

# RECLAMATION

*Managing Water in the West*

*Report DSO-05-04*

## Guidance on Sampling, Transportation, and Analysis of Materials



*Dam Safety Technology Development Program*



U.S. Department of the Interior  
Bureau of Reclamation  
Technical Service Center  
Denver, Colorado

December 2005

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**BUREAU OF RECLAMATION**  
**Technical Service Center, Denver, Colorado**  
**Materials Engineering and Research Laboratory, 86-68180**

**Report DSO-05-04**

**Guidance on Sampling, Transportation,  
and Analysis of Materials in Drains**

**Dam Safety Technology Development Program**  
**Denver, Colorado**

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## **Mission Statements**

The mission of the Department of the Interior is to protect and provide access to our Nation's natural and cultural heritage and honor our trust responsibilities to Indian Tribes and our commitments to island communities.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.



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# Introduction

The topic of monitoring seepage sediments in dams is a recurring one. There is no guidance on how to sample, transport, and analyze sediment samples from drains in any Bureau of Reclamation (Reclamation) document. Examples of sediments that may be present in a drain are soils, clay to sand size sediments, biological growths and films, and precipitates.

Investigators often need guidance to estimate costs and specify sampling procedures for dam safety monitoring programs. Without guidance, investigators may sample critical material and handle it inappropriately, which requires resampling and loss of time and resources. This brief guidance document provides advice on how to effectively use the Technical Service Center (TSC) to help you determine what is fouling a drain. This report includes a discussion of drains, inspections, materials, sampling, transportation, testing, costs, and TSC contacts.

This document should be used as a practical guide, as the title suggests. It should not be considered complete or a definitive dissertation on microbiology and sampling.

## Drains in Dams

There are several drain types in dams and appurtenant structures. For a comprehensive reference on drains, refer to the Reclamation publication *Drains for Dams and Associated Structures*, published in 2004 and *White Paper on the Impacts of Aging of Seepage Control/Collection System Components on Seepage Performance*, published in 2000 by McCook.

A few common drain types include:

- Concrete dams
  - formed drains
  - cracks
  - foundation drains
  - drilled vertical drains
  
- Embankment dams
  - chimney drains
  - downstream drainage blankets
  - toe drains

- drainage trenches
- drainage tunnels
- pressure relief wells
  
- Drainage systems for appurtenant structures
  - drilled foundation drains
  - underdrains in trenches
  - backfill at base of walls

## Inspection Activities

It is likely that deposited materials will be discovered in drains during operation and maintenance (O&M) inspections and activities. Dam inspections are conducted by the Dam Safety Office, the Technical Service Center, and regional and area offices as part of Comprehensive Facility Reviews (CFR) and Periodic Facility Reviews (PFR). Annual Facility Inspections are performed in years in which a CFR or PFR is not scheduled. Drain inspections are typically performed using closed circuit television (CCTV) equipment.

If deposited materials are present, they will be revealed during inspections or monitoring of the drain. The sampling is typically scheduled for a later date or routine O&M activities. Instructions to O&M personnel should be clear and concise. Figures 1 to 12 contain photographs submitted by Denise Hosler, 86-68220, and Chuck Cooper, 86-68130, showing examples of biofouling, biofilms, bacterial growths, mineralization, sediments, and vegetation found in drains during inspections.

## Material Found in Drains

### Sediments

Sediments may be transported in seepage and collected at outfalls and drain outlets. Sediments may be evidence of piping or internal erosion of a structure, which may have serious structural consequences. Evidence of erosion of dam or foundation materials surrounding a drain requires immediate attention. Continued erosion could result in partial or complete dam failure. If piping is suspected, a sample of the suspected eroding material should be collected by drilling or excavation.

Samples of any significant or unusual buildup of sediments in a drain should be petrographically examined to provide evidence for piping within the dam or



**Figure 1.**—The weep hole shows an example of sulfur (yellow) and phosphorous (white) related biofilm.



**Figure 2.**—The weep shows an example of a sulfate (black) related biofilm.



**Figure 3.**—The outlet shows an example of biofouling.



**Figure 4.**—The weep hole shows an example of sulfur (yellow) and phosphorous (white) related biofilm.

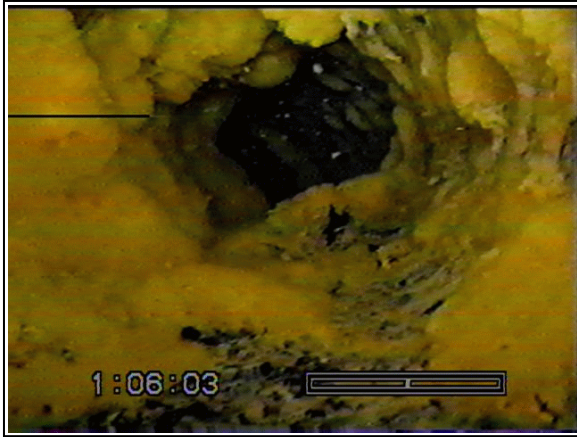


**Figure 5.**—The weir shows an example of biofouling.



**Figure 6.**—The bacterial growth is partially covering the inside of 8-inch diameter HDPE pipe.

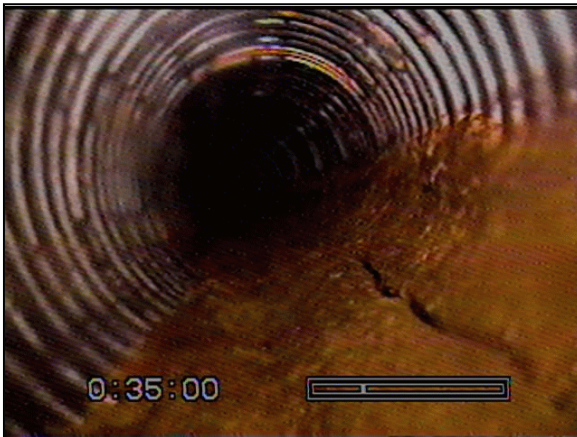




**Figure 7.**—Bacterial grow is shown completely covering the 8-inch diameter HDPE pipe.



**Figure 8.**—Bacterial grow is shown completely covering the 8-inch diameter HDPE pipe.



**Figure 9.**—Bacterial growth is shown covering the 8-inch diameter HDPE pipe invert.



**Figure 10.**—Calcium carbonate precipitate was observed covering the 18-inch pipe interior about 60 ft upstream of the outfall exit portal of a toe drain.



**Figure 11.**—Sediments were observed fouling the invert portion of a 12-inch diameter HDPE pipe.



**Figure 12.**—Vegetative growth shown fouling 8-inch diameter HDPE pipe.



foundation. This type of examination is most effective if accompanied by a companion sample of material adjacent to the drain for comparison.

## Mineralization and Encrustations

The accumulation of minerals deposited in a drain or the material surrounding a drain hinders water from exiting or being removed from a structure. An excellent discussion of mineralization and encrustation and groundwater constituents can be found in the *Ground Water Manual* (Reclamation, 1995) and Driscoll (1986).

## Biofilms and Drains

Biofilms are composed of populations or communities of microorganisms adhering to environmental surfaces. These microorganisms form slimelike mats, which bacteria adhere to, causing fouling. Biofilms may be found on essentially any environmental surface in which sufficient moisture is present. Their development is most rapid in low flowing systems where adequate nutrients are available, such as drains. The following sections discuss some biofilms that affect drains.

### Iron-Related Bacteria

A common bacterially rich organic slime observed in Reclamation structures is composed of iron-related bacteria. Iron-related bacteria films can be sticky and cause drain blockage. Any of the following symptoms suggest the presence of iron-related bacteria or other microflora:

- Orange, red, brown, and black colored slime
- Reduced water flow
- Slimy deposits blocking main lines and laterals
- Unpleasant odor in water
- Slimy, rusty deposits in water collection systems
- Severe staining on concrete surfaces
- Oil-like films on surface water
- White flocking, like finely shredded tissue paper, floating in water

Iron-related bacteria are a diverse group of microorganisms widely distributed in nature. They are found naturally in fresh and salt waters and in soils. Iron-related bacteria are nuisance microorganisms capable of transforming dissolved iron and manganese to an insoluble form that can cause severe fouling and plugging in pipes, plumbing, well pumps, treatment plants, and distribution systems.

They tend to grow much faster and in greater quantities when the temperature rises in a drain or when exposed to air. The result of the iron-related bacteria converting soluble iron from a soluble state ( $\text{Fe}^{2+}$ ), to the insoluble form ( $\text{Fe}^{3+}$ ) is referred to as “red water.” It is in this stage that iron and manganese become

deposited on the outside of the bacteria cell sheaths and the slimes they produce. The bacteria cell sheaths and slimes become encrusted with iron and manganese.

### **Sulfate-Reducing Bacteria**

Sulfate-reducing bacteria live in oxygen-deficient water. They reduce sulfur compounds, producing hydrogen sulfide gas in the process. Hydrogen sulfide gas is foul smelling and highly corrosive. Sulfur-reducing bacteria are more common than iron bacteria. The most obvious sign of a sulfur bacteria problem is the distinctive “rotten egg” odor of hydrogen sulfide gas. Sulfate-reducing bacteria occur in waters where oxygen is absent and sufficient dissolved organic materials are present (Cullimore, 1992)

Iron-related bacteria may coexist with sulfate-reducing bacteria. Iron-related bacteria and sulfate-reducing bacteria contamination are often difficult to tell apart because the symptoms are similar. Sulfur-reducing bacteria often live in complex symbiotic relationships with iron-related bacteria, so both types may be present.

### **Sulfur-Oxidizing Bacteria**

Sulfur-oxidizing bacteria require oxygen to grow and convert sulfides to sulfuric acid or hydrogen sulfide to sulfates. They can be colorless, purple, or green.

### **Algae**

Algae are small single or simple multicellular plant-like organisms that grow in the presence of light by photosynthesis. Algae occur in shallow wells or drains where there are adequate nutrients.

### **Heterotrophic Bacteria**

Heterotrophic bacteria are able to utilize organic materials as their principle source of energy and carbon for survival, growth, and synthesis.

## **Sampling**

During the course of an inspection, it may be determined that drains are plugged or deposition of material in outfall or seeps is reducing the effectiveness of drains or otherwise causing a problem. Good digital photographs can help the field, office, and laboratory personnel communicate.

Those who conduct drain maintenance should develop a sampling plan in consultation with project engineers and field personnel. A discussion of the problem and how to implement the plan will help define the critical issues before sampling, so that the proper personnel can make appropriate analyses. Often, the issue is: how much sample needs to be collected or under what conditions should

the sample be shipped to provide the analyst with what he or she needs to conduct an effective analyses.

Information provided with submitted samples should include a clear statement defining the problem and/or the information sought; names of Project, Area, Region, and/or Technical Service Center personnel familiar with the problem; sample location information including amount and location of the deposit; knowledge of the type of material previously taken from drains, if known; any other relevant data; and the required deadline for results.

Appropriate personnel in the Technical Service Center or your contract laboratory should be contacted with any questions concerning type, quantity, selection, preparation, and shipment of representative samples. Submitted samples should be representative of the material intended for analyses. The analyst should be able to provide a complete cost estimate for the recommended work to be performed.

Upon arrival of samples in the laboratory, the analyst will determine which tests are to be performed based on the purpose of the examination and previous communication with project personnel. Photographs of submitted samples will be provided upon request. Because more than one analysis may be performed on a sample, enough material for each procedure should be submitted.

## **Inorganic Material**

If the material contaminating the drain appears to be sediments (mineral and soil material), then no special precautions are usually needed regarding holding times.

Every effort should be made to obtain a representative sample, that is, a sample or group of samples selected to typify the larger population.

If laboratory identification of a precipitate is required, a representative sample, at least 1 teaspoon or 50 grams, should be sent to a qualified laboratory for examination. Typically, calcium carbonate deposits can be easily identified by application of a mixture of 3:1 distilled water to hydrochloric acid as described in Reclamation's *Engineering Geology Field Manual* (1991), page 43. Calcium carbonate is also easily and inexpensively determined by microscopic examination.

Water carrying suspended sediments can be sampled by taking a water sample. The sample should be a sufficient volume to allow at least 1 teaspoon or 50 grams of sediment to settle.

Excessive amounts or unusual materials in a drain may require a sample. Ensure that all particle sizes are represented by taking a sample large enough to ensure an adequate population of all particle sizes. If only a limited amount of material is

available, take everything. If abundant material is available, then an average sample can be assembled by taking a scoop from 30 different parts of the sample to yield a representative sample free of grouping and segregation error.

Reclamation's *Concrete Manual* (1992) appendix, ninth edition, designation 7, page 511, offers guidance on the amount of material required with respect to particle size. A typical sand sample, with particle sizes ranging from 0.074 to 4.75 mm, should weigh about 500 grams (1 pound) or equal about a pint of material. The weight or volume requirement increases with increasing particle size.

## Organic Material

If the material contaminating the drain is suspected to be organic, an analyst should be contacted in advance of field sampling to ensure the sample is properly handled and preserved so that it survives the trip to the laboratory undamaged. Usually, the analyst recommends that the organic material sample is placed in a clean container, transported in an insulated picnic cooler with sealed "blue ice" containers, and shipped to the laboratory as soon as possible to reduce the holding time. Shipping organic material is like transporting fresh lettuce a long distance. Planning needs to be done to ensure the lettuce arrives in good condition. Planning should include making sure a qualified analyst is on duty to accept the shipment. About 500 mL (1 pint) of fresh material is required for a positive identification. Denise Hosler and Fred Nibling, 86-68220, are the TSC's current analysts and contacts for biological identification.

Denise Hosler, 86-68220, has described an aseptic technique for sampling water:

Have latex gloves and isopropyl alcohol on hand. After putting on gloves, wash hands and sample bottle and top with some alcohol. Open the sample bottle as close to the sampling location as possible taking care not to contaminate the bottle top by facing the cap in the bottom up position or leaving the cap off for excessive time, to reduce the chance of airborne bacterial contamination. Triple-rinse the bottle with sample water then fill the bottle with sample water and cap. Label each bottle with the sample location and place in cooler. If requested in advance, 86-68220 will prepare a cooler with sample bottles and send it to the collection site.

Water samples should be collected in clean, 500 mL nalgene bottles using aseptic techniques and placed in an iced cooler and shipped immediately to the laboratory. Collect the samples early in the work week so the water can be cultured upon arrival.

Biofilms and slimes should also be collected in clean, nalgene bottles or stout plastic bags using aseptic techniques and placed in an iced cooler

and shipped immediately to the laboratory. Collect the samples early in the work week so the water can be microscopically examined upon arrival.

## **Transportation**

### **Transporting Inorganic Materials to the Laboratory**

Ship the samples by any reasonable means in a competent container directly to the Bureau of Reclamation Materials Engineering Research Laboratory Petrographic Laboratory or your contract laboratory:

Materials Engineering Research Laboratory (call for current laboratory location)  
Mail code 86-68180  
Denver Federal Center  
6th and Kipling  
Denver CO 80225  
(303) 445-2374

### **Transporting Organic Materials to the Laboratory**

As soon as possible, store the labeled samples in a picnic cooler. Use sealed “blue ice” cartridges to chill the cooler and samples. Ship the samples OVERNIGHT EXPRESS directly to the Bureau of Reclamation Ecological Research and Investigations Group or your contract laboratory. It is necessary to contact the analyst prior to sampling and shipment to ensure the sample is properly received in the laboratory.

Ecological Research and Investigations Group (call for current laboratory location)  
Mail code 86-68220  
Denver Federal Center  
6th and Kipling  
Denver CO 80225  
(303) 445-2200

# Testing of Materials

## Inorganic Materials

Soil and soil-like materials from drains are petrographically examined to determine mineralogical composition, organic fraction, and origin usually for documentation purposes. Soil and soil-like material in a drain is analyzed to identify the mineralogical composition and to detect the presence of minerals and rock types that determine origin, occurrence, and history of the sample. If a sample of the construction or foundation material surrounding the drain is submitted for examination, the samples can be compared for common mineralogical composition.

The petrographic examination of soils generally includes a description of the submitted sample and a determination of the mineralogical composition and estimated volume percentages.

The soil and soil-like material analysis results can be applied to the material in the field only to the extent that the submitted sample represents that material.

## Organic Materials

Two approaches are generally considered. One is to sample the water that has passed over the biofilm using aseptic techniques to reduce sample contamination (Denise Hosler, personal communication). The second technique is to remove some of the slime or biofilm from the original site for microscopic examination.

The Ecological Research and Investigations Group (ERIG) laboratory performs bacterial activity reaction tests and light microscope examinations. The ERIG laboratory performs analytical testing for water, solid samples, and hazardous wastes, research and special studies to solve environmental, operation and maintenance, and engineering problems.

## Examples

The appendix contains two memoranda of laboratory examinations of inorganic and organic materials as an example of the TSC work. Both technical memoranda were regarding material in a vault at Wasco Dam, Oregon. The first is an example of inorganic petrographic analysis of sediments and provides mineralogical composition and loss on ignition test results, which indicates a significant organic content. The second is an example describing bacterial and

light microscope results of filamentous bacteria. Both examples represent the quality of service provided by the TSC.

## Costs

The website <http://www.usbr.gov/tsc> provides information regarding engineering services provided by the Technical Service Center as well as information on personnel, business practices, and billing rates. Please call for an estimate and personal service. The Client and Support Services Office (86-68010) can facilitate the work and any financial arrangements.

## 2006 TSC Laboratory Contacts

Materials Engineering Research Laboratory  
Bureau of Reclamation  
PO Box 25007, 86-68180  
Denver CO 80225-0007  
Group Manager Bill Kepler  
(303) 445-2386  
(303) 445-6341 FAX  
Key personnel: Doug Hurcomb (ext. 2336)

Ecological Research and Investigations Group  
Bureau of Reclamation  
PO Box 25007, 86-68220  
Denver CO 80225-0007  
Group Manager G. Chris Holdren  
(303) 445-2200  
(303) 445-6328 FAX  
Key personnel: Denise Hosler (ext. 2195) and Fred Nibling (ext. 2202)

Client and Support Services Office  
PO Box 25007, 86-68010  
Denver CO 80225-0007  
Manager Christi Young  
(303) 445-2561  
(720) 544-0507 FAX

## References

Bureau of Reclamation, *Engineering Geology Field Manual*, Second Edition, Volume 1, 1991.

Bureau of Reclamation, *Concrete Manual*, Ninth Edition, 1992.

Bureau of Reclamation, *Ground Water Manual*, Second Edition, U.S. Government Printing Office, 1995.

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Driscoll, F.G, *Groundwater and Wells*, Second Edition, Johnson Division, St. Paul, Minnesota, 1986.

Ford H.W., *Iron Ochre and Related Sludge Deposits in Subsurface Drain Lines*, Circular 671, Florida Cooperative Extension Service, 1993.  
[http://edis.ifas.ufl.edu/BODY\\_AE026](http://edis.ifas.ufl.edu/BODY_AE026)

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Lennox, J.E., *A Manual of Biofilm related exercises, A Biofilm Primer*  
<http://www.personal.psu.edu/faculty/j/e/jel5/biofilms/primer.html>

McCook, D.K., *White Paper on the Impacts of Aging of Seepage Control/Collection System Components on Seepage Performance*, 2000. ASDSO/FEMA

University of Alaska Fairbanks, Water Treatment Plant, Iron Bacteria  
<http://www.uaf.edu/fs/water/ironbact.html>



# Appendix

## **Drain samples submitted for examination and testing**

Earth Sciences and Research Laboratory Referral No. 8340-03-17: “Sediment Sample Physical Properties and Petrographic Examination Results—Wasco Dam, Oregon,” with selected figures

Ecological Research and Investigations Group Memorandum dated September 2, 2003, “Report of Bacterial and Chemical Testing of the Vault at Wasco Dam,” with selected attachments



D-8340  
PRJ-13.00

## MEMORANDUM

To: Manager, Geotechnical Engineering Group 2, D-8312  
Attention: J. Gagliardi

From: Doug Hurcomb  
Geologist, Earth Sciences and Research Laboratory Group

Subject: Sediment Sample Physical Properties and Petrographic Examination Results - Wasco Dam Sediment Monitoring – Wasco Dam, Juniper Division, Wapinitia Project, Oregon

Earth Sciences and Research Laboratory Referral No. 8340-03-17

Petrographic referral code: 03-05

## INTRODUCTION

One sediment sample from the vault box left of outlet works, Wasco Dam, Oregon, was submitted to the Earth Sciences and Research Laboratory (ESRL) by J. Gagliardi, Geotechnical Engineering Group 2, for examination. The sediment was sampled in July 2002 during an annual examination of Wasco Dam after some earthquake activity near the dam-site. The material was deposited by the drain system and formed a cone of sediments. The dam safety office project plan document dated February 13, 2003, referred to “algae and iron bacteria” clouding the water in the vault. The sample was submitted to the ESRL in February 2003 and assigned the laboratory index No. 19S-73.

Physical properties tests and the petrographic examination were conducted in the ESRL.

The purpose of the petrographic examination was to determine the mineralogical composition of the sediment. Physical properties and loss on ignition (LOI) testing were performed on the sample. Advice on biological identification was requested.

## PHYSICAL PROPERTIES TEST RESULTS

The physical properties tests were performed following procedures described in: USBR 5330, Performing Gradation Analysis of Fines and Sand Size Fraction of Soils, including Hydrometer Analysis; USBR 5350, Determining the Liquid Limit of Soils by the One-Point Method; USBR 5360, Determining the Plastic Limit and Plasticity Index of Soils; and USBR 5430, Determining Moisture, Ash, and Organic Content of Soils.

Sediment sample 19S-73 was determined to be Silty Sand (SM) with no plasticity. Figures 1 and 2 contain the particle size and grain size distribution test reports and selected physical properties (test results not included here).

### PETROGRAPHIC EXAMINATION AND RESULTS

The submitted sediment was examined megascopically, microscopically, by X-ray diffraction and by some qualitative physical and chemical tests. The percent organic content of the sediment was determined by loss on ignition (LOI).

The as-received sediment sample 19S-73 was wet and grayish brown. When air dried the material was loose and sandy and forms friable aggregates. Sediments consist of silt and sand size particles up to about 1.5 mm in diameter. The angular to chiefly subrounded sand size particles were composed of glassy volcanic particles, altered volcanic particles, kaolin(?), feldspar, magnetite, and unidentified altered crystals with miscellaneous minerals in the finer sizes (figure 3). Examination of the unwashed silt size particles revealed high amounts of amorphous volcanic glass.

X-ray diffraction analysis results revealed kaolinite, feldspar, and tridymite with minor hematite and trace quartz.

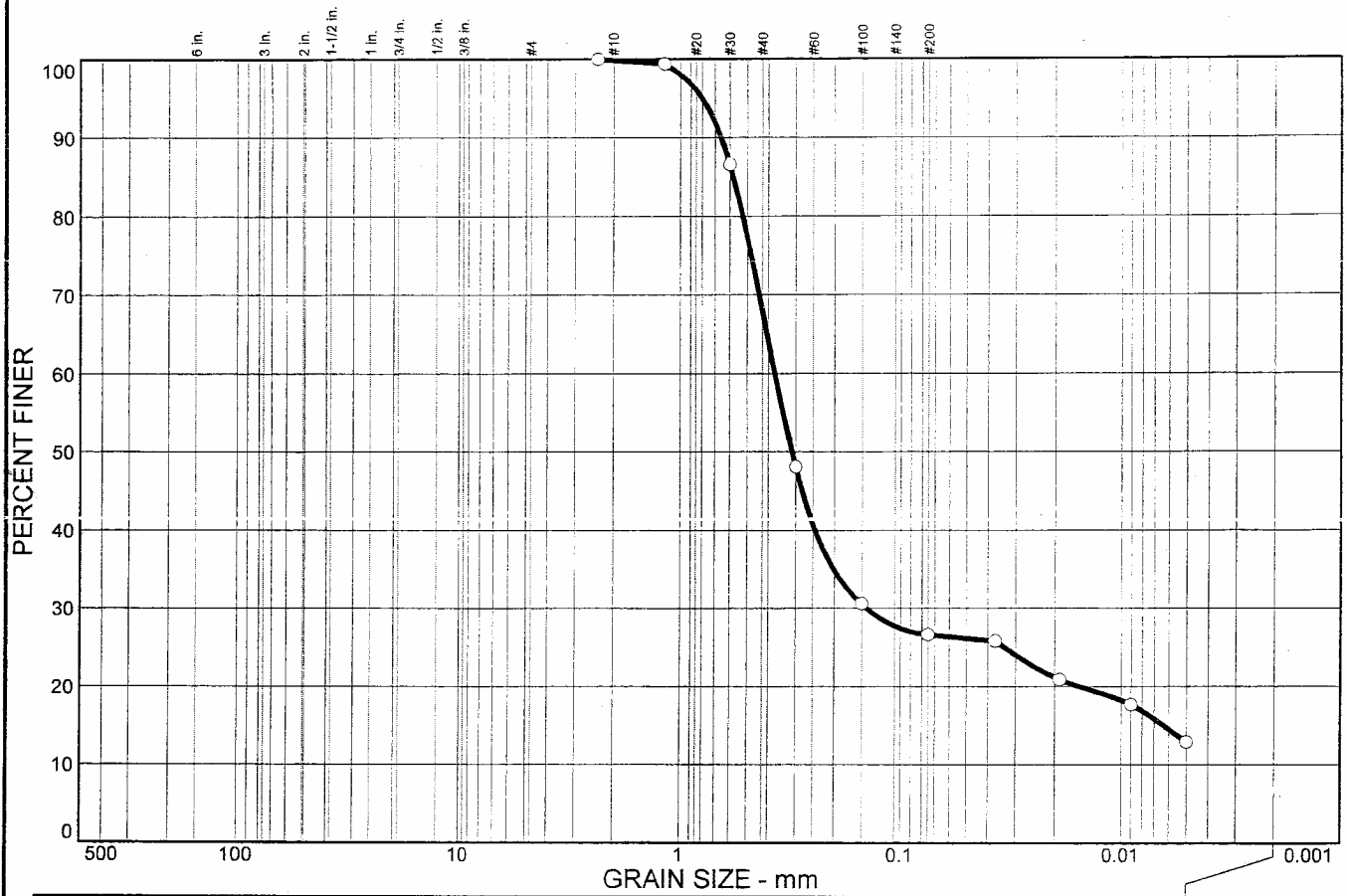
LOI was determined in the petrographic laboratory using a method similar to USBR 5430, Determining Moisture, Ash, and Organic Content of Soils. About 50 gm of air dried sediment was held at 400 degrees C for 8 hours. The percent organic content was determined to be 8.0 percent.

### BIOLOGICAL IDENTIFICATION

Biological identification of any organic materials in the submitted sample cannot be performed due to the condition of the sample. If a biological identification is desired, the Ecological Research and Investigations Group, D-8220, may be able to provide assistance. Contact Denise Hosler (303 445-2195) or Fred Nibling (303 445-2202) for more information.

Attachments

# PARTICLE SIZE DISTRIBUTION TEST REPORT



% COBBLES	% GRAVEL		% SAND			% FINES	
	CRS.	FINE	CRS.	MEDIUM	FINE	SILT	CLAY
0.0	0.0	0.0	0.1	31.9	41.4	26.6	

SIEVE SIZE	PERCENT FINER	SPEC.* PERCENT	PASS? (X=NO)
#8	100.0		
#16	99.4		
#30	86.6		
#50	48.1		
#100	30.6		
#200	26.6		

**Soil Description**

Silty sand

**Atterberg Limits**

PL= NP      LL= NL      PI= none

**Coefficients**

D<sub>85</sub>= 0.578      D<sub>60</sub>= 0.373      D<sub>50</sub>= 0.312  
D<sub>30</sub>= 0.142      D<sub>15</sub>= 0.0063      D<sub>10</sub>=  
C<sub>u</sub>=

**Classification**

USCS= SM      AASHTO= A-2-4(0)

**Remarks**

Moisture content = 46.9%

\* (no specification provided)

Sample No.: 73      Source of Sample:  
Location: vault box, left of outlet works

Date: 03/13/03  
Elev./Depth: sediment

**BUREAU  
OF  
RECLAMATION**

Project: Wapinitia Project  
Feature: Wasco Dam  
Project No: 19S

Figure 1

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GRAIN SIZE DISTRIBUTION TEST DATA

---

Project: Wapinitia Project  
Feature: Wasco Dam  
Project Number: 19S

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Sample Data

---

Source:

Sample No.: 73

Elev. or Depth: sediment

Sample Length (in./cm.):

Location: vault box, left of outlet works

Description: Silty sand

Date: 03/13/03

PL: NP

LL: NL

PI: none

JSCS Classification: SM

AASHTO Classification: A-2-4(0)

Testing Remarks: Moisture content = 46.9%

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Mechanical Analysis Data

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Sieve	Size, mm	Percent finer
# 8	2.360	100.0
# 16	1.180	99.4
# 30	0.600	86.6
# 50	0.300	48.1
# 100	0.150	30.6
# 200	0.075	26.6

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Hydrometer Analysis Data

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Size, mm	Percent finer
0.0370	25.8
0.0190	20.9
0.0090	17.7
0.0050	12.9

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Fractional Components

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Gravel/Sand based on #4

Sand/Fines based on #200

% COBBLES =                    % GRAVEL =

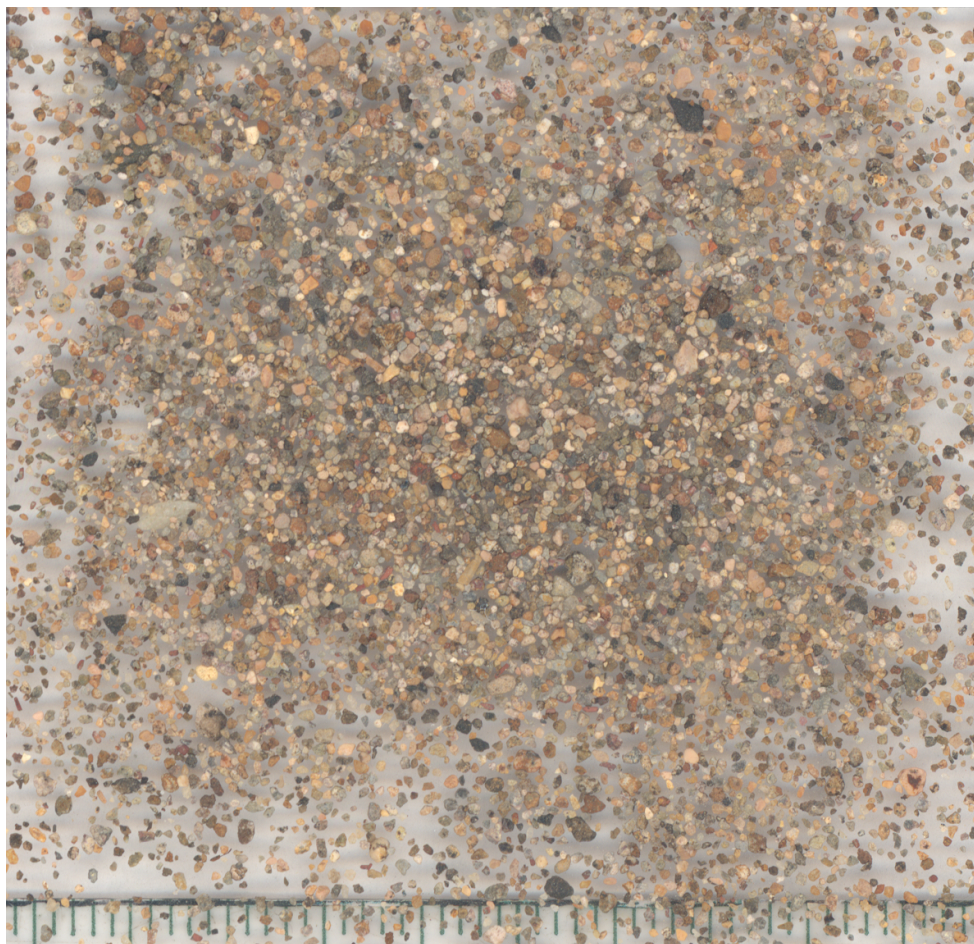
% SAND = 73.4 (% coarse = 0.1      % medium = 31.9      % fine = 41.4)

% FINES = 26.6

D85= 0.58    D60= 0.37    D50= 0.31

D30= 0.14    D15= 0.01

Figure 2



**Figure 3. Washed Sand Size Sediments from ESRL Test Procedure USBR 5330.** The scanned image provides visual documentation of the coarser sediments in sample 19S-73 for future reference. Note the millimeter scale.







# United States Department of the Interior

BUREAU OF RECLAMATION  
PO Box 25007  
Denver, Colorado 80225-0007

IN REPLY REFER TO:

D-8220  
RES-1.10

## MEMORANDUM

Date: September 2, 2003

To: Jack Gagliardi,  
Geotechnical Engineering Group 2

From: Denise Hosler,  
Ecological Research and Investigations Group

Subject: Report of Bacterial and Chemical Testing of the vault at Wasco Dam

On June 9, 2003 water and sediment samples were collected from the sediment vault that collects the flow from the left abutment toe drain. The samples arrived cool and in good condition. There were two 1-liter cubitainers of water collected at the top of the vault and 4 baggies of sediment collected from the sediment trap at the bottom of the vault. Per client request, three of the four sediment samples were composited and tested for metal and bacterial content. The fourth was sent for asbestos analysis, since the pipe at Wasco Dam contains asbestos.

Bacterial tests were begun on June 11, 2003 and conducted using Biological Activity Reaction Tests (BART) and light microscopy. The results of the microbiology, asbestos, and chemical analyses performed are attached on the following pages. There were no asbestos fibers found in the sediment, and the chemistry was consistent with the microbiological testing. The bacterial activity is occurring in the sediment and/or trap at the bottom of the vault, in part, due to the flow rates through the vault.

As we discussed, this effort was in response to an earthquake event that occurred in June of 2002 and caused considerable clouding of the water. At the time of this sampling event, the vault water was reported to be clear. These data may be used to reflect current conditions, and in the event of any seismic event, a repeat sampling event is recommended. In light of the current data results, I believe that the cloudy water observed in June 2002, was most likely the result of abiotic sediments released into a relatively high flow system after the earthquake event.

I thank you for the opportunity to participate on this project, and if you have any questions regarding this report, please contact me at 303-445-2195.

Sincerely,

Denise M. Hosler  
Ecological Research and Investigations

Attachments

**Cultural Biological Activity Reaction Test (BART) methods:** BART methods (heterotrophic culturing) and their application are explained in the report appendix. Briefly, BART tubes contain a dehydrated selective or differential culture medium selected for the microbial group of interest (iron-related bacteria, etc.), and a plastic ball in a 15-mL tube. Adding sample water hydrates the medium, and a redox gradient forms between the ball and the medium in the bottom. Interpretation is based on observation of the medium appearance and time it takes for a reaction to occur.

BART cultures demonstrated positive results for Iron Related Bacteria (IRB), Slime Forming Bacteria (SLYM), and to a lesser extent Sulfate Reducing Bacteria (SRB). The bacterial growth reaction is noted in Table 1, the number of days until the appearance of bacterial growth listed in Table 2. Based upon the days until appearance or days of delay before cultures appear, the populations or colony forming units per milliliter may be estimated using Table 3.

**Table 1. BART Reactions**

Sample Location	IRB = Iron Related Bacteria	SRB = Sulfate-reducing Bacteria	SLYM = Slime-forming Bacteria	ALGAE
Vault Water	GC, BR	CL	CL, DS, SR, PB	0
Vault Water (Duplicate)	GC, BR	CL	CL, DS, SR, PB	0
Trap Water	BC, BG, FO, BR	CL, BB, BA	DS, SR, BL	OB

- IRB: GC = Pseudomonads, BC, BR, BG = positive for Iron related bacteria, FO = anaerobic bacteria.
- SRB: CL = anaerobic bacteria, BB = Dense slime and sulfate-reducing consortium, BA = complex bacterial consortium with sulfate-reducing bacteria present.
- SLYM: CL = Slime Forming bacteria, DS, SR = Dense slime-forming bacteria, PB = *Fluorescing Pseudomonads*, BL = *Pseudomonads* and enterics.
- ALGAE: OB = Positive for *Diatoms and Desmids*

The vault water was sampled from the top of the four-foot deep water and the trap water was the water drained from the sediment material taken from the sediment trap located at bottom of the vault.

### Light Microscopy

Slides prepared with the filamentous bacteria found in the IRB BARTs were colorless lacking Mn or Fe staining and had abundant, irregularly separated small cells in sheaths lacking partitions. These are probably *Thiothrix* and also some iron-stained and incrustated bacteria noted in the vault sediment were probably *Leptothrix*.

**Table 2. BART Reactions**

The BART reaction time is classified as 'aggressive' if bacterial growth occurs within two to four days, 'moderate' if bacterial growth occurs between six to eight days, and non-aggressive for reaction times greater than eight days. The classification for Algae is longer due to the slower growth rates. Algal growth may be considered aggressive up to sixteen days and is considered non-aggressive if it appears after twenty-five days. All types of bacteria exhibited more aggressive growth and variety in the trap water.

Sample Location	BART Type and Days to Reaction**						
	IRB 1*	IRB 2	SRB 1*	SRB 2	SLYM 1	SLYM 2	ALGAE
Vault Water	3	5	0	0	3	5	0
Vault Water (Duplicate)	3	5	0	0	3	5	0
Trap Water	1	4	3	4	3	0	1

- IRB 1 = the first iron related (IRB) reaction, positive for Iron related and
- IRB 2 = the second observed, *Pseudomonads*.
- SRB 1 = the first sulfate-reducing bacteria observed, SRB consortium
- SRB 2 = sulfate-reducing bacteria as anaerobic bacteria.
- SLYM 1 = slime-forming bacteria.
- SLYM 2 = *Fluorescing pseudomonads*.
- ALGAE = Positive for *Diatoms and Desmids*

\*\* These are days until a reaction occurred (days of delay or time lag) after inoculation. A '0' indicates no reaction.

**Table 3. Relationship between DD and log CFU/ml for BART used**

Time lag (DD)	IRB	SRB	SLYM
0.5	6.6	6.6	6.8
1.0	6.0	6.0	6.6
1.5	5.8	5.8	5.8
2.0	5.0	5.0	5.6
3.0	4.0	4.6	4.6
4.0	3.6	4.0	3.0
5.0	3.0	3.6	2.6
6.0	2.0	3.0	2.0
7.0	2.0	2.0	1.0
8.0	2.0	2.0	1.0

DD are expressed as days until a reaction occurs. The CFU/ml conversions are expressed as log CFU/ml. Thus, for a culture in an IRB-BART tube (results vary among types), DD 2 =  $10^5$  CFU.

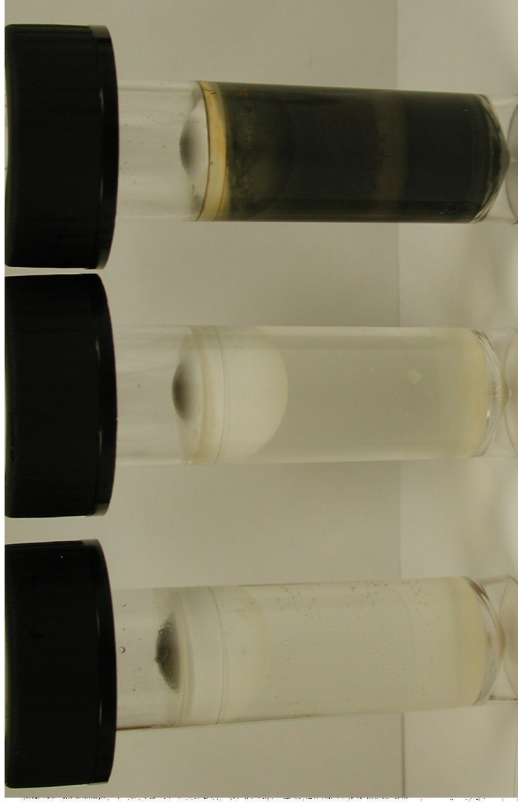
From: DBI, 1999. Biological Activity Test (BART™) User Manual. DBI, Regina, Saskatchewan. Up-to-date at URL <http://dbi.sk.ca>

The IRB reaction types tended to be those of aerobic and facultative anaerobic heterotrophs. Heterotrophic bacteria are present at  $10^{3.0-4.0}$  CFU/ml in water samples

tested and  $10^{6.0}$  in trap water. The heterotrophic bacteria present in the SLYM-BART are present at  $10^{3.6-5.0}$ , and sulfate-reducing bacteria (SRB) at  $10^{4-4.6}$  (when present). Days of delay are converted to cells per milliliter values using the relationship in Table 3. The Algae Bart are not included in this table, however, the immediate presence of algae seen in the trap water puts it in the aggressive growth category of greater than  $10^6$  CFU/ml.



Iron Related Bacteria (IRB) - Wasco vault waters on left and trap water far right.



Sulfate Reducing Bacteria (SRB) - Wasco vault waters on left and trap water far right.



Slime Forming Bacteria (SLYM) - Wasco vault waters on left and trap water far right.



Iron Related Bacteria (IRB) - Wasco trap water far and vault waters on right.