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Deliverable summary

This deliverable summarizes first transport experiments and their analysis in soil and aquifer materials from the Emilia Romagna aquifer, Italy, as well as in local soil from the campus of the Weizmann Institute, Rehovot, Israel. Azithromycin (AZT), which has been reported to be found in other Italian aquifers, was tested first. Analysis of AZT sorption in batch experiments indicated strong sorption to both Weizmann and Bologna soils. Study of transport of AZT in sand and Weizmann soil columns demonstrated that under all reasonable environmental conditions, the AZT is not mobile in soil even after very long periods of flow. These studies were followed by investigation of two relatively mobile contaminants. One compound, considered very stable and mobile, is a complex of gadolinium (Gd-DTPA) that is used as a magnetic resonance imaging (MRI) contrasting agent. The second compound examined in initial experiments is carboplatin, an anticancer drug. Results from these initial experiments are being used as the foundation for further investigation and final deliverables (end of project) that are related to the study of transport, fate and risk assessment of these compounds in the environment, at the field scale.

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ANALYSIS OF SELECTED ECS AT FIELD SITES

1. Introduction

This deliverable summarizes first transport experiments and their analysis in soil and aquifer materials from the Emilia Romagna aquifer, Italy, as well as in local soil from the campus of the Weizmann Institute, Rehovot, Israel. Azithromycin (AZT), which has been reported to be found in other Italian aquifers, was tested first. Analysis of AZT sorption in batch experiments indicated strong sorption to both Weizmann and Bologna soils. Study of transport of AZT in sand and Weizmann soil columns demonstrated that under all reasonable environmental conditions, the AZT is not mobile in soil even after very long periods of flow.

These studies were followed by investigation of two relatively mobile contaminants. One compound, considered very stable and mobile, is a complex of gadolinium (Gd-DTPA) that is used as a magnetic resonance imaging (MRI) contrasting agent. The second compound examined in initial experiments is carboplatin, an anticancer drug. Results from these initial experiments are being used as the foundation for further investigation and final deliverables (end of project) that are related to the study of transport, fate and risk assessment of these compounds in the environment, at the field scale.

Abbreviations appearing in the text: AZT – azithromycin; **DDW** – double distilled water; **EC** – emerging contaminant; **MRI** – magnetic resonance imaging; **PV** – pore volume

2. Transport or retention of Azithromycin in sand and soils

2.1 Introduction

Azithromycin (AZT) is the first clinically developed azalide antibiotic, a subclass of macrolide antibiotics derived from erythromycin (Bright et al., 1988; Miguel and Barbas, 2003). Azithromycin differs chemically from erythromycin with a methyl-substituted nitrogen atom incorporated into the lactone ring. This 15-membered expanded lactone ring has improved acid stability compared to erythromycin (Miguel and Barbas, 2003; Zhang et al., 2009). Azithromycin also has a high bacteriostatic action in front of a wide spectrum of pathogenic bacteria and thus in recent years has become one of the most widely prescribed human antibiotics (Timoumi et al., 2014; Maier and Tjeerdema, 2018). Demand for azithromycin has also increased for use as a veterinary antibiotic for disease control and for promotion of animal growth (Tong et al., 2009).

AZT has been recognized as an emerging contaminant in recent years (EPA, 2009; Verlicchi et al., 2012). A large number of publications and reviews on different aspects of AZT, including its solubility and stability (Timoumi et al., 2014; Wang et al., 2014), assessment of fate (Gobel et al., 2007; Topp et al., 2016), and environmental risk (Verlicchi et al., 2012) have increased the concern/interest on this emerging contaminant. Antibiotics released into the environment have attracted special attention due to their serious impact on the ecosystem and major concern regarding emergence of drug-resistant bacteria.

Persistence of AZT has been reported worldwide in both fresh and seawater, as well as in sediments (Jones-Lepp et al., 2012; Maier and Tjeerdema, 2018; Mirzaei et al., 2018). AZT has been reported to be present, and is well studied, in wastewater treatment plant effluent (Gobel et al., 2005; Koch et al., 2005; Yasojima et al., 2006; Ghosh et al., 2009; Tong et al., 2009) and also in sewage sludge (Okuda et al., 2009). Mirzaei et al. (2018) reported significant differences in the concentrations of 13 different antibiotics including AZT, in two rivers receiving and not receiving treated wastewater originating from treatment plant effluents. Wastewater treatment plants (WWTPs) are recognized as one of the main sources of these pharmaceutically active compounds into both aquatic and terrestrial environments (Biel-Maeso et al., 2017). Land application of wastes from medicated animals or people, effluents from pharmaceutical manufacturing, and irrigation with reclaimed wastewater may also contribute significantly to environmental emissions of pharmaceutically active compounds such as antibiotics.

Once released into aquatic systems or after entering the soil system, AZT and all of its known derivatives generally display recalcitrant behavior. Maier and Tjeerdema (2018) reported a high affinity of AZT for soil based on sorption-desorption studies. Researchers have attempted different extraction methods for AZT from sediments/soils: for example, Okuda et al. (2009) reported pressurized liquid extraction with either water or methanol and ultrasonic solvent extraction using mixture of methanol-water; Biel-Maeso et al. (2017) reported pressurized hot water extraction (solid phase extraction).

To the best of our knowledge, the fate of AZT in soil is unclear and transport or mobilization of AZT in soil has not been successfully attempted or reported to date. This study reports the transport of AZT to determine its mobility under special environmental conditions.

2.2 Materials and methods

2.2.1 Column experiments for fully saturated conditions - This setup was applied mostly for experiments with soil from Weizmann campus, Israel. The soil was packed into vertical polycarbonate columns, and fully saturated with water by a peristaltic pump from the bottom of the column (Figure 1).

Chemicals - Azithromycin dihydrate ($C_{38}H_{72}N_2O_{12}\cdot 2H_2O$; molecular weight 785.0), potassium phosphate dibasic (K_2HPO_4), potassium phosphate monobasic (KH_2PO_4), ethylenediaminetetraacitic acid disodium salt dihydrate ($Na_4EDTA\ 2H_2O$) were purchased from Sigma-Aldrich. Organic solvents (ethanol and methanol), formic acid (98%) were purchased from Avantor J.T. Baker HPLC grade. Acetonitrile (HPLC grade) was purchased from Bio-Lab Ltd., Israel. Ammonia solution (25%) was purchased from Merck, Germany. Double-deionized water (DDW, 18.2 MΩ·cm) was used in all experiments.

Preparation and detection of Azithromycin - A stock solution of AZT (1000 mg L^{-1}) was prepared by dissolving azithromycin dihydrate salt in methanol. For working concentrations of AZT during experiments, stock solution was diluted with DDW. The concentration of AZT in solution was measured by LC-MS (Micromass Quattro micro API Mass Spectrometer) from Waters (TC) Israel Limited. All samples were passed through 0.22 μm PTFE filters before measurement with LC-MS. Azithromycin and their common derivatives are reported (Maier and Tjeerdema, 2018).

2.2.2 Experimental setup

Batch experiments - Batch experiments of AZT sorption to porous material were conducted as a benchmark for column experiments. Experiments were carried out in 40 mL glass vials with screw caps at a ratio of 2 g sand/soil in 10 mL AZT solution (1 mg L^{-1} concentration). Three AZT solution were checked. The first was DDW with addition of any substance; the second was EDTA solution that was based on report of Tong et al. (2009) that 0.75 mg mL^{-1} Na_4EDTA preventing antibiotics from forming complexes with metallic ions, while analyzing AZT in wastewater samples. The third solution contained phosphate buffer and was based on a report by Moreno et al. (2009) that in used 0.1 M phosphate buffer (pH 8.0) with aqueous solution of AZT for stability studies of AZT.

Column experiments - Quartz sand (~0.4-0.6 mm diameter), purchased from Unimin Corporation was used for packed bed column experiments. Being a relatively inert and homogeneous porous medium, sand was used as a benchmark experiment. Prior to use the sand was washed in 1% HCl, followed by repeated washing with DDW and dried at 105 °C. Sand was packed into vertical polycarbonate columns (20 cm × 3.2 cm), and saturated with background solution/DDW at the rate of 0.5 mL min^{-1} from bottom to top using a peristaltic pump. The saturation phase lasted for 10 pore volumes (PV). Following saturation, AZT suspended in background solution (1 mg L^{-1}) was pulsed into the saturated column at a constant rate of 1.0 mL min^{-1} from bottom to top for approximately 52 PV. The emerging effluent was collected into polypropylene tubes by a fraction collector. At the end of sample injection, the column continued to be pulsed with background solution for another 30 PV.

Extraction of AZM - For the extraction of AZT, 1 mL of water was added to soil/sand and vortexed for 10 s before the addition of 10 mL of methanol. Vials were capped, sonicated for 60 min at 40 °C and centrifuged for 10 min as described above. Concentration of AZT in the extract was analyzed with LC-MS. The procedure was repeated once more and the supernatants pooled. Recovery for AZT following this procedure was reported to be ~31% by Topp et al. (2016).

2.3 Results and discussion

2.3.1 Sorption of AZT on porous media - batch experiments

Sand, Weizmann Institute soil (characteristics reported by Goykhman et al., 2018 and referred in the text as soil 1) and aquifer material from Emilia Romagna (referred in the text as soil 2) were examined. The characteristics of Emilia Romagna aquifer material (as a mixture of four different samples) are shown in Table 1.

Table 1. Characteristics of Emilia Romagna aquifer material.

Properties		Methods	Italian Soil
Size	>50 μm	Hydrometer	99.98% ± 0.5%
	2-50 μm		0.015 ± 0.008% Silt
	< 2 μm		0.005 ± 0.001% Clay
pH		DDW	8.59 ± 0.05
		DDW+$CaCl_2$	8.05 ± 0.05
Porosity			
Organic matter (%)		Loss on ignition	0.6% ± 0.05%
CEC composition [meq /100 g]		Na^+	0.55
		Mg^{2+}	1.524
		K^+	0.67
		Ca^{2+}	8.62
Total CEC [meq / 100g]		Ammonium replacement	11.36
Soil carbonates (Qualitative)		3N HCl-effervescence	Highly calcareous
Soil carbonates (Quantitative) CO_3-C%		Gravimetric method	0.118 ± 0.02
Dissolved Organic Carbon [ppm]		UV at 274 nm	0.035 ± 0.005

Figure 1 shows the effect of the components in the background solution, i.e. DDW, Na_2EDTA and phosphate buffer on the retention of AZT in sand/soil matrices. Both the DDW and the Na_4EDTA were not found to stabilize AZM in solution, and remain attached to sand/soil matrixes. For the DDW and EDTA solutions, retention on the porous media was in all cases found to be ~100%. The 0.1 M phosphate buffer (pH 8.0) was found to stabilize AZT in solution, with only ~12% trapped in sand. However, AZT is retained/trapped by both types of soil tested even though both types of soil contain high percentages of sand in composition.

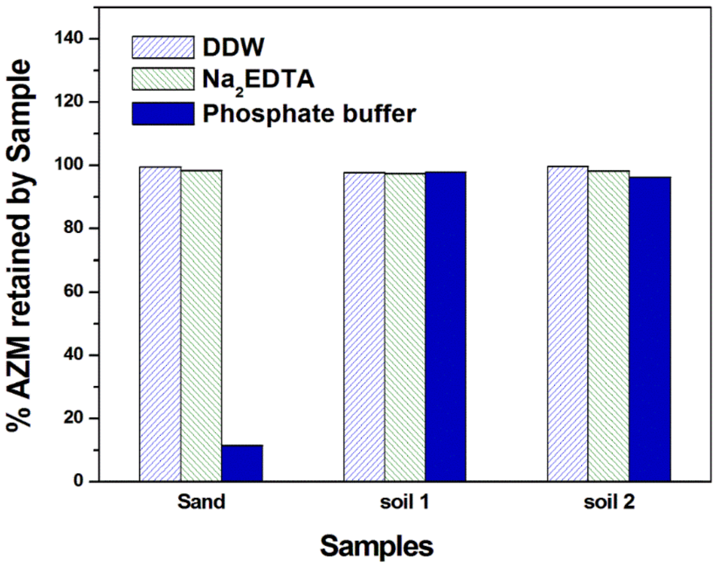


Figure 1. Retention of AZT by sand and soils as a function of the background solution.

2.3.2 Mobility of AZT in sand/soil

Azithromycin in aqueous solution was found to show low mobility in sand and in two types of soil. Transport of AZT was studied in sand columns. Fig. 2 shows the breakthrough curve for transport of AZT in sand with different composition of background solutions: DDW only, 0.75 mg mL^{-1} Na_4EDTA and 0.1 M phosphate buffer (pH 8.5). Results are shown as breakthrough curves in terms of relative concentration (C/C_0) as a function of pore volume (PV). In accord with the batch experiments, the presence of phosphate buffer (pH 8.5) was found to play a dominant role in mobilizing and transporting AZT in saturated sand columns, in comparison to aqueous solution of AZT and in the presence of Na_2EDTA as a chelating agent.

Figure 3 shows the retention profile of AZT at different depths along the sand column, after the transport experiment of AZT in a sand column with 0.1 M phosphate buffer (pH 8.5). The retention profile shows that the overall retention in the sand column was ~5% in of the total mass of AZT injected into the column. For the sand column with the phosphate buffer solution, a mass balance of > 90% was found; however for any realistic scenario, i.e., either transport in soil or environmental aqueous solutions (not buffered or with regulated pH), no transport is expected.

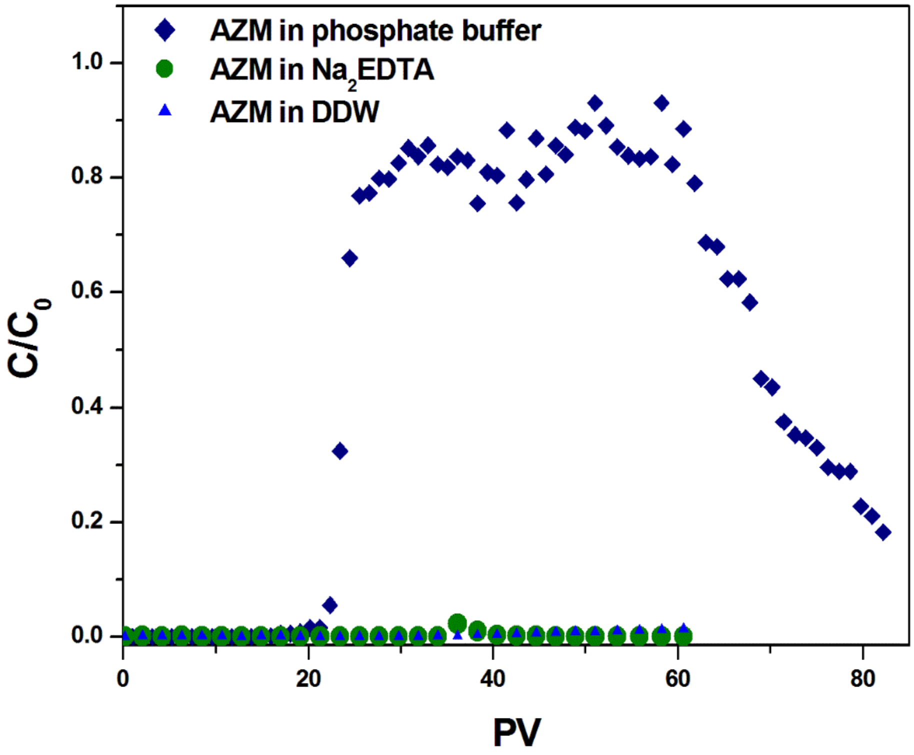


Figure 2. Transport of azithromycin (AZT) in sand column influenced by components of background solution: phosphate buffer pH 8.5; Na$_2$EDTA; DDW. Sample injection continued for ~52 PV followed by washing of the column with respective background solutions.

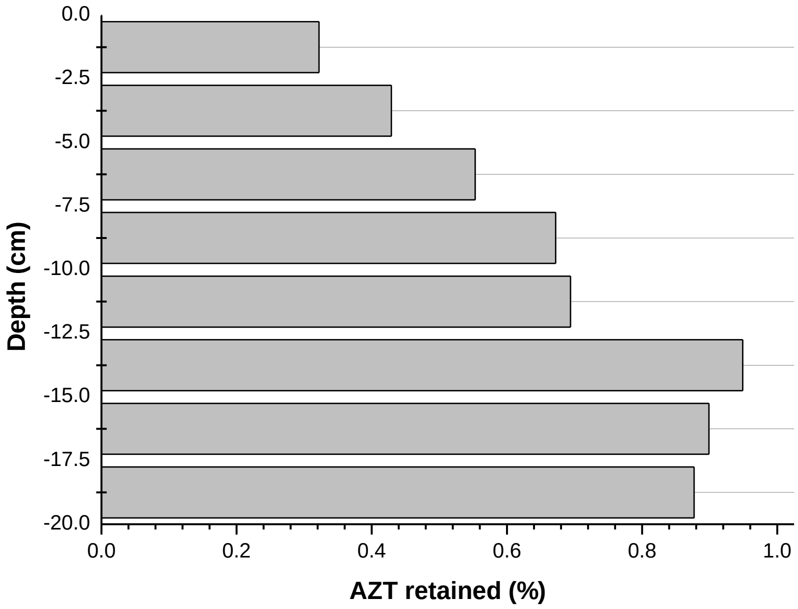


Figure 3. Retention profiles of azithromycin (AZT) at the end of column experiment, at different depths of sand column, reported as the % mass fraction retained.

2.4 Conclusion

The main findings of the present study are the high affinity of AZT for soil matrices, and lower mobility under most environmental conditions. Therefore, the detection of AZT in an aquifer is probably due to preferential flow or direct injection of contaminated water to the aquifer, and not through transport of recharge water in soil and aquifer material.

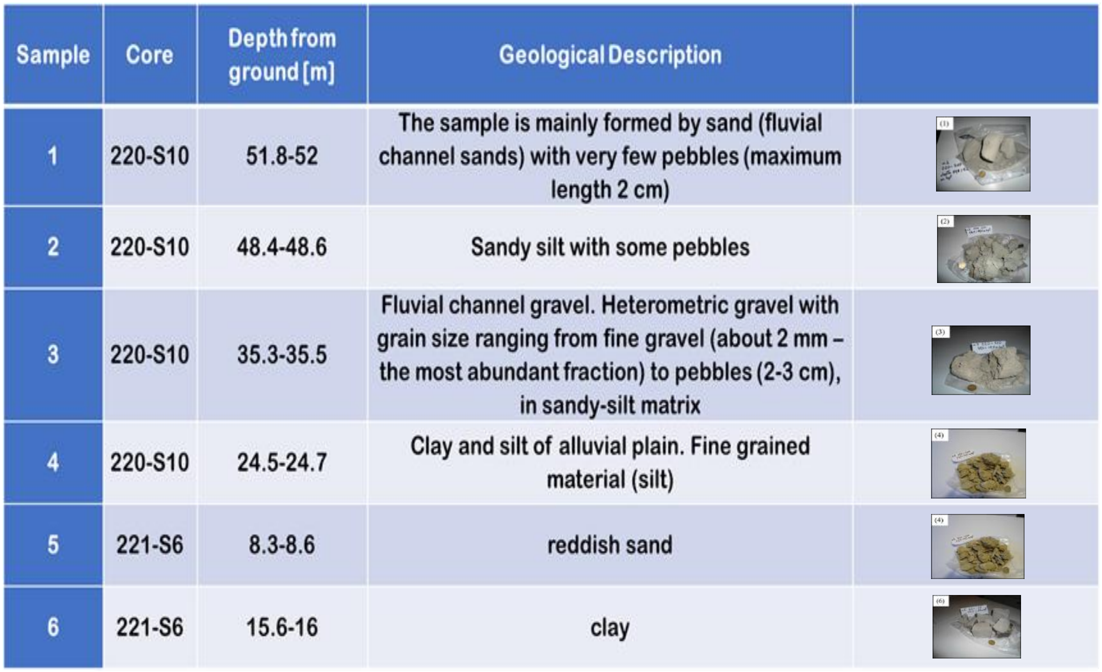
3. Transport of Gd-DTPA and Carbo-Pt in Italian soil

We received samples of aquifer material from Emilia Romagna aquifer (Bologna area) Italy. These samples are being used to test contaminant transport in real porous media from the Bologna aquifer, which is the overall goal of the project. However, the amount of aquifer material received is very limited and therefore a protocol was developed to test the transport of potential contaminants before using the samples from Italy.

The first step is testing the behavior of the contaminants in sand. Then we test the transport in Weizmann soil followed by running small-scale batch sorption isotherms. Only based on the results of these tests, and after discussion of the results with the other teams of the project, do we move forward to perform transport experiment with the Italian soil samples.

A description of the samples, and from what layer/depth they were taken, is given in Table 2. As can be seen, the samples are taken from different geological layers and have different properties. Because of the small amount and after discussion with the Italian team, samples 1-4 were combined to give a mixture that can be used for a set of several experiments that will allow comparison of the behavior of different contaminants in this soil.

Table 2. Characterization of aquifer porous media from Emilia Romagna, Italy.



Sample	Core	Depth from ground [m]	Geological Description	
1	220-S10	51.8-52	The sample is mainly formed by sand (fluvial channel sands) with very few pebbles (maximum length 2 cm)	
2	220-S10	48.4-48.6	Sandy silt with some pebbles	
3	220-S10	35.3-35.5	Fluvial channel gravel. Heterometric gravel with grain size ranging from fine gravel (about 2 mm – the most abundant fraction) to pebbles (2-3 cm), in sandy-silt matrix	
4	220-S10	24.5-24.7	Clay and silt of alluvial plain. Fine grained material (silt)	
5	221-S6	8.3-8.6	reddish sand	
6	221-S6	15.6-16	clay	

The first breakthrough experiments with the Emilia Romagna aquifer material are depicted in Fig. 4. Two relatively mobile contaminants were used: the first is a complex of gadolinium (Gd-DTPA) that is used as an MRI contrasting agent. The complex is considered very stable and mobile. The second compound that was used for the first experiments is carboplatin, an anticancer drug (for more details, see Goykhman et al. 2018). As can be seen in Figure 4, both compounds move through the soil quite quickly. The Gd complex shows tracer-like behavior with a classical breakthrough pattern. The carboplatin shows a similar pattern but clear retardation was observed. Both compounds elute completely from the soil column, indicating the potential of these compounds to move and spread in the aquifer.

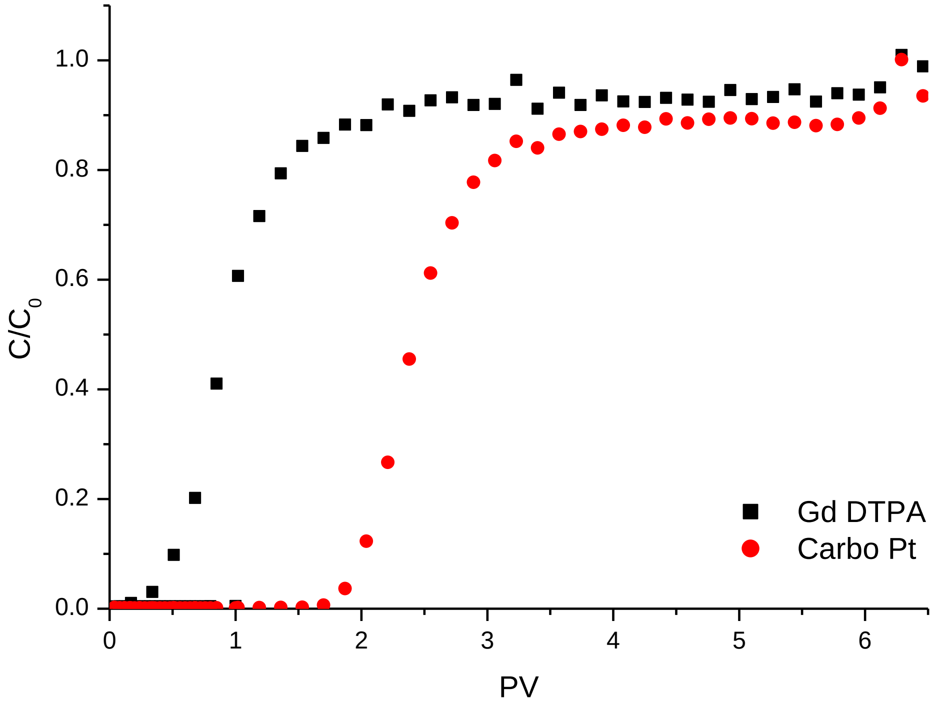


Figure 4. Transport of carboplatin, and Gd-DTPA in soil the Italian Soil (mixture of samples 1-4).

This part of the work is continuing and more results are expected in the last part of the project and final Deliverables.

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