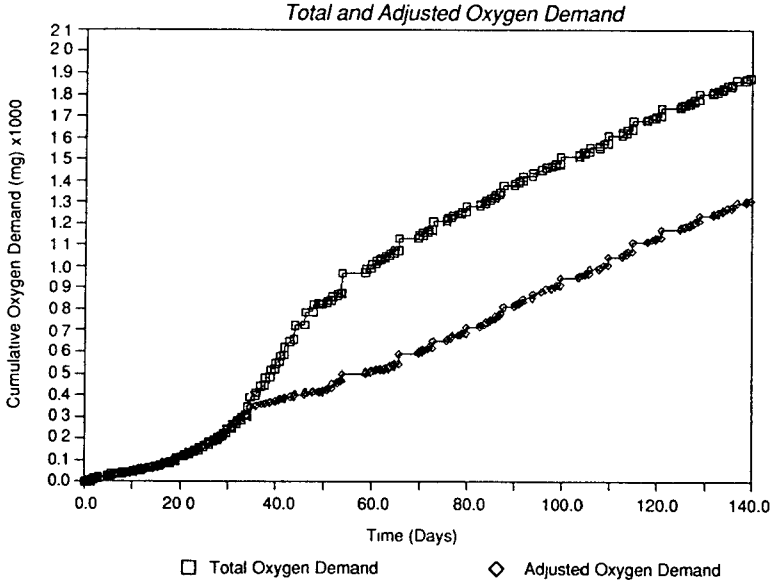
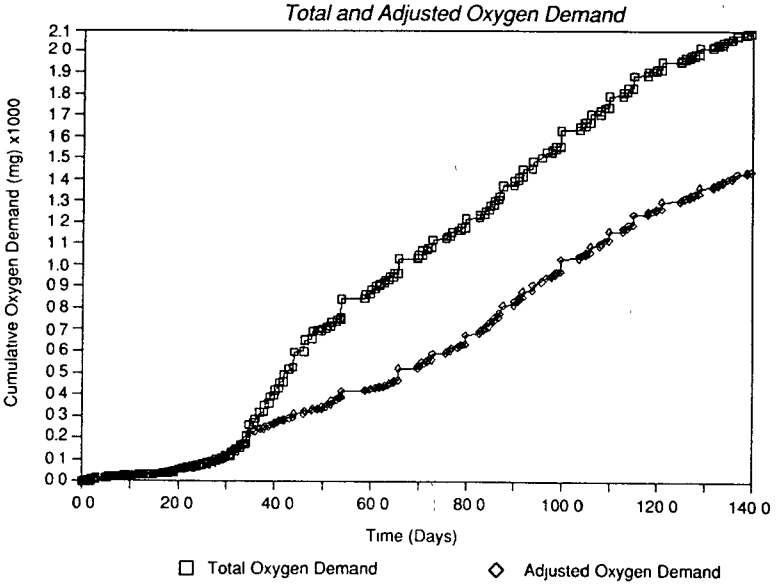


Figure 7. Cumulative Oxygen Demand, Column C

Total and Adjusted Oxygen Demand



the system due to degassing. The total and adjusted oxygen demand curves demonstrated the potential of hydrogen peroxide, at 100.0 mg/l to rapidly decompose resulting in the production of pure oxygen. The slopes of the cumulative adjusted oxygen demand curves (Figures 5-7) during the 100.0 mg/l hydrogen peroxide injection period is less than the slope at lower hydrogen peroxide concentration periods during the study. A decrease in the oxygen consumption rate indicates that inhibition of bacterial respiration during this period may have occurred. The hydrogen peroxide concentration was reduced to 30.0 mg/l due to the inefficient use of available oxygen and the apparent reduction in the rate of oxygen consumption.

Effluent available oxygen concentration during the 100.0 mg/l hydrogen peroxide injection averaged 24.6 mg/l. The aqueous solubility of pure gaseous oxygen at 12 C is approximately 43.0 mg/l. The results of the column study suggested that the oxygen bubbles did not have sufficient time to diffuse from the gaseous phase into the aqueous phase.

In an attempt to optimize the utilization of oxygen in the system, an oxygen mass balance on the columns was used to compute an influent hydrogen peroxide concentration (69 mg/l) below which degassing would not occur. This approach assumed that maximum solubility of dissolved oxygen could be achieved. Influent hydrogen peroxide concentration was increased to 60.0 mg/l. Shortly thereafter, gas bubbles were observed in the effluent line. As discussed previously, this data indicated that the retention time of the oxygen bubble in the system was inadequate for oxygen gas to diffuse into the aqueous phase.

During the later stages of the study, degassing was noted to occur at hydrogen peroxide concentrations that previously did not result in degassing. It appears that this may have been due primarily to enzymatic decomposition associated with the additional biomass in the system and the short retention time of the bubbles in the column. Influent hydrogen peroxide concentration during the remainder of the experiment was incrementally adjusted as the oxygen demand changed with time.

The average cumulative total oxygen demand from the columns was 1940 $\pm$ 127 mg oxygen and the average cumulative total oxygen demand, adjusted for the oxygen degassing was 1360 $\pm$ 67 mg O2. During the various operating scenarios (i.e. varying influent hydrogen peroxide concentration) of this study, degassing accounted for approximately 30% of the total oxygen demand.

The slope of the cumulative adjusted oxygen demand curve in Figures 5-7 represents the overall rate of oxygen consumption during the study period. Linear regression analysis of the cumulative adjusted oxygen demand versus time, after the 100.0 mg/l hydrogen peroxide injection, are as follows:

Column	Slope (mg oxygen/day)	r^2
A	11.4	0.99
B	10.4	0.99
C	13.5	0.99

Gas chromatograph analysis of the initial and final aquifer material [19] and the column effluent was performed to calculate an approximate mass balance of hydrocarbons in the system, (Table 2). Based on the mass of aquifer material in each column, the initial and final average fuel carbon (FC) concentrations, and an estimate of the effluent FC, the amount of FC degraded was estimated using equation 13. Based on the average of all three columns, 36% of the initial mass of fuel carbon leached from the

Table 2 - Hydrocarbon Mass Balance

	COLUMNS		
	A	B	C
Aquifer Material Analyses			
Mass (kg)	0.479	0.475	0.474
Initial [FC](1) (mg/kg)	906.0	906.0	906.0
Initial mass FC(2) (mg) (M_I)	434.0	430.0	429.0
Final [FC] soil(3) (mg/kg)	136.0	60.0	76.0
Mass FC final(4) (mg) (M_F)	65.0	29.0	36.0
Column Effluent Analyses			
Effluent FC aqueous(5) (mg) (M_E)	154.0	150.0	161.0
Estimated FC Degraded			
Mass FC degraded(6) (mg) (M_D)	215.0	251.0	232.0

(1) Average of triplicate analyses of contaminated aquifer material from which the column material was derived, (790.0, 1045.0, and 884.0 mg/kg) initial fuel carbon concentration in column aquifer material = 906.0 mg/l.
(2) Mass FC initial = 906.0 (mg/kg) X Soil mass (kg).
(3) Average of replicate analyses for each column of fuel carbon in the final aquifer material.
(4) Mass FC final = Final [FC] mg/kg X Soil mass(kg).
(5) Estimated FC in column effluent.
(6) Mass FC biodegraded, $M_I - M_F - M_E$.

aquifer material, 10% remained on the aquifer material, and 54% degraded.

$$M_D = M_I - M_F - M_E \quad (13)$$

where; M_D = fuel carbon biodegraded;
M_I = fuel carbon initial;
M_F = fuel carbon final; and
M_E = fuel carbon in column effluent.

The empirical carbon and hydrogen content of the aviation gasoline was determined to be approximately 2.16 parts hydrogen per part carbon, or roughly 85% carbon and 15% hydrogen [12]. The ideal stoichiometric biological conversion of hydrocarbon to carbon dioxide and water is approximately 3.48 parts oxygen per part hydrocarbon as described in the following equation:

$$CH_{2.16} + 1.54\ O_2 \text{ ----------> } CO_2 + 1.08\ H_2O \quad (14)$$

The ratio of the estimated oxygen consumed to estimated aviation gasoline degraded in this study was greater than the stoichiometric conversion ratio, (Table 3). Measured conversion ratios greater than the stoichiometric prediction was likely to occur due to errors in the mass balance analysis and the abiotic oxygen demand. It was not possible to differentiate between biotic and abiotic oxygen demand in this study.

Table 3 - Conversion Ratios (O_2(mg) / Aviation Gasoline (mg))

	COLUMNS A	B	C
Mass O_2 consumed[1] (mg)	1322.0	1307.0	1434.0
Mass Aviation gasoline[2] degraded (mg)	253.0	295.0	274.0
Conversion Ratio mg O_2/mg Av. gasoline	5.23	4.43	5.23

(1) Adjusted Cumulative Oxygen Demand, Figures 5-7
(2) Mass Aviation gasoline degraded = Mass FC Degraded/(0.85)

Colorimetric hydrogen peroxide analysis of the column effluent was performed to determine the persistence of hydrogen peroxide in the contaminated aquifer material. Prior to terminating the experiment, hydrogen peroxide was injected at 50.0, 100.0, and 200.0 mg/l. These concentrations were injected for 7, 10, and 13 hours, respectively, prior to collecting effluent samples. Breakthrough of hydrogen peroxide, for all three concentrations, was less than 11% (Table 4). The introduction of phosphate as a nutrient into the columns was expected to minimize the non-enzymatic catalysis of hydrogen peroxide. Therefore, the rapid decomposition of hydrogen peroxide in this system appeared to be due to enzymatic catalysis, demonstrating that the column media was biologically active.

Table 4 - Hydrogen Peroxide Breakthrough

	COLUMNS								
	A	B	C	A	B	C	A	B	C
[H2O2]i (mg/l)	50.0			100.0			200.0		
[H2O2]e (mg/l)	ND	ND	ND	9.6	10.4	2.5	16.5	17.0	9.3
H2O2 Breakthrough (%)	<10	<10	<10	9.6	10.4	2.5	8.3	8.5	4.7

[H2O2]i, [H2O2]e - influent and effluent hydrogen peroxide concentration, respectively

Detection limits:
$[H2O2]_{50.0}$ = 5.0 mg/l, $[H2O2]_{100.0,\ 200.0}$ = 2.5 mg/l

SECTION 6

FIELD STUDY

BACKGROUND

Twenty years ago, aviation gasoline (25,000 gal.) spilled into a shallow, sandy, water table aquifer at the U.S. Coast Guard Station in Traverse City, Michigan. The aviation gasoline migrated to the water table and spread laterally. Soluble components of the hydrocarbon plume migrated longitudinally in the direction of ground water flow and eventually moved off the Coast Guard Station and contaminated a large number of domestic water wells in a residential area, refer to Figure 8. The spill site was cored extensively to determine the distribution of contamination in the subsurface. The majority of the contamination was found to be distributed within a narrow interval between 15 and 17 feet below the land surface (Table 5) [22d] corresponding to the seasonal low and high water table at the site.

Table 5 - Vertical Distribution of Contamination 50 feet Downgradient From The Injection Wells

Depth Interval (feet below surface)	Fuel Hydrocarbons (mg/kg aquifer)
15.1-15.5	< 11
15.5-15.8	39
15.8-16.2	2370
16.2-16.5	8400
16.5-17.2	624
17.2-17.5	< 13
18.0-18.3	< 13

In 1988, the U.S. Coast Guard and the U.S. EPA began the operation of a pilot scale in-situ bioremediation project in the area of the original spill [15,17a-f]. A series of five deep wells (I1-I5) were used to inject clean water beneath the plume area in an effort to raise the water table and subsequently saturate the contaminated "smear zone". Raising the water table was performed in order to allow the delivery of soluble nutrients to the targeted zones of contamination. Five chemical feed wells (CF1-CF5) were used to inject nutrients and hydrogen peroxide in the shallow, contaminated layer (Figures 9,10). A series of downgradient monitoring wells (DG-8, -25, -37, -49B, -61, -109)

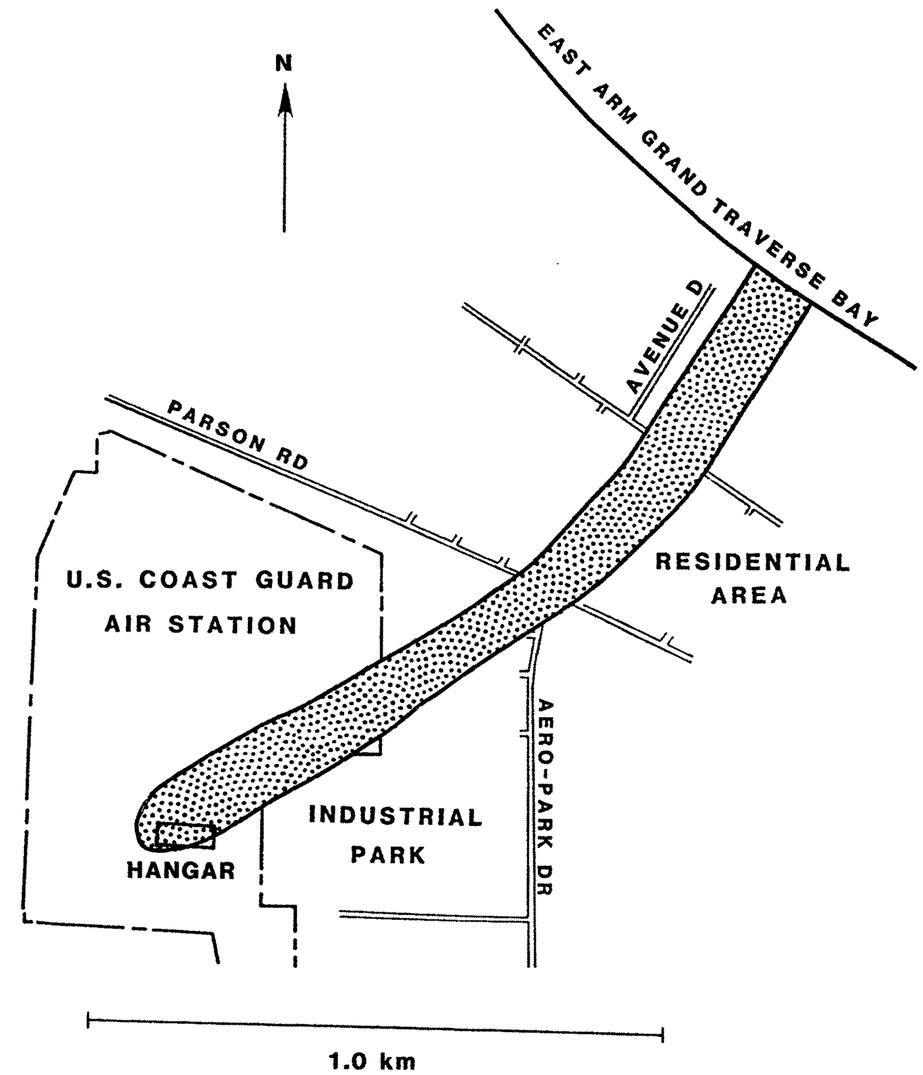


Figure 8 - Aviation Gasoline Plume, U.S. Coast Guard Station, Traverse City, Michigan

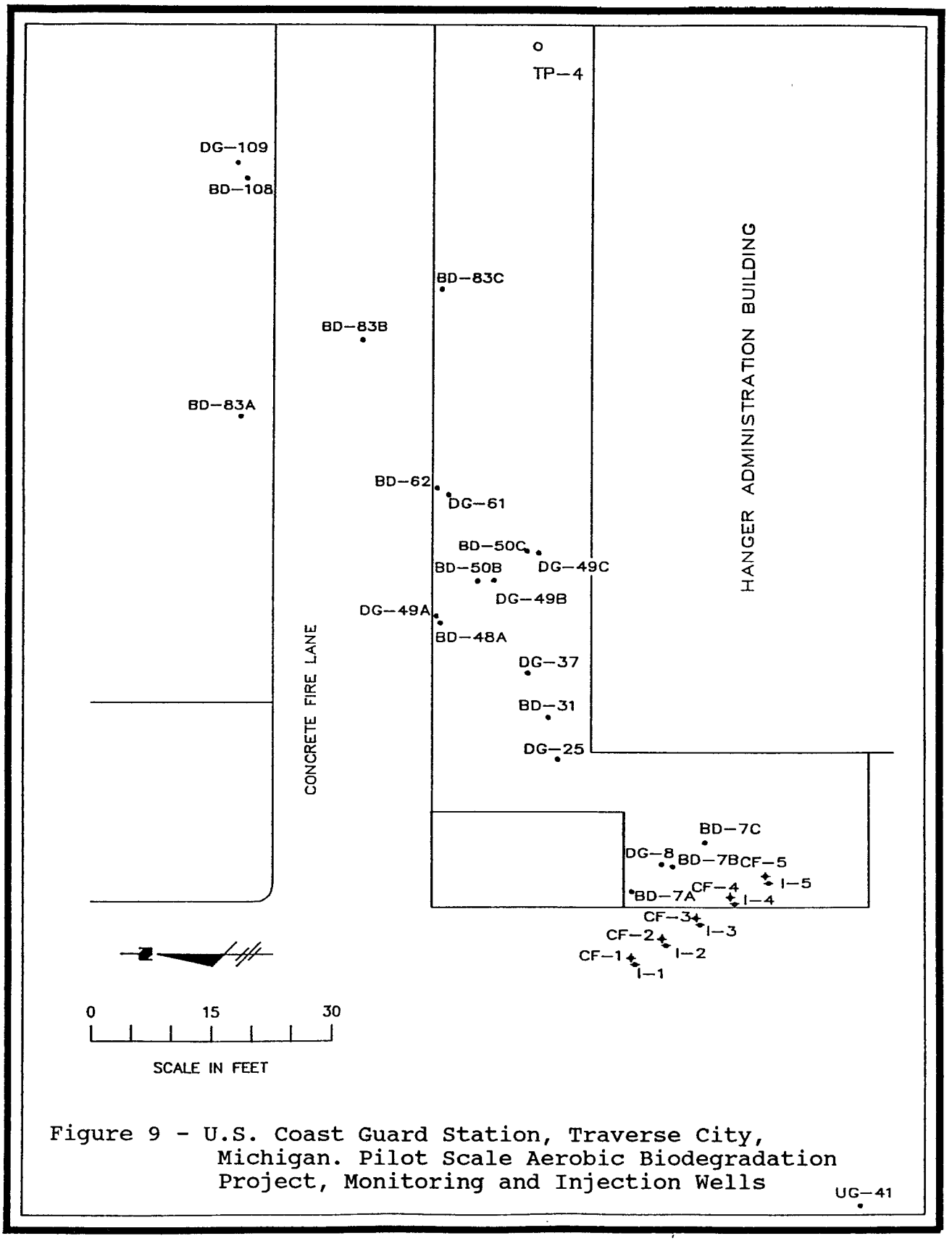
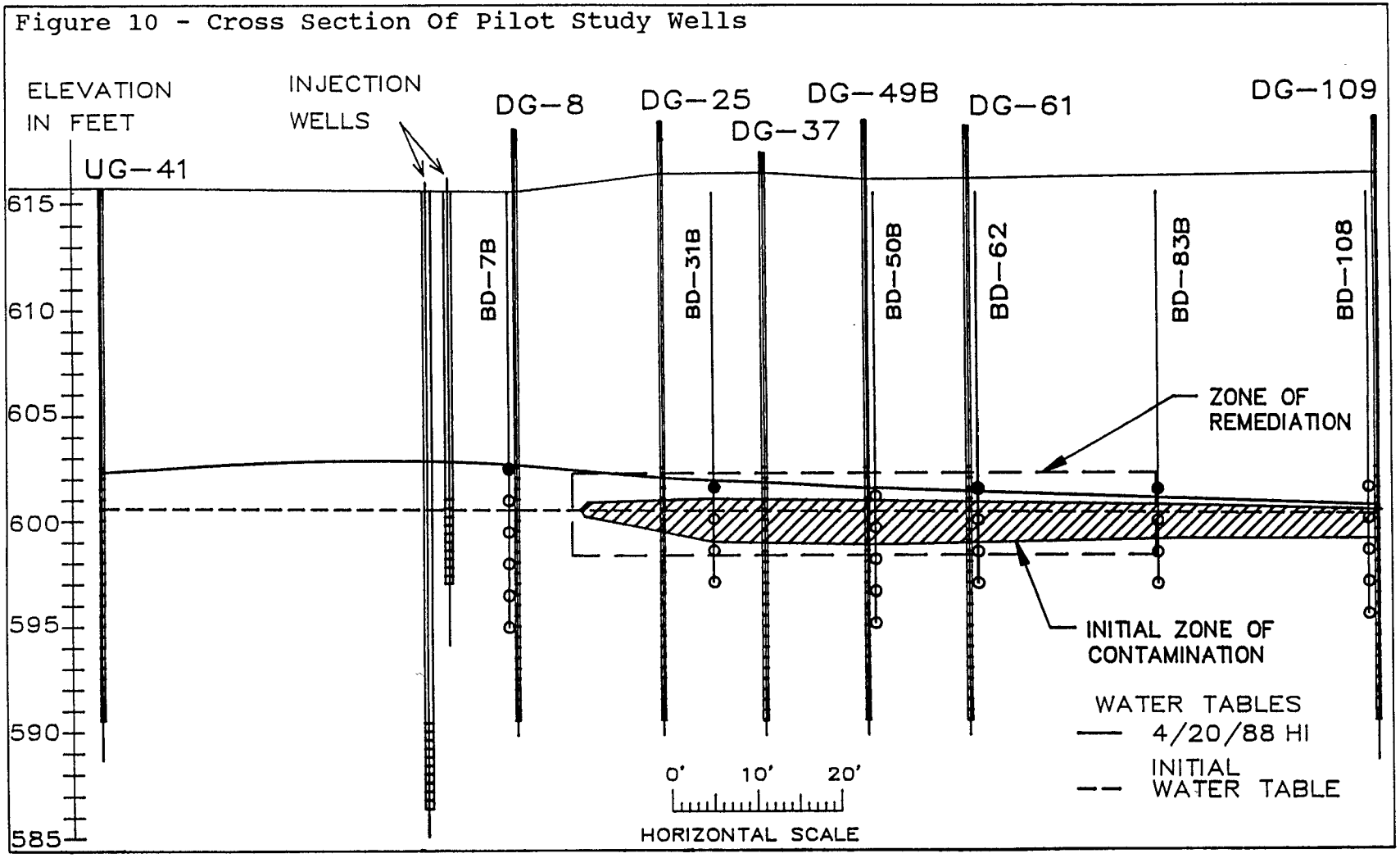


Figure 9 - U.S. Coast Guard Station, Traverse City, Michigan. Pilot Scale Aerobic Biodegradation Project, Monitoring and Injection Wells

Figure 10 - Cross Section Of Pilot Study Wells



and subsurface sampling lines (BD-7A,B,C, BD-31, BD-48A, BD-50A,B,C, BD-62, 83-A,B,C, and BD-108) were installed to monitor the performance of bioremediation. The sampling lines have several vertical sampling ports (1 foot stainless steel screens) at one foot intervals. The sampling ports are numbered sequentially starting at one and increasing with depth (i.e. BD-7B1, BD-7B2, BD-7B3...).

Nutrient and oxygen injection began in March 1988. Nutrient enriched water was injected at a total flow rate of approximately 11 gpm in the five chemical feed wells. The nutrients in the injected water contained approximately 380 mg/l ammonium chloride, 190 mg/l disodium phosphate, and 190 mg/l potassium phosphate. A liquid oxygen source was used to inject approximately 40 mg/l dissolved oxygen. Approximately 3 months later (June 1988), hydrogen peroxide was injected according to the schedule given in Table 6. Prior to hydrogen peroxide injection, phosphate breakthrough had occurred in the nested monitoring wells. This was necessary to complex the iron found in the aquifer material with phosphates and therefore, minimize the iron catalyzed hydrogen peroxide decomposition reaction. Due to problems associated with the chemical feed system, including hydrogen peroxide stratification in the feed tank, the concentration of hydrogen peroxide that was injected varied considerably. Therefore, it was difficult to compare the concentration of dissolved oxygen in the injected water with parameters measured in the monitoring wells.

Table 6 - Oxygen - Hydrogen Peroxide Injection Schedule

Date	Time(days)	Cumulative Time(days)	Oxygen Source	Concentration
3/2/88	90	90	Liquid Oxygen	40(1)
6/2/88	5	95	Hydrogen Peroxide	50
6/7/88	7	102	" "	110
6/14/88	64	166	" "	250
8/17/88	106	272	" "	500
12/3/88	179(2)	451	" "	750

(1) Concentration as dissolved oxygen
(2) 179 days as of 5/31/89

Both soil gas and ground water analyses were used to investigate the fate of hydrogen peroxide in the in-situ bioremediation field scale pilot study. Results of the laboratory study indicated that a significant fraction of hydrogen peroxide injected into the subsurface may decompose prematurely and result in the liberation of oxygen gas into the unsaturated zone. This

was evaluated in the field by analyzing soil gas in the injection area for the concentration of oxygen and comparing it to the background soil oxygen concentration. Soil gas samples were collected at various depths and locations in the vicinity of the chemical injection wells.

SECTION 7

MATERIALS and METHODS

FIELD STUDY

Soil gas was sampled in the vicinity of the injection area and analyzed for the oxygen concentration. Soil gas samples were obtained using a series of stainless steel tubes (3/8 in. I.D.) that could be coupled together and driven into the subsurface to various depths. Due to problems associated with driving and retrieving the coupled sampling tubes, soil gas sampling mainly occurred in the 0-10 ft range. Soil gas was pumped into a sample vessel which contained an oxygen detector using a hand held positive displacement pump. The oxygen detector is a GasTech Model LO2 OxyTechTor galvanic cell which measures the concentration of oxygen from 0-100% $\pm$ 5% of the reading. Oxygen was also measured in the headspace in ground-water monitoring wells by lowering the detector to various levels within the well.

Ground-water analyses for available oxygen (i.e. [DO] + 0.471[H2O2]) performed throughout the pilot study [15,17a-f] offered the opportunity to evaluate the performance of the bioremediation system. Available oxygen from both the dissolved oxygen and the hydrogen peroxide was measured using the Winkler azide modification method (EPA Method No. 360.2)

SECTION 8

RESULTS

FIELD STUDY

During the field investigation (8/89), hydrogen peroxide was injected (11 gpm) into ground water at 750 mg/l. The water table in the injection area was approximately 13.5-14.5 feet below grade. There were several impervious areas (asphalt, concrete) around the injection area that limited the locations where soil gas samples could be collected. Nevertheless, numerous soil gas samples were retrieved and analyzed for the concentration of oxygen. This data was used to map the horizontal distribution of oxygen as a function of depth (3, 6, 9-10 ft.) (Figures 11-13). The concentration of oxygen in the unsaturated zone, as indicated in these figures, was clearly greater than both atmospheric and background soil oxygen concentrations, 20.9% and 20.7%, respectively. This indicated that a significant amount of oxygen was lost from the system and was not available for bioremediation of hydrocarbon in the ground water. These figures also indicated that the oxygen concentration increases with depth. Unlike the injection area, the oxygen concentration in areas further downgradient decreased with depth (Table 7). The X and Y coordinates in Table 7 are based on a cartesian coordinate system with the ordinate at injection well I-3.

The concentration of oxygen in the headspace of the wells was obtained by lowering the oxygen detector into the monitoring wells. Although the concentration of oxygen in the monitoring wells may not be representative of the concentration of oxygen in the unsaturated zone, elevated oxygen concentrations demonstrate the rapid rate of hydrogen peroxide decomposition. The same general trend occurred in the wells as in the soil gas; the oxygen concentration in the injection area increased with depth, and the oxygen concentration downgradient of the injection area decreased with depth.

The concentration of available oxygen in ground water downgradient from the injection zone clearly indicated that oxygen was being delivered to the system. The concentration of available oxygen in downgradient sampling ports, plotted as a function of time, are presented in Appendix A. It was difficult to predict the rate at which hydrogen peroxide decomposed or the fraction of injected oxygen that was liberated from the saturated zone. This was largely due to the variability of the influent hydrogen peroxide concentration as a function of time.

Table 7 - Oxygen Concentration Profile Downgradient of the Injection Area

Sample No.	Location[1] X(ft)	Y(ft)	Depth (ft)	Oxygen (%)
1	55	0	3	18.9
			6	17.7
			9	17.6
2	61	26	6	8.6
			10	4.4
			14	1.2
3	109	0	3.28	14.5
			6.56	11.6
			9.84	8.5
			13.12	5.1
4	280	0	1	19.9
			2	13.3
			3	12.0
			4	10.2
			6	8.1

(1) X and Y coordinates indicate both downgradient and lateral distances, respectively from injection wells.

Figure 11. Percent Oxygen in the Unsaturated Zone, In-Situ Bioremediation Pilot Study, Aviation Gasoline Fuel Spill, (3 ft.) Traverse City, Mich., (8/89)

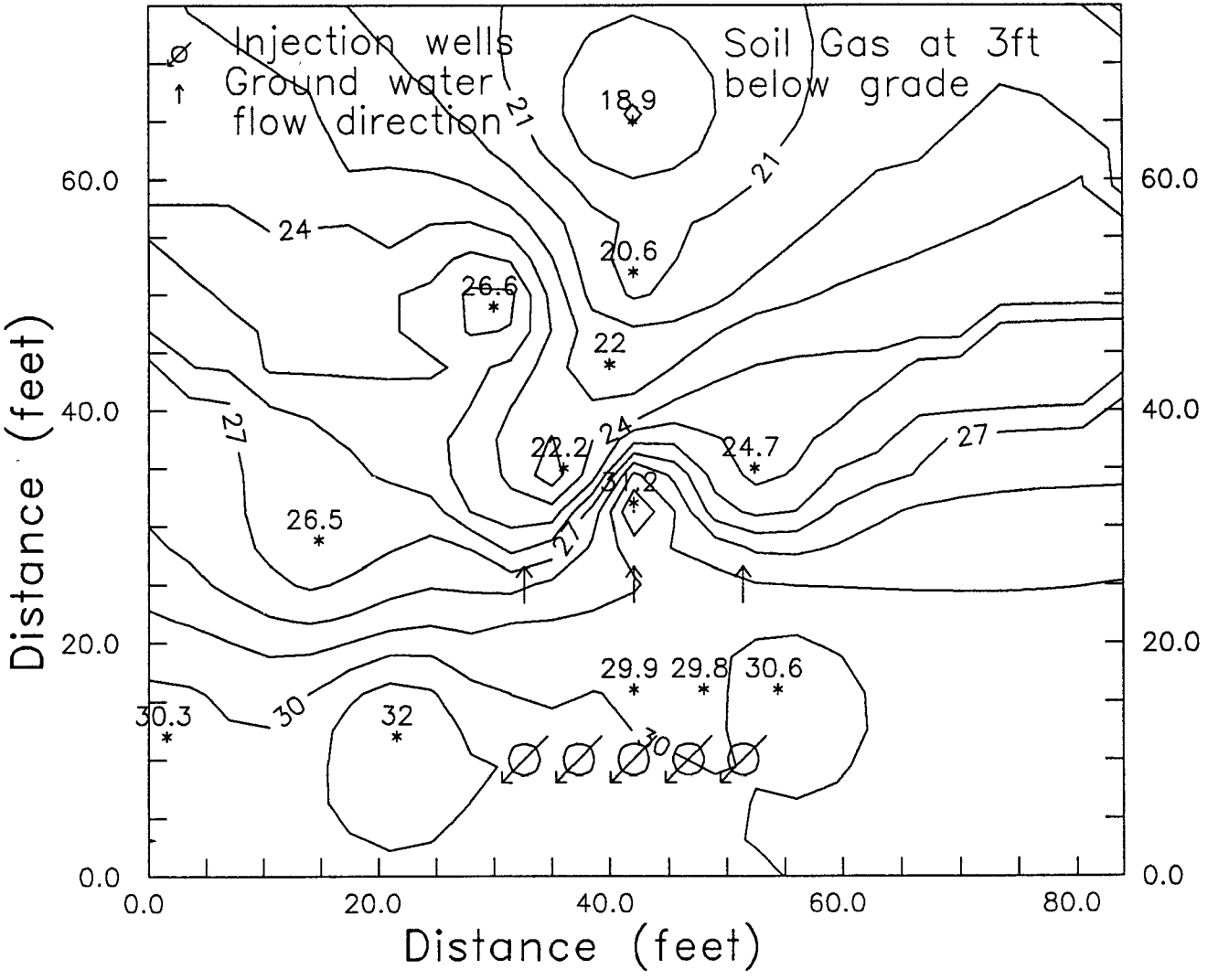


Figure 12. Percent Oxygen in the Unsaturated Zone In-Situ Bioremediation Pilot Study, Aviation Gasoline Fuel Spill, (6 ft.) Traverse City, Mich., (8/89)

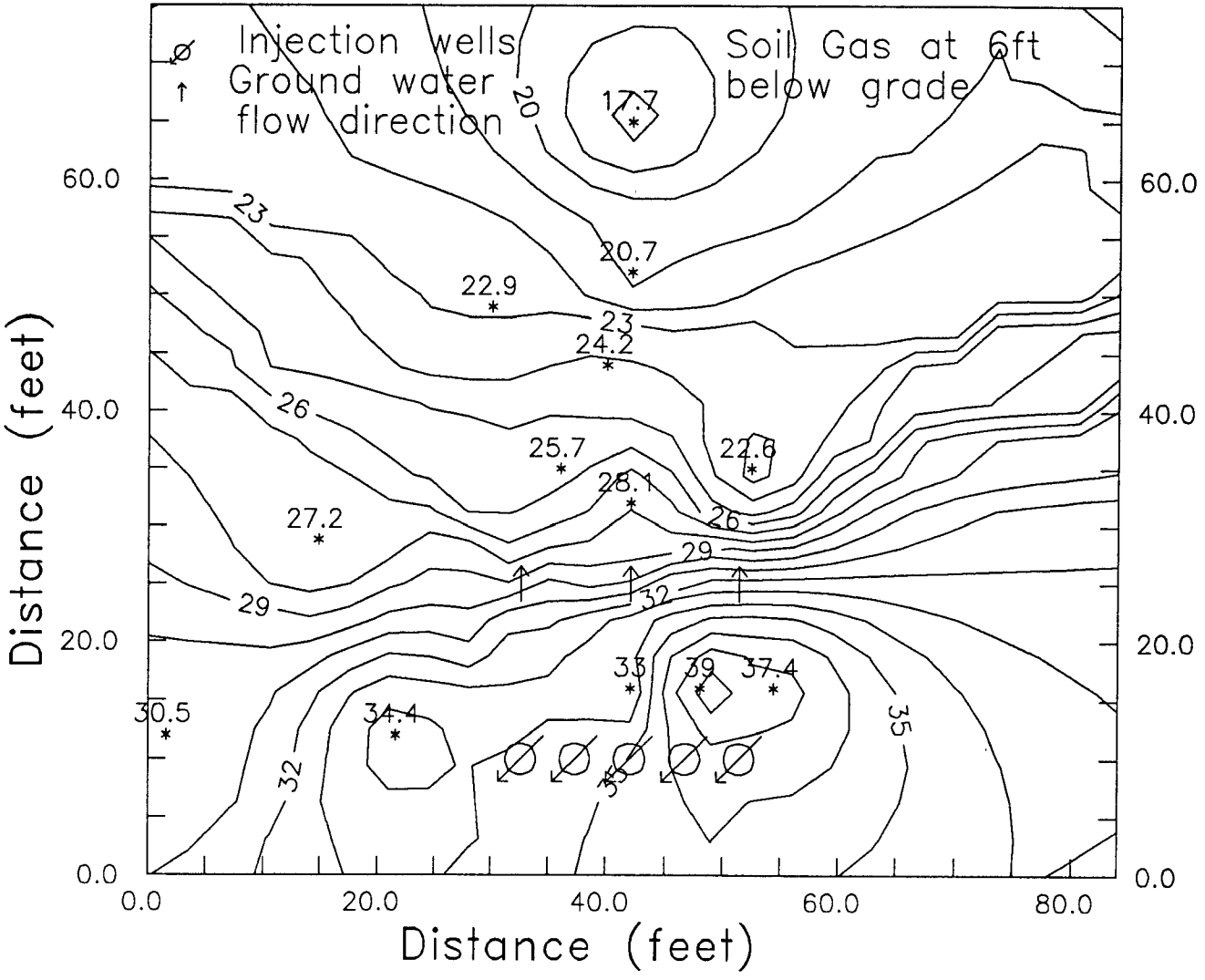
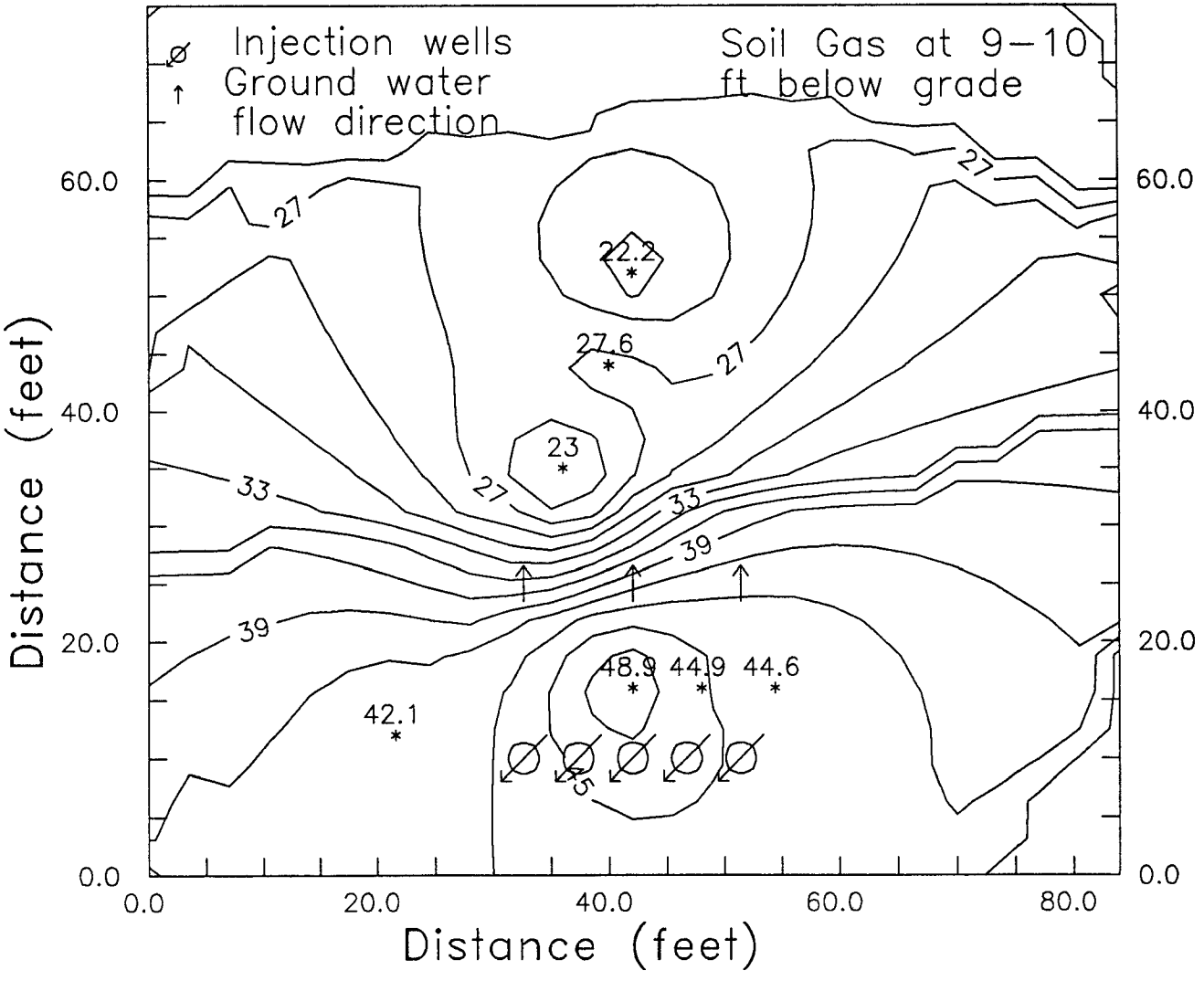


Figure 13. Percent Oxygen in the Unsaturated Zone In-Situ Bioremediation Pilot Study, Aviation Gasoline Fuel Spill, (9-10 ft.) Traverse City, Mich., (8/89)



SECTION 9

DISCUSSION

Results of the field investigation support observations from the laboratory study: hydrogen peroxide decomposed resulting in the liberation of oxygen at a rate faster than the oxygen could be utilized biologically and solubilized into aqueous phase. Subsequently, oxygen gas was liberated from the ground water into the unsaturated zone. The oxygen gas which was liberated into the unsaturated zone could be considered an oxygen sink from the biodegradation process in the saturated zone. This sink introduces a considerable element of uncertainty in estimating how much oxygen is actually delivered to the system and utilized in the biodegradation process. Consequently, predicting the amount of hydrocarbon degraded based on the amount of hydrogen peroxide delivered to the system is inaccurate. Additionally, the data from this study clearly dispells the concept that hydrogen peroxide will only decompose as a function of the biological demand.

Similar to the laboratory study, precautions were taken in the field scale pilot study to minimize iron driven decomposition reactions of hydrogen peroxide by introducing disodium phosphate and potassium phosphate. Therefore, decomposition of hydrogen peroxide was expected to be attributed mainly to catalase, (i.e. enzymatic decomposition).

Two areas addressed in the laboratory study that were not investigated in the field study are microbial inhibition due to hydrogen peroxide and the stoichiometry of the degradation of hydrocarbon based on the amount of oxygen consumed. An accurate mass balance of oxygen and hydrocarbon is critical to make an assessment of both processes. Neither an oxygen nor a hydrocarbon mass balance could be accurately achieved at field scale. Therefore these two areas could not be investigated. An important note to make however, is that the conversion ratios found in the column study represent minimum values due to the oxygen and hydrocarbon mass balance of a carefully controlled system. In a field scale system, it is speculated that the amount of oxygen required will be significantly greater due to short circuiting of the oxygen with repect to the contaminant plume.

The decision to use hydrogen peroxide as a supplemental source of electron acceptor in bioremediation is an issue of economics and safety. Historically, many bioremediation practitioners have utilized hydrogen peroxide under the assumption that it does not rapidly decompose and that the entire amount of oxygen injected into the subsurface contributes to bioremediation. Prior to selection of hydrogen peroxide as a source of oxygen for bioremediation systems, alternative sources should be considered. These alternatives include liquid oxygen and gaseous oxygen either shipped in or produced on-site, i.e. oxygen generation via molecular sieve. Additionally, when

estimating the costs associated with using hydrogen peroxide, consideration should be given to the fraction of oxygen that is lost from the system.

SECTION 10

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SECTION 11

APPENDIX

Field Data - Available Oxygen in Ground Water

FIGURES **PAGE**

Fig. A.1 - Available Oxygen, Sampling Port 7A

Traverse City, Mich., Aviation Gas Spill

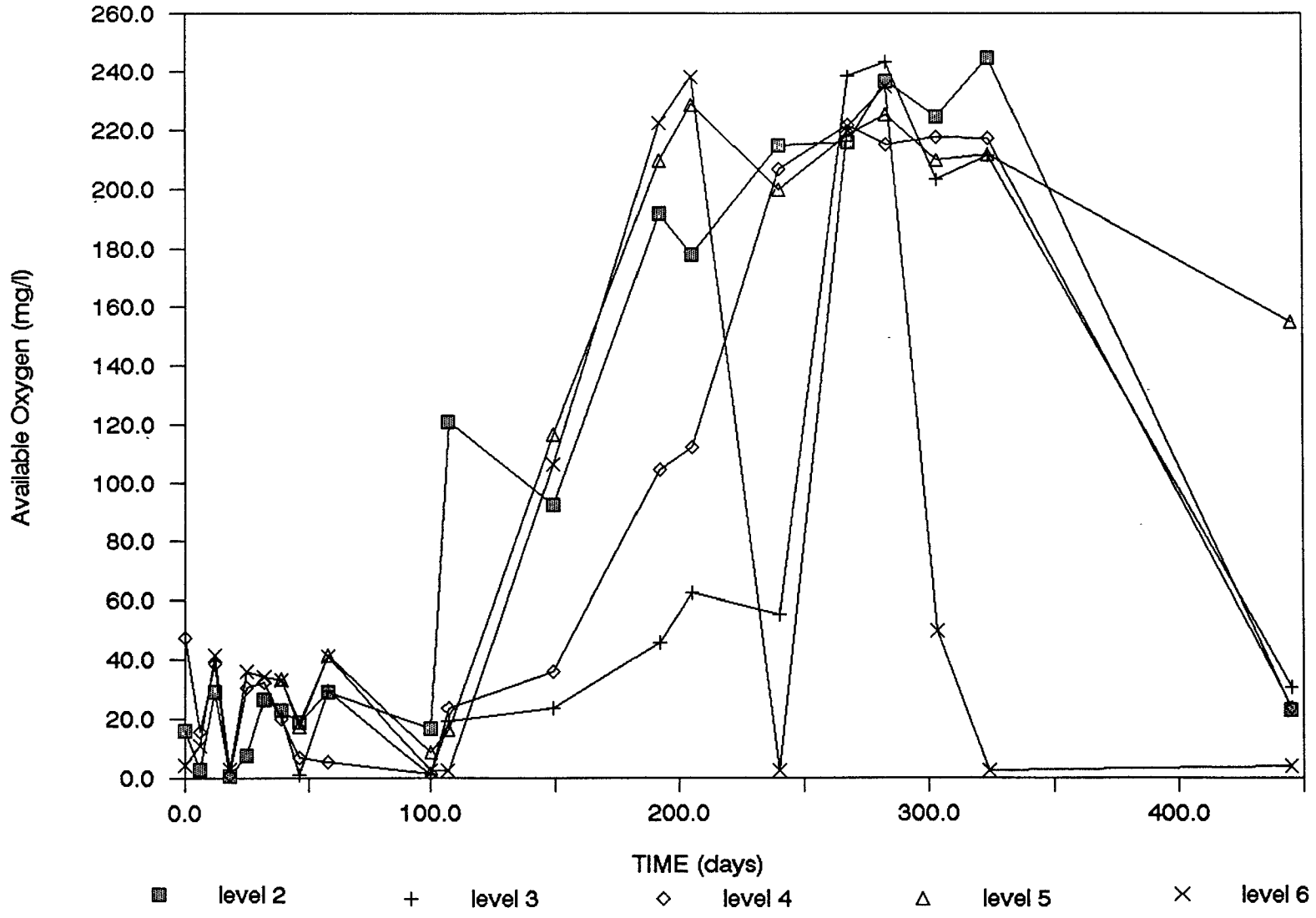


Fig. A.2 - Available Oxygen, Sampling Port 7B

Traverse City, Mich., Aviation Gas Spill

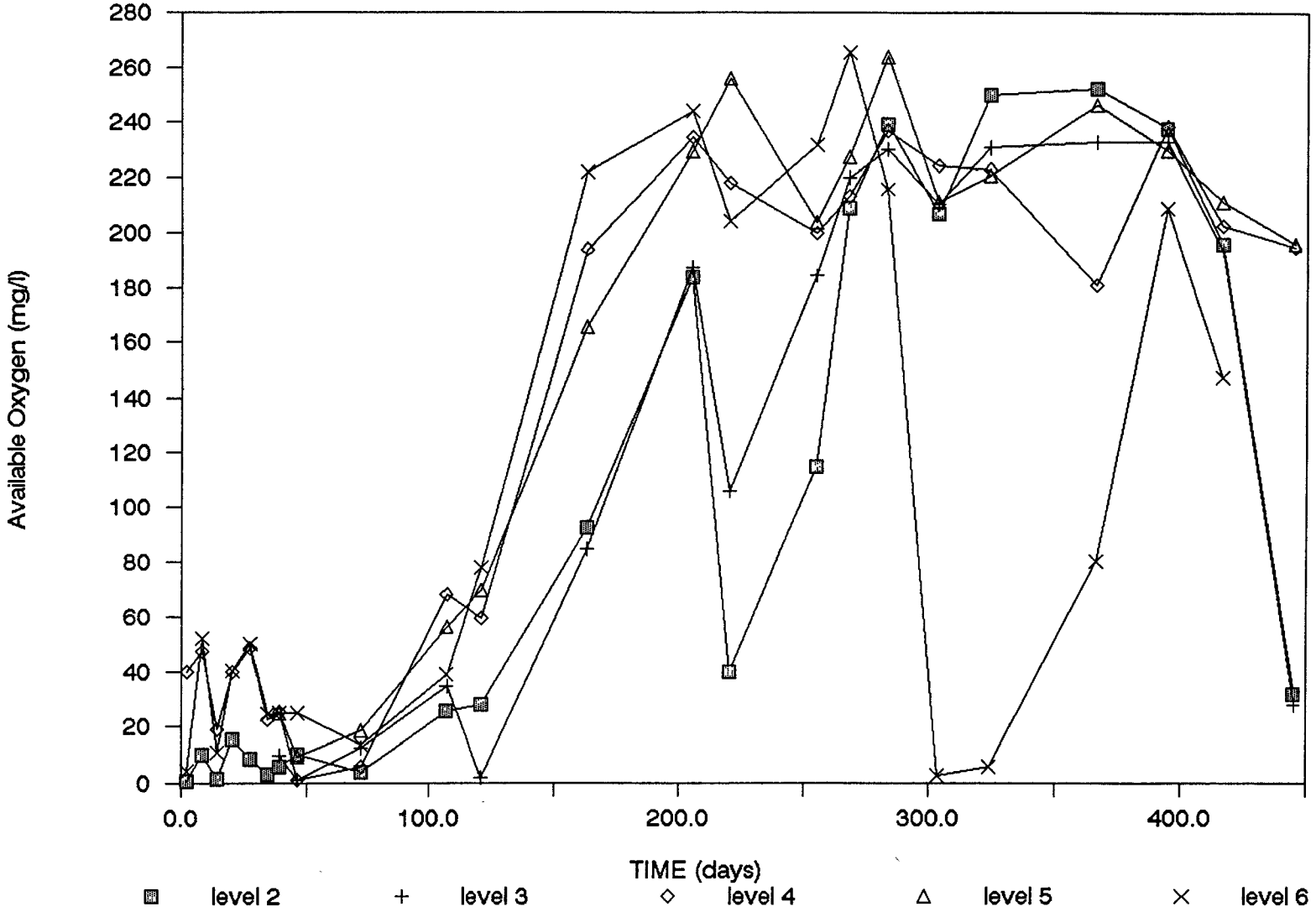


Fig. A.3 - Available Oxygen, Sampling Port 7C

Traverse City, Mich., Aviation Gas Spill

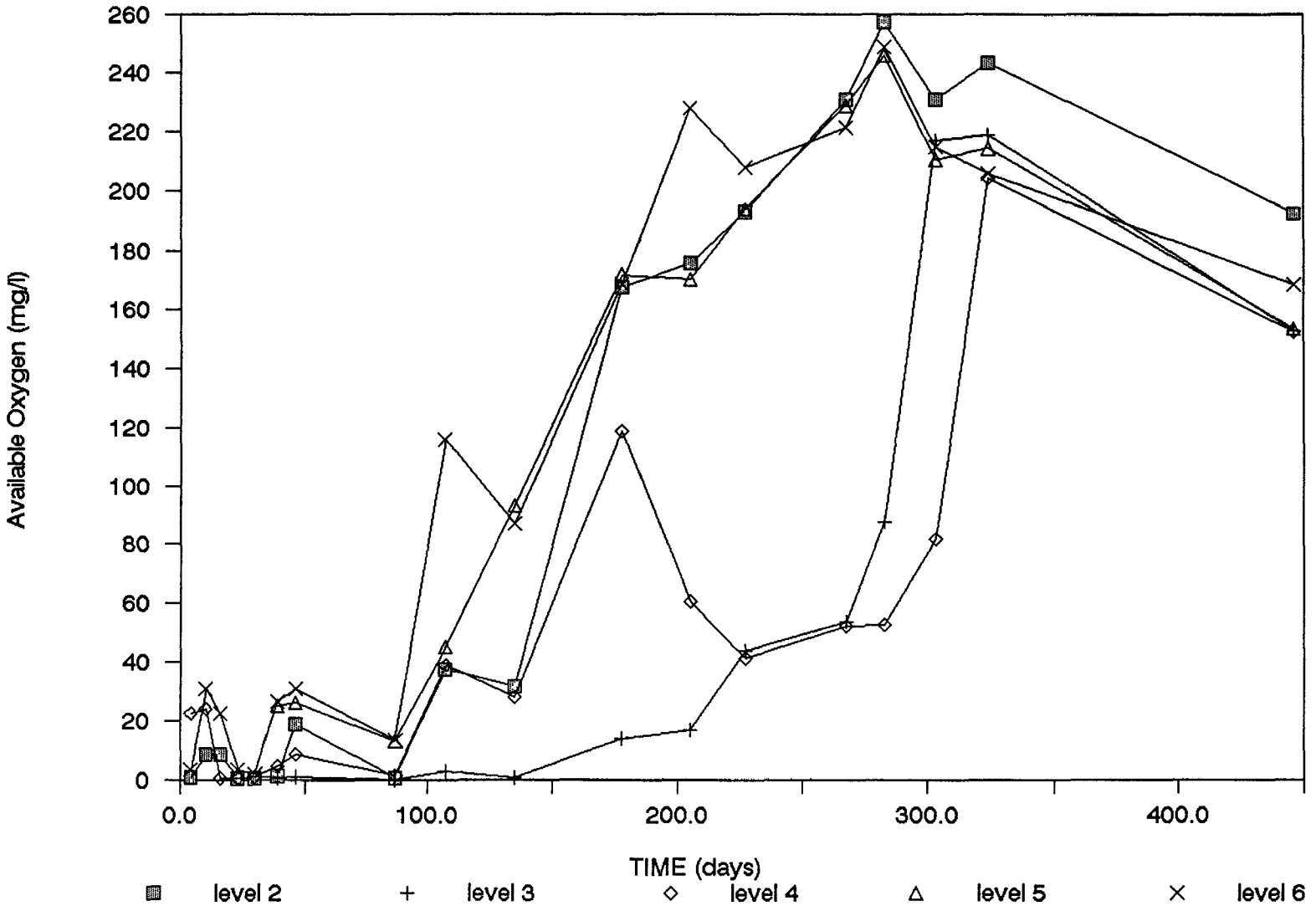


Fig. A.4 - Available Oxygen, Sampling Port 31

Traverse City, Mich., Aviation Gas Spill

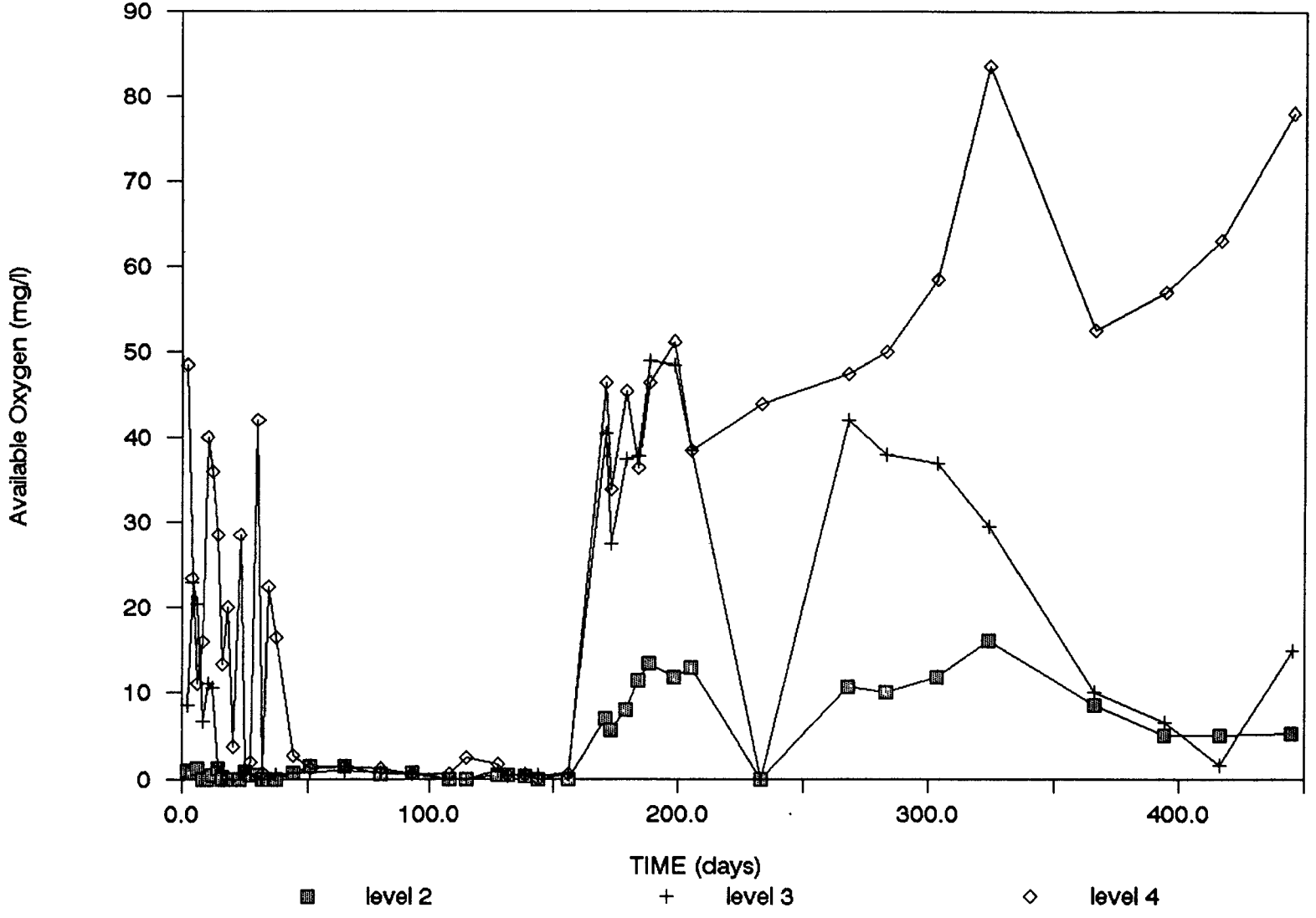


Fig. A.5 - Available Oxygen, Sampling Port 48A

Traverse City, Mich., Aviation Gas Spill

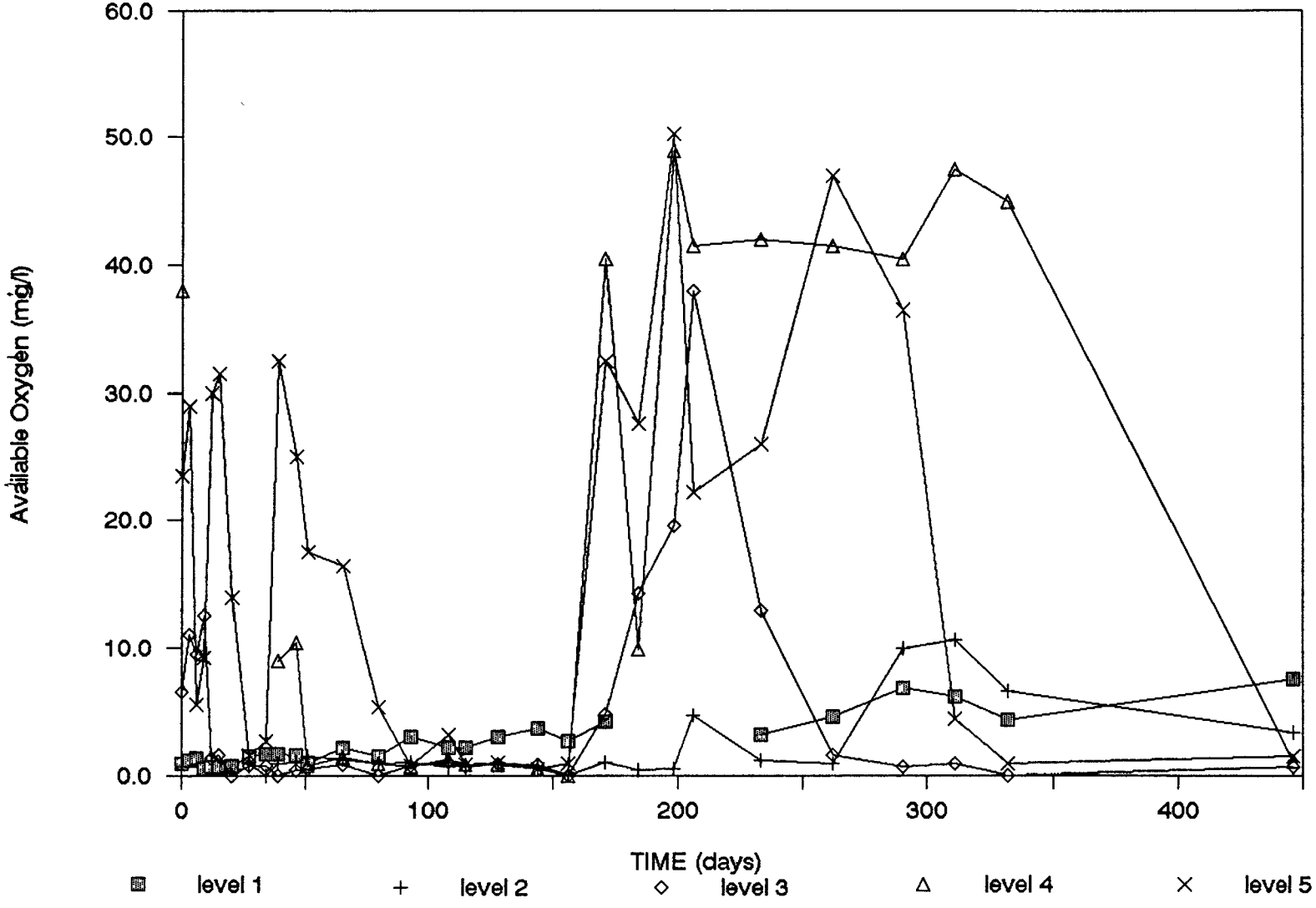


Fig. A.6 - Available Oxygen, Sampling Port 50B

Traverse City, Mich., Aviation Gas Spill

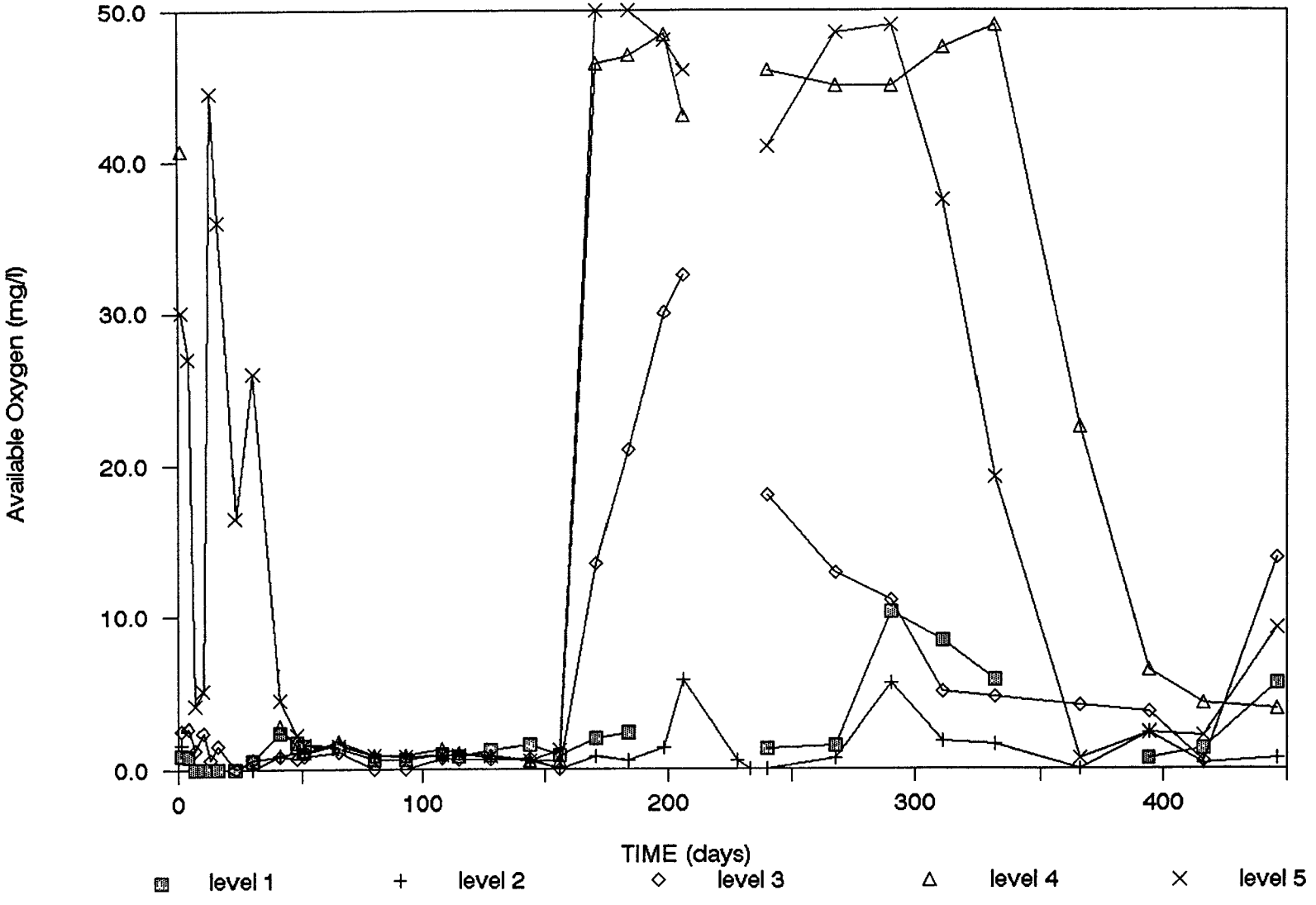


Fig. A.7 - Available Oxygen, Sampling Port 50C

Traverse City, Mich., Aviation Gas Spill

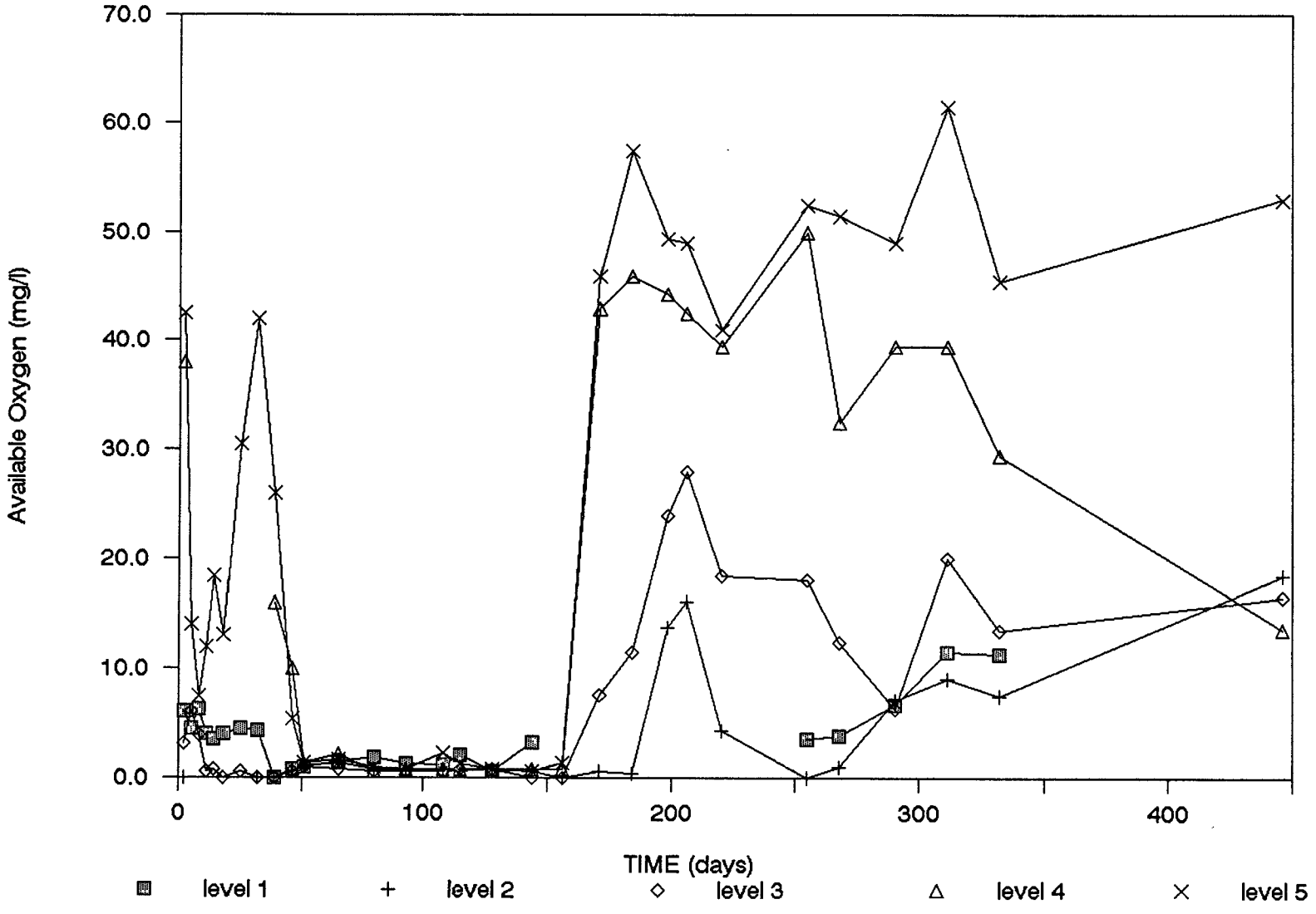


Fig. A.8 - Available Oxygen, Sampling Port 62

Traverse City, Mich., Aviation Gas Spill

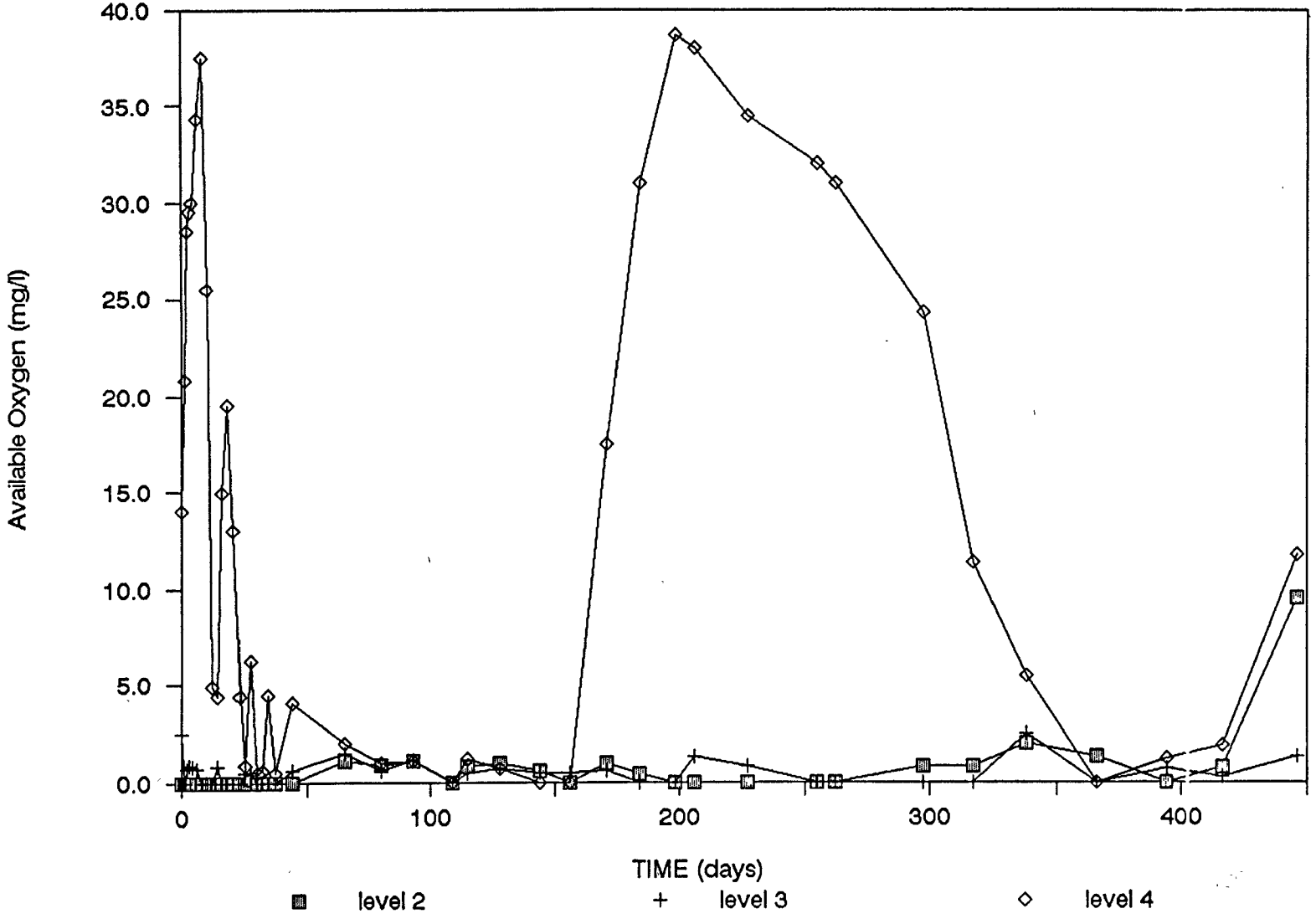


Fig. A.9 - Available Oxygen, Sampling Port 83A

Traverse City, Mich., Aviation Gas Spill

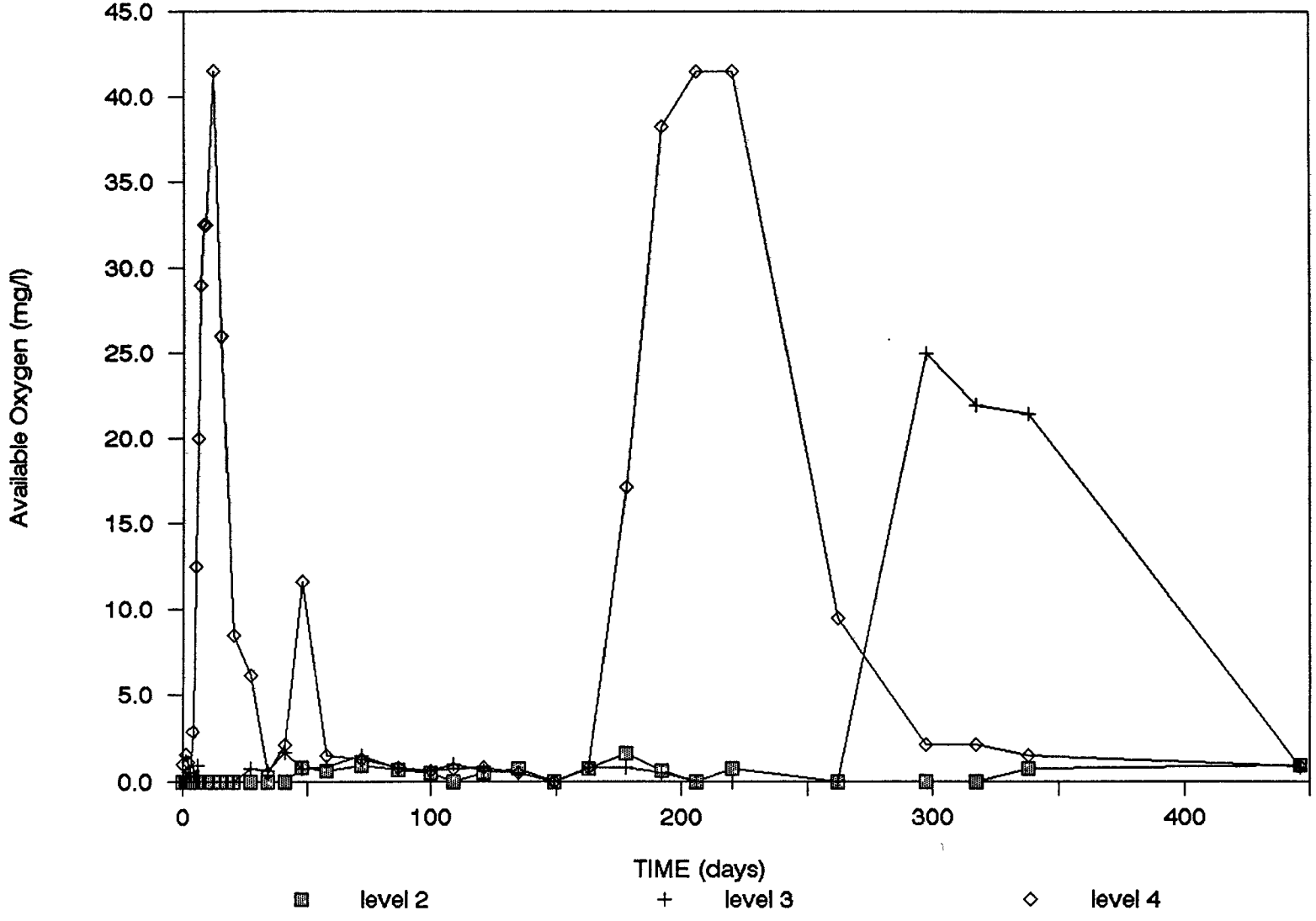


Fig. A.10 - Available Oxygen, Sampling Port 83B

Traverse City, Mich., Aviation Gas Spill

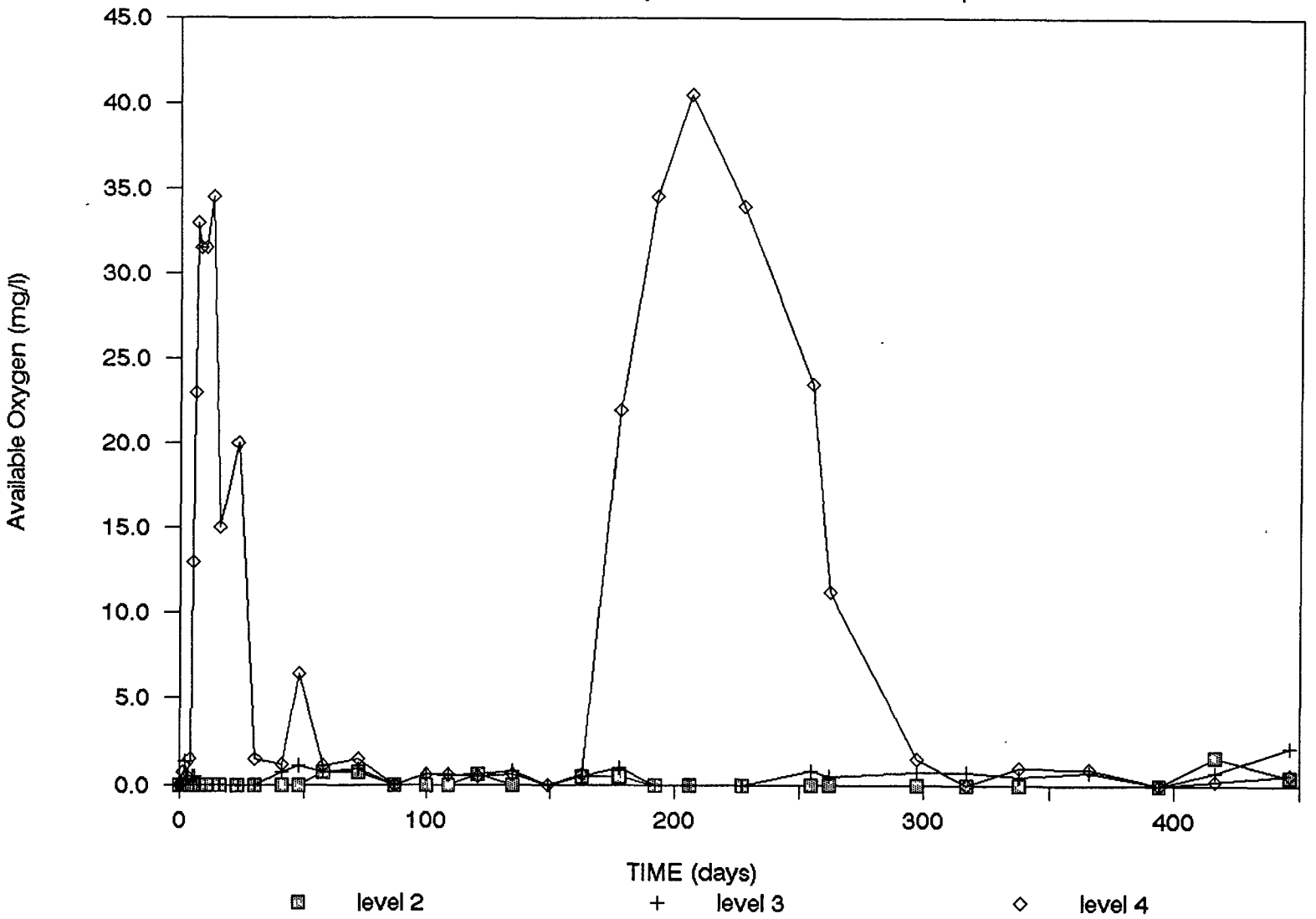


Fig. A.11 - Available Oxygen, Sampling Port 83C

Traverse City, Mich., Aviation Gas Spill

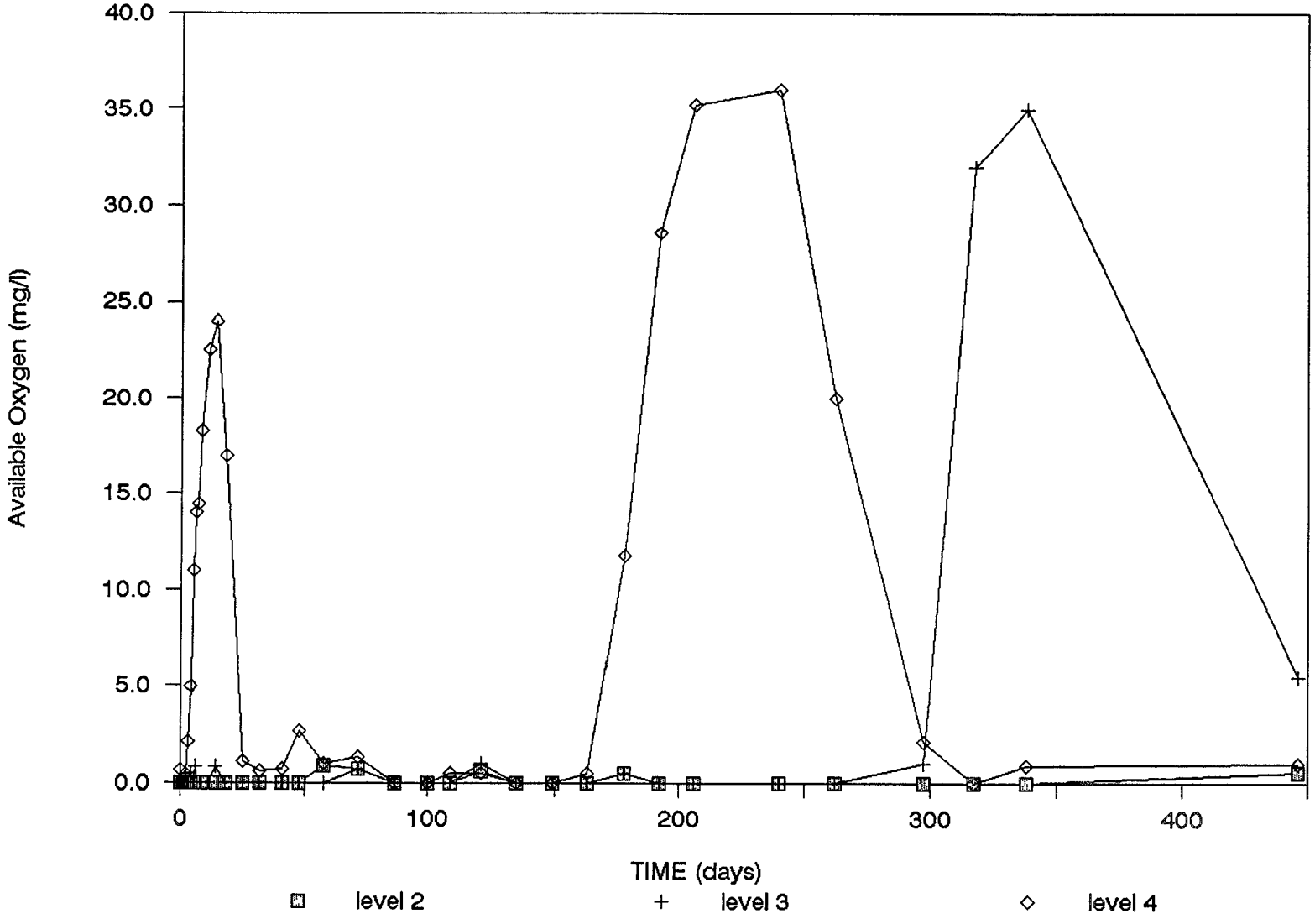
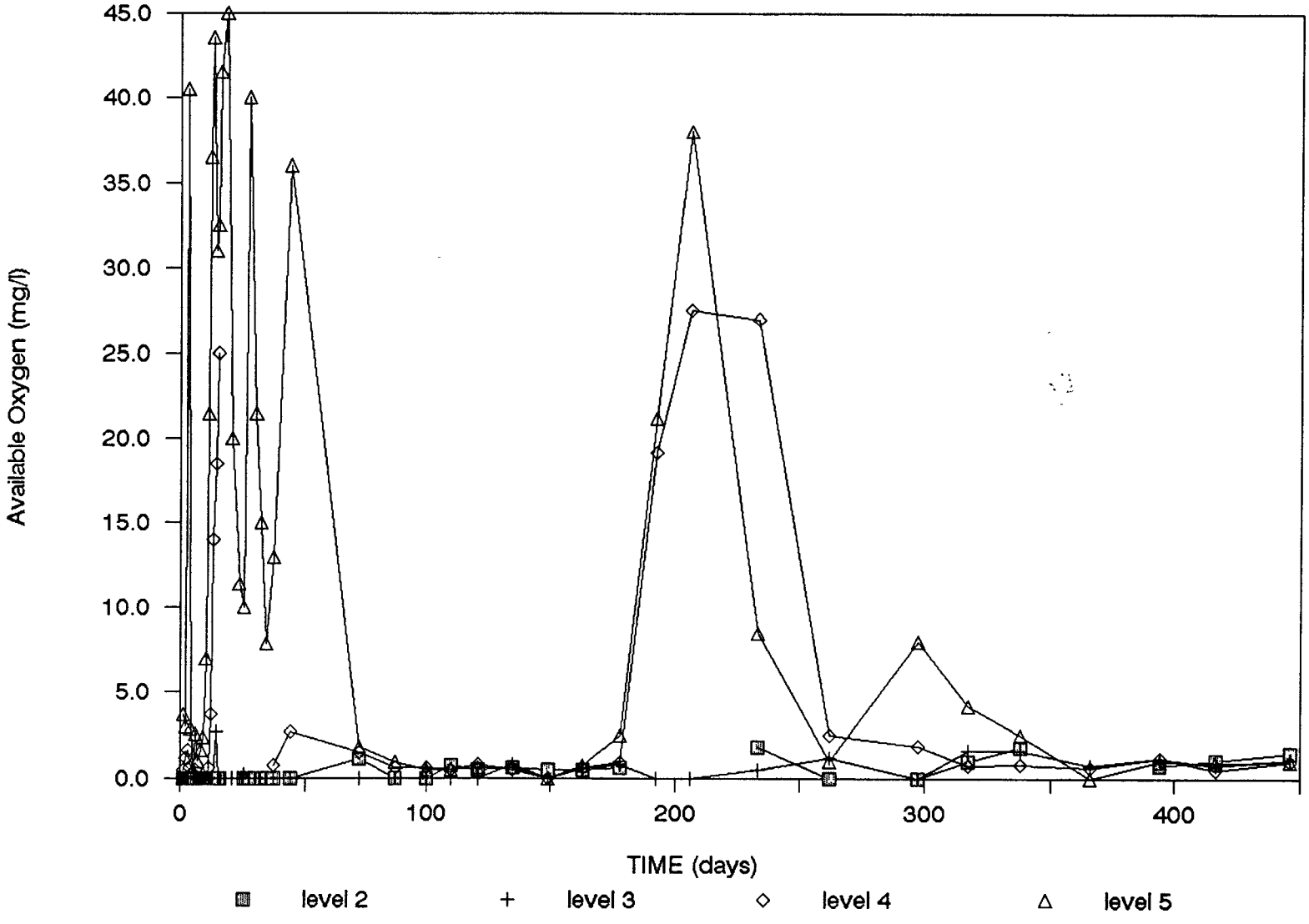


Fig. A.12 - Available Oxygen, Sampling Port 108

Traverse City, Mich., Aviation Gas Spill



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