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Characterization and Isolation of Constituents Causing Red Coloration in Desert Arroyo Seepage Water

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Abbreviations

Da	dalton
DI	deionized water
DOC	dissolved organic carbon
EPA	U.S. Environmental Protection Agency
ESR	electron spin resonance
g	grams
GAC	granular activated carbon
IR	infrared
m^2/g	meters squared per gram
µg/L	micrograms per liter
µL	microliters
µm	micrometers
mg/L	milligrams per liter
mL	milliliters
mS/cm	millisiemens per centimeter
NMR	nuclear magnetic resonance
SC	specific conductivity
UV	ultraviolet
vis	visible
VOCs	volatile organic compounds
ZVI	zero-valent iron

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Abstract

Surface water in swamps and bogs is often colored yellow or brown from dissolved humic matter. While common in humid regions, such coloration in natural water is rare in arid areas. However, groundwater seeping from the Mancos Shale at numerous locations throughout much of its depositional basin in Colorado, New Mexico, and Utah has a distinctive yellow-to-red coloration. Field relationships indicate that the color is derived from constituents released from the Mancos Shale. This study used extraction and chemical oxidation methods to characterize the nature of the yellow/red color and to isolate the color-forming constituents from the inorganic matrix. Filtering and dialysis tests indicate that the coloration is due to moieties smaller than 1000 daltons. Tests with peroxide and bleach indicate that a portion of the color-forming constituents are readily oxidized. These tests and extractions by a variety of solid substrates suggest that the color-forming constituents are humic materials in the form of fulvic acid. A method was derived by which the color was concentrated by a factor of about 5, while the salt content was reduced by a factor of about 50. Analysis of the isolate has not yet been conducted.

1.0 Introduction

Groundwater seeping from the Mancos Shale at numerous locations throughout much of its depositional basin in Colorado, New Mexico, and Utah is saline with specific conductivity (SC) values often exceeding 20 millisiemens per centimeter (mS/cm). The seepage has near neutral pH, elevated concentrations of major ions, dissolved organic carbon (DOC), nitrate (NO_3), selenium (Se), and uranium (U) (Table 1). The high levels of these constituents were referred to as natural contamination by Morrison et al. (2012). The chemical signatures of the seepage are dominated by chemical exchange with the Mancos Shale and are likely affected by weathering processes.

Table 1. Concentrations of Constituents in Selected Mancos Shale Seeps

Region	**Delta, Colorado**	**Montrose, Colorado**	**Green River, Utah**	**Shiprock, New Mexico**
Area	Delta Reservoir	Houston Gulch	Cisco	Many Devils Wash
Sample Location	DRS3	HGS	S36	EF19
Sample Date	11/8/2010	11/3/2010	11/11/2010	3/21/2012
pH	7.25	7.36	7.67	7.18
SC (mS/cm)	22.8	22.8	24.5	30.6
Alkalinity (mg/L as $CaCO_3$)	1040	690	479	680
Calcium (mg/L)	410	450	396	408
Sodium (mg/L)	5900	2550	6500	8560
Magnesium (mg/L)	600	3300	840	1080
Potassium (mg/L)	34.6	47.0	23.6	56.8
Iron (mg/L)	<1	<8	<1	<0.1
Sulfate (mg/L)	15,100	22,500	17,700	17,500
Nitrate (mg/L)	76.0	1370	841	3180
Chloride (mg/L)	798	102	436	1250
Selenium (µg/L)	100	890	4100	1560
Uranium (µg/L)	137	354	123	156
DOC (mg/L)	44	67	24	34
Color (Pt-Co units)	350	575	84	211

The Mancos Shale was deposited during the Late Cretaceous Epoch in the offshore and open-marine environment of the epicontinental Western Interior Seaway (Johnson 2003). Mancos Shale contains clayey to sandy to calcareous silt-shale with minor limestone, marlstone, bentonite, and sandstone beds (Noe et al. 2007). Near-surface portions of Mancos have undergone chemical changes from weathering processes, chiefly pyrite oxidation to iron hydroxide minerals and oxidative loss of organic matter. Weathered Mancos Shale contains gypsum ($CaSO_4 \cdot 2H_2O$) and calcite ($CaCO_3$); the amounts of both decrease with increasing depth. However, weathering of Mancos Shale can occur without any obvious change in appearance. In a study of the weathering of organic matter in the Mancos Shale, Leythaeuser (1973) found that core samples with a similar dark-gray appearance had lower concentrations of organic carbon in the shallowest zones, likely due to weathering. In a study of black shale in Germany, Littke et al. (1991) also showed that organics can be weathered without a change in appearance.

A unique feature of the Mancos seepage water is its color. The water is typically colored light yellow to deep red (Figure 1). The color varies with the thickness of the water column. In a streambed fed from a Mancos seep, a light yellow color was observed where the water was only 1 to 2 inches deep; however, in pools of 8 to 20 inches deep fed from the same seep, the color was red with the darkest red colors in the deepest pools. The red color of the water provides a readily identifiable indication of natural contamination in desert arroyos in the arid southwestern U.S. (DOE 2011). For ease of discussion, the term “red water” will be used throughout this paper to refer to the colored seepage water that may range from light yellow to deep red.

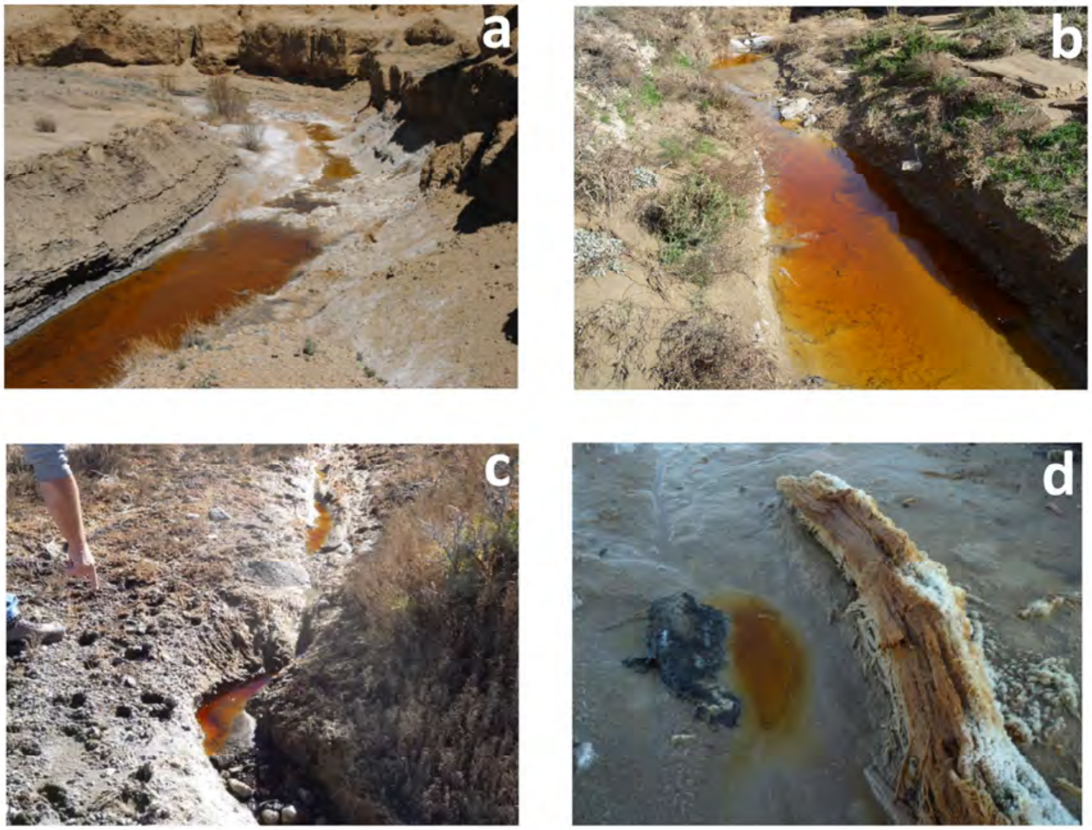

Figure 1. Red Water Occurrences at (a) Many Devils Wash (b) Salt Creek Wash at location SCWS (c) Houston Gulch near Montrose, Colorado, and (d) Whitewater Creek near Grand Junction, Colorado.

Although red coloration in natural water is often caused by oxidized Fe, no Fe was detected in the seep samples. The samples have elevated concentrations of DOC, which suggests that the color is related to organic complexes. Dissolved humic material often imparts yellow and brown colors to surface waters and is especially abundant in humid climates (Foster 1950, Curtis and Schindler 1997). Although similarities exist, the water in the Mancos seeps has a deeper red color compared to the typical yellow and brown colors of water in heavily vegetated swamps and ponds. A search of the literature revealed no investigations of the red-colored water that occurs in these desert arroyos.

The natural contamination derived from the Mancos Shale is of interest because of implications to the feasibility of groundwater cleanup at several sites managed by the U.S. Department of Energy's Office of Legacy Management that are located in Mancos Shale terrane. The red color of the seep water is a unique property that has not been characterized. The purpose of this study was to characterize the chemical nature of the red coloration in several groundwater samples collected near Shiprock, New Mexico (Figure 2) and develop methods to separate the color-forming constituents from the water so that more detailed analysis could be conducted. Methods used to characterize the color-forming constituents included filtration, dialysis, solid-phase extraction, and chemical treatments. Isolation was accomplished using two different materials: Supelite DAX-8 resin (DAX-8) and diethylaminoethylcellulose (DEAE).

2.0 Methods and Materials

2.1 Sample Collection

Samples were collected from seeps in three desert arroyos near Shiprock, New Mexico: Many Devils Wash, Salt Creek Wash, and Upper Eagle Nest (Figure 2). Sample EF19 from Many Devils Wash was collected from a hand-dug well completed with slotted PVC well screen. Other samples were collected from holes dug by hand into the seepage areas. The holes were purged and allowed to refill to ensure that recent seepage was being sampled. Samples, ranging in color from yellow to red, were collected in Nalgene carboys using a peristaltic pump and kept cool until analysis (Figure 3). Some samples were field filtered through in-line 0.45-micrometer (µm) filters (Geotech Dispos-a-filter 7305004).

2.2 Materials

A variety of commercially available solid substrates were used to attempt separations of color from the solutions. Screening-level extraction tests were conducted with zero-valent iron (ZVI), NSR-1 resin, Diphonix resin, Silicalite, polyvinylpolypyrrolidone (PVPP), gelatin, OnGuard II, Ambersorb, granular activated carbon (GAC), coconut-shell charcoal, bone charcoal, DEAE, and DAX-8. For comparison, some of the tests were conducted on solutions made from commercial humic acid (Sigma Aldrich: H1, 675-2) and tannic acid standards (Hach: 79114). The solid substrates were used "as is" without any washing or conditioning, except where noted. The properties of these materials are summarized in Table 2.

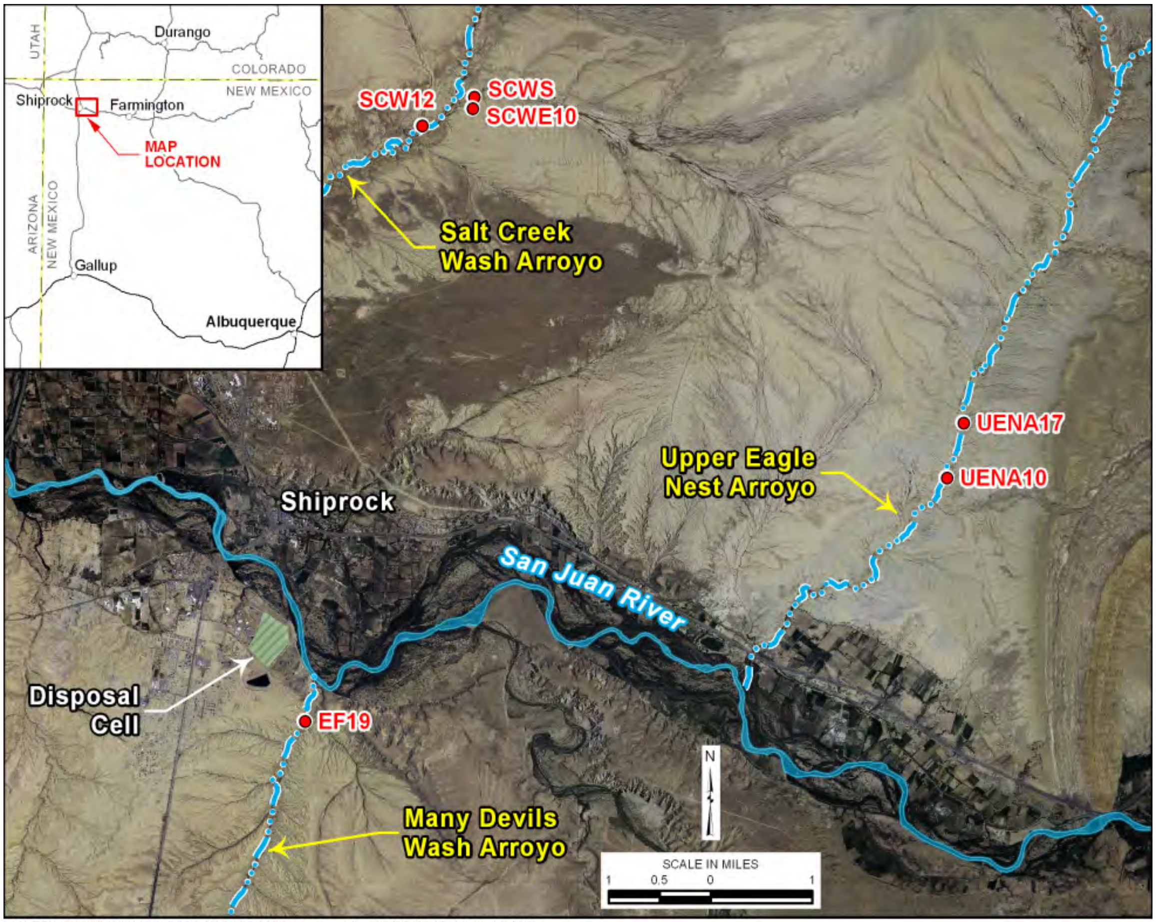

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Figure 2. Location Map

Figure 3. Color Variation in Samples EF19, SCWS, and SCWE10.
Field Filtered through 0.45 µm Filter

Table 2. Properties of Solid-Phase Extractants

Product	Supplier	Mesh Size	Constituents[a]	Composition
Zero-Valent Iron	Peerless	-8/+20	VOCs, metals, U	cast iron
NSR-1 Resin	Dow Chemical	-14/+40	NO_3	triethylamine strong base anion, styrene divinylbenzene macroporous quaternary amine functional groups
Diphonix Resin	Eichrom	-20/+50	metals	polystyrene divinylbenzene with diphosphonic and sulfonic functional groups
Silicalite	UOP	powder	low-molecular-weight organics	SiO_2
PVPP (polyclar vt)	morebeer.com Concord, CA	140 µm	polyphenols	polyvinylpolypyrrolidone
Gelatin	morebeer.com	powder	colloids	collagen
OnGuard II Ba	Dionex	granular	SO_4, CrO_4	styrene-based, sulfonic acid resin, Ba form
OnGuard II Na	Dionex	granular	alkaline earth and transition metals	styrene-based, sulfonic acid resin, Na form
OnGuard II H	Dionex	granular	alkaline earth and transition metals	styrene-based, sulfonic acid resin, H form
Ambersorb 563	Rohm & Haas	-20/+50	VOCs, metals	carbonaceous adsorbent
Ambersorb 572	Rohm & Haas	-20/+50	VOCs, metals	carbonaceous adsorbent
AC1240 GAC	Siemens	-12/+40	organics, odor	activated bituminous carbon
UC1240 GAC	Siemens	-12/+40	organics, color	activated bituminous carbon
Coconut-Shell Charcoal	Fisher Scientific	granular	low-molecular-weight organics	steam-activated carbon
Bone Charcoal	Cercona	granular	U, VOCs	calcined raw bone meal with phosphate binder
DEAE	Sigma Aldrich	fibrous	anions, proteins	cellulose fibers
DAX-8	Sigma Aldrich	-40/+60	humic/fulvic acids, color	acrylic ester

[a] Constituents designed to be removed from the aqueous phase

ZVI is granular cast iron processed from automotive industry byproducts that has been fired at high temperature to remove cutting oils. ZVI has been used to remove U and chromium (Cr) from contaminated groundwater (Naftz et al. 2002) and to degrade volatile organic compounds (VOCs) *in situ* (Orth and Gillham 1996). The ZVI had a grain size of -8/+20 mesh and was from Peerless Metal Powders & Abrasive (Detroit, Michigan). Dowex NSR-1 resin, obtained from Dow Chemical (Midland, Michigan), is designed primarily to remove NO_3 from potable water (Dow Chemical 2012). Diphonix, obtained from Eichrom (Darien, Illinois), is a cation-exchange resin designed to remove of a variety of metals from water, including cobalt (Co), copper (Cu), Cr, Fe, lead (Pb), Mn, nickel (Ni), plutonium (Pu), U, and zinc (Zn) (Eichrom 2012). Silicalite, obtained from UOP Inc. (Des Plaines, Illinois), is a hydrophobic molecular sieve with the composition silicon dioxide (SiO_2), that has been used to remove low-molecular-weight polar organic compounds from water, including acetic acid, acetaldehyde, butanol, 1,4-dioxane, ethyl acetate, chloroform, crotonaldehyde, hexane, methanol, pentane, phenol, and propanol (Flanigan et al. 1978; Chriswell et al. 1983). PVPP and gelatin are used to remove color in some industrial processes. They are also used as “fining” agents to remove organic compounds, improve clarity, and adjust flavoring in the beer- and wine-making industry.

OnGuard II resins are proprietary products of the Dionex company (Dionex 2012). OnGuard II Ba, OnGuard II H, and OnGuard II Na are styrene-based, sulfonic acid resins in the barium (Ba), hydrogen (H), and sodium (Na) forms, respectively. They are supplied as granular material encased in small (1 cubic centimeter of solid phase) flow-through cartridges. OnGuard II is used to remove interfering ions from solutions prior to chemical analysis. OnGuard II Ba is designed to remove sulfate (SO_4) and chromate (CrO_4). OnGuard II H and OnGuard II Na are designed to remove alkaline earths (e.g., Ba, calcium [Ca], and magnesium [Mg]), and transition metals (e.g., Co, Cr, Cu, Fe, Mn, molybdenum [Mo], and Zn).

Ambersorb 563 (Am563) and 572 (Am572) are proprietary carbonaceous adsorbents of the Rohm and Haas Company (now part of Dow Chemical). Ambersorb is no longer available commercially and our samples were obtained in 1994. Ambersorb was produced by controlled pyrolysis of highly sulfonated styrene divinylbenzene ion exchange resins (Choma and Jaroniec, 1997; Padhye et al. 2010). Am572 was supplied in the form of spherical beads with a size range of 0.297 to 0.841 millimeters and a surface area of 1100 meters squared per gram (m^2/g). Am563 is also in the form of spherical beads of the same size range as Am572 but has a surface area of 550 m^2/g (Padhye et al. 2010). Ambersorb resins were tested because of their proven ability to remove organic constituents and color from aqueous media. Am563 has been used to remove VOCs (1,1-dichloroethane, trichloroethene, vinyl chloride, and others) from contaminated groundwater (EPA 1995). Because of its affinity for organic complexing agents such as ethylenediaminetetraacetic acid (EDTA), Baytak and Türker (2006) used Am572 to concentrate EDTA complexes of Pb and Ni from aqueous solutions. Kendüzler and Türker (2002) used Am572 to remove Fe, Mn, and Zn chelates from aqueous solutions. Kendüzler and Türker (2002) also cite references to research that used Am572 to remove thiodiglycol, Geosmin, 2-methylisoborneol, and taste and odor compounds from water. Both Am563 and Am572 have been used to remove methyl tertiary butyl ether (MTBE) from groundwater (Davis and Powers 2000). Am572 is also an efficient solid-phase extraction media for nitrosamines (Padhye et al. 2010).

GAC is used extensively in the oil refining and gas processing industries and in aqueous applications. GAC samples AC1240 and UC1240 were obtained from the Siemens Corporation (Roseville, Minnesota). UC1240 is primarily used to remove organic contaminants, and for decolorization, amine purification, glycol purification and chemical purification. AC1240 is used for drinking water treatment, groundwater remediation, wastewater treatment, and industrial process water treatment to remove chlorine (Cl_2), chloramine, organic contaminants, pesticides, taste, and odor. GAC is widely used for color removal in the food processing industry. Coconut shell and bone charcoal are forms of activated carbon that are used to remove small molecular weight organic compounds from drinking water (Padhye et al. 2010). Bone charcoal was obtained from Cercona of America, Inc. (Dayton, Ohio) and coconut shell charcoal from Fisher Scientific (Fair Lawn, New Jersey).

Isolation of the color-forming constituents was conducted with two substrates: DEAE, (Sigma-Aldrich: D3764) and DAX-8 (Sigma-Aldrich: 20278), an acrylic ester resin. Miles et al. (1983) used DEAE to isolate humic constituents from river water. They favored it over the polystyrene divinylbenzene compounds such as Ambersorb, in part to alleviate issues of carbon release during the isolation procedure. DEAE is used in laboratory chromatography and ion exchange columns. Amberlite XAD-8 (XAD-8), a non-ionic acrylic ester resin (Thurman and Malcolm 1981), has been used extensively to isolate humic substances. XAD-8 was recommended by the International Humic Substances Society to be used as a standard for the isolation of humic substances; however, XAD-8 is no longer manufactured. DAX-8 was proposed as a substitute for XAD-8 for isolating humic material, and was found to be slightly more sorptive than XAD-8 (Peuravuori et al. 2002). DAX-8 is used in the paper industry to treat pulp waste.

2.3 Analytical Methods

Except where noted, all chemicals were reagent grade. Laboratory-purified deionized water (DI) had a resistance of 18.2 megaohms. DOC was analyzed by digesting a filtered sample in persulfate at 105 ^{o}C to liberate carbon dioxide (CO_2) and measuring the resulting pH change, after removing inorganic carbon in acid (Hach 1989). Organic carbon is reported as DOC if it passes a 0.45 µm filter. Color was determined on filtered samples by absorbance of light at 420 nanometers (nm) and comparison to a platinum-cobalt (Pt-Co) standard (Hach 1989). Uranium was measured on acidified samples by laser-induced kinetic phosphorescence (Chemchek undated). Selenium was measured on acidified samples by inductively coupled plasma-optical emission spectroscopy (Perkin Elmer Optima 7000 DV). Specific conductivity and pH were measured with sondes. Selected groundwater samples were analyzed for VOC concentrations by gas chromatography using U.S. Environmental Protection Agency (EPA) Method 8260.

2.4 Oxidation

A method for acid-peroxide oxidation was adapted from a method described by Chemchek (undated) that is used to remove organics and chloride (Cl) in samples prior to U analysis. The method destroys organics by oxidizing them to CO_2 and oxidizes chloride to chlorine; both CO_2 and Cl_2 are driven off by heating. Three milliliters (mL) of concentrated nitric acid (HNO_3) and 0.5 mL of 30% hydrogen peroxide (H_2O_2) were added to 10 mL of sample in a 250 mL beaker. The sample was brought to sub-boiling temperatures on a hot plate until dry. The addition of nitric acid and hydrogen peroxide and heating to dryness was conducted two additional times. The solids that remained were reconstituted to 20 mL with DI resulting in a solution with dilution factor 2 relative to the starting sample.

Oxidation was also attempted by adding 100 microliters (µL) of Clorox bleach (6.0% NaOCl yielding 5.7% available Cl_2) to 20 mL of sample in a glass vial. The sample was agitated by hand and left to rest 10 minutes before adding another 100 µL of bleach. Bleach additions continued up to a total of 1 mL. An initial color measurement was made and color was measured after each bleach addition.

2.5 Dialysis

Samples were filtered (0.45 µm) and placed in 1 liter Nalgene bottles. Dialysis “bags” were prepared by clamping the ends of tubular dialysis membranes (Spectrum Laboratories SpectraPor). The membranes had a pore size that allowed passage of molecules with a maximum molecular mass of 1000 daltons (Da). Prior to use, the membranes were soaked in DI for 30 minutes and then rinsed to remove sodium azide that was used for membrane preservation. The bags were filled with DI and suspended in the sample. The bags were approximately 8 inches long and held 100 mL of DI. The Nalgene bottles containing the dialysis bags were capped and placed in a refrigerator at 4 °C for 20 days. Analyses were conducted on the solutions both in and out of the dialysis bags.

2.6 Solid-Phase Separations

An OnGuard II cartridge was connected to a syringe and conditioned by passing 2 mL of sample through the cartridge and discarding the effluent. Over a period of 10 minutes, an additional 25 mL of sample was passed through the cartridge and the effluent collected in a plastic centrifuge tube. The process was repeated with a new cartridge to treat a total of 50 mL of sample.

Extractions by other solid substrates were performed by adding a known weight (usually 2 g) of solid substrate to a known volume (usually 40 mL) of colored groundwater sample in a 50 mL plastic centrifuge tube and agitating end-over-end for a prescribed length of time (usually 24 hours). pH values were adjusted in some tests using hydrochloric acid (HCl) or sodium hydroxide (NaOH). Following extraction, samples were centrifuged (usually 10 minutes at 3300 rpm) and then vacuum filtered through a 0.45 µm filter. Deionized water was used as the sample for some tests to determine the loss of carbon from the solid substrates.

2.7 Color Isolation

Isolation of color-forming constituents was conducted with DEAE and DAX-8. Each of these solid substrates was pretreated following a procedure adopted from Miles et. al (1983). For pretreatment, 50 grams (g) of material was placed in a beaker with 1 liter of 0.5 molar (M) HCl. The mixture was stirred on a magnetic stir plate for 1 hour followed by filtering through Whatman no. 1541-125 filter paper (22 µm) in a Buchner funnel, and then rinsed with DI until the pH of the filtrate was near neutral. The filter cake was transferred to a beaker and stirred for 1 hour with 1 liter of 0.5 molar NaOH. This mixture was again filtered through the Buchner funnel and rinsed with DI until the pH was neutral. Pretreated solids were stored in 125-mL amber Nalgene bottles at 4 °C.

To isolate the color-forming constituents, 500 mL of a colored groundwater sample was mixed with about 2 g (wet weight) of pretreated DEAE or DAX-8. The pH of some of the groundwater samples was adjusted using 1M HCl or 1M NaOH prior to mixing with the solids. The mixture was stirred for about 15 hours, filtered through Whatman no. 1541-125 filter paper in a Buchner funnel, rinsed with approximately 100 mL of DI, and the filter cake transferred to a 50 mL plastic centrifuge tube. For elution off of the substrate, 20 or 40 mL of 0.1M NaOH was added and the mixture was stirred end-over-end for 1 hour. The suspension was centrifuged and decanted. In some tests, the solids were treated a second time with 0.1M NaOH. The residue following decantation was syringe-filtered through a 0.45 µm nylon acrodisk filter and pH was lowered either by adding concentrated HCl or passing the aqueous phase through a Dionex OnGuard II H resin cartridge. For pH adjustment using OnGuard II H, the solution was syringed through the cartridge at a rate of 10 mL/minute, after discarding the first 5 drops. Samples collected at various points through the process were analyzed. Controls were run with DEAE or DAX-8 in 500 mL of DI or pH-adjusted DI to evaluate release of carbon from the solid substrates.

3.0 Results

Groundwater samples were subjected to a series of tests intended to characterize and isolate the constituents responsible for the red color. Samples used in the analyses were collected from desert arroyos that had surface water pools that ranged in color from light yellow to deep red (Figure 1). While still distinctly colored, when placed in sampling containers these waters appeared lighter shades of yellow, orange, and red (Figure 3). The qualitative colors specified in this section refer to the color in the sample containers. Because results of preliminary tests indicated that sample color may be related to humic matter, commercial samples of humic acid and tannic acid were analyzed for comparison.

3.1 Volatile Organic Compound Analytical Results

VOC concentrations in nine samples of groundwater collected from Many Devils Wash, Salt Creek Wash, and Upper Eagle Nest arroyos were less than the method detection limit with one exception. A sample from Salt Creek Wash had a toluene concentration of 0.14 micrograms per liter (µg/L). Because the concentration was only slightly above the detection limit of 0.1 µg/L, it may be due to sampling or laboratory contamination.

3.2 Filtration and Dialysis

Filtration was conducted on an unfiltered sample of EF19 to determine if any coloration is associated with particulate matter. Filtering through 0.22, and 0.1 µm filters did not visibly alter the color from the 0.45-µm filtrate. The color values for the filtrates were 203, 197, and 210 Pt-Co units, indicating that the smaller mesh filters did not remove additional color-forming constituents. DOC values of the three filtrates were also similar to each other, and the unfiltered sample, indicating that organic carbon passed freely through all three filters (Table 3).

Table 3. Results of Filtering of Sample EF19

Filter (µm)	DOC (mg/L)	Color (Pt-Co units)
none	43	–
0.45	42	203
0.22	30	197
0.1	45	210

Dialysis membranes can assess the size of smaller moieties than can filters. The dialysis membranes can differentiate a molecular mass of 1000 Da. A Da is equivalent to 0.9997 mass units. Most humic acid molecules exceed 1000 Da. A 1000 Da dialysis bag containing DI was immersed in each of samples EF19, SCWS, and SCWE10.

The water in the dialysis bags was colorless initially but was visibly colored after 20 days of immersion (Figure 4) and the water in the bags appeared identical to the color of the surrounding solution. The color values shown in Table 4 confirm that the constituents causing the color had migrated through the dialysis bags. The DOC values in and out of the dialysis bag for each test were also similar, suggesting that the color was associated with dissolved carbon and that all of the DOC was less than 1000 Da. Uranium and Se, two of the natural contaminants from Mancos Shale, also migrated freely through the membranes. The U and Se could be in ionic forms or combined with organic molecules.

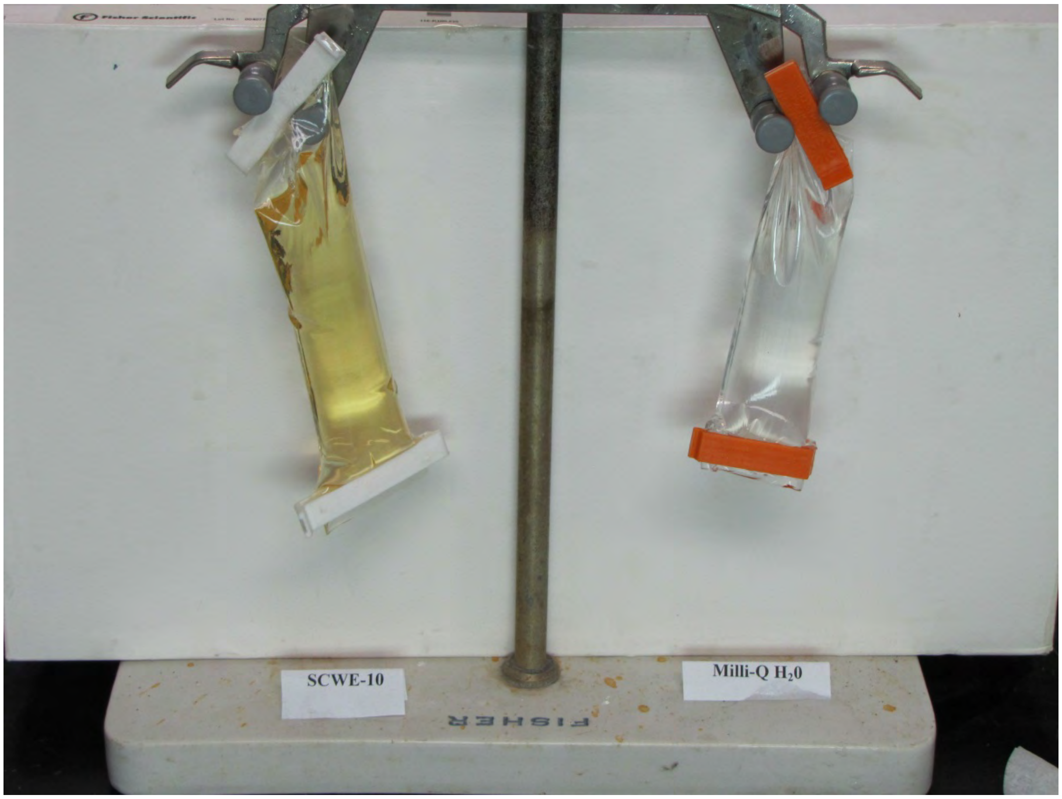

Figure 4. Color in a Dialysis Bag Before (Right) and After (Left) 20 Days of Immersion in Sample SCWE10

Table 4. Dialysis Results After 20 Days of Immersion

Test No.	Sample	pH	DOC (mg/L)	Color (Pt-Co units)	U (µg/L)	Se (µg/L)
1	EF19 (out of bag)	7.77	28	170	127	1430
	EF19 (in bag)	7.93	28	183	113	1440
2	SCWS (out of bag)	7.72	67	418	124	2810
	SCWS (in bag)	7.91	81	427	122	2810
3	SCWE10 (out of bag)	7.83	70	568	128	1050
	SCWE10 (in bag)	7.99	67	540	157	1040
4	Humic Acid (out of bag)	8.44	55	3830	<4	<20
	Humic Acid (in bag)	8.45	3.5	<25	<2	<10

A 200 milligrams per liter (mg/L) humic acid solution was used to help evaluate the veracity of the dialysis tests. As expected, humic acid molecules were too large to migrate through the 1000 Da membrane. The color of the outer solution was 3830 Pt-Co units while the color in the dialysis bag after 20 days immersion was less than 25 Pt-Co units. Most of the DOC was associated with the humic acid molecules and remained outside the bag (Table 4).

3.3 Oxidation Treatments

Acid/peroxide oxidation was used to determine if the color of the samples could be changed by altering or destroying dissolved organics. The method is designed to oxidize organics and drive them off as CO_2. The oxidation was conducted on five different samples of colored water and in all cases the DOC concentrations decreased (Table 5). No quantitative color analyses were performed but the samples were colorless after the treatments. The results suggest that the sample color was due to the DOC fraction that was altered or destroyed by oxidation.

Table 5. Nitric Acid/Hydrogen Peroxide Oxidation Results[a]

Sample	[b]DOC	[c]DOC	[b]Qualitative Color	[c]Qualitative Color
EF19	42	30	Yellow	Colorless
SCWS	180	36	Yellow	Colorless
SCW12	740	220	Yellow	Colorless
UENA10	2200	37	Yellow	Colorless
UENA17	380	41	Yellow	Colorless

[a] Tests conducted using 3 mL HNO_3 and 0.5 mL H_2O_2 in 10 mL of sample
[b] Before treatment
[c] After treatment

Bleach was also used to oxidize DOC. Various amounts of bleach were added to a 20-mL split of colored sample SCWS. The SCWS sample became less colored with bleach additions. The color value decreased from 464 to 245 Pt-Co units following the first bleach addition and decreased monotonically with subsequent bleach additions (Table 6). These results suggest that some of the color is due to DOC fractions that are readily oxidized with bleach.

Table 6. Results of Bleach Addition to 20 mL of Sample SCWS

Total Bleach Added (µL)	Color (Pt-Co units)
0	464
100	245
200	204
300	173
400	155
500	140
600	129
700	117
800	110
900	101
1000	97

3.4 Extraction by Solid Phases

Screening-Level Testing. Screening-level tests were conducted with a variety of solid-phase extractants. Each extractant was manufactured to remove specific groups of analytes from water. The screening tests were conducted with sample EF19; this sample was yellow before the extractions. The colors following extractions are provided in Table 7. The two Ambersorb resins and bone charcoal were the most effective at removing color. Dowex NSR-1 resin removed a portion of the color. Based in part on these tests, Am563 and Am572 were selected for additional testing.

Table 7. Color Change in 40 mL of EF19 Treated with Solid-Phase Extractants

Solid-Phase Extractant	Mass (g)	Qualitative Color After Treatment
None (Control)	–	Yellow
Peerless Zero-Valent Iron	1.98	Yellow
Dowex NSR-1 Anion-Exchange Resin	2.04	Very Pale Yellow
Eichrom Diphonix Resin	1.62	Yellow
Ambersorb 563	1.48	Colorless
Ambersorb 572	1.95	Colorless
Silicalite Powder	2.37	Yellow
S115 Molecular Sieves	1.81	Yellow
Coconut Shell Charcoal	0.17	Yellow
Cercona Bone Charcoal	2.22	Colorless
Polyvinylpolypyrrolidone	1.99	Yellow
Gelatin	2.01	Yellow

Screening-level tests were also conducted with DEAE and DAX-8. Both DEAE and DAX-8 decreased the color value (Table 8). Resulting pH values were more than 8. Because of the similar composition to XAD-8, and data presented by Thurman (1986; Figure 4-3) that indicated an increase in color and DOC removal at pH values less than 3 for XAD-8, DAX-8 was expected to remove humic matter more efficiently at lower pH values. Therefore, a split of DAX-8 that had been adjusted to pH 2 was also tested. Lowering the pH to 2 improved the color removal with DAX-8 (Table 8). These results reinforce the importance of pH on procedures designed to remove and isolate humic matter from aqueous solutions as noted by previous researchers (e.g., Miles et al. 1983).

Table 8. Screening-Level Tests of DEAE and DAX-8[a]

Substrate	[b]Color (Pt-Co units)	[c]Color (Pt-Co units)	[b]pH	[c]pH	[b]SC (mS/cm)	[c]SC (mS/cm)
DEAE	648	347	7.62	8.29	35.1	34.3
DAX-8	648	538	7.62	8.15	35.1	36.4
DAX-8 at pH 2	536	107	2.03	2.17	38.9	39.4

[a] Tests were conducted using 2 g solids in 40 mL of SCWE10
[b] Before treatment
[c] After treatment

OnGuard II. Testing was conducted to evaluate the effect of inorganic ions on sample color. OnGuard II cartridges are used to remove inorganic ions, mainly alkaline earth metals, from solution. A sample of EF19 was filtered (0.45 µm) and then treated with each of the three OnGuard II resins. In all three tests, the color of the solution after treatment was similar to the color before treatment and DOC concentrations were not significantly affected (Table 9). The results indicate that the ions removed by these resins do not affect color.

Table 9. Results With Dionex OnGuard II Resin Cartridges

Sample	Resin	[a]DOC (mg/L)	[b]DOC (mg/L)	[a]Qualitative Color	[b]Qualitative Color
EF19	OnGuard II Ba	42	46	Yellow	Yellow
	OnGuard II H	42	43	Yellow	Yellow
	OnGuard II Na	42	42	Yellow	Yellow
	none	-	42	Yellow	Yellow

[a] Before treatment
[b] After treatment

Ambersorb. Ambersorb extractions were conducted on groundwater samples EF19 and SCWE10. Control samples, which had no Ambersorb, were analyzed to establish the initial values for the extraction tests. Both Am563 and Am572 removed color from sample EF19 as indicated by the color value decreasing from 179 to less than 25 Pt-Co units (Table 10). Am572 removed most of the color from sample SCWE10, but Am563 was slightly less effective with a decrease of only 81%, from 670 to 128 Pt-Co units. Am563 and Am572 reduced color in the humic acid solutions by 43% to 61% (Table 10). Neither Ambersorb resin completely removed the DOC. In fact, at least 29% of the DOC remained in the samples except for the tannic acid standard. Tannic acid was not colored but was analyzed to determine the efficiency of DOC removal by Ambersorb. Most of the DOC from tannic acid was removed by both Am563 and Am572.

Specific conductivity values of EF19 decreased only slightly by contact with Am563 (Table 10). The decrease could indicate a small amount of adsorption of ions; however, the relatively small changes may be due to instrument variation. Specific conductivity of the humic acid and tannic acid standards increased slightly, suggesting desorption of ions from the Ambersorb (the Ambersorb was not washed prior to these tests). The pH values changed slightly during the extractions, probably in response to release or uptake of H^+ by the solid-phase surfaces. A small amount of DOC (1.8 and 1.9 mg/L) and ions (specific conductivity values of 0.60 and 0.83 mS/cm) was released from both Am563 and Am572 resins when leached by DI (Table 10). Uranium was not removed by Am563, suggesting that U does not influence the color. About 28% of the U was removed by Am572, indicating some affinity of U for this resin. Selenium concentrations were lowered slightly by both resins, suggesting that they are selective to Se or that a small fraction of Se is tied to the color-forming compounds.

Table 10. Ambersorb Resin Extraction Results [a]

Sample	Sorbent	pH	DOC (mg/L)	Color (Pt-Co units)	SC (mS/cm)	U (µg/L)	Se (µg/L)
EF19	None	7.79	36	179	30.4	139	1800
	Am563	7.80	21	<25	29.3	143	1700
	Am563[b]	7.81	22	<25	29.9	146	1700
	Am572	8.29	28	<25	31.4	98	1750
SCWE10	none	7.81	94	670	31.9	159	773
	Am563	7.87	50	128	31.8	161	746
	Am572	8.00	30	<25	32.9	116	703
HA1000	none	8.90	260	19,900	0.32	-	-
	Am563	6.79	160	11,400	0.43	-	-
	Am572	9.23	94	7720	0.52	-	-
HA100	none	6.85	28	1520	0.04	-	-
	Am563	4.02	8.1	422	0.16	-	-
	Am572	8.91	8.7	440	0.31	-	-
TA1000	none	3.50	495	<25	0.04	-	-
	Am563	3.08	2.1	<25	0.28	-	-
	Am572	6.71	3.5	44	0.22	-	-
DI	Am563	3.51	1.8	<25	0.60	<0.4	<10
DI	Am572	8.80	1.9	<25	0.83	<0.4	<10

[a] Tests were conducted using 2 g solids in 40 mL of sample
[b] Duplicate test
HA100 = 100 mg/L humic acid solution
HA1000 = 1000 mg/L humic acid solution
TA1000 = 1000 mg/L tannic acid solution

Tests were conducted to determine the effect of color removal by Ambersorb over a pH range of 2 to 12. Most of the color of EF19 was removed at all pH values with both Am563 and Am572 as indicated by color values that decreased from 203 to less than 25 Pt-Co units for most of the tests (Table 11). Color removal was slightly less effective with Am563 at pH values more than 8. Specific conductivity and concentrations of DOC, U, and Se had similar trends for both Am563 and Am572. Evaluation of the DOC removal over this pH range was impacted by the release of carbon from the Ambersorb at low pH values. At a pH of 2, DOC increased from 42 to 130 for Am572 and to 140 mg/L for Am563. Specific conductivity increased at both high and low pH values due to the additions of acid and base. Large decreases in U concentrations occurred at pH values more than 10, likely due to precipitation of U hydroxide minerals. The large decreases in U concentrations that occurred at low pH values are not understood. Selenium concentrations typically decreased by about 7%, indicating partial sorption to the Ambersorb over the entire pH range.

Tests were conducted in DI to determine if pH variations caused leaching of carbon from Am572. The Am572 was used, as received, without washing prior to the tests. The DI had a DOC concentration of less than 1 mg/L prior to adding the resin. In all cases, even without adding acid or base (pH 8.86), carbon was leached into the water (Table 12). The release of carbon from the resin complicates determination of the carbon uptake in the tests. For this reason, we implemented a method using DEAE, recommended by Miles et al. (1983), to isolate the color-forming constituents from the groundwater samples (see Section 3.5).

Table 11. Ambersorb Resin With EF19 at Varying pH[a]

Sorbent	pH	[b]DOC (mg/L)	[c]DOC (mg/L)	[b]Color (Pt-Co)	[c]Color (Pt-Co)	[b]U (µg/L)	[c]U (µg/L)	[b]Se (µg/L)	[c]Se (µg/L)	[c]SC (mS/cm)
Am572	2.22	42	130	203	<25	139	86	1800	1650	36.1
	6.92	42	41	203	<25	139	37	1800	1630	31.0
	7.53	42	31	203	<25	139	137	1800	1670	30.8
	8.04	42	16	203	<25	139	125	1800	1690	30.2
	9.68	42	18	203	<25	139	110	1800	1660	30.4
	11.72	42	21	203	<25	139	12	1800	1690	37.0
Am563	2.06	42	140	203	<25	139	92	1800	1660	37.9
	3.71	42	29	203	<25	139	2	1800	-	31.2
	7.13	42	15	203	<25	139	31	1800	1700	30.9
	7.87	42	16	203	31	139	154	1800	1670	30.6
	9.23	42	15	203	45	139	112	1800	1710	30.8
	11.72	42	22	203	25	139	<2	1800	1670	36.8

[a] Tests were conducted using 2 g solids in 40 mL of EF19
[b] Before treatment
[c] After treatment

Table 12. Carbon Release from Am572 in Deionized Water at Varied pH[a]

pH	[b]DOC (mg/L)	[c]DOC (mg/L)
1.78	<1	12
3.83	<1	2.4
4.79	<1	2.5
5.93	<1	2.6
8.86	<1	4.0
11.04	<1	4.8

[a] Tests were conducted using 2 g solids in 40 mL of DI
[b] Before treatment
[c] After treatment

Granular Activated Carbon. Two types of GAC (AC1240 and UC1240) were tested. Both forms of GAC removed the color while removing less than 50% of the carbon (Table 13). Uranium concentrations remained nearly the same before and after treatment. Selenium concentrations decreased slightly, similar to results of the Ambersorb resins.

Table 13. Removal of Color from Sample EF19 with GAC[a]

GAC	[c]pH	[b]Qualitative Color	[c]Qualitative Color	[b]DOC	[c]DOC	[b]U	[c]U	[b]Se	[c]Se
AC1240	8.38	Yellow	Colorless	42	31	139	132	1800	1670
UC1240	8.24	Yellow	Colorless	42	28	139	142	1800	1650

[a] Tests were conducted using 2 g solids in 40 mL of EF19
[b] Before treatment
[c] After treatment

3.5 Isolation of Color-Forming Constituents

The purpose of isolating the color-forming constituents is to provide a concentrated solution of color-forming constituents that can be investigated using a variety of organic analysis methods. The best isolates are those that have high concentrations of color-forming constituents and low concentrations of other constituents. Thus, the most useful isolates are those with high color values and low specific conductivity values.

DEAE and DAX-8 were used to isolate and concentrate the color-forming constituents from the SCWE10 groundwater sample. These substrates were selected because they removed some of the color from samples in screening tests (Table 8) and literature sources indicated that they are effective for isolation of dissolved humic materials (Miles et al. 1983, Peuravuori et al. 2002, McDonald et al. 2004). DEAE is less likely to yield carbon, requires less conditioning, and binds humic materials more reversibly than the polystyrene divinylbenzene compounds such as Ambersorb (Miles et al. 1983). XAD-8 was selected by the International Humic Substances Society and has been used extensively for isolation of humic substances. XAD-8 is no longer manufactured, and it has inherent issues including numerous preparatory steps. DAX-8 is now being used by some researchers in lieu of XAD-8 (McDonald et al. 2004).

Color isolation using DEAE was conducted on splits of sample SCWE10 adjusted to pH values of 2, 4, 6, 8, and 10. The isolate for pH value 2 had a color value of 3430 Pt-Co units, which is more than 5 times the value of the original SCWE10 water (Table 14). All isolates up to pH 8 were more highly colored than the original SCWE10 sample. DOC was concentrated to 120 mg/L in the pH 2 isolate, but this represents an increase by a factor of only 1.5 from the original SCWE10. Thus, the method appears to isolate the color-forming constituents more effectively than it does the entire DOC inventory, suggesting that color may be tied to a specific fraction of the DOC. The salt content decreased by about threefold in the isolates, as indicated by a decrease in specific conductivity values from 35.1 to about 10 mS/cm (Table 14). After elution with NaOH, the pH values were adjusted by adding HCl. HCl and NaOH accounted for the salt content, as indicated by the value of 9.5 mS/cm in the method blank.

Table 14. Color Isolation from SCWE10 Water Using DEAE and pH Adjusted with HCl [a]

Sample	Color (Pt-Co units)	pH	SC (mS/cm)	DOC (mg/L)	U (µg/L)	Se (µg/L)
SCWE10[b]	648	7.62	35.1	78	174	1270
pH 2	3430	4.31	10.2	120	353	98.6
pH 4	1280	3.79	9.39	45	888	63.0
pH 6	836	3.72	9.99	32	<4	46.3
pH 8	936	6.40	11.4	38	<4	73.0
pH 10	81	6.23	10.6	<10	<4	14.6
DI Control	<25	4.11	9.5	<10	<4	<10
pH2 2nd OH[c]	582	-	-	-	-	-
pH4 2nd OH[c]	188	-	-	-	-	-
pH6 2nd OH[c]	146	-	-	-	-	-
pH8 2nd OH[c]	211	-	-	-	-	-
pH10 2nd OH[c]	37	-	-	-	-	-
DI Control 2nd OH[c]	<25	-	-	-	-	-

[a] Tests were conducted using 2 g DEAE in 500 mL sample. Eluted with 20 mL NaOH. pH adjustments made with HCl.
[b] Original sample of SCWE10
[c] Second elution with 20 mL NaOH

Color-forming constituents were not completely removed from the DEAE by a single elution with 20 mL of NaOH. Color values of up to 582 Pt-Co units were present in the second elutions with an additional 20 mL of NaOH (Table 14). DOC was highest in the pH 2 samples, indicating that the most highly colored isolates also have high carbon. The wide range of U concentrations in the isolates, from less than 4 to 888 µg/L, was unexpected and the cause is unknown. Selenium concentrations averaged about 50 µg/L in the isolates, much lower than the starting concentration of 1270 µg/L (Table 14).

To minimize the addition of salt from HCl, a set of tests were conducted using OnGuard II H resin to adjust the pH values following elution. The results, shown in Table 15, were from tests conducted in the same manner as those with results shown in Table 14, except for the use of OnGuard II H in lieu of HCl and the samples were eluted with a single batch of 40 mL NaOH rather than two separate 20 mL batches. The use of the OnGuard II H resin was effective, as indicated by specific conductivity values that were less than 4.0 mS/cm for the isolate. The effectiveness is also demonstrated by comparing the method blanks for tests that used HCl (Table 14) and OnGuard II H resin (Table 15); these had specific conductivity values of 9.5 and 3.86 mS/cm, respectively.

Table 15. Color Isolation from SCWE10 Water Using DEAE and pH Adjusted Using OnGuard II H Resin[a]

Sample	Color (Pt-Co units)	pH	SC (mS/cm)	DOC (mg/L)	U (µg/L)	Se (µg/L)	Cl (mg/L)	NO_3 (mg/L)	SO_4 (mg/L)
SCWE10[b]	648	7.62	35.1	78	174	1270	2410	1820	21,600
pH2	1710	2.05	3.72	64	471	55.6	12.9	16.6	537
pH4	588	2.04	3.20	24	5.6	38.1	12.2	14.6	466
DI pH2[c]	<25	2.01	3.86	4.6	<4	<10	322	2.5	49.6

[a] Tests used 2 g DEAE in 500 mL of SCWE10. Eluted with 40 mL NaOH; pH adjustments with OnGuard II H resin.
[b] Original sample of SCWE10
[c] DI adjusted to pH 2, control

Color-forming constituents from sample SCWE10, pretreated to a pH of 2, were isolated with DAX-8 using a similar procedure as was used with DEAE. The isolate, using DAX-8, had an increase in color from 648 to 3070 Pt-Co units, an increase in DOC from 78 to 320 mg/L, and a decrease in specific conductivity from 35.1 to 0.68 mS/cm (Table 16). Thus, DAX-8 produced a better isolate, having more color and less ions than DEAE. The isolate was analyzed by ion chromatography to determine the concentrations of specific anions. Concentrations of Cl, NO_3, and SO_4 were reduced by more than 99% (Table 16). The U concentration in the isolate was 20% less than in the sample.

Table 16. Color Isolation from SCWE10 Using DAX-8[a]

Sample	Color (Pt-Co units)	pH	SC (mS/cm)	DOC (mg/L)	U (µg/L)	Se (µg/L)	Cl (mg/L)	NO_3 (mg/L)	SO_4 (mg/L)
SCWE10[b]	648	7.62	35.1	78	174	1270	2410	1820	21,600
pH2	3070	2.49	0.68	320	140	34	3.6	2.8	49.1
DI pH2[c]	<25	2.79	0.007	5.9	<4	<10	4.2	<2.5	3.0

[a] Tests used 2 g DAX-8 in 500 mL of sample. Eluted with 40 mL NaOH; pH adjustments with OnGuard II H resin.
[b] Original sample of SCWE10
[c] DI adjusted to pH 2, control

4.0 Discussion

Groundwater collected from seeps and shallow wells in Mancos Shale terrane at a number of locations in Colorado, New Mexico, and Utah has a distinctive yellow to red coloration derived from the Mancos Shale (Morrison et al. 2012). The goal of this study was to provide insights into the nature of the color-forming constituents. Colored water samples were characterized using size separation and solid-phase extraction methods, and the color-forming constituents were concentrated using chemical isolation methods. The water samples have high concentrations of dissolved solids, dissolved organic matter, metals, and trace elements. Any of these constituents, or combinations of them, could contribute to the coloration.

4.1 Organic Carbon in the Mancos Shale

The DOC concentrations found in seeps issuing from the Mancos Shale in Colorado, New Mexico, and Utah ranged from 2.9 to 280 mg/L and often exceeded 50 mg/L; concentrations were particularly high in colored samples (DOE 2011). These DOC concentrations far exceed the norm for natural groundwaters, which commonly range from 0.2 to 15 mg/L with a mean of 0.7 mg/L (Thurman 1986). There are unusually high concentrations of DOC in some groundwater associated with petroleum and oil field brines that can be as high as 1000 mg/L (Thurman 1986). DOC concentrations in samples used in this study ranged from 42 to 2200 mg/L (Table 5).

Field relationships indicate that elevated concentrations of Na, SO_4, Se, U, and the constituents causing the red coloration (DOC) are being leached from Mancos Shale. Leythaeuser (1973) analyzed samples of Mancos Shale collected during coring of an outcrop in Utah and found that the organic carbon content decreased from about 1.5% to 1% due to weathering processes in the outer 10-ft interval. Thus, there is potential for loss of carbon to shallow groundwater during weathering. The mechanisms responsible for transfer of Mancos Shale carbon to DOC have not been investigated. The kerogen component in the Mancos is largely contained within marine dark-gray shale. Stuermer and Payne (1976) noted significant structural differences between marine and terrestrial fulvic acid. Marine fulvic acid has fewer aromatic groups, lower molecular weights, and more than 10 times the amount of nitrogen than terrestrial. These properties may help identify processes responsible for the release of humic-like material from the Mancos Shale.

4.2 Humic Material

A survey of the literature indicated that many worldwide occurrences of colored water are caused by dissolved humic matter. Humic matter is known to cause yellow and brown coloration in many natural waters, particularly in humid climates. Colored (mostly yellow) natural waters have been referred to by a variety of names including humnolimnic acids, humic substances, yellow substances, *gelbstoff*, *gilvin*, or simply color; and a variety of measurement units including Hazen units, color (Pt-Co units), g440, and Abs (1 cm-1) have been used in measurements of color (Cuthbert and Girogio 1992; Kalle 1996; Sieburth and Jensen 1968). An unusual groundwater that occurs in oil shale beds has concentrations of water-soluble humic matter up to 58,000 mg/L (Dyni 1996). This groundwater, referred to as “trona water,” is black and is associated with sodium bicarbonate derived from the mineral trona [$Na_3(CO_3)(HCO_3)\cdot 2H_2O$] that is present in the same geologic formation as the oil shale.

Humic matter is a ubiquitous component of the natural environment, resulting largely from the decay of plant materials. Humic materials are polymeric constituents of organic-rich soils and peat, and often impart a brown or yellow color to natural waters. Humic substances are divided into three groups based on solubility: humic acid, fulvic acid, and humin. Humic acid is soluble in basic solutions but precipitates at low pH. Humic acid is not well defined and typically is a mixture of aromatic and heterocyclic structures. Fulvic acid is soluble under all pH conditions. Humic materials that cause coloration in many natural streams are characterized by molecular weights ranging from 1500 to 5000 for humic acid and from 600 to 1000 for fulvic acid (Malcolm 1990). Humin is defined as the water insoluble portion of humic material. Tannic acid is a water-soluble constituent derived from tannin that is composed largely of degraded tree bark and leaf litter. Tannins have properties similar to humic substances. Tannin produced from tannic acid has been shown to be an effective substrate for uranium extraction (Sakaguchi and Nakajima 1987).

Humic matter contains thousands of organic compounds, any of which could influence the color of an aqueous solution. Color changes can be caused by relatively subtle alteration of dissolved humic matter. For example, a tannic acid solution made in DI had a light yellow color but when made in 0.05 M sodium bicarbonate solution at the same concentration, the solution turned dark brown after a few hours. Thurman (1986 and references provided therein) notes that aquatic humic substances show an increase in color by a factor of two or more as pH is increased from 2 to 13. Thurman (1986) also notes that humic acid absorbs more light at a wavelength of 660 nm than does fulvic acid and is thus colored more deeply red.

4.3 Extraction of Color

The red color is not due to particulate matter, as indicated by its passage through filters down to 0.1 µm. Passage of the colored water samples through various OnGuard II resins did not result in color removal. From these data, we surmise that the color is not directly caused by the high concentrations of inorganic ions, although ions in combination with organic molecules are not excluded. Low Fe concentrations in our samples exclude Fe as a direct cause of the color. Uranium was not removed by Am563 or GAC, suggesting that U does not influence the color. The color is not caused by VOCs, as indicated by low (mostly nondetect) VOC concentrations.

The red color is completely in a dissolved form, as indicated by its passage through a 1000 Da dialysis bag. Humic acid is composed of large carbon chains that do not pass through the 1000 Da membrane; thus, the constituents causing the color in the arroyo samples are smaller than humic acid. Some humic substances including fulvic acid have molecular units smaller than 1000 Da. It is possible that the color is caused by fulvic acid or other low-molecular-weight organic molecules and may be complexed with metals or major ions. In summary, these results suggest that the color is caused by DOC that is likely in the form of dissolved humic-like substances.

Cross-linked polymeric styrene-based resins (Am-563 and Am572), DEAE, and DAX-8 effectively extracted the color. These substrates are well suited to extracting organic constituents from water. Elevated DOC concentrations and color in the DAX-8 and DEAE isolates support an organic origin for the color. GAC was effective at removing all the color from the samples. Thurman (1986) indicated that GAC removes humic matter but is not efficient at removing large humic acid molecules from aqueous solutions because of restricted pore sizes. Thus, the tests with GAC provide additional evidence that the color is caused by relatively small moieties.

Coconut-shell charcoal, PVPP, and Silicalite are used to remove low-molecular-weight organic compounds (such as phenolic compounds) from water. The screening-level tests indicated that these substrates did not remove color, suggesting that the color is not from these phenolic organics. In contrast, bone charcoal, which should also remove low-molecular weight organics, did cause color removal. DEAE also has been shown to remove the colorless low-molecular weight organic compounds from aqueous solution, whereas XAD-8, an acrylic ester resin similar to DAX-8, does not concentrate the low-molecular weight compounds (Miles et al. 1983).

4.4 Recommendations for Analysis of Isolates

Isolation of the color-forming compounds was accomplished using DEAE or DAX-8. Analysis of the isolate has not yet been attempted. Although not definitive, the preliminary characterization data from this study suggests that the color-forming constituents are humic materials with molecular weight less than 1000 Da, but likely have molecular weights more than phenolic-like compounds. The low molecular weight excludes most humic acid moieties, but the constituents could be in the group of humic materials commonly referred to as fulvic acid.

Analysis methods often used to characterize humic materials in soil science include elemental (C-H-N-S-O) analysis; absorption spectrophotometry in the ultraviolet (UV), visible (vis), and infrared (IR) regions; electron spin resonance (ESR); nuclear magnetic resonance (NMR); spectrofluorometry; electrometric titrations; molecular weight measurements; viscosity; electron microscopy; thermal analysis; x-ray analysis; radiocarbon dating; and chemical degradation (Schnitzer 1982). The spectral methods provide information on functional groups, whereas ESR and NMR provide information on free radical groups.

Since our goal is to identify constituents that are the source of the coloration, the choice of the samples to be analyzed is of paramount importance. The samples from this study had a range in color from colorless to yellow to deep red. The colorless samples had similar elemental chemistry to the colored samples, being high in concentrations of DOC, U, and Se. Identifying differences in the organic fractions of the colorless and colored samples may help to understand the origin of the color. Some of the extractions effectively removed color but much of the DOC remained in solution. Comparison of the non-extracted DOC that does not color the solution to the extracted portion may also provide insight into the nature of the color-forming constituents. A variety of solid-phase substrates removed color and it is likely that the different substrates remove different fractions of organic compounds. Therefore, analysis of the solutions from various extractants could help identify the compounds responsible for the color.

A tiered approached to analysis is recommended. Low-cost analytical methods (e.g., UV-vis spectrometry) should be used on a wide range of colored and non-colored samples and isolates to identify any distinctive signatures. Selected samples should be characterized using commonly applied IR and NMR methods. Continuing this tiered approach, more detailed (and costly) analyses could then be applied, if deemed necessary.

5.0 References

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