The Pennsylvania State University

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THE ROAD TO BROOK TROUT RECOVERY: CRAB-SHELL RESTORES ALKALINITY TO WATERSHEDS IMPACTED BY ACID DEPOSITION

A Thesis in

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by

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ABSTRACT

The effectiveness of crab shell versus traditional limestone rock for the passive treatment of acid deposition was tested in a series of batch microcosm, continuous-flow column, and field-scale road-side ditch and underdrain experiments. Contact with crab shell in batch microcosms quickly increased the pH of acid impacted stream water from 3.1 to 7.6, and increased the alkalinity from 0 to 37.2 mg/L as $CaCO_3$ in as little as 6 hours. With increasing contact time, the pH and alkalinity continued to increase, reaching a maximum of 8.2 and 136.8 mg/L as $CaCO_3$, respectively, after a 10 days of treatment. Under continuous-flow conditions, crab shell increased the pH of the water from 3.87 to 9.2 in just 2 days and then slowly returned to a circum-neutral pH of 7.5 for the remainder of the 54-day study. In comparison, columns containing limestone were able to increase the pH to a maximum of 8.55 by day 24, but then lost neutralizing capacity, ending at a pH of 6.01 after 54 days. Alkalinity followed the same trend as pH, increasing from 0 mg/L as $CaCO_3$ to an average of 634 mg/L as $CaCO_3$ in columns containing crab shell, yet only reaching a maximum of 22.8 mg/L as $CaCO_3$ in columns containing limestone. Low levels of aluminum (0.6 mg/L) were easily removed from solution by crab shell, but broke through in columns containing limestone, eventually reaching influent concentrations within 55 days. Fermentation of crab shell released low levels of bioavailable ammonium (NH_4^+) into the water (< 18 mg/L as N), which may be helpful for restoring biological diversity in nutrient-deficient watersheds. Minimum loading criteria for crab shell and limestone to neutralize the acidic water examined in this laboratory study were determined to be 0.2 – 0.9 g/L and 13 – 60 g/L, respectively. Field-scale road-side ditches with crab shell in underdrains showed increases in pH, alkalinity, and aluminum removal of 1.19 units, 111 mg/L as CaCO3, and 0.466 mg/L, respectively, over a 3 month period. In comparison, treatment with limestone in ditches and in underdrains resulted in lower net changes in pH, alkalinity, and aluminum removal of 0.64, 6.13 mg/L as CaCO3, and 0.146 mg/L, respectively. This work suggests that crab shell may be an effective alternative substrate for the restoration of waters impacted by acid deposition due to its ability to provide excess alkalinity, remove metals, and provide trace nutrients, all with lower mass requirements and thus a smaller footprint than limestone.

Keywords: acid deposition; acid rain; crab shell; chitin; brook trout; aluminum removal

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NOTATION

PTS	Passive Treatment Systems
HRT	Hydraulic retention time
ANC	Acid-neutralizing capacity
N:P	Nitrogen to Phosphorous Ratio
TAN	Total Ammonia Nitrogen
CMC	Acute Toxic Criterion
TOC	Total Organic Carbon
ALD	Anoxic Limestone Drains
SAPS	Successive Alkalinity Producing Systems
DON	Total Organic Nitrogen
DOC	Dissolved Organic Carbon
EPA	Environmental Protection Agency
PFBC	Pennsylvania Fish and Boat Commission
ORP	Oxidation/Reduction Potential

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1. INTRODUCTION

Acid deposition due to precipitation became a major problem in the northeastern United States following the industrial revolution. As the pH of rain and snowmelt has decreased over time, the habitats of vitally import species have been detrimentally altered. In Northwestern Pennsylvania, native populations of brook trout have been reduced due, in part, to increases in acid precipitation. In this thesis, it is offered that the acidification of tributaries feeding into wilderness trout streams can be remediated, and downstream waters potentially buffered, through the use of road-side ditch underdrains filled with a novel, alkalinity-bearing substrate: crab shells.

In this thesis, crab shells, which are waste products from the seafood industry, were tested as an alternative substrate to limestone, which is the conventional material used for treating acid deposition. The stream of interest, the South Branch of Kinzua Creek, located in McKean County, PA, is plagued by low alkalinity, high acidity, low pH, and low, but significant, concentrations of the toxic metal aluminum. To test whether crab shells could successfully restore Kinzua Creek water more efficiently than limestone, a series of batch microcosm tests, continuous-flow columns, and field-scale experiments were performed.

A literature review of the problem of acid deposition, effects on fish, and potential passive treatment systems is presented in Chapter 2. Chapter 3 describes the experimental laboratory setup and the methods used to determine alkalinity and acidity, ammonium, metals, and anions concentrations. In Chapter 4, the results of the laboratory experiments (batch microcosms and continuous-flow columns) are presented. Chapter 5 discusses the results of those experiments and how they might affect the ecosystem of Kinzua Creek by increasing the pH, alkalinity, and ammonium from crab shell fermentation. In Chapter 6, a presentation of the field-scale study results is provided. Finally, in Chapter 7, are the conclusions of the laboratory study, their significance to the field of environmental engineering, and suggestions for future work.

2. LITERATURE REVIEW

2.1. ACID DEPOSITION

Acid deposition is a major problem in the United States and is mostly due to anthropogenic activities, land use practices, and low buffering capacities of natural bedrock. The problem is particularly of concern in the northeastern United States which has shown progressively decreasing pH and alkalinity of surface waters as recorded over the last 30 years (Driscol et al. 2001) (Figure 2-1). Northern Pennsylvania is an area that experiences some of the highest acid deposition rates in the nation due to the low natural buffering capacity of the surrounding bedrock (Herlihy et al. 1993). The Commonwealth of Pennsylvania, as of 2002, has “135 miles of chronically acidified streams dues to acid rain” (Schmidt, K.L. & Sharpe, W.E. 2002).

In a logical progression, the biota in many of the impacted areas has also declined with the pH in the waters that harbor them. This is not only due to the overall trend of lowered pH values in stream waters, but also due to the liberation of toxic metals that would not normally be bioavilable under neutral pHs. This has many researchers concerned for the fate of certain macroinvertebrate and fish species which are sensitive to metals accumulation (Baker, et al. 1982).

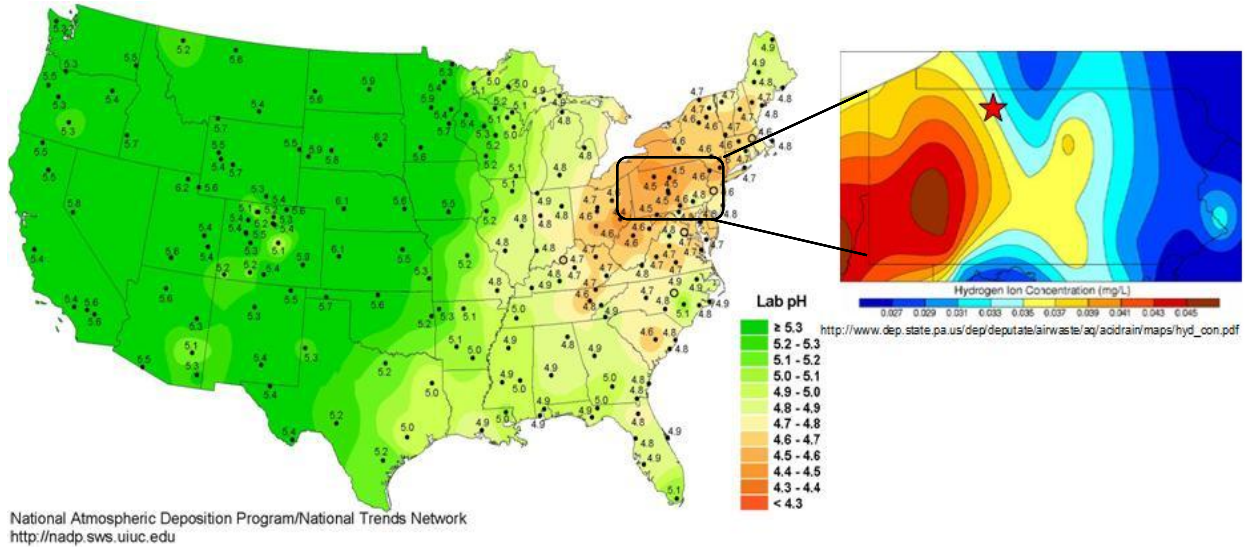


Figure 2-1. Acid Rain Deposition. Hydronium ion (H^+) concentration map made from pH measurements in the continental USA. Expanded detail shows McKean County, Pennsylvania (★), the location of Kinzua Creek watersheds, which were examined in this study. (Taken from: http://www.dep.state.ps.us/deputate/airwaste/aq/acidrain/maps/hyd_com.pdf and http://nadp.sws.uiuc.edu).

2.2. ALUMINIUM IN STREAM WATERS

Although few geological studies have characterized the area specific to the site of this study, Northern Pennsylvania's bedrock is similar to certain areas in Centre Country, Pennsylvania, which has been shown to be deficient in calcite ($CaCO_3$) and other carbonates usually considered as sources of alkalinity (Kirby et al., 2008). Kirby et al. (2008) analyzed grab samples from a geologically similar area to Kinzua Creek in Central Pennsylvania, and the clay mineral illite, among others, was found. Also in the Kirby et al. study, the weathering of illite ($KAl_2(Si_3,Al)O_{10}(OH)_2$) to kaolinite ($Al_2Si_2O_5(OH)_4$) by preferential stream flowpaths, and the drop in pH caused by acid deposition, is implicated in the release of aqueous (dissolved) aluminum hydroxides by the following reaction:

$$Al_2Si_2O_5(OH)_4 + 5H_2O \leftrightarrow 2Al(OH)_3^\circ + 2H_4SiO_4^\circ \qquad \textbf{(Eqn 2-1)}$$

Aluminum solubility is controlled by pH: as the pH rises, it is removed as a hydroxide precipitate ($Al(OH)_3$) (Robinson-Lora & Brennan, 2009). Surface waters with alkalinity less than 200 ueq/L are considered sensitive to acid deposition and the subsequent liberation of aluminum (Cleveland, 1991). Aluminum affects the function of fish gills creating respiratory and ion regulatory dysfunctions (Poléo, 1994). The Environmental Protection Agency (EPA) established chronic and acute toxicity aluminum concentrations for freshwater ecosystems: 0.750 mg/L for acute exposure and 0.087 mg/L for chronic exposure (EPA, 2004).

2.3. KINZUA CREEK

The South Branch of Kinzua Creek (hereafter referred to as Kinzua Creek), located within the Allegheny National Forest in Northern Pennsylvania's McKean County, is managed as a wilderness trout stream by the Pennsylvania Fish and Boat Commission (PFBC). Wilderness trout streams are a valuable resource in Pennsylvania, providing angling opportunities in remote areas with minimal human impacts.

Analysis of raw water taken from Kinzua Creek in February 2009 revealed that the average pH was 4.71 ± 0.44, average alkalinity was 4.25 ± 0.07 mg/L as $CaCO_3$, and average aluminum concentration was 0.53 ± 0.07 mg/L (Table 4-1). The preliminary pH and aluminum analysis of the raw water suggests a problem, not only for the ecology of Kinzua Creek, but also for the state of Pennsylvania. According to the Pennsylvania Fish and Boat Commission, recreational fishing of streams like Kinzua Creek and its tributaries generates more than $1 billion for Pennsylvania annually (PA Fish and Boat Commission, 2010). In 2010 alone, 3,959,700 trout were stocked into Pennsylvania waters with the majority (2,599,600) stocked into streams (PA Fish and Boat Commission, 2010). If the acid deposition problem continues, fish kills may become more prevalent during times of high flow rates into streams like Kinzua Creek. Many wilderness trout streams, including Kinzua Creek and its tributaries, support brook trout (*Salvelinus fontinalis*) populations which are a component of that $1 billion revenue.

2.4. BROOK TROUT

The brook trout is an ecologically and economically important species in the commonwealth of Pennsylvania. However, recent studies have demonstrated population declines throughout the Northeastern U.S. (Hudy et al., 2008). Declining brook trout populations have contributed to their recent listing as a “species of greatest conservation need” and to their addition to the Pennsylvania Wildlife Action Plan (Pennsylvania Game Commission and Pennsylvania Fish and Boat Commission, 2008). Much of the decline and/or extirpation of brook trout populations has largely been attributed to habitat loss due to acid deposition and the problems therein (Herlihy et al., 1993). Thus, improving water quality in these tributaries may restore important brook trout spawning and rearing habitat to the Kinzua Creek watershed.

Brook trout, according to Baker and Christensen (1991), are considered an “acid tolerant” fish species. Brook trout are able to tolerate chronic acidic conditions and also thrive relatively well under episodic acidification. Although the brook trout exhibit this amazing tolerance, the number of brook trout per 0.1 hectare in the Northeastern United States was found to be 215 in non-acidic streams, 82 in episodically acidic streams, and 46 in chronically acidic streams (Baker et al., 1996). Although the brook trout may be able to tolerate acidic deposition in their habitat, they are susceptible to toxic effects due to liberation of toxic aluminum, which is most evident in streams with lowered pHs (Baker et al., 1996). Furthermore, brook trout are better able to rebound from episodic acidification when water quality is restored quickly. Cleveland et al. (1991) found that after exposing brook trout to low pH/high aluminum conditions for 7 days and then restoring the water quality for 120 hours afterwards, the brook trout were better able to recover, spawn, and hatch than when continuously exposed.

2.5. USE OF PASSIVE TREATMENT SYSTEMS

Episodic acidification is caused by the high flow rates a stream experiences during storms or snowmelt. These high flow rates are not only due to addition of precipitation directly to the stream but also due to road run-off. In order to combat the increased acidity associated with high flow rates, passive treatment systems (PTS) have been used. PTS rely on "chemical and biological processes to treat acidity with little to no mechanical manipulation" (Schmidt et al., 2002). They are more common in watershed restoration projects because they are relatively inexpensive to build and operate after placement.

There are two categories for PTS treatments (Figure 2-2): Category I and Category II. Category I methods aim to raise pH and alkalinity through the: 1) liming of watersheds and wetlands; 2) addition of limestone to groundwater wells; and 3) anoxic limestone drains (ALD). Category II methods are more invasive to the surrounding areas and generally more costly. These methods aim to not only raise the pH and alkalinity but also remove metals through: 1) the addition of aerobic and anaerobic wetlands; and 2) successive alkalinity producing systems (SAPS).

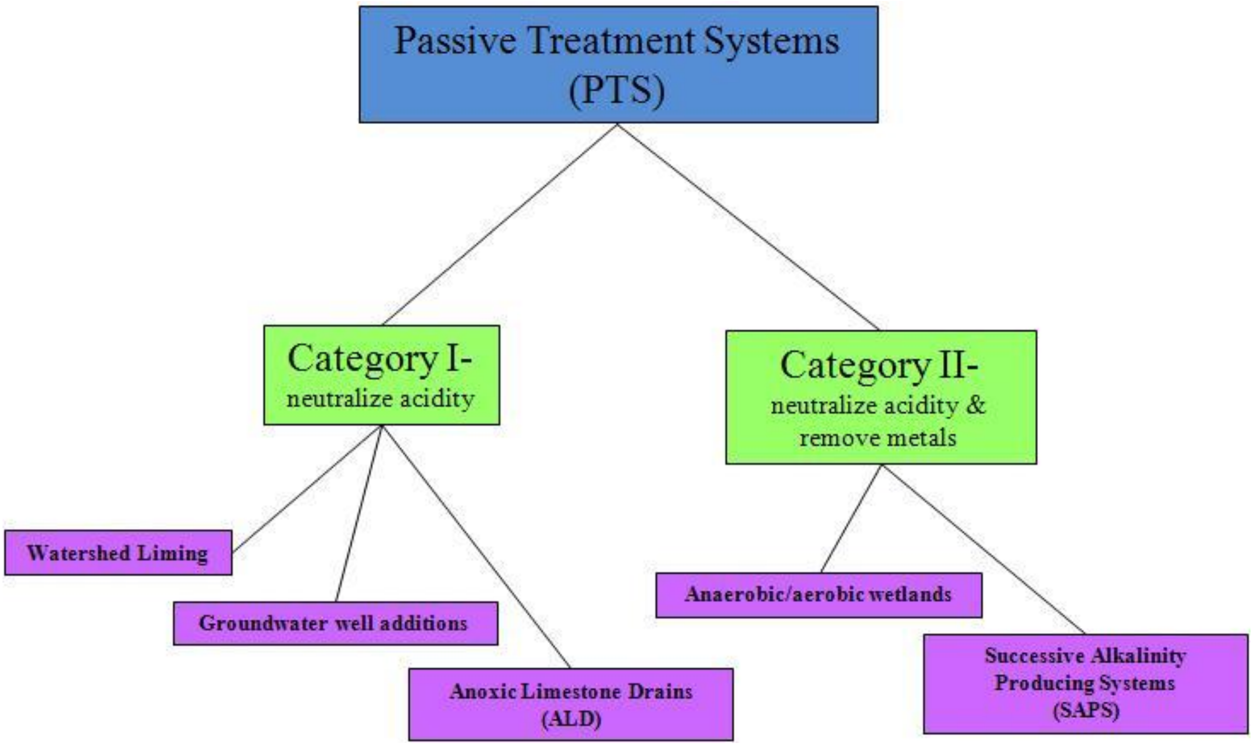


Figure 2-2. Passive Treatment Systems- Categorically Determined by mode of action. Adapted from Schmidt et al., 2002

2.6. USE OF LIMESTONE IN PASSIVE TREATMENT SYSTEMS

Traditionally, limestone rock has been used in these PTS as an alkaline agent to neutralize the acidity of the stormwater. Limestone works well to increase the pH of acidic waters, but its acid neutralizing capacity (ANC) has come under question for the continuous buffering of acidic waters. Limestone treatments work initially to restore pH values to circumneutral levels, yet a consistently higher pH is not always possible due to increased precipitation at certain times of the year (Ormerod et al. 2009). This would suggest that the buffering capacity of limestone is not effective enough to maintain higher pH values year-round. Although these types of limestone treatments can elevate pH, they have had conflicting success in their ability to restore fish species. In studies by Menendez et al. (1972) and Clayton et al. (1998) recolonization of reproducing fish populations was observed in streams which had little to no recruitment before the use of limestone. Yet others found no change in fish communities following treatment with limestone, although said events were successful in raising stream pH (Eggleton et al. 1996, Simmons & Doyle 1996, Bradley & Ormerod 2002, LeFevre & Sharpe 2002, McClurg et al. 2007).

Limestone mainly becomes a problem during episodic high precipitation events when the limestone treatments cannot neutralize the acid rapidly enough (Wheatherley et al. 1988). Furthermore, researchers in Southwestern Pennsylvania utilized limestone sand at twice the amount needed to treat an annual calculated load for the stream of interest (Keener et al. 2005). The study concluded that the double application of limestone did not produce any additional improvements in the stream water quality, did not halt the remobilization of aluminum, and did not aid in the recruitment of macroinvertebrates. Not only did these researchers not attain the type of success they were expecting for water quality with a double application of limestone, they later discovered that they had overestimated the mass of limestone required in the applications. Based upon an equation established by Clayton (1998) for the loading amount of limestone based on pH, the researchers added 26% more limestone than what was calculated to be needed. They attributed this phenomenon to the increased flows during its application. During monitoring, notable quantities of limestone were observed on the stream banks. The higher flows rate may have deposited the limestone onto these higher elevations and it wasn’t until another high flow rate event was the limestone again able to become effective. Also, substrate samplers filled with equivalent amounts of limestone by volume were placed in the streambed and sampled for macroinvertbrate densities. A negative correlation was found between the amount of limestone collected in the sampler and macroinvertbrate densities. Keener and Sharpe attribute this to the samplers becoming a hindrance to habitation by inundating the area macroinverbrates would normally occupy with limestone.

In light of previous findings, a passive treatment system must then be designed to be able to handle chronic and episodic acid deposition, provide consistent alkalinity for buffering acidic pH, prevent the remobilization of aluminum, and have a smaller footprint as to not inhibit attractive habitat conditions.

2.7. NUTRIENTS

The pH of stream waters may be able to be restored, but biodiversity in and around streams still remains the key to the brook trout’s recovery. Baldigo and Lawrence (2001) measured the density of brook trout in severely acid-impacted streams, non-acid-impacted streams, and the habitat conditions of those streams that would be attractive to brook trout. They found that, although there were lower densities of brook trout in severely impacted streams which had pH values similar to Kinzua Creek, the habitat conditions of the trout were the most important factor in population densities. It was apparent from the findings of Baldigo and Lawrence (2001) that undercut banks, stream flow, and channel width were the conditions that attracted brook trout. This study proposes that a circum-neutral pH value is not the only factor that will enhance brook trout recruitment to Kinzua Creek. In addition to stream characteristics which are hard to control (i.e., habitat conditions found in Baldigo and Lawrence (2001)), nutrients within a watershed are vital in creating attractive habitat conditions for brook trout.

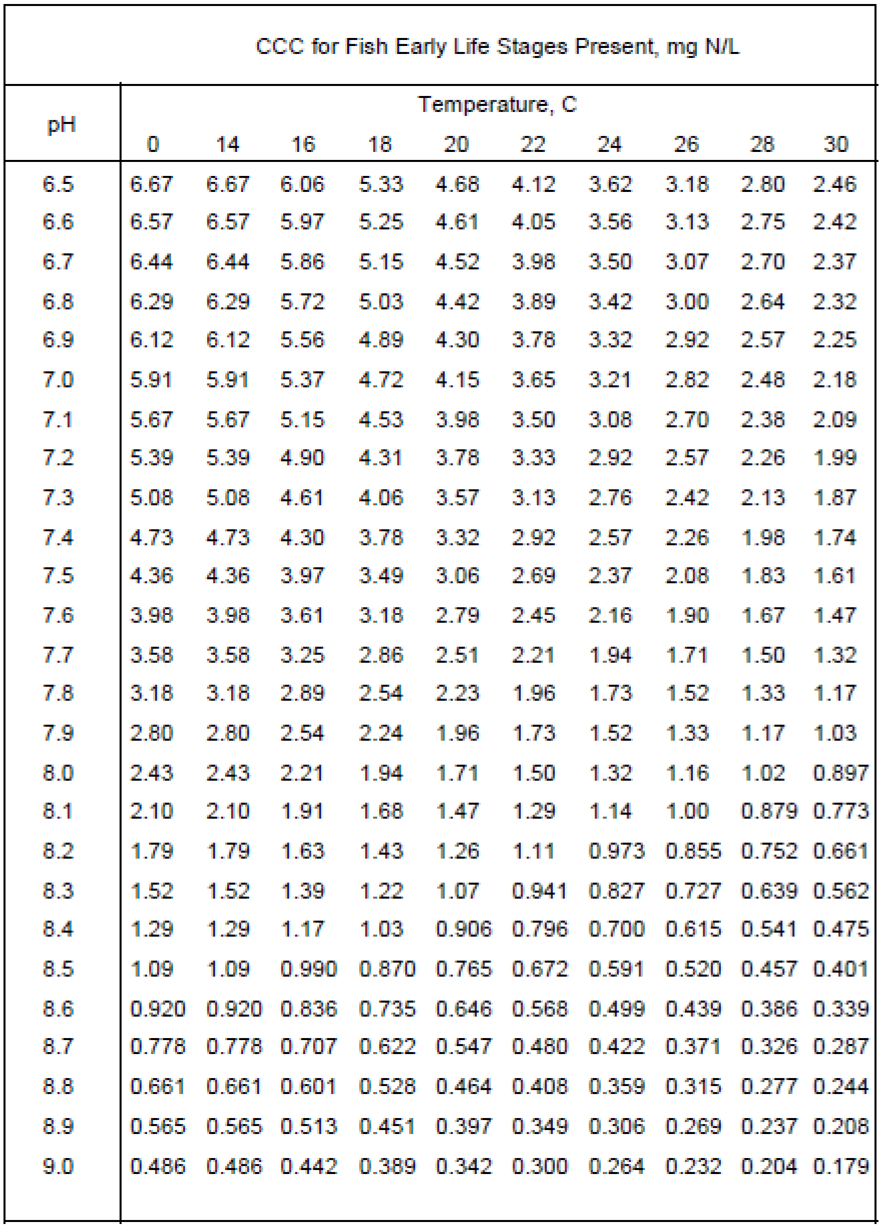
Watersheds require the macronutrients nitrogen and phosphorous to be able to support aquatic life (Smith & Tran, 2010). Phosphorous is usually the most limiting nutrient in watersheds, and thus is in higher demand over nitrogen by plants and animals that make their homes near or in the streams.

Excess nutrients can be problematic, however. An overabundance of nitrogen, as well as the addition of phosphorous in excess of nitrogen, can lead to eutrophication. For this reason, state water quality standards specify that phosphorous should generally be below 0.1 mg/L, nitrate levels should be below 1.0 mg/L (0.23 mg/L as N), and ammonia in streams should be less than 0.03 mg/L (0.023 mg/L as N) in unpolluted freshwater bodies (EPA, 1986). Watersheds which are deficient in these nutrients are more likely to experience shifts in macroinvertebrate and other biological communities.

In this study, ammonium (NH_4^+) and ammonia (NH_3), or total ammonia nitrogen (TAN, NH_4^+ and NH_3 together), are important nutrients due to their potential release during crab shell fermentation. Toxic limits of nitrogen to various aquatic species have been established by the EPA (Table 2-3). The speciation of NH_4^+/NH_3 is pH (pKa = 9.3) and temperature dependant at different life stages of fish; therefore, the EPA (Document EPA-822-R-99-014) has established limits of acute toxicity (CMC) based on pH and life stage of fish present in a watershed by equation 2-2:

$$CMC = \left(\frac{0.275}{1+10^{7.204-pH}} + \frac{39.0}{1+10^{pH-7.204}}\right) \qquad \textbf{(Eqn. 2-2)}$$

Table 2-1. EPA Chronic Criterion for Ammonia in Surface waters with early life stage species inhabitants



CCC for Fish Early Life Stages Present, mg N/L

pH	Temperature, C									
	0	14	16	18	20	22	24	26	28	30
6.5	6.67	6.67	6.06	5.33	4.68	4.12	3.62	3.18	2.80	2.46
6.6	6.57	6.57	5.97	5.25	4.61	4.05	3.56	3.13	2.75	2.42
6.7	6.44	6.44	5.86	5.15	4.52	3.98	3.50	3.07	2.70	2.37
6.8	6.29	6.29	5.72	5.03	4.42	3.89	3.42	3.00	2.64	2.32
6.9	6.12	6.12	5.56	4.89	4.30	3.78	3.32	2.92	2.57	2.25
7.0	5.91	5.91	5.37	4.72	4.15	3.65	3.21	2.82	2.48	2.18
7.1	5.67	5.67	5.15	4.53	3.98	3.50	3.08	2.70	2.38	2.09
7.2	5.39	5.39	4.90	4.31	3.78	3.33	2.92	2.57	2.26	1.99
7.3	5.08	5.08	4.61	4.06	3.57	3.13	2.76	2.42	2.13	1.87
7.4	4.73	4.73	4.30	3.78	3.32	2.92	2.57	2.26	1.98	1.74
7.5	4.36	4.36	3.97	3.49	3.06	2.69	2.37	2.08	1.83	1.61
7.6	3.98	3.98	3.61	3.18	2.79	2.45	2.16	1.90	1.67	1.47
7.7	3.58	3.58	3.25	2.86	2.51	2.21	1.94	1.71	1.50	1.32
7.8	3.18	3.18	2.89	2.54	2.23	1.96	1.73	1.52	1.33	1.17
7.9	2.80	2.80	2.54	2.24	1.96	1.73	1.52	1.33	1.17	1.03
8.0	2.43	2.43	2.21	1.94	1.71	1.50	1.32	1.16	1.02	0.897
8.1	2.10	2.10	1.91	1.68	1.47	1.29	1.14	1.00	0.879	0.773
8.2	1.79	1.79	1.63	1.43	1.26	1.11	0.973	0.855	0.752	0.661
8.3	1.52	1.52	1.39	1.22	1.07	0.941	0.827	0.727	0.639	0.562
8.4	1.29	1.29	1.17	1.03	0.906	0.796	0.700	0.615	0.541	0.475
8.5	1.09	1.09	0.990	0.870	0.765	0.672	0.591	0.520	0.457	0.401
8.6	0.920	0.920	0.836	0.735	0.646	0.568	0.499	0.439	0.386	0.339
8.7	0.778	0.778	0.707	0.622	0.547	0.480	0.422	0.371	0.326	0.287
8.8	0.661	0.661	0.601	0.528	0.464	0.408	0.359	0.315	0.277	0.244
8.9	0.565	0.565	0.513	0.451	0.397	0.349	0.306	0.269	0.237	0.208
9.0	0.486	0.486	0.442	0.389	0.342	0.300	0.264	0.232	0.204	0.179

Taken from EPA, “1999 Update for Ambient Water Quality Criteria for Ammonia”.

2.8. EUTROPHICATION

Nitrogen and phosphorous are the two most vital nutrients in eutrophication with phosphorus being the most limiting nutrient in aquatic ecosystems (Sharma et al. 2009). Sharma, et al. (2009) found that it was phosphate (PO_4^{3-}) that played the biggest role in addition of phosphorous to waters experiencing eutrophication. They also found that there is a hierarchy with regards to preferred forms of nitrogen for eutrophication by microorganisms. According to their results, nitrate (NO_3^-) is the most favorable form, followed by nitrite (NO_2^-), and ammonium (NH_4^+). In most plants, however, ammonia is the preferred nitrogen source since it does not require further reduction before use.

Eutrophication is marked by algal (particularly cyanobacterial) ‘blooms’. These blooms are the major cause of decreased water quality from excess nutrients being fed into surface waters. Some of the water quality issues include toxicity to aqueous life and food web alterations. Cynaobacteria, blue-green algae, are the most notorious bloom formers (Paerl et al. 2001). Other nutrients and chemical factors play a role in algal blooms as well (i.e., dissolved oxygen, temperature, iron concentrations) but the overarching factor implicated in the majority of algal blooms observed in freshwater ecosystems with varying physical conditions is a nitrogen to phosphorous ratio (N:P) of lower than 29:1 (Sharma et al., 2009, and Smith, 1983). Flett et al (1980) found that the key ratio for nitrogen-fixing cyanobacteria eutrophication in lakes is a N:P of 10:1 or lower.

2.9. USE OF CRAB SHELL IN PTS

In this study, the use of crab shell was evaluated as an alternative source of alkalinity to neutralize acid precipitation and supply additional buffering capacity to downstream watersheds.

Crab shells are composed primarily of calcium carbonate ($CaCO_3$), protein, and chitin. Crab shell has a higher surface area than limestone, making the biogenic $CaCO_3$ more available for dissolution into acid-impacted waters. When applied for the treatment of acid mine drainage, the $CaCO_3$ in crab shells has previously been shown to rapidly neutralize acidity and remove dissolved metals as hydroxide- or carbonate-precipitates (Daubert & Brennan, 2007; Robinson-Lora & Brennan, 2010). The protein and chitin in the shells provide respectively rapid and slow-release of nutrients such as nitrogen.

Crab shells release ammonium due to fermentation. This fact could be beneficial to aquatic life living in nutrient-deficient waters. Yet, the toxic nature of ammonium/ammonia at certain concentrations above those which are acceptable in natural waters (Table 2-3) could pose a threat to fish, as well as contribute to eutrophication. The release of fatty acids, ammonia, and the rapid dissolution of $CaCO_3$ all contribute to elevated alkalinity with crab shells (Korte et al. 2008). As long as the ammonia concentrations are within acceptable levels, toxicity and eutrophication should not be a concern, and the ammonia should only affect the alkalinity which, when lacking, is the root cause of problems associated with acid deposition.

The solid nature of crab shell makes it easily applied in a variety of settings, while its particle size and non-swelling nature help to maintain porosity and prevent clogging in continuous-flow systems (Brennan, 2003). Due to the abundance of crab shell, being mainly a waste product of the seafood industry, means availability is not limited and costs are relatively low ($0.60/lb, JRW Bioremediation, LLC). Although crab shell has not been directly evaluated in treating acid deposition in streams, our lab has extensive experience using crab shell as a treatment material to mitigate a variety of pollutants, including the high acidity and metals contents of mine impacted waters at both the lab and field scales (Venot et al., 2008). All of these factors make crab shells an attractive substrate for treating contaminated water systems where nutrients and space may be limiting (Vera et al., 2001; Brennan et al., 2006).

2.10. HYPOTHESIS

It was hypothesized that crab shell would neutralize pH, enhance alkalinity, and remove dissolved metals from waters affected by acid deposition and provide better treatment for affected streams for a lower cost and at a smaller footprint than limestone. To test this hypothesis, a series of microcosm (batch) tests and continuous-flow column experiments were conducted with acid rain water collected from a tributary to the South Branch of Kinzua Creek. These experiments explored the hypothesis through the completion of the following main tasks:

1. Determine if crab shell will neutralize pH, enhance alkalinity, and remove dissolved metals from waters affected by acid precipitation more efficiently than limestone through laboratory testing.
2. Measure any added undesirables to the water as a byproduct of crab shell treatment.

The results of the above tasks were used to guide field-scale testing of crab shell for the treatment of acid deposition in the Kinzua Creek watershed. Ken Anderson of the Pennsylvania Fish and Boat Commission oversaw the field study using ditch design from The Center for Dirt and Gravel Studies at Penn State. To support the field activities, the following subtasks were also completed in this study and are presented in Chapter 6 for completeness:

1. Calculate the loading criteria of crab shell necessary to achieve efficient treatment.

2. Determine a breakdown of costs for the treatment of acid deposition in Kinzua Creek with crab shell as opposed to other, more common alternatives.

3. MATERIALS & METHODS

3.1. CHEMICALS

All chemicals used in this study were reagent grade or higher quality. ChitoRem® SC-20 (minimally processed crab shell), derived from Dungeness crab (JRW Bioremediation, LLC, Lenexa, KS), was used as the crab shell source. Previous work indicates that SC-20 contains ~10% chitin ($C_8H_{13}NO_5$), ~12% protein ($C_{16}H_{24}O_5N_4$), and ~78% mineral matter (35% as CaO) and has a specific surface area of 14 m^2/gram (Robinson-Lora & Brennan, 2010). The water used in the microcosm experiments was obtained from a tributary to the South Branch of the Kinzua Creek, just upgradient of the proposed crab shell PTS at approximately 41,43'34.29"N and 78,45'4.15"W. The water was collected on January 11, 2009, into 5 polypropylene jerricans and carboys with minimal headspace, transferred immediately to the laboratory, and held at room temperature (22 ± 2°C) in the dark until the experiments were initiated. Within 3 days of collection, the water from each collection vessel was analyzed for pH, acidity, alkalinity, ammonia, anions, and metals (Table 1). Silica sand (16-20 mesh, Badger Mining Corp., Berlin, WI) was used as an inert packing material in the column experiments and was washed overnight in 0.25 M nitric acid, thoroughly rinsed with deionized water, and dried at $105^{o}C$ before use. The purpose of this acid wash was to prevent any metallic residues on the sand from leaching into the water during the experiment. The limestone used in the column study (#10 aggregate, New Enterprises Stone & Lime, Tyrone Forge, PA) was selected based on its use in the companion field study and had a reported composition of 50-55% $CaCO_3$ and 35-45% $MgCO_3$.

3.2. MICROCOSM TESTS

Microcosm tests were used to rapidly assess the ability of crab shell to achieve remediation of acid precipitation. For the microcosm experiment, 0.045 g of crab shell was added to 24 replicate 50 mL non-sterile polypropylene centrifuge tubes. This mass of crab shell was selected based on previous studies which found that a loading of 1 g/L was effective at neutralizing acid mine drainage (Robinson-Lora & Brennan, 2010). After adding the crab shell, the vessels were filled with 45-mL of Kinzua Creek water and sealed with 5 ml air headspace (Figure 3-1). Negative controls (without added crab shell) were also established. The microcosms were incubated at room temperature (22 ± 2°C) in the dark, and shaken horizontally on an orbital shaker for a total time of 10 days. Periodically during the course of the experiment (at 0, 1.5, 4, 6, 12, and 24 hours, and 2, 4, and 10 days), duplicate active microcosms and singlet controls were sacrificed and the aqueous contents analyzed for pH, acidity, alkalinity, ammonium, anions, and metals.

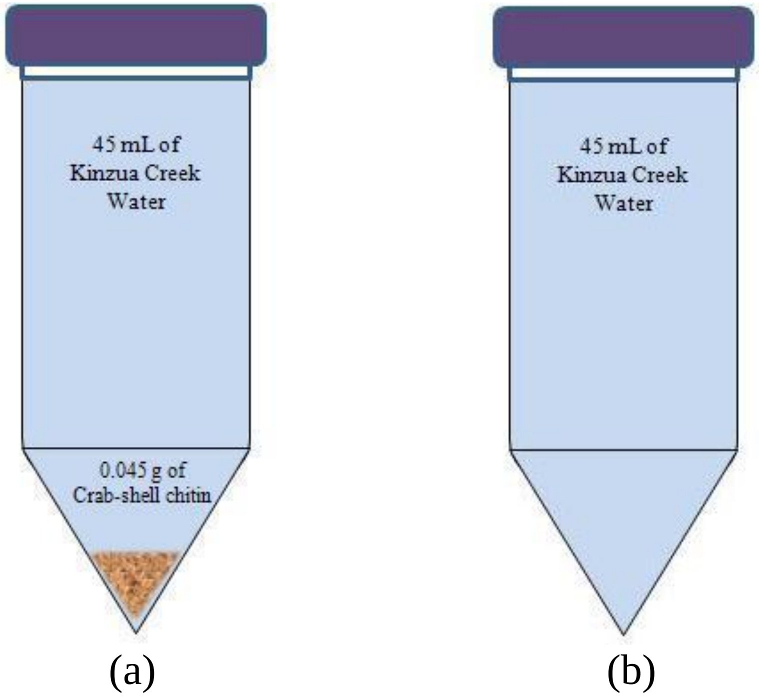


Figure 3-1. **Microcosm Set-up.** Active microcosm, (a), with 45 mL of Kinzua Creek water and 0.045 mg of crab shell, and control microcosm, (b), with 45 mL of Kinzua Creek water only.

3.3 CONTINUOUS-FLOW COLUMN SETUP

Column studies were conducted to quantify acidity reduction rates, metal removal capacities, and confirm suitable retention times when crab shell and/or limestone is used as a barrier material for acid precipitation treatment. Four, 1-foot long, 3-cm inner diameter, clear, Schedule 40 PVC columns with matching end caps (United States Plastic Corp.) were washed with laboratory-grade detergent and tap water, rinsed with deionized water, and air dried prior to use. Polycarbonate stopcocks with luer fittings (Cole-Parmer) were attached to the end-caps to allow for sampling of the influent and effluent water. The columns were wet-packed in free-standing Kinzua Creek water. Packing of each column took an average of 45 minutes to complete, with the crab shell-containing columns packed last. The columns were placed in-line with a continuous feed of Kinzua Creek water directly after packing was completed. The columns were packed with the following materials (where the percentages listed are by volume): 1) 100% sand only (control); 2) 100% limestone only; 3) a mixture of 50% crab shell and 50% sand; and 4) a mixture of 50% crab shell and 50% limestone (Figure 3-2). The mass of materials packed into each column is provided in Table 3-1.

Table 3-1. Masses of packing materials used in the continuous-flow columns.

Column	Contents	Mass (grams)
1	Sand	661.6
2	Limestone	730.5
3	50% Crab shell, 50% Sand	106.6 (crab shell) 160.0 (sand)
4	50% Crab shell, 50% Limestone	106.6 (crab shell) 182.6 (limestone)

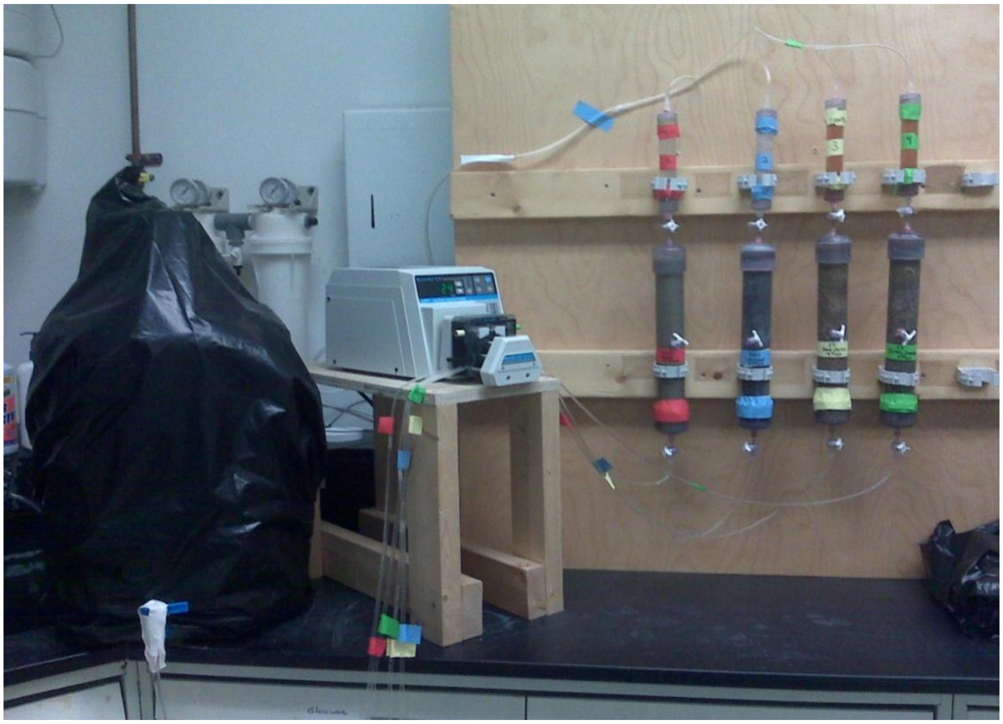


Figure 3-2. Continuous-flow columns in the laboratory. Black bag is covering Kinzua Creek raw water being continuously fed through pump to columns. Columns are (left to right) 100% Sand, 100% Limestone, 50% Crab Shell, 50% Sand, and 50% Crab Shell, 50% Limestone. Sampling ports above columns collect effluent water for analysis.

A multichannel peristaltic pump was used to deliver Kinzua Creek water through the columns at rate of 0.5 mL/min, resulting in a hydraulic residence time (HRT) of 6 hours, which is representative of local runoff flow during an average storm event for the tributaries that were used in the field study. Aqueous samples were collected from the effluent sampling ports of each column every 1 to 7 days, depending on the observed rate of remediation, and analyzed for pH, acidity, alkalinity, ammonium, anions, and metals. All samples were taken in singlet from each column and analyzed along with a sample of the influent water. The columns were run for a total of 55 days, which was the time it took for all the collected water to be exhausted. Throughout the experiment, the columns were kept at room temperature (22 ± 2°C) in the dark.

3.4. ANALYTICAL METHODS

Electrodes were used to measure pH (Accumet® BASIC, AB15 connected to a Thermo-ORION pH probe) and ammonia concentrations (ISE ORION 9512) which were non-detect < 1 mg/L, and standard laboratory techniques were used to measure hot acidity and alkalinity titrations (Methods 2310 and 2320; APHA, 1998). The endpoint for alkalinity titrations was pH 4.5 and the endpoint for acidity titrations was 8.3. Anions were measured using a Dionex DX-100 ion chromatograph (IC) with an AG4A IonPac analytical column and an AS4A guard column according to standard laboratory techniques (Methods 4110). Detection limits were determined experimentally by running a standard curve from 1 mg/L to 100 mg/L of a combined stock under the same conditions as sample analysis. Any values below or above those end points are extrapolated values. Samples for anion analysis were filtered with a 0.2 um filter and stored at 4°C until analyzed. Samples for metals analysis were preserved with 70% nitric acid and stored at 4°C until analyzed. Dissolved metals were analyzed on a Perkin-Elmer Optima 5300 ICP (inductively coupled plasma emission spectrometer) by the Materials Research Institute on the Penn State University campus using standard laboratory techniques (Methods 3500, Part C). Detection limits for metals analysis were as follows: aluminum and manganese, 10 ug/L; calcium, 200 ug/L; iron, 50 ug/L; and sodium, 200 ug/L.

4. RESULTS

4.1.RAW WATER ANALYSIS

Before microcosm testing was initiated, preliminary testing was done on the 5 samples of Kinzua Creek raw water (Table 4-1). A table of the individual results can be found in Appendix B-1.

Table 4-1. Raw water quality analysis of a tributary to the South Branch of Kinzua Creek impacted by acid deposition. Concentrations are quadruplicate averages.

Analyte	Concentration
pH	4.71 ± 0.44
Acidity (mg/L as $CaCO_3$)	27.0 ± 0.07
Alkalinity (mg/L as $CaCO_3$)	4.25 ± 0.07
Aluminum (mg/L)	0.53 ± 0.01
Ammonium (mg/L as N)	< 1.0
Calcium (mg/L)	0.96 ± 0.08
Chloride (mg/L)	< 1.0
Iron (mg/L)	0.01 ± 0.01
Manganese (mg/L)	0.28 ± 0.01
Nitrate (mg/L)	2.6 ± 0.3
Phosphate (mg/L)	2.7 ± 0.2
Sodium (mg/L)	0.40 ± 0.08
Sulfate (mg/L)	9.3 ± 0.5

4.2.MICROCOSM EXPERIMENT

4.2.1. TEN DAY MICROCOSM EXPERIMENT

In the microcosm experiment, an overall trend of increasing pH and alkalinity and decreasing acidity over the ten days was observed, reaching final values of 8.20 ± 1.27 for pH, 136.80 ± 27.01 mg/L as $CaCO_3$ for alkalinity, and -100.3 ± 5.52 mg/L as $CaCO_3$ for acidity (Figure 4-1).

TAN was below detection (< 1 mg/L) until day 10 when it reached a value of 4.80 ± 3.73 mg/L as N, which corresponds to 0.25 ± 0.19 mg/L of free ammonia (NH_3).

The average anion content increased from 2.11 ± 0.59 mg/L to 90.75 ± 1.37 mg/L for chloride (Cl^-), but remained relatively constant for NO_3^- (2.55 ± 0.08 mg/L) and PO_4^{3-} (3.05 ± 0.37 mg/L) over the ten days. Sulfate (SO_4^{2-}) reached a maximum concentration of 21.86 ± 0.25 mg/L at the 6-hour time point but had an average concentration of 16.37 ± 1.39 mg/L throughout the experiment (Figure 4-2). Average metals concentrations at the end of the experiment were 0.37 ± 0.44 mg/L Al, <0.01 mg/L Fe, 0.05 ± 0.04 mg/L Mn, 37.37 ± 6.01 mg/L Ca, and 40.35 ± 1.20 mg/L Na (Figure 4-3). A summary of water quality measurements for the 10-day microcosm experiment can be found in Table 4-3.

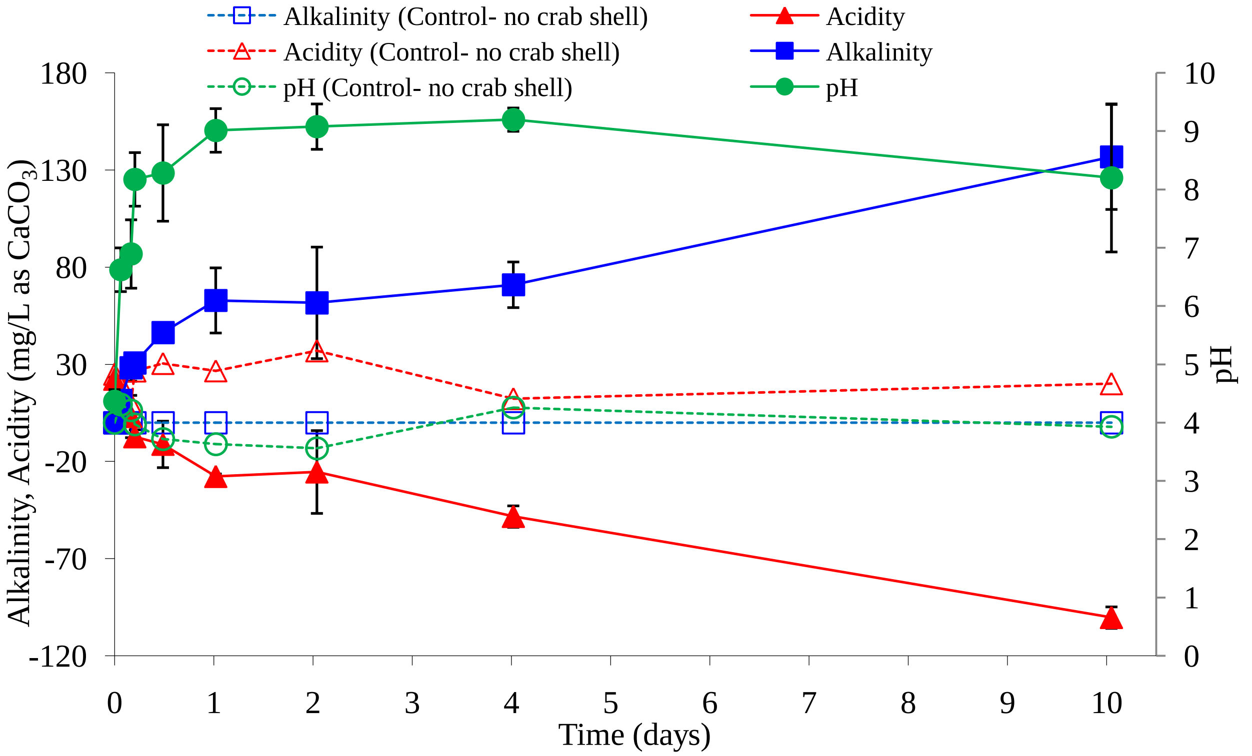


Figure 4-1. pH and alkalinity of 10-day microcosm experiment treating acid-precipitation impacted water. Actives are dulpicate averages; controls are in singlet; error bars represent one standard deviation.

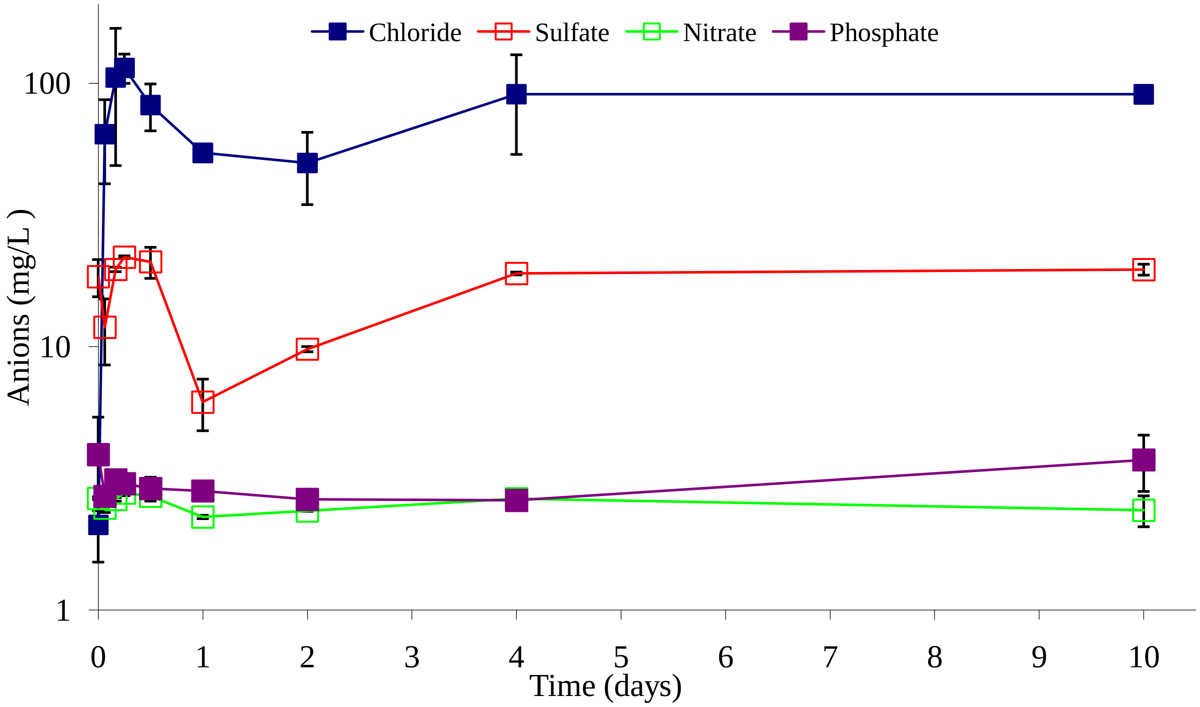


Figure 4-2. Anion analysis of 10-day microcosm experiment treating acid-precipitation impacted water. Data points are duplicate averages with error bars representing one standard deviation.

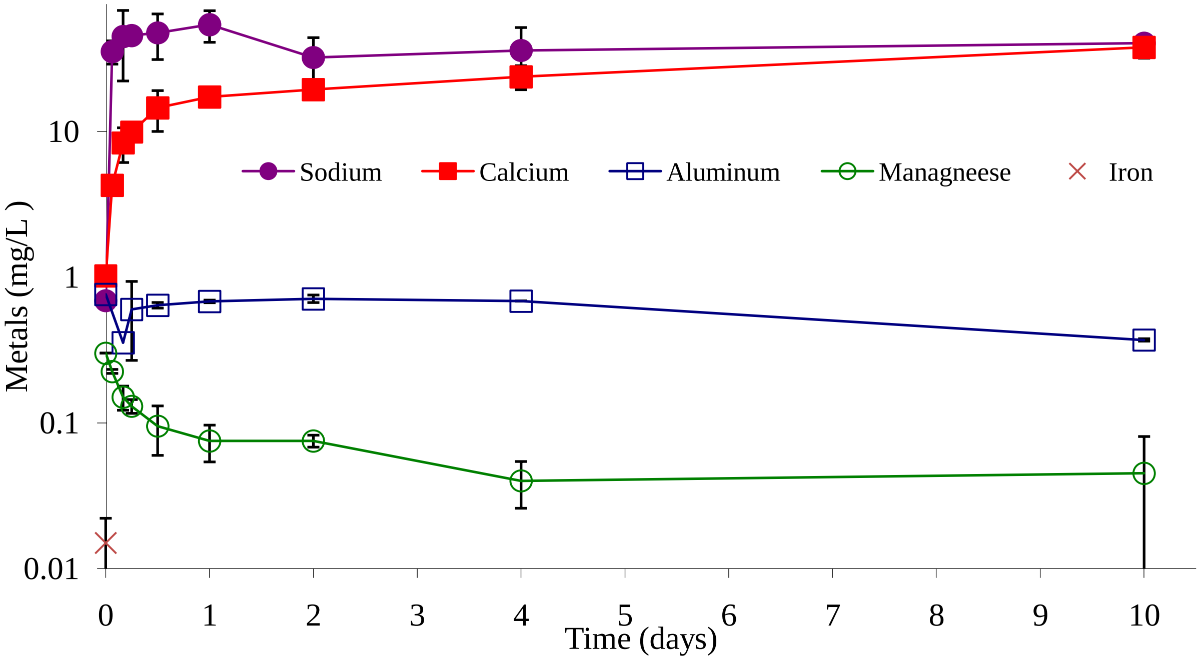


Figure 4-3. Metals analysis of 10-day microcosm experiment treating acid-precipitation impacted water. Data points are duplicate averages with error bars representing one standard deviation.

Table 4.3. Water quality analysis of the 10-day microcosm experiment testing crab shell treatment of acid-impacted waters.

Analyte	0 Hour	10 Days
pH	4.37 ±0.20	8.20 ±1.27
Alkalinity (mg/L as $CaCO_3$)	0.00	136.8 ±27.0
Acidity (mg/L as $CaCO_3$)	22.35 ±1.06	-100.3 ±5.52
TAN (mg/L as N)	0.00	4.80 ±3.41
Chloride (mg/L)	2.11 ±0.59	89.78 ±1.37
Nitrate (mg/L)	2.65 ±0.03	2.39 ±0.32
Phosphate (mg/L)	3.89 ±1.51	3.72 ±0.90
Sulfate (mg/L)	18.44 ±3.00	19.61 ±0.94
Aluminum (mg/L)	0.760	0.37 ±0.44
Iron (mg/L)	0.02 ±0.01	<0.01
Manganese (mg/L)	0.300	0.05 ±0.04
Calcium (mg/L)	1.02 ±0.03	37.75 ± 6.01
Sodium (mg/L)	0.63 ±0.04	40.35 ±1.20

4.2.2. SIX HOUR MICROCOSM EXPERIMENT

To better understand the chemical changes that were occurring upfront at early times in the 10 day microcosm experiment, a shorter microcosm experiment was conducted under the same conditions, but with more frequent sampling points at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, and 6 hours. That experiment yielded similar results: pH and alkalinity rose from 3.13 ± 0.01 to 7.62 ± 0.81 and 0.00 to 37.15 ± 7.14 mg/L as $CaCO_3$, respectively. Acidity decreased from 24.30 ± 4.38 to 3.10 ± 10.61 mg/L as $CaCO_3$. Total ammonia nitrogen (TAN) was non-detect until hours 1, 1.25, and 1.5 when it measured 6.12 ± 4.76, 3.27 ± 3.17, and 0.45 ± 1.59 mg/L as N. Total free ammonia measurements for those 3 timepoints were <0.001 mg/L as N. After 1.5 hours, TAN became non-detect again for the remainder of the experiment. Analysis of metals concentrations showed Al removal from a starting concentration of 0.77 ± 0.01 to 0.625 mg/L over the 6 hours of the experiment. Ca and Na increased in the system from 0.02 ± 0.01 to 10.97 ± 3.30, and from 0.66 ± 0.02 to 22.55 ± 4.60 mg/L, respectively. Fe and Mn were below the limit of detection (0.01 mg/L) throughout the 6 hour microcosm experiment. All tables and figures for the 6-hour microcosm experiment can be found in Appendix B-2.

4.3. CONTINUOUS-FLOW COLUMN EXPERIMENT

The behavior of crab shell in the column study followed patterns similar to those observed during its use as a substrate for the treatment of acid mine drainage (Robinson-Lora and Brennan, 2009). Within the first 2 hours of continuous-flow treatment with crab shell, pH and alkalinity rapidly increased to 8.1 and 1627 mg/L as $CaCO_3$, respectively, with corresponding decreases in acidity (Figures 4-4 & 4-5). This was followed by a decrease in alkalinity over the next two days to a local minimum of 241 mg/L as $CaCO_3$, although pH continued to increase during this period to a maximum of 9.2. After this initial period, alkalinity slowly rose over the next 30 days to a maximum of 1045 mg/L as $CaCO_3$, and then slowly decreased to 70 mg/L as $CaCO_3$ by the end of the experiment at 55 days.

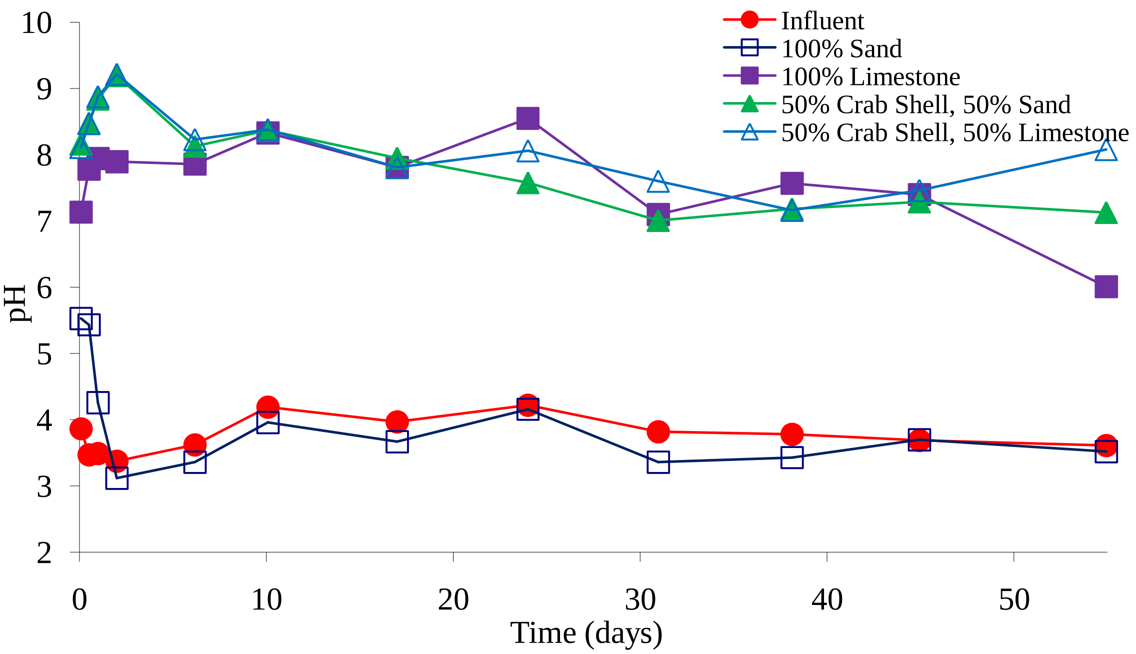


Figure 4-4. pH changes in continuous-flow column studies over 55 days treating acid-precipitation impacted waters. Data points are singlet measurements.

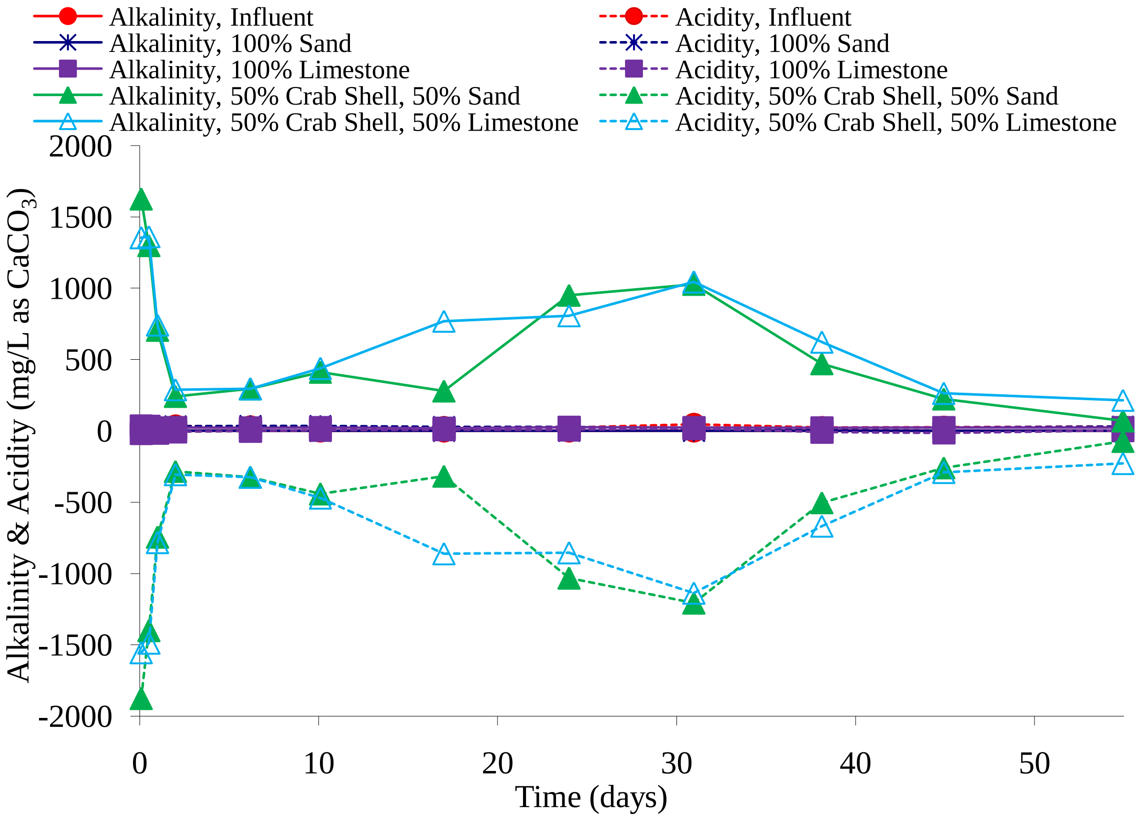


Figure 4-5. Alkalinity and acidity changes in continuious-flow column studies over 55 days treating acid-precipitation impacted waters. Data points are singlet measurements.

The initial rapid neutralization and buffering was likely caused by crab shell fines in the system, which readily dissolved in the acidic influent water. Similarly, salt dissolution from the surface of the crab shells is likely responsible for peak sodium concentrations (4,606 mg/L) within the first 2 hours of treatment, followed by an exponential decrease to 3.3 mg/L at 10 days as the salts were washed out of the system, eventually reaching a final background value of 1.5 mg/L by the end of the experiment (Figure 4-6).

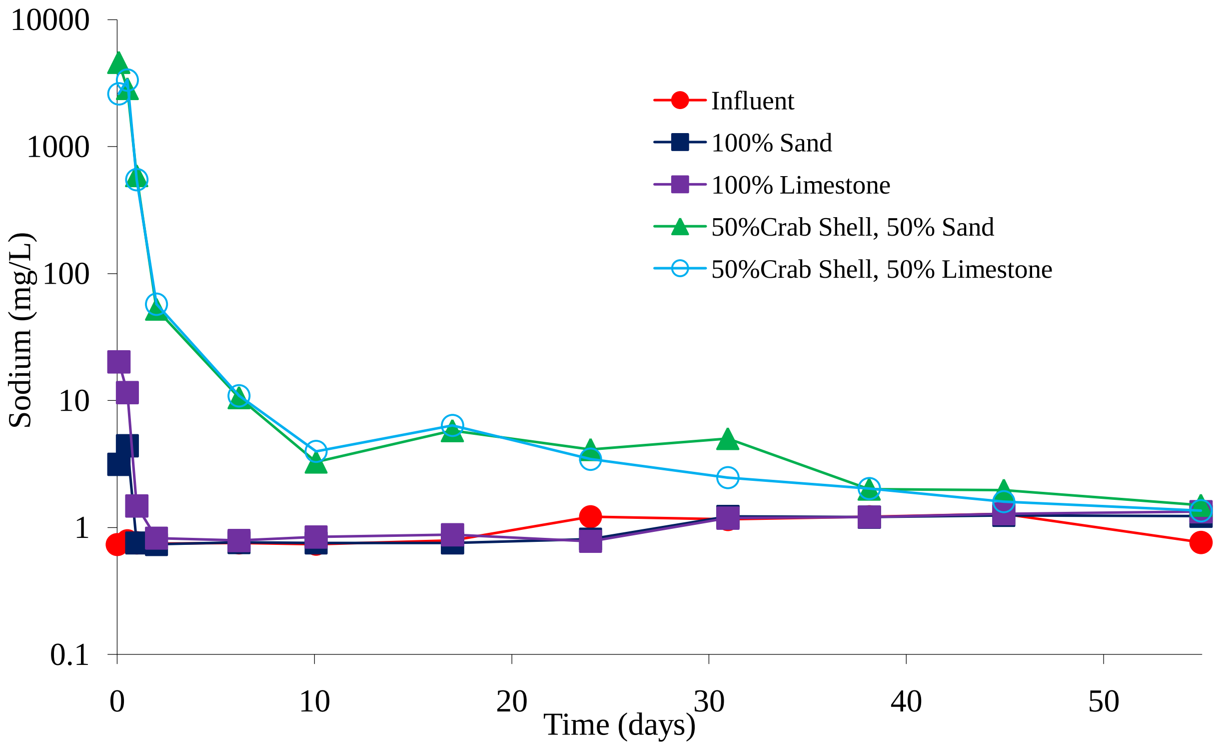


Figure 4-6. Sodium concentrations in continuous-flow columns over 55 days treating acid-precipitation impacted water. Data are singlet measurements.

Rapid fermentation of available protein in the crab shells likely caused the initial spike in TAN (16 mg/L as N) at 2 hours, followed by a more gradual fermentation of chitin over time which resulted in a maximum concentration of 18 mg/L as N at 31 days, gradually falling to 8 mg/L as N by day 55 (Figure 4-7). It is important to note that the speciation of nitrogen in the columns would have been predominantly as the bioavailable ammonium (NH_4^+), rather than the more toxic ammonia gas (NH_3) due to intra-column pH values below the acid dissociation constant of ammonium (pKa = 9.3). The measured total ammonia nitrogen (TAN) and calculated free ammonia (NH_3) values based on pH are shown in Table 4-4.

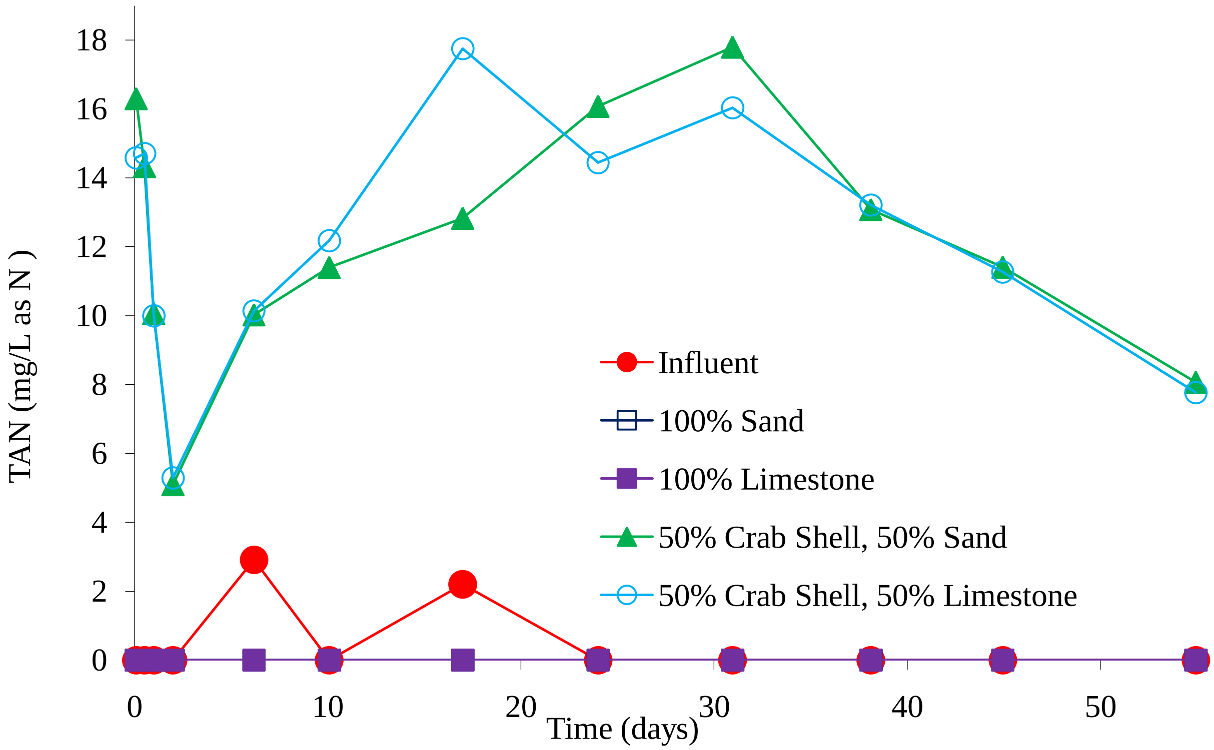


Figure 4-6. Total ammonia nitrogen (TAN) concentration as nitrogen in continuous-flow column experiments over 55 days treating acid-precipitation impacted water. Data are singlet measurements.

Although effluent pH values were similar in systems containing either limestone or crab shells, the concentrations of alkalinity, sodium, ammonium, and calcium (Figure 4-7) were much higher in systems containing crab shell. In fact, it is clear that the crab shell was controlling the overall values of these analytes, even in systems when crab shell was combined with limestone. Nevertheless, aluminum was removed to below detection (< 0.01 mg/L) in all active treatments until day 24 when it began to break through in columns containing limestone only, returning to influent values by day 55 (Figure 4-8). Except for an isolated point at day 24, columns containing crab shell maintained aluminum at levels below detection (10 ng/L) throughout the entire experiment. A summary of water quality measurements for the initial influent (t = 2 hours) and final effluent (t = 55 days) for each column is provided in Table 4-5.

Table 4-4. Total ammonia nitrogen (TAN) and free ammonia (NH_3) concentrations in the effluent of columns containing crab shells throughout the continuous-flow column experiments as a function of pH.

Timepoint	**pH**	**50% Crab Shell, 50% Sand**		**pH**	**50% Crab Shell, 50% Limestone**	
(days)	**(-)**	**TAN (mg/L as N)**	**NH_3 (mg/L as N)**	**(-)**	**TAN (mg/L as N)**	**NH_3 (mg/L as N)**
0.1	8.16	16.31	0.77	8.11	14.59	0.62
0.5	8.48	14.33	1.34	8.47	14.72	1.36
1	8.84	10.07	1.94	8.88	10.01	2.06
2	9.20	5.11	1.79	9.22	5.30	1.91
6	8.13	10.03	0.45	8.23	10.15	0.56
10	8.37	11.41	0.85	8.38	12.19	0.93
17	7.95	12.83	0.38	7.81	17.76	0.39
24	7.58	16.08	0.21	8.06	14.45	0.55
31	7.01	17.81	0.06	7.60	16.04	0.22
38	7.18	13.09	0.07	7.16	13.22	0.07
45	7.29	11.41	0.08	7.46	11.28	0.11
55	7.13	8.08	0.04	8.08	7.78	0.31

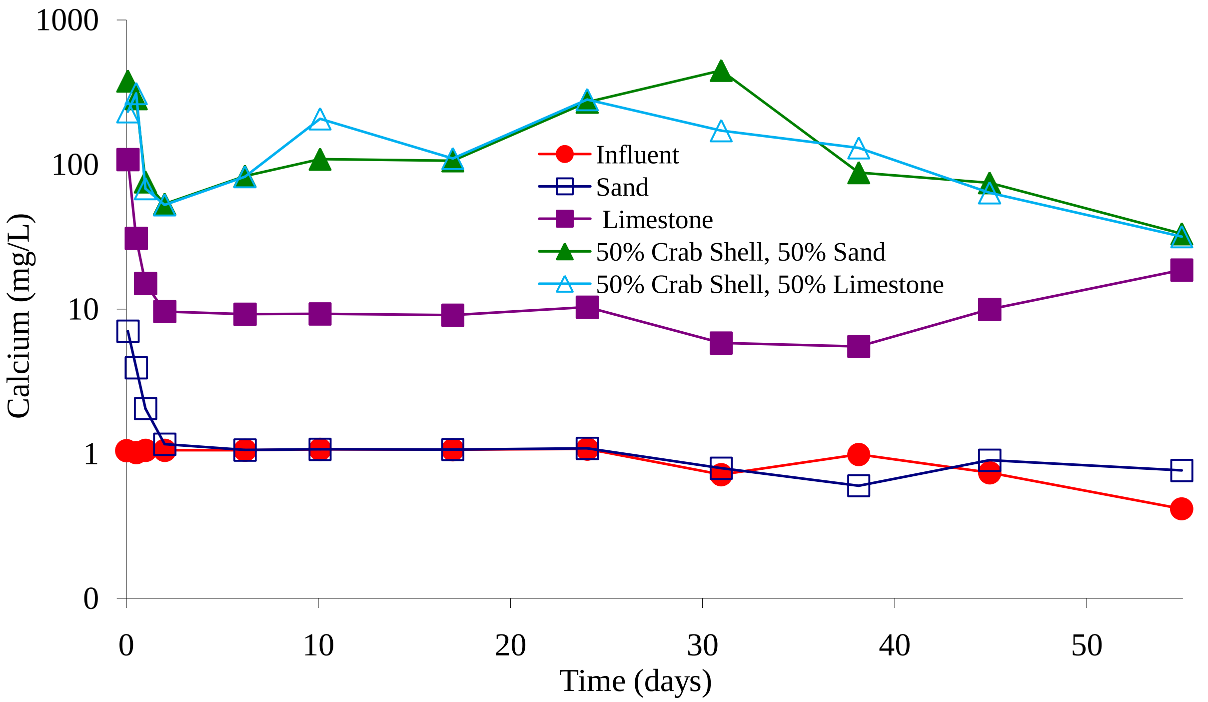


Figure 4-7. Calcium concentrations in the effluent of continuous-flow columns over 55 days treating acid-precipitation impacted water. Data points are singlet measurements.

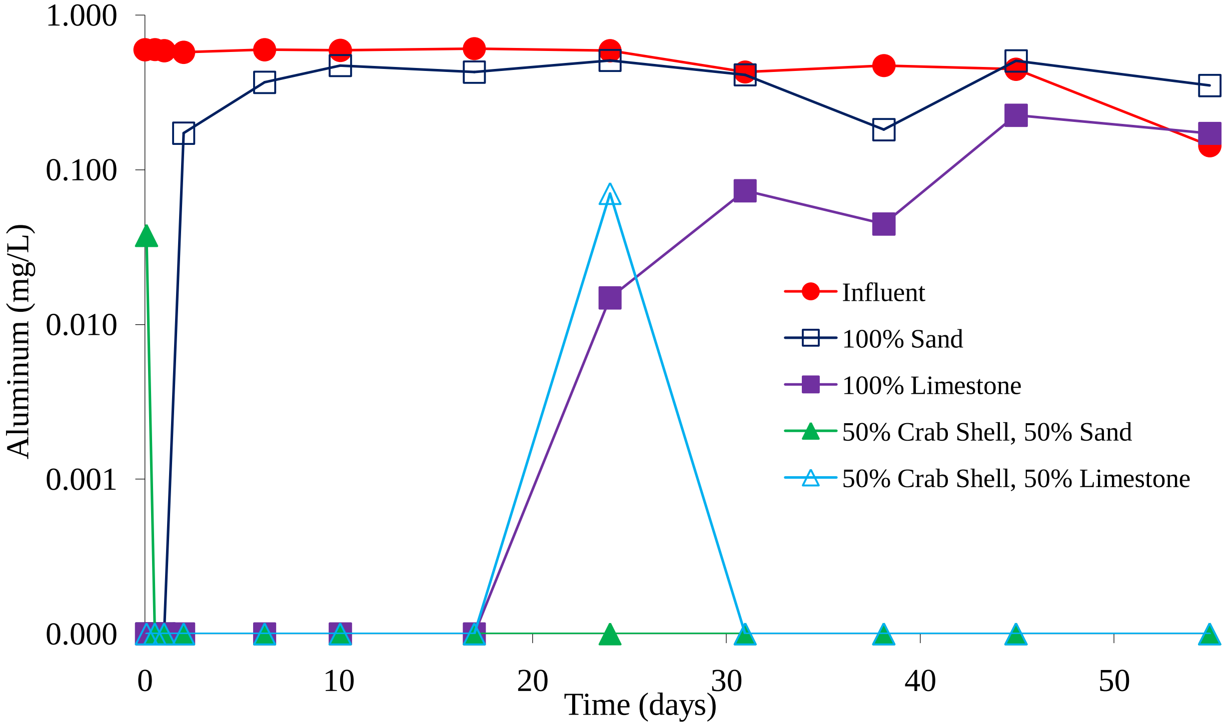


Figure 4-8. Aluminum concentrations in the effluent of continuous-flow columns over 55 days of treating acid-precipitation impacted water. Data points are singlet measurements.

Table 4.5. Initial (t = 2 hours) and final (t = 55 days) water quality analysis of continuous-flow columns testing different substrates for the treatment of acid-precipitation impacted water.

	Influent		**100% Sand**		**100% Limestone**		**50% Crab Shell, 50% Sand**		**50% Crab Shell, 50% Limestone**	
Analyte	2 Hours	55 Days	2 Hours	55 Days	2 Hours	55 Days	2 Hours	55 Days	2 Hours	55 Days
pH	3.87	3.61	5.53	3.52	7.14	6.01	8.16	7.13	8.11	8.08
Alkalinity (mg/L as CaCO$_3$)	0.00	0.00	15.35	0.00	35.2	22.7	1626.8	70.4	1355	215
Acidity (mg/L as CaCO$_3$)	27.4	29.4	6.8	31.3	-17.7	2.9	-1876.8	-73.5	-155	-228
TAN (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	16.31	8.08	14.59	7.78
Aluminum (mg/L)	0.596 ±0.186	0.143 ±0.135	<0.01	0.35	0.00	0.172 ±0.04	0.037 ±0.00	<0.01	<0.01	<0.01
Iron (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	1.402 ±0.20	<0.01	0.684 ±0.20	<0.01
Manganese (mg/L)	0.057 ±0.24	0.11 ±0.02	<0.01	0.208 ±0.04	<0.01	<0.01	0.611	0.014	0.359	<0.01
Calcium (mg/L)	1.05 ±0.06	0.417 ±0.05	7.05 ±0.04	0.77 ±0.12	108.1 ±1.88	18.66 ±3.29	376 ±12.6	33.1 ±4.74	231 ±2.64	31.7 ±5.87
Sodium (mg/L)	0.735 ±0.426	0.762 ±0.427	3.51 ±.045	1.23 ±1.08	20.2 ±1.41	1.34 ±1.10	4605 ±140	1.50 ±1.12	2604 ±23.4	1.36 ±1.11

4.3.1. CONSERVATIVE TRACER TESTS

A sodium chloride tracer solution was used to determine the HRT, effective pore volume, and dispersion coefficient for the columns (Table 4-5). The nominal flow rate was 0.5 mL/min throughout the tracer tests and entirety of the 55 day continuous-flow column experiment, although the measured flow rate varied slightly throughout the experiment for each column. Calculations for HRT, dispersion number, and effective pore volume are described Appendix C-1. The tracer response curves are shown in Appendix C-2. According to Metcalf & Eddy (2003), a dispersion number less than 0.05 indicates a plug-flow reactor with low dispersion, while 0.05-0.25 qualifies as moderate dispersion. Ideally in continuous-flow column experiment, low dispersion numbers are desirable. When a column is acting under plug flow conditions, all of the substrate is contacting all the water for an equivalent amount of time throughout the entire column. The dispersion numbers for all the columns in this experiment indicate moderate dispersion. The moderate dispersion numbers in the column experiments, especially in the case of the limestone column, indicate that the columns may not have been packed uniformly, and thus all of the influent water might not have been contacting all of the substrates for an equivalent amount of time. At its shorter retention time and higher dispersion number, the 100% limestone column may have exhausted prematurely. The retention time and dispersion numbers of the control column (sand) and 50% crab shell, 50% sand column are similar, however, indicating that comparisons in water treatment can indeed be made. Unfortunately, the tracer test for the final 50% crab shell, 50% limestone column failed, so an effective pore volume similar to that of the 50% crab shell, 50% sand column will be assumed (288.5 mL).

Table 4-6. Conservative tracer test results for the 55-day continuous-flow column experiment.

Description	Retention time (hr)	Dispersion number (-)	Dispersion Number Description	Flow rate during tracer test (mL/min)	Effective pore volume (mL)
100% Sand	12.4	0.066	Moderate Dispersion	0.440	326.4
100% Limestone	7.5	0.143	Moderate Dispersion	0.411	185.7
50% Crab Shell, 50% Sand	11.2	0.085	Moderate Dispersion	0.429	288.5

5. DISCUSSION

5.1. ALKALINITY, PH, AND BUFFERING CAPACITY

In all experiments containing crab shell, pH and alkalinity rapidly increased (within hours) as acidity decreased. Increasing calcium and magnesium ion (Ca^{2+} and Mg^{2+}) concentrations throughout the experiments indicate that the increase in alkalinity was directly attributable to the dissolution of calcium and magnesium carbonates from the crab-shell particles (Figure 5-1). The other component of alkalinity (3 – 20%) released from the crab shells is most likely in the form of fermentation products (i.e., volatile fatty acids). Although not measured here, acetate is known to be the primary product of chitin fermentation (Brennan et al., 2006), and its contribution to alkalinity has been well documented.

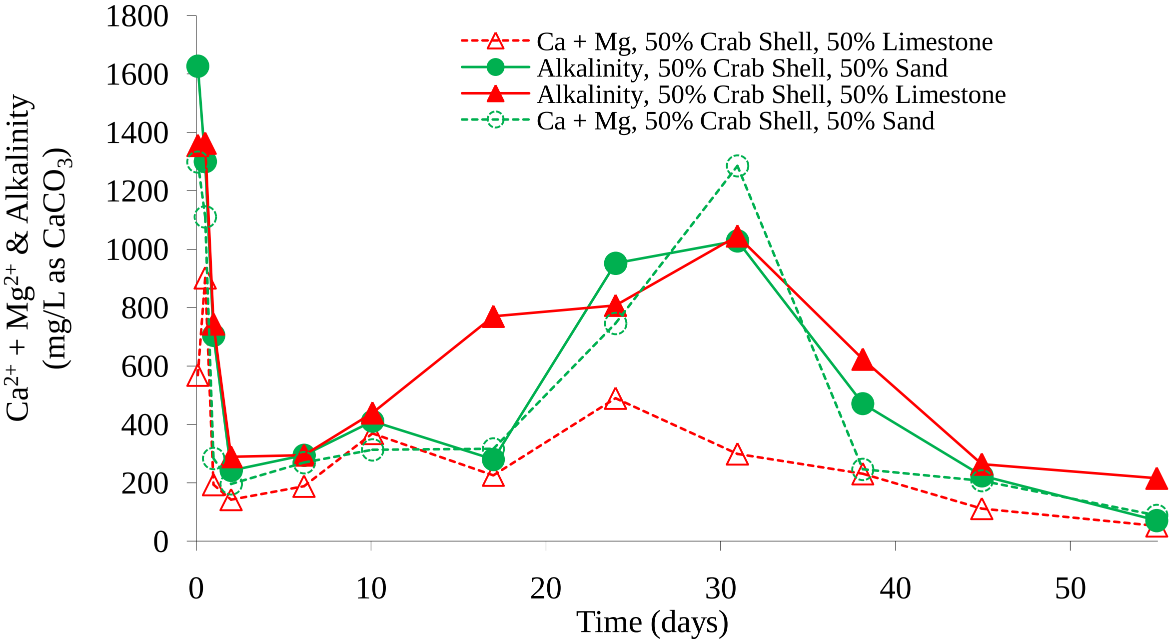


Figure 5-1. Calcium and magnesium changes with alkalinity in continuous-flow column experiments treating acid-precipitation impacted waters. Data points are singlet measurements.

While limestone alone was capable of raising the pH to circum-neutral values similar to crab shell, its ability to raise alkalinity and buffer the system was 1 to 2 orders of magnitude less than that of crab shell. This can be attributed to the greater dissolution of calcium carbonate from the crab shell, as is clear from the Ca^{2+} concentration profiles of the different materials (Figure 4-7). The greater release of $CaCO_3$ and the resulting greater buffering capacity of crab shell may be due to its greater surface area: 14 m^2/g versus < 0.5 m^2/g for limestone (Robinson-Lora & Brennan, 2009). Based on surface area alone, 28 times more limestone mass would be needed than crab shell to provide the same buffering capacity. This larger mass requirement translates into a larger treatment system footprint and greater total treatment cost.

The excess alkalinity generated by the crab shell in the 10-day microcosm experiment indicates that even less material could have been used to effectively treat the same volume of water. Over the course of the 10-day microcosm experiment, alkalinity in the crab shell-treated active microcosms was able to neutralize the existing acidity plus provide excess alkalinity ranging from 1.5 to 7.8 times, according to the following equation (Eqn. 5-1):

$$Alkalinity_{excess,t} = \frac{Alkalinity_{active,t} + Acidity_{control,t}}{Acidity_{control,t}} \qquad \textbf{(Eqn. 5-1)}$$

On average, each microcosm could have contained 3.5 times less crab shell to treat the same amount of raw water, reducing the required crab shell loading from 1 g/L to 0.3 g/L. Note that the required loading decreases with increasing HRT, from 0.64 g/L for 1.5 hours to 0.13 g/L at 10 days.

Similar to the findings of the microcosm experiments, the continuous-flow columns generated excess alkalinity as well. The alkalinity generated by crab shell only column was on average 27 times greater than that required to treat the influent acidity. In comparison, the excess alkalinity in the limestone column was on average only 2 times greater than needed to treat the acidity of the same water. The difference in alkalinity generation between the two amendments is even more striking when considering that their masses in their individual columns were not equivalent: the limestone column contained approximately 7 times more limestone by mass than the crab shell column, yet the crab shells produced 40 times more alkalinity than limestone. When normalized by mass, and disregarding the initial rapid alkalinity production period observed during the first 6 days, we find that the minimum loading required to neutralize the acidity of the influent water ranges from 13 to 60 g/L for limestone and from 0.2 to 0.9 g/L for crab shell (Appendix A-1). Cost-estimate calculations based on these loading criteria can be found in Appendicies A-2 and A-3.

The greater alkalinity generation of the crab shell microcosms and columns is of particular importance for this study. While both limestone and crab shells have the potential to increase the pH in the local area of treatment, it is the excess alkalinity generated by the crab shells that will continue buffering downstream waters. Waters treated with crab shell will experience far more acid-neutralizing capacity (ANC) that would not be possible with the same mass of limestone.

5.2. METALS REMOVAL

Throughout the 55-day continuous-flow column experiment, aluminum was removed from the water in the crab shell-containing columns, but broke through by day 24 in the 100% limestone column (Figure 4-9). With starting concentrations of 0.53 ± 0.01 mg/L of aluminum found from the raw water analysis, and the acute toxic limit of aluminum as 0.087 mg/L, removal of this particular metal is of extreme concern in this study.

It is asserted that the removal of aluminum is directly caused by addition of crab shell as a substrate. Furthermore, the mode of action for removal of aluminum by the crab shell is due to the continued generation of alkalinity; a simple rise in pH accounts for aluminum forming hydroxides ($Al(OH)_3$) and precipitating out of solution. As stated previously, limestone cannot generate excess alkalinity to the levels of crab shell. Therefore, the continual generation of excess alkalinity from the crab shell as a substrate will facilitate the removal of aluminum from acidic influent waters to Kinzua Creek.

5.3. AMMONIUM/AMMONIA RELEASE FROM CRAB SHELL

5.3.1. TOXICITY OF AMMONIUM/AMMONIA

The difference in the release of undesirable compounds with a crab shell PTS versus a limestone PTS is the ammonium/ammonia (TAN) generation from the fermentation of crab shells. With the pK_a of $NH_4^+/NH_3 = 9.3$, higher pH values would result in lower NH_4^+/NH_3 ratios in stream waters. This is evident from the continuous-flow column experiments where the largest increase of NH_3 naturally followed the highest pH values. The NH_3 toxicity for freshwater fish has been reported as 3.38 mg/L as N (USEPA, 1999). A study by Brinkman, 2009, found that “survival, growth, and biomass were not significantly affected at 7.44 mg NH_3-N/L or lower concentrations but were reduced at 16.8 mg NH_3-N/L. The chronic value based on lethal and sublethal endpoints was 11.2 mg NH_3-N/L.” A definitive concentration of NH_3 toxicity is difficult to establish due to the nature of NH_3 excretion in fish. As with most metabolites in aquatic macro organisms, fish rely on a gradient to expel NH_3. Stocked fish will have a naturally higher tolerance to ambient NH_3 concentrations because they are held in tanks in closer proximity to one another than they would be once released into streams. It has been suggested that the EPA NH_3 toxicity concentration is a protective measure for younger wild fish that would be more susceptible to lower concentrations earlier in their life stage.

TAN generation for the 55 day continuous-flow column experiment showed an initial rapid increase within the first few time points sampled. On days 1 and 2, NH_3 concentrations corresponding to these TAN values were slightly above the CMC values for early life stage fish (Table 5-1). After these time points, NH_3 generation fell to levels below the CMC. Although the elevated levels found on days 1 and 2 were local values for the effluent of the continuous-flow columns, dilution effects would likely decrease these values in the field. The NH_3 values in this study never rose to the lower EPA limit of 3.88 mg/L as N, which would constitute chronic exposure. The nature of the PTS in the field study (crab shell-lined roadside ditches) would need to be evaluated for episodic releases of NH_3 by factoring in temperature and overall pH.

Table 5-1. CMC values corresponding to TAN measurements observed in the 55 day continuous-flow column experiment.

Timepoint	**50% Crab Shell, 50% Sand**	**Toxic Limit**	**50% Crab Shell, 50% Limestone**	**Toxic Limit**
(days)	**NH_3 (mg/L as N)**	**NH_3 (mg/L as N)**	**NH_3 (mg/L as N)**	**NH_3 (mg/L as N)**
0.1	0.77	4.13	0.62	4.55
0.5	1.34	2.24	1.36	2.27
1	1.94	1.15	2.06	1.08
2	1.79	0.67	1.91	0.65
6	0.45	4.38	0.56	3.61
10	0.85	2.75	0.93	2.70
17	0.38	6.17	0.39	7.96
24	0.21	11.7	0.55	5.01
31	0.06	23.9	0.22	11.4
38	0.07	20.2	0.07	20.6
45	0.08	17.7	0.11	14.1
55	0.04	21.3	0.31	4.82

Baldigo & Lawrence (2001) found that habitat conditions affected brook trout population density more than water chemistry. The highest densities of brook trout in their study were found in areas with more bank undercuts, grasses, higher stream flow, and gravel fines in the stream bed. These densities held true even in strongly acidified waters ($pH \leq 4.77$). This finding suggests that the TAN release from the crab shell PTS might have more of an effect on the brook trout populations by supplying bioavailable nitrogen to downstream aquatic vegetation which will increase brook trout recruitment in the area. TAN by crab shells could affect the amount of grasses recruited to the area by supplying nitrogen which would possibly have been limited before crab shell PTS additions. Ochoa-Hueso and Manrique (2010) found that anthropogenic additions of nitrogen in the form of fertilizers (about 20 kg of nitrogen per hectare per year) were favored by annual grasses which grow near stream banks. However, an excess of TAN may be counterproductive to brook trout recruitment and a native balance of foliage (Ochoa-Hueso and Manrique, 2010). Further investigation would be required to determine the effects of additional TAN on downstream biota and brook trout recruitment.

5.3.2. EUTROPHICATION

It has been suggested that the ratio of nitrogen to phosphorous must be less than 29:1 for eutrophiciation by cyanobacteria to occur (Sharma et al., 2009). The intrinsic values for NO_3^- and PO_4^{3-} for Kinzua Creek yield an N:P value of about 5:1. Analysis of the microcosm anions (NO_3^- and PO_4^{3-}) and TAN data (calculated in terms of nitrogen and phosphorous) yields a maximum N:P of about 2:1 by the end of the 10-day microcosm experiment (Figure 5-2), which is actually an improvement in water quality in terms of eutrophication potential. The findings of Flett et al. (1980) suggest that a N:P of 10:1 or less would select for eutrophication by nitrogen-fixing bacteria. If the use of crab shells in PTS did select for nitrogen-fixing bacteria, those microbes could possibly add more NH_3 to the stream ecosystem through the conversion of atmospheric N_2 to NH_3, than would be generated by the crab shell alone. However, from the N:P observed in the 10-day microcosm experiment, this is not likely to happen.

Inspection of the ratios throughout the 10-day microcosom experiment for phosphorous and nitrogen show that, although there is some phosphourous associated with the crab shell surafce which is transient (Robinson-Lora and Brennan, 2009), the ratio remains relitively constant until the genration of ammonium, which adds to total nitrogen in the system. Therefore, N:P ratios can be etablished for the the column experiments by simply adding in TAN data (as nitrogen) and considering the N:P to be constant and intrinisic for Kinzua Creek throughout the 55 days (Figure 5-3).

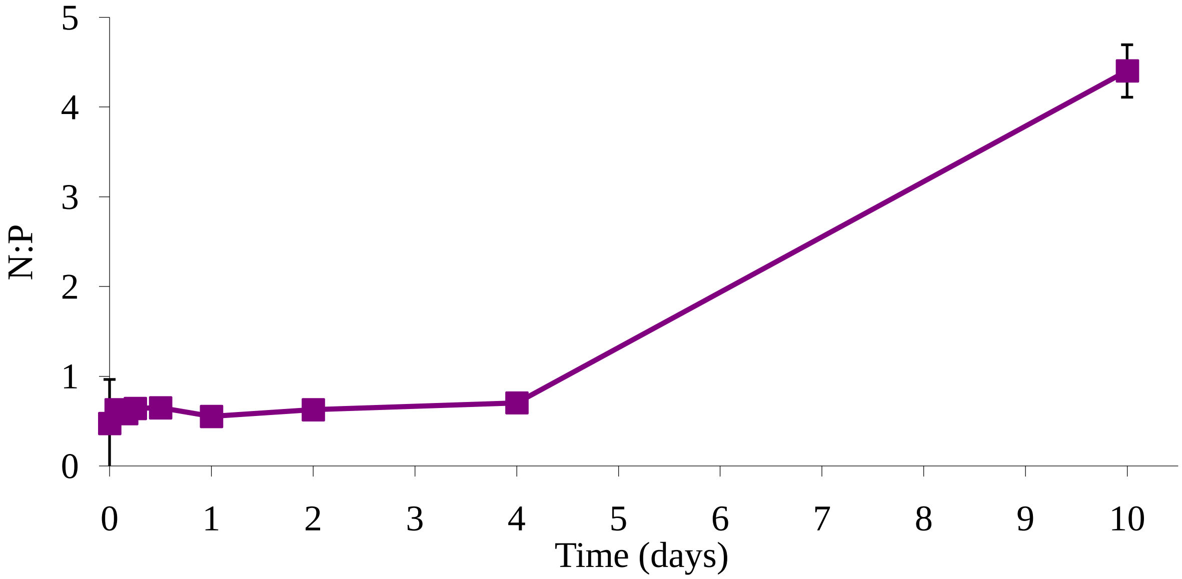


Figure 5-2. N:P over time in the 10-day microcosm experiment treating acid-precipitation impacted water. Nitrogen data is addition of TAN (as nitrogen) and nitrate (as nitrogen). Phosphorous data is phosphate (as phosphorous). Data points are duplicate averages and error bars represent one standard deviation.

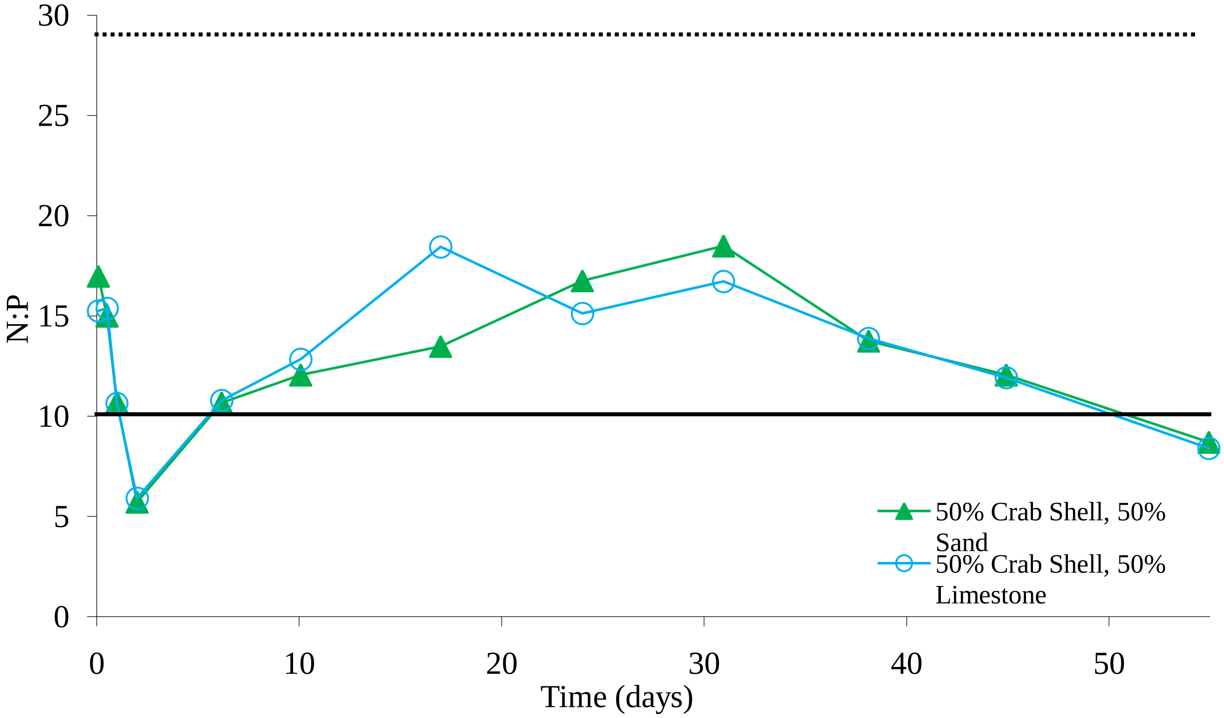


Figure 5-3. N:P for the continuous-flow column experiment over 55 days treating acid-precipitation impacted water. Data show the addition of background NO_3^- (as nitrogen, determined from anion analysis of microcosm experiment) added to TAN concentrations divided by background phosphate (as phosphorous) concentrations. Dashed line shows 29:1 ratio determined by Sharma et al (2009), solid line shows 10:1 ratio determined by Flett et al (1980) for nitrogen-fixing bacteria.

The continuous-flow column experiments showed a larger generation of TAN over 55 days, and once the data is compared as a ratio to the background concentrations native to Kinzua Creek, it is seen that over the length of the experiment, the ratio exceeds the 10:1 for nitrogen-fixing bacteria during the majority of TAN generation and also remains below the 29:1 theorized for cyanobacterial eutrophication. Based upon the ratios established by Flett, et al. (1980) for eutrophication by selection of nitrogen-fixing bacteria and Smith (1983) for selection by cynaobacteria, it appears as if only cyanobacteria would be of concern. Furthermore, averaging the ratios over the 55 days of the continuous-flow column experiment still shows that eutrophication by nitrogen-fixing bacteria would not be probable (both columns averaged 13:1). However, a better investigation in the field would be necessary to confirm the assertion that cyanobacteria would cause eutrophication. Based solely on the N:P found in this study, it would appear that the possibility exists. Yet, eutrophication in surface waters is a complex issue with several significant variables which were not addressed in these experiments. Additionally, brook trout densities have been more significantly linked to habitat conditions making those variables of most interest for further investigation in conjunction with the ratios found in this study.

6. FIELD STUDY

6.1. FIELD SITES

The field study portion of this work was completed by Ken Anderson, Fisheries Biologist II with the Pennsylvania Fish and Boat Commission. The field data recorded to date was gathered by Ken Anderson, and monitoring of the different treatment sites is ongoing at the time of this thesis.

Three impaired tributaries of the South Branch of Kinzua Creek in the Allegheny National Forest on Forest Road 279 (FR 279), Wetmore and Hamlet Townships, McKean County, PA (Figure 6-1), were treated in-concert with routine Forest Service road maintenance by constructing innovative PTS. The purpose of these PTS was to supply buffering capacity to the watershed via the stormwater management mechanism of the road. Two substrates were used and evaluated for their effectiveness in buffering stormwater runoff: limestone and crab shell. An additional tributary watershed served as a control during the monitoring phases of the project. The laboratory results of this study were used to guide the design of the field-scale PTS.

Driving surface aggregate (DSA) was applied to all the field sites. As a practice, the Fish and Boat Commission routinely applies DSA to unpaved roads, like those providing access to the tributaries of Kinzua Creek used in the field study. Developed by Penn State University's Center for Dirt and Gravel Road Studies, DSA is a mixture of crushed stone and has a unique particle size gradation designed to maximize packing density and produce a durable road surface that performs better than conventional aggregates. According to DSA specifications (PA State Document, Publication 447, Section 400), the DSA materials are derived from crushed rock (no silt or clay may be added), 98% of fines (#200) must be crushed rock, and the mixture must have a pH range of 6 to 12.45. Compared to alternatives, benefits of DSA include: denser, stronger road surface, greater resistance to traffic abrasion, fewer soil particles at road surface which produces less traffic dust and less water pollution because surface run-off contains less silt and clay fines.

The ditches were installed beginning on June 30th, 2009, by North Wind, Inc., and took 2 ½ weeks to complete. Geotextile fabric was placed under the substrate (limestone or crab shell) in the ditches and crab shell underdrains were constructed to deliver the treated water directly to the stream (Figure 6-2.). The crab shell used for the field study was purchased from JRW Bioremediation and the limestone was purchased from Quality Aggregates, Inc. DSA, from New Enterprises, Inc., was applied to the road surface at every site. Tributaries marked 1.1, 1.2, and 1.3 are crab shell treatment sites, 2.1 and 2.2 are limestone treatment sites, 3.1, 3.2, and 3.3 are DSA only sites, and 4.1 served as a control site.

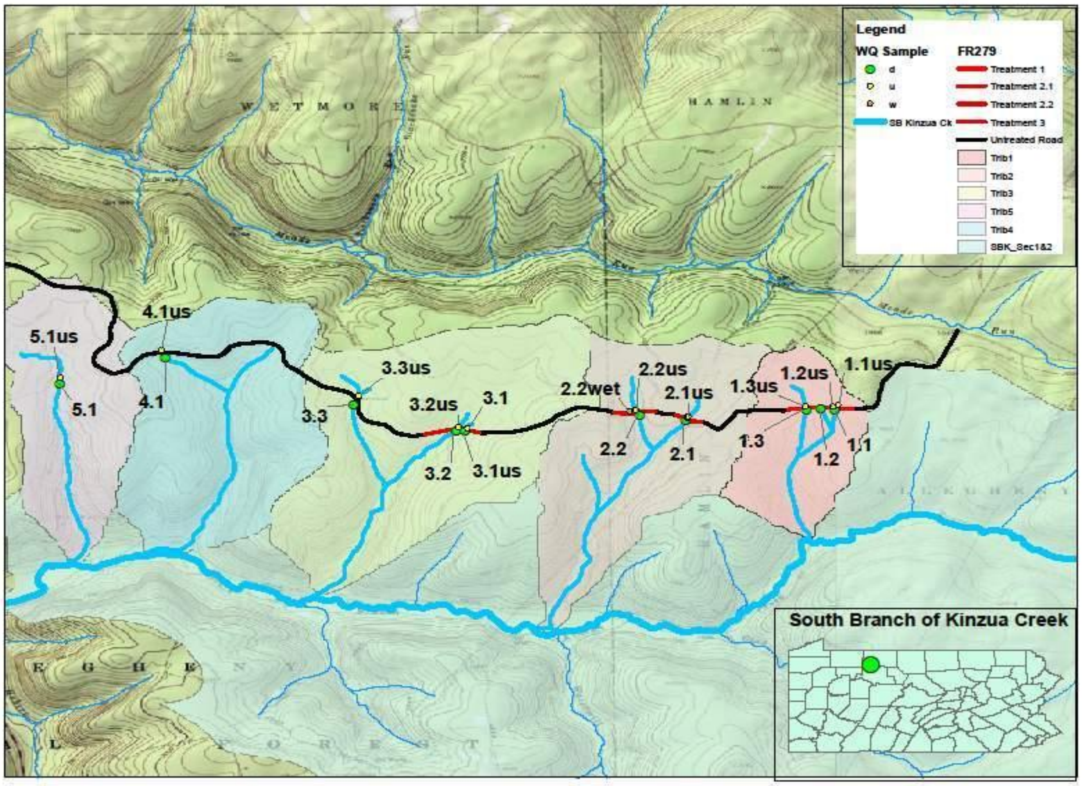


Figure 6-1. Treatment sites for field study of PTS with crab shell or limestone as substrates. Sites 1 were lined with crab shell, 2 with limestone, 3 DSA only, and 4 was a control.

6.2. DITCH CONSTRUCTION

The Penn State Center for Dirt and Gravel Road Studies provided evaluation for the construction of the limestone and crab shell ditches and underdrains (Figures 6.2, through 6.5). Ditches were constructed along side of FR 279 with a 6-in depth and a 12-in width. The bottom of the ditches sat on top of a geotextile-wrapped underdrain (18-in wide x 18-in deep), which was filled with either #1 AASHTO limestone choked with 4-in of #10 limestone sand (Figure 6.2) or #1 AASHTO sandstone choked with 6-in of #10 sand mixed with crab shell (Figure 6.3). Each underdrain had a 4” perforated plastic pipe with a filter sock running throughout its length. A summary of dimensions for the active treatment ditches are presented in Table 6.1.

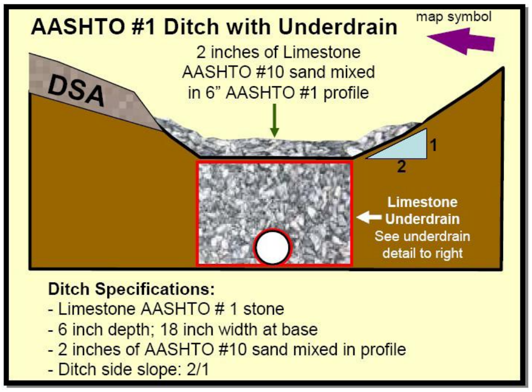


Figure 6.2. Limestone ditch and underdrain cross section detail. Taken from PSU Center for Dirt and Gravel Road Studies report.

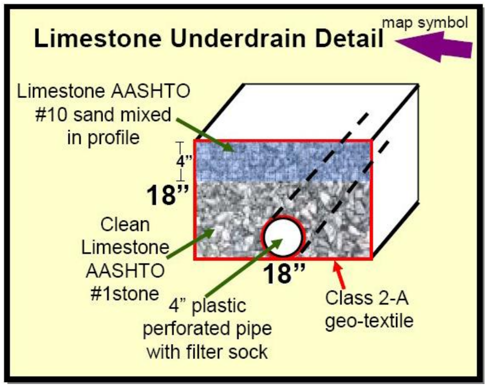


Figure 6.3. Limestone underdrain cross section detail. Taken from PSU Center for Dirt and Gravel Road Studies report.

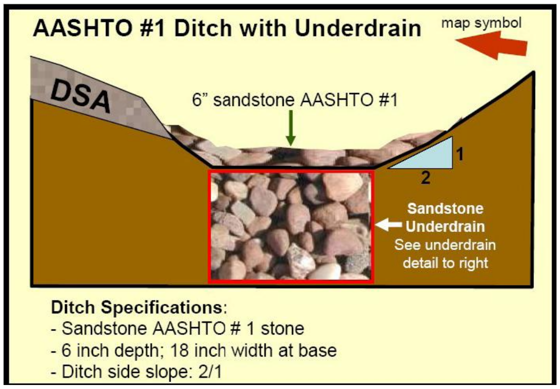


Figure 6.4. Crab shell ditch and underdrain Cross section. Taken from PSU Center for Dirt and Gravel Road Studies report.

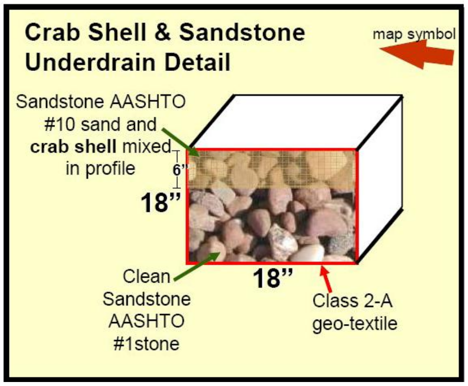


Figure 6.5. Crab Shell underdrain cross section. Taken from PSU Center for Dirt and Gravel Road Studies report.

Table 6.1. Active treatment ditch dimensions for field study.

Treatment	Length (feet)		Width (inches)	Substrate Depth (inches)	Total Depth (feet)
Crab Shell	700	**Ditch**	12	-	0.5
		Underdrain	18	6	1.5
Limestone	1878	**Ditch**	12	2	.5
		Underdrain	18	4	1.5

The control site (site 4.1) had 1-ft wide ditches only (no underdrains) filled with a 6-in layer of #1 sandstone. All ditches were sloped 2/1. The ditches and underdrains were constructed to filter water from road run-off into the ditches and down through the substrate-filled underdrains. After making contact with the substrate, the treated water runs through the underdrain pipe and is carried downgradient to a culvert where it then enters the tributary waters.

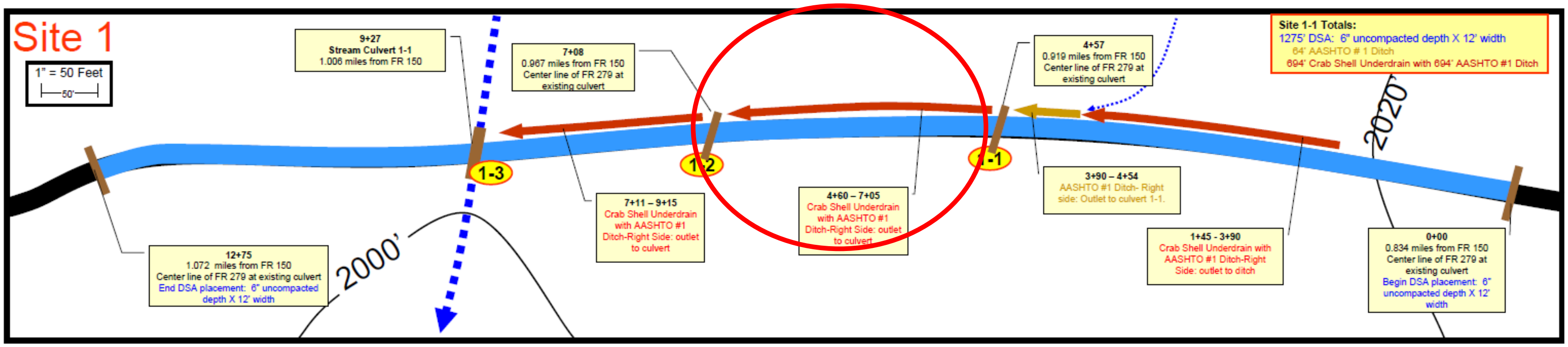


Figure 6.6. Detail of crab shell treatment sites. Site 1-2 was used for presentation of field data (indicated by circle). Top arrows indicate ditches with crab shell underdrains, solid vertical lines indicate culverts, and the solid thick line is FR 279.

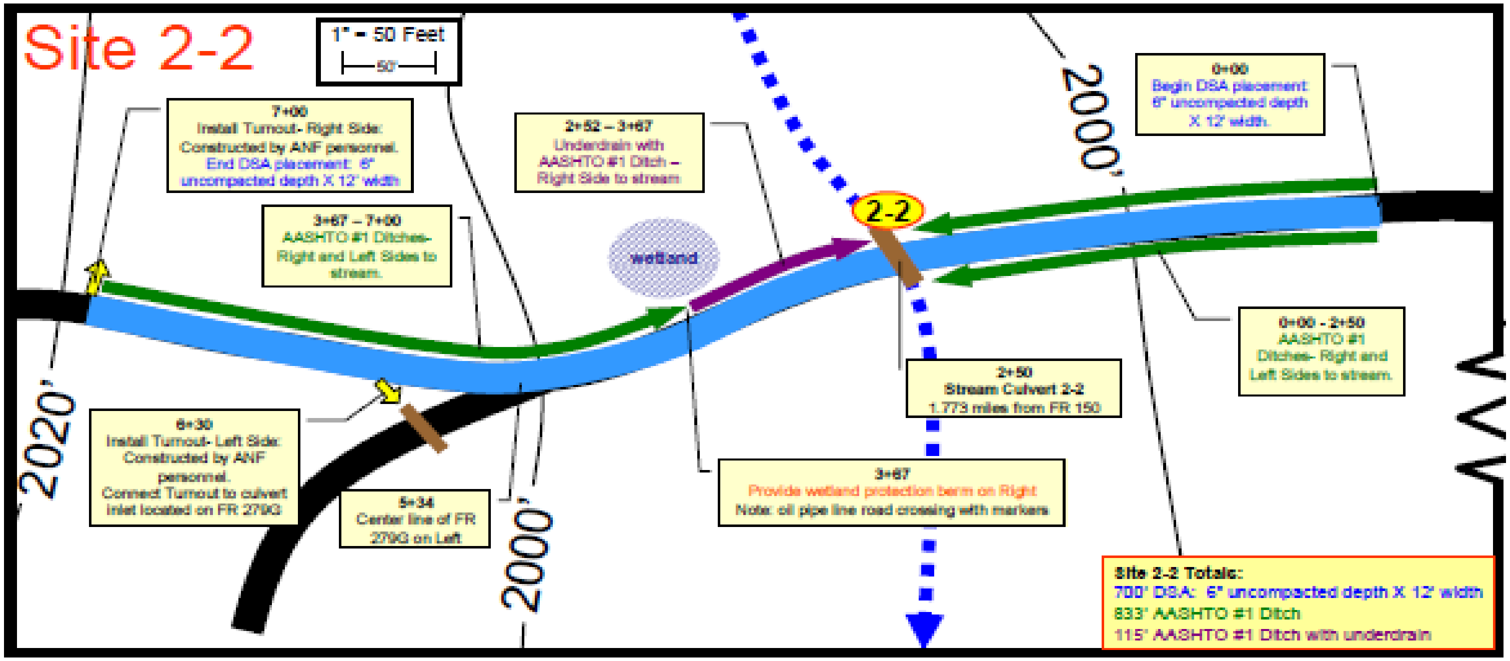


Figure 6.7. Detail of limestone treatment site used for analysis of field data, site 2-2. Middle arrow is limestone ditch next to wetland area, Arrows at either end are limestone ditches, solid vertical lines indicate culverts. The solid thick line is FR 279.

6.3. FIELD DATA

6.3.1. MATERIALS AND METHODS

Pre-treatment monitoring began on April 1st, 2009, and continued until June 16th, 2009. Post-treatment monitoring occurred from August 11th, 2009, through November 12th, 2009. pH data was collected more frequently and for a longer duration: from April 11th 2008, through July 2010.

pH was measured with a Myron Ultrameter II Model 6P in the field. Alkalinity was determined according to Standard Methods 2320B, and metals were determined according to Standard Methods 200.8. Analysis of metals and alkalinity was done by Analytical Systems Inc. Laboratories (ASI) in Brookville Pa.

6.3.2. FIELD WATER QUALITY RESULTS

For the field study, the different treatment areas were not receiving the same flow; therefore, sites from each treatment were chosen for their flow similarities. For the crab shell treatment, site 1.2 was chosen, and for limestone, site 2.2 due to their similar proximity to wetland areas, thus keeping the substrates under relatively uniform moisture conditions. The average of sites 3.1, 3.2, and 3.3 were used for DSA comparisons, and site 4.1 was used for the control data. Values for the pre-treatment water quality data are averages for that treatment site over a 3 month period from April 2009- June 2009 (Table 6-2) and post-treatment data was collected for 3 months after ditch installation (Table 6-3). pH data was collected for approximately one year (1 year before treatment and 1 year after treatment). After treatment was initiated, the crab shell ditches showed the greatest increase in pH and then maintained consistently higher pH values during treatment monitoring (Figure 6.8). Measurements for each analyte were taken at the point where the treated water exited the piping in the culverts, before the treated water entered the stream.

Table 6.2. Pre-treatment water quality at the field sites used for the different substrates. Sites used were Control (4.1), Crab Shell Treatment (1.2), Limestone Treatment (2.1), and DSA only (3.1-3.3).

Analyte	**Control**	**Crab Shell**	**Limestone**	**DSA**
pH	5.04 ±0.27	5.39 ±1.45	5.22 ±0.48	4.82
Alkalinity (mg/L as $CaCO_3$)	0.00	2.53 ±3.5	0.200 ±0.45	0.07 ±0.27
Aluminum (mg/L)	0.31 ±0.22	0.92 ±0.39	0.25 ±0.18	0.32 ±0.20
Calcium (mg/L)	1.10 ±0.13	1.76 ±0.18	1.43 ±0.34	1.19 ±0.68
Iron (mg/L)	0.06	12.4 ±16.9	0.24 ±0.29	0.15 ±0.48
Sodium (mg/L)	0.25	1.95	0.230	0.56 ±0.21
Manganese (mg/L)	0.04 ±0.10	0.26 ±0.01	0.12 ±0.05	0.17 ±0.10
TAN (mg/L)	N/A	0.090	N/A	N/A

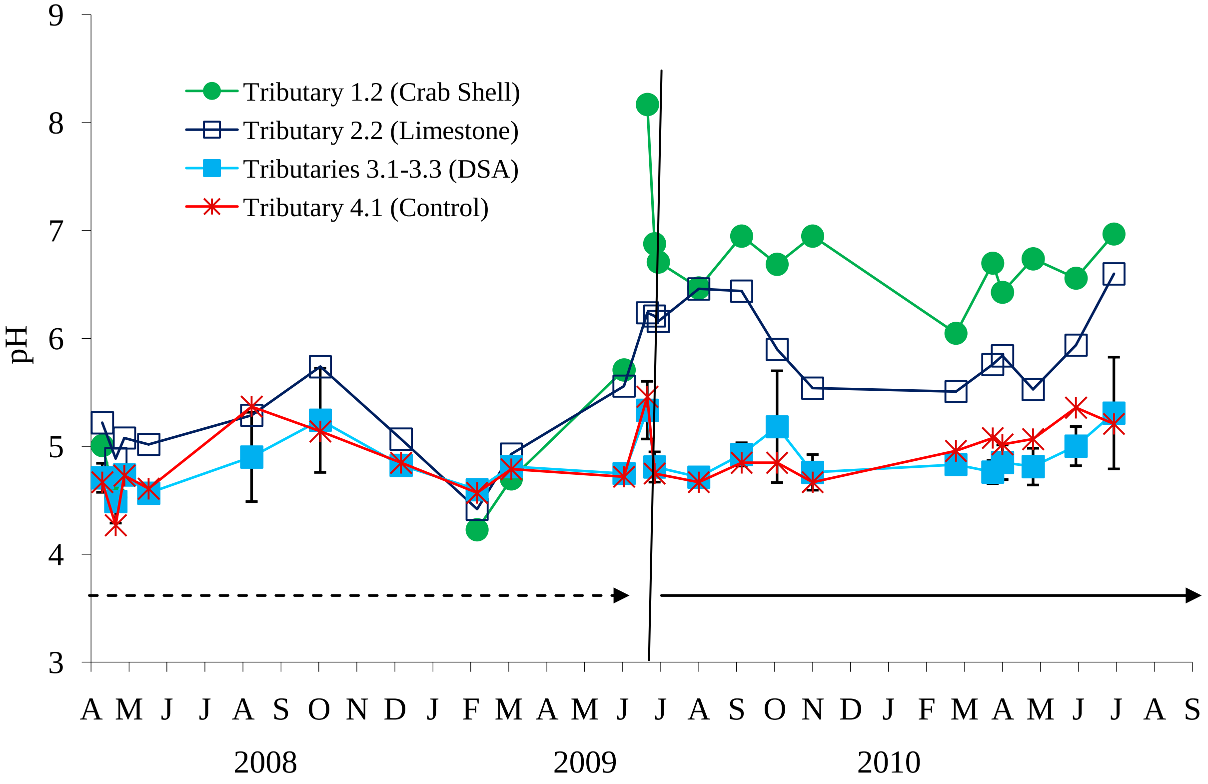


Figure 6.8. pH measurements in tributaries to Kinzua Creek treated with different acid-neutralization techniques. Crab shell, limestone, and control data are singlet measurements, DSA is triplicate averages; error bars represent one standard deviation. Dashedarrow indicates pre-treatment measurements while solid arrow indicates post-treatment measurements. Solid vertical line indicates time of treatment installation.

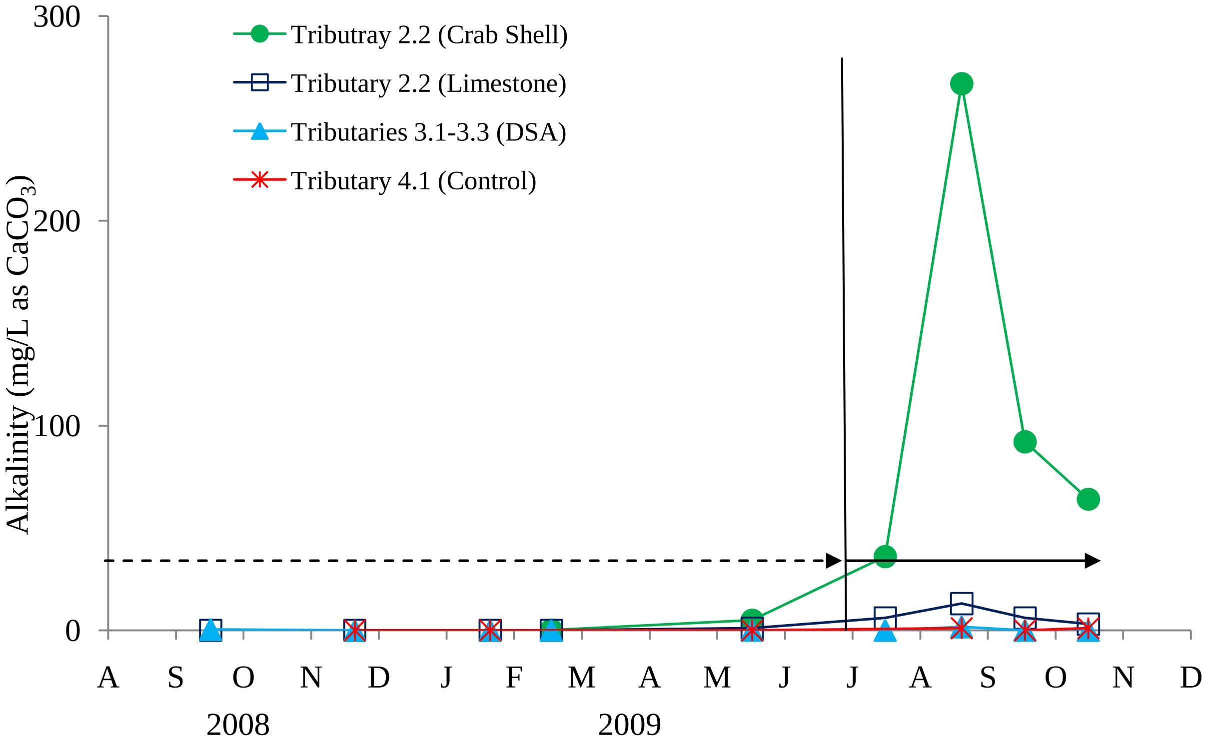


Figure 6.9. Alkalinity measurements in tributaries to Kinzua Creek treated with different acid-neutralization techniques. Data points are singlet measurements. Dashed arrow indicates pre-treatment measurements while solid arrow indicates post-treatment measurements. Solid vertical line indicates time of treatment installation.

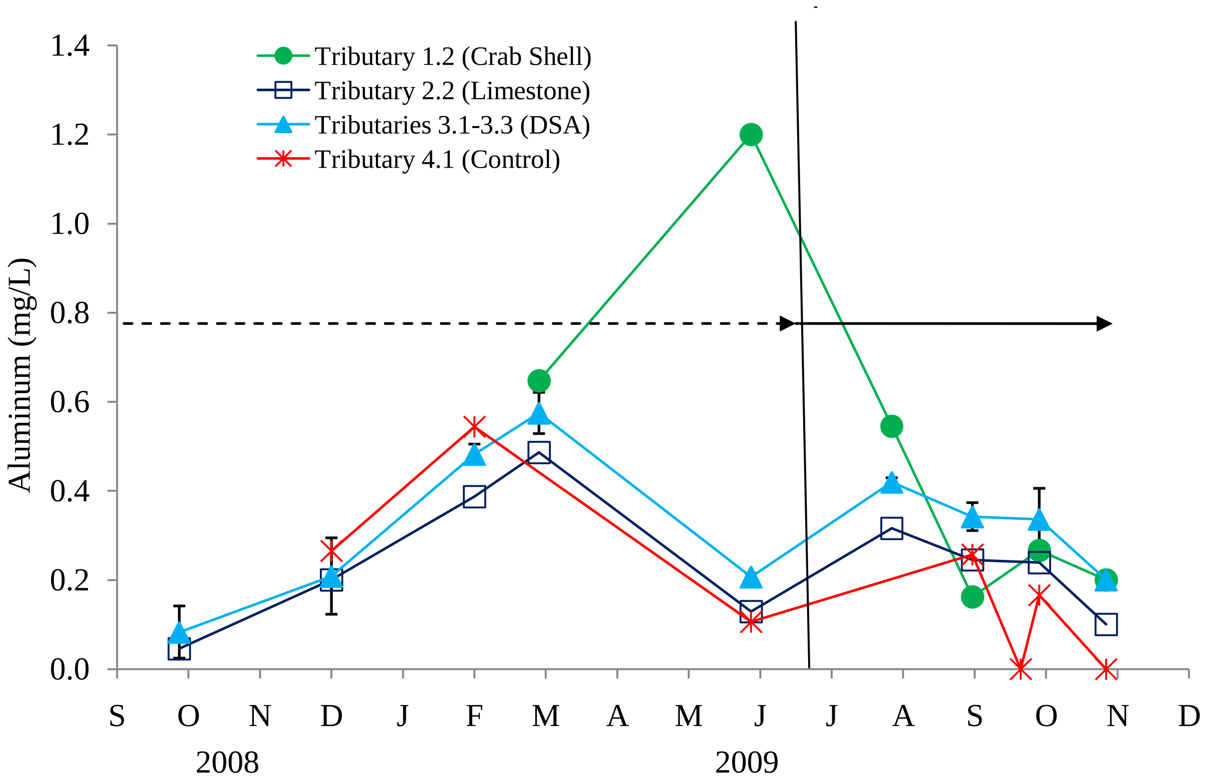


Figure 6.10. Aluminum concentrations in tributaries to Kinzua Creek treated with different acid-neutralization techniques. DSA site values are triplicate averages with error bars representing one standard deviation. All other data points are singlet measurements. Dashed arrow indicates pre-treatment measurements while solid arrow indicates post-treatment measurements. Solid vertical line indicates time of treatment installation.

After installation of the ditches, crab shell showed increases over the 3 or 6-month monitoring period in: pH to a value of 6.66 ±0.28; alkalinity to a value of 115 ±104 mg/L as $CaCO_3$; and aluminum removal of 0.293 mg/L (down from a starting value of 0.924 ±0.39 mg/L) (Table 6.3). Limestone ditches were less effective, with an average annual value of 5.94 ±0.37 for pH, 7.00 ±4.24 mg/L as $CaCO_3$ for alkalinity, and aluminum concentration of 0.225 ±0.09 mg/L (down from 0.250 ±0.45 mg/L). Aluminum concentrations in the field seem to track with pH, with lower pH values corresponding to higher aluminum concentrations, as would be expected due to enhanced dissolution under acidic conditions.

Table 6.3. Post-treatment water quality values in tributaries to Kinzua Creek for the different acid-neutralization techniques. Values are averages from 3 months after treatment with one standard deviation.

Analyte	Control	Crab Shell	Limestone	DSA
pH	5.11 ± 0.12	6.66 ±0.23	5.94 ±0.37	4.85
Alkalinity (mg/L as $CaCO_3$)	0.67 ± 0.58	115 ±144	7.00 ±4.24	0.42 ±0.79
Aluminum (mg/L)	0.14 ±0.13	0.29 ±0.17	0.23 ±0.09	0.32 ±0.89
Calcium (mg/L)	1.17 ± 0.12	40.1 ±34.2	3.43 ±1.29	1.00 ±0.22
Iron (mg/L)	0.33 ±0.36	4.54 ±2.28	0.43 ±0.26	0.16 ±0.19
Sodium (mg/L)	0.25 ±0.01	0.97 ±0.40	0.28 ±0.04	0.51 ±0.30
Manganese (mg/L)	0.10 ±0.04	1.44 ±1.60	0.12 ±0.04	0.18 ±0.08
TAN (mg/L)	0.240	0.09	0.41	0.11 ±0.03

It is to be noted, however, that the control and DSA sites also had an increases in alkalinity. The alkalinity value for the control site before installation of the ditches was 0.00 mg/L as $CaCO_3$ and after treatment that value increased to 0.667 ±0.577. It follows that the control site also had a decrease in aluminum (from 0.305 ±0.22 down to 0.141 ±0.13 mg/L, Figure 6.5). Yet, the DSA site saw a slight increase in aluminum from 0.318 ±0.20 to 0.324 ±0.89 mg/L despite an increase in alkalinity from 0.071 ±0.27 to 0.417 ±0.79 mg/L as $CaCO_3$. Even with the generation of alkalinity at the control and DSA sites, aluminum removal was still less than the crab shell and limestone sites (Figure 6.10).

The net change of analytes in presented in Table 6.4. The net change was determined by subtracting the value after treatment, with respect to the control value, from the value after treatment, with respect to the control value (Eqn 6-1).

$$Net\ Change\ of\ Analyte = ((Analyte_{after} - Control_{after}) - (Analyte_{before} - Control_{before})) \qquad \text{(Eqn 6-1.)}$$

Crab shells were able to remove an average of 0.466 ±0.13 mg/L of aluminum, add an average of 112 ±100 mg/L as $CaCO_3$ alkalinity, and raise the pH by average of 1.19 ± 1.02. Limestone did not successfully remove aluminum (there was aluminum removal at the control site, Figure 6.10), only brought the pH up by an average of 0.64, and could only add an average of 6.13 ±3.22 mg/L as $CaCO_3$ alkalinity.

Table 6.4. Net change of analytes for each treatment in the field study from the values before treatment to after treatment.

Analyte	Crab Shell	Limestone	DSA
pH	1.19 ±1.02	0.64	-0.04 ±0.15
Alkalinity (mg/L as $CaCO_3$)	112 ±100	6.13 ±3.22	-0.321 ±0.05
Aluminum (mg/L)	-0.466 ±0.13	0.140	0.170 ±0.02
Calcium (mg/L)	38.3 ±34.0	1.94 ±0.96	-0.251 ±0.45
Iron (mg/L)	-8.15 ±15.0	-0.083 ±0.40	-0.263 ±0.65
Sodium (mg/L)	-0.985 ±0.39	0.045 ±0.02	-0.052 ±0.07
Manganese (mg/L)	1.12 ±1.57	-0.055 ±0.03	-0.048 ±0.05
TAN (mg/L)	N/A	N/A	N/A

TAN was not measured before installation of the ditches, but was measured after. The value for TAN at the crab shell site was of most concern, but was not a significant value compared to the limestone site, which produced more TAN. More investigation is needed to explain why this was the case. Since the TAN value was lower after treatment for the crab shell ditches, eutrophication due to crab shell fermentation does not appear to be a concern at site 1.2. Furthermore, based on anecdotal evidence from the Fish and Boat Commission, no algal blooms were seen at any of the treatment sites.

A drop in Oxidation/Reduction Potential (ORP) was observed after installation of the treatments (Figure 6.11), indicating the possibility of an O_2 sag at the confluence of the treated water and the stream waters. An O_2 sag could create an oxygen limited system which may have difficulty supporting aerobic aquatic life. Due to dilution of the treated water by the stream water, however, the effects of this ORP decrease would be lessened. Furthermore, complete mixing of the combined waters may occur far enough downstream so that the drop in ORP is not only lessened, but moot by the time the waters reach brook trout populations. Measurements of the downstream waters would be needed to determine the point of complete mixing.

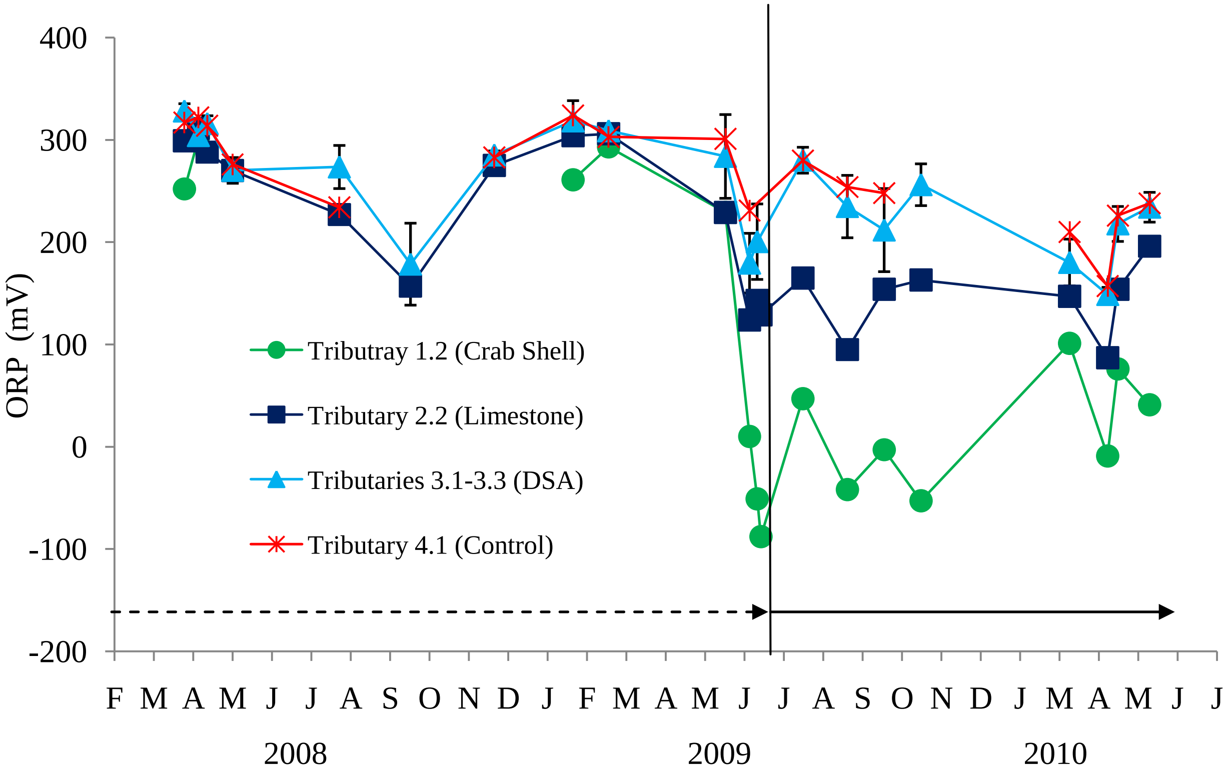


Figure 6.11. Oxidation/Reduction potentials (ORP) in tributaries to Kinzua Creek treated with different acid-neutralization techniques. DSA site values are triplicate averages with error bars representing one standard deviation. All other data points are singlet measurements. Dashed arrow indicates pre-treatment measurements while solid arrow indicates post-treatment measurements. Solid vertical line indicates time of treatment installation.

Actual costs for the field study can be found in Appendix A-3.

6.3.3. BROOK TROUT RECRUITMENT RESULTS

The PA Fish and Boat Commission used EPA Method EPA 841-B-99-002 to evaluate habitat conditions which would be favorable for brook trout. Based on a scale of 125 as a minimum (least favorable conditions) and 200 as a maximum (most favorable conditions), each site used in the field study scored between 160-180. Therefore, brook trout recruitment based on favorable habitat conditions is approximately the same for each site and water quality should then play the most important role in recruitment.

The control site had 15 brook trout before the study was initiated, but saw a decrease over the monitoring period to 10 individuals with no early stage trout. The crab shell site had adult brook trout before treatment and also saw recruitment approximately 1000 feet downstream of site 1.3 with an additional 2 early life stage individuals. Also, 2 new species were seen (creek chub and black nose dace) in the same area, 1000 feet downstream of treatment site 1.3. None of the other sites had any species of fish present before or after treatment. Biological assessment continues at the sites by Clarion University in collaboration with the PA Fish ad Boat Commission.

7. CONCLUSIONS, ENGINEERING SIGNIFICANCE, AND FUTURE WORK

7.1. CONCLUSIONS

Based on the results of batch microcosms and continuous-flow column experiments comparing crab shell to limestone rock for the passive treatment of water impacted by acid precipitation, the following conclusions can be made:

- Although both crab shell and limestone were found to be effective for increasing pH to circum-neutral values, crab shells provided much more buffering capacity (several hundreds of mg/L as $CaCO_3$).
- Fermentation of crab shells released low levels of ammonium (NH_4^+) into the water (< 18 mg/L as N), which may be helpful for restoring biological diversity in nutrient-deficient watersheds.
- Addition of TAN to stream waters due to fermentation of crab shells does not appear to pose a significant threat for anthropogenic eutrophication.
- Low levels of aluminum (0.6 mg/L) were easily removed from solution by crab shell, but broke through in columns containing limestone, eventually reaching influent concentrations within 55 days.
- Based upon excess generation of alkalinity of the microcosm experiment, a loading equation for the minimum amount of crab shell substrate needed to treat influent waters was established.

7.2. ENGINEERING SIGNIFICANCE

- Use of crab shells as a substrate for treating acid-impacted waters in a passive treatment system would be preferential over limestone due to the excess alkalinity generated.
    - As shown, the excess alkalinity generated by the crab shells would add more buffering capacity to downstream waters over the alkalinity generated by limestone.
- In terms of cost, crab shells are more expensive than limestone, but may last longer in passive treatment systems (Appendix A-5 and Table A-3.1).
- Field testing is necessary before implementation of a passive treatment system due to the variability in not only water quality, but in treatment success based upon stream flow and ditch dimension.
- Use of crab shells will continually remove aluminum as long as the substrate is generating alkalinity.
    - Replacing the substrate may be necessary if alkalinity generation becomes exhausted.

7.3. FUTURE WORK

Further research which would be useful for evaluating and optimizing passive treatment systems for the remediation of acid deposition using crab shell as a substrate include:

- Using episodic flow rates which reflect those seen in the field. This could provide a better estimate for the minimum loading of crab shell and overall costs.
- Plant uptake of nutrients released from crab shell, such as nitrogen, which could determine whether brook trout recruitment would be related to the enhancement of habitat (for example, N-uptake by aquatic grasses).
- Microbial community analysis to determine the key microorganisms which could possibly add to eutrophication.

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APPENDIX A

A-1 MINIMUM CRAB SHELL LOADING DATA MICROCOSM EXPERIMENT

The following figure (A-1.1) was developed from the results of the microcosm experiments' excess alkalinity. Excess alkalinity can be calculated from equation 5-1 (below and in text).

$$Alkalinity_{excess,t} = \frac{Alkalinity_{active,t} + Acidity_{control,t}}{Acidity_{control,t}} \qquad \textbf{(Eqn. 5-1)}$$

Table A-1.1. Minimum loading equation table. Values were used to develop loading equation A-1.1.

Microcosm Treatment Time	Overtreatment	Minimum Loading
(days)	**(excess alkalinity)**	**(1 g/overtreatment)**
6 hours	2.16	0.46
12 hours	2.52	0.40
1 day	3.36	0.30
4 days	6.73	0.15
10 days	7.84	0.13
Averages	3.53	0.41

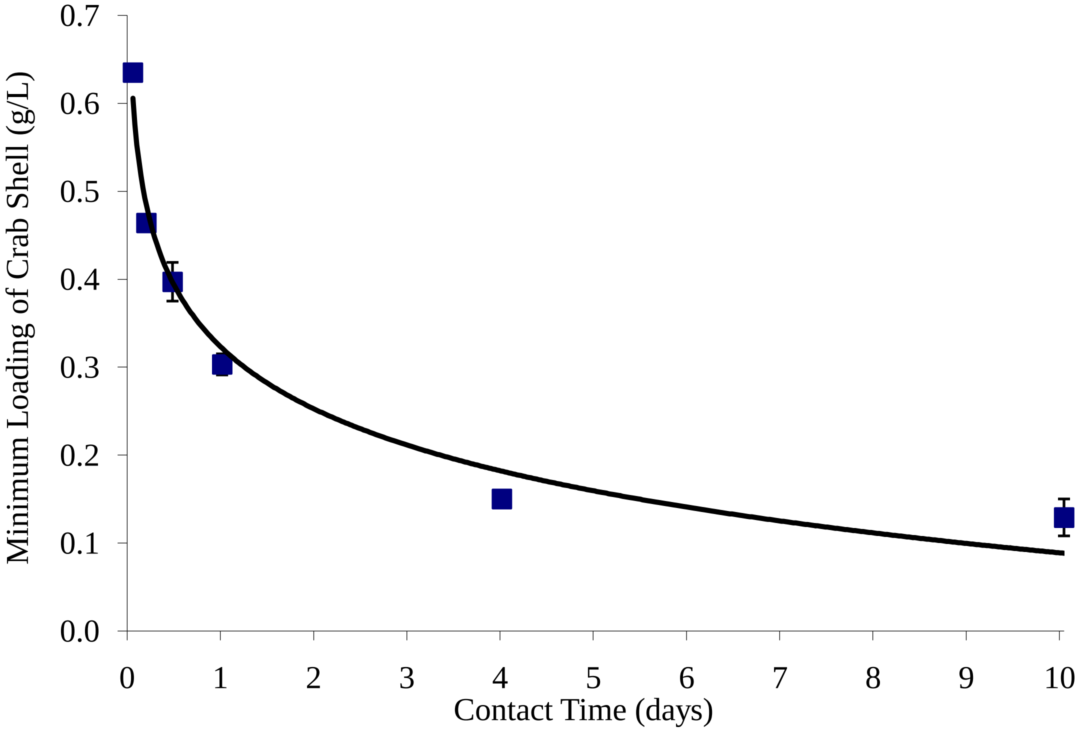


Figure A-1.1. Equation for loading capacity of crab shell based on contact time of water with the crab shell. Based on values from Table A-2.1.

A-2. MINIMUM CRAB SHELL LOADING EQUATIONS MICROCOSM EXPERIMENT

A regression analysis was performed with the data to determine a predictive empirical equation for this relationship:

$$\text{Minimum crab shell loading (g/L)} = -0.10 \ln(\text{HRT(days)}) + 0.321 \qquad \textbf{(Eqn. A-2.1)}$$

This logarithmic loading equation could be used to develop a preliminary design for the mass of crab shell required in a PTS, given a specified HRT and pore volume. For example, in a PTS ditch line similar to site 1.2 in the field study, with underdrain dimensions 700’ x 0.5 x 1.5’ with an effective porosity, n, of 0.33, and a discharge, Q, of 1.1 L/s (0.038 ft^3/s, which is mid-range for this site, measured in the field during a month with average flow, April), the following calculations apply:

Effective pore volume of ditch, V_n = (700’ x 0.5’ x 1.5’) x (0.33) = 173.25 ft^3

HRT = V_n/Q = (173.25 ft^3)/(0.038 ft^3/s) = 4559.21 s = 1.27 hr = 0.05 days

Minimum crab shell loading (g/L) = -0.10 ln(0.05 days) + 0.321 = 0.78 g/L

Assuming continuous flow for 1 year yields a total acid rain volume of:

$$\frac{1.1L}{yr} \times \frac{3600s}{d} \times \frac{365d}{yr} \times 1yr = 1{,}445{,}400L$$

The minimum crab shell required to treat this volume of water is:

$$\frac{0.78g\ \text{crabshell}}{L} \times \frac{1kg}{1000g} \times 1{,}445{,}400 = 1127kg \times \frac{2.2lb}{1kg} = 2479lb$$

We routinely recommend that crab-shell chitin be mixed uniformly with #10 sand on a 1:4 ratio by mass to ensure sufficient hydraulic conductivity. In this example then,

2479 lb x 4 = 9916 lb sand would also be required.

It should be noted, however, that this is the minimum mass required to neutralize the acidity, and would theoretically provide no additional buffering capacity for downstream waters. For additional alkalinity production, a greater loading of crab shell should be considered. However, these calculations also assume continuous flow conditions year round, which is unlikely to occur in ephemeral ditch lines; therefore, this design would be expected to impart excess alkalinity to downstream waters during periods of flow, as well as last considerably longer than 1 year. Finally, it should be noted that equation A-1.1 was developed based on the acidity of the water at this particular site (27 mg/L as $CaCO_3$, determined from initial raw water analysis). Sites with different acidities may have different treatment requirements; therefore, treatability testing is recommended prior to developing a design.

A-3. COST ANALYSIS FOR CRAB SHELL USE IN ROAD-SIDE DITCH UNDERDRAINS

Based on the minimum loading equation, the cost associated with using crab shells as a substrate in road-side ditch underdrains can be determined as follows:

For a ditch line similar to site 1.2 in the field study, with underdrain dimensions 700’ x 0.5 x 1.5’ and the minimum loading value associated with those dimensions as 2,479 lbs of crab shell (Appendix A-2),

2,479 lbs of crab shell x 0.60 $/lb = $1487.40

However, treatment capacities (calculated from the continuous-flow column experiment) can be established to provide a cost analysis based on substrate treatment. These capacities are based on the amount of Kinzua Creek raw water treated by a given mass of substrate before breakthrough of a certain analyte. For all substrates, aluminum is used to normalize the treatment capacity calculations. Based on cost estimates of $0.60/lb for crab shell and $0.006/lb for limestone, breakthrough of aluminum for the 100% limestone column occurred at day 25 and, after 55 days of treatment, the crab shell only column did not see breakthrough of aluminum. The mass of substrate in each column can be found in Table 3-1, the cost to treat a given volume of Kinzua Creek raw water is calculated in Table A-2.1. The grade of crab shell used in this study produces a price of $3.55/lb. There is a grade a crab shell that has not been processed to remove the associated water. That value is reflected in Table A-2.1.

Table A-2.1. Substrate treatment capacity and associated cost based on laboratory column studies.

Substrate	Treatment Capacity (L Kinzua Creek water treated per kg substrate)
100% Limestone	0.56
100% Crab Shell	> 1.18

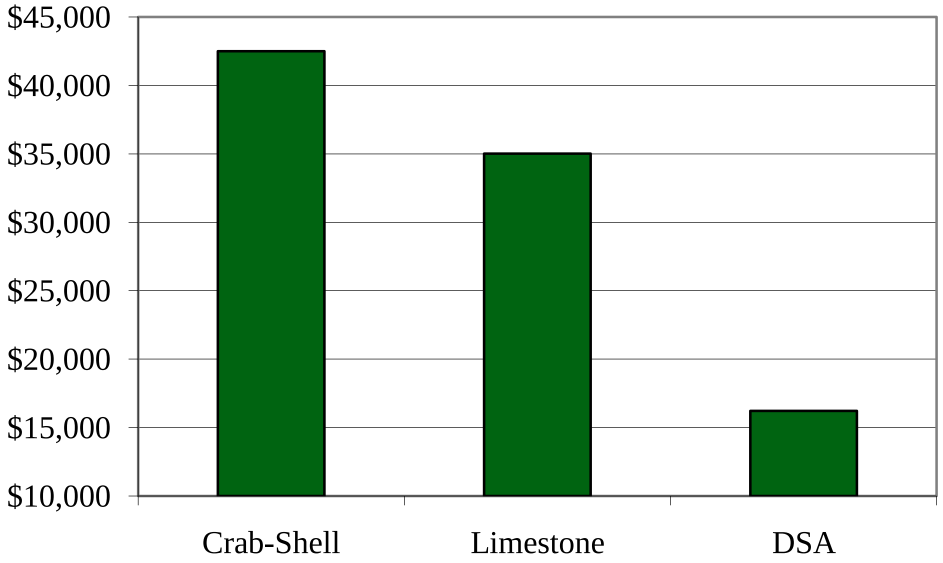


Figure A-2.1. Total actual construction costs associated with the field study.

APPENDIX B

B-1. INITIAL KINZUA CREEK RAW WATER RESULTS

Kinzua Creek raw water was delivered in 3 carboys and 2 jerricans to room 5A Sackett Buidling, University Park, PA, January 11, 2009. The results of initial pH, alkalinity/acidity, and ammonium/ammonia analysis can be found in Table B-1.1.

Table B-1.1. Initial analysis of Kinzua Creek raw water.

Sample ID	pH	Alkalinity	Acidity	TAN
	(-)	(mg/L as CaCO3)	(mg/L as CaCO3)	(mg/L as N)
Carboy 1	5.32	14.0	11.3	0.00
Carboy 2	4.74	3.00	24.3	0.00
Carboy 3	4.47	0.00	42.2	0.00
Jerrican 1	4.32	0.00	30.2	0.00
Jerrican 2	10.3	235	-213	0.00

B-2. SIX-HOUR MICROCOSM EXPERIMENT RESULTS

To better understand the chemical changes that were occurring upfront at early times in the 10 day microcosm experiment, a shorter microcosm experiment was conducted under the same conditions (1 g crab shell/L, shaken in the dark at 22 ± 2°C), but with more frequent sampling points at 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 3.5, 4, 5, and 6 hours. The results are discussed in Chapter 4 and presented below.

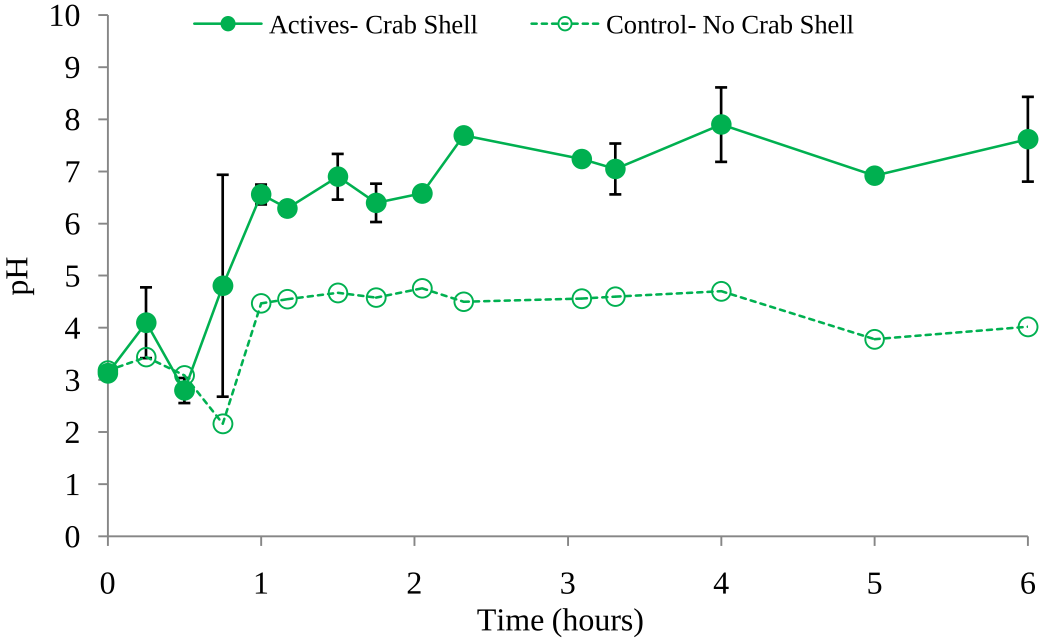


Figure B-2.1. pH measurements for the 6-Hour Microcosm Experiment testing the effectiveness of crab shell for the remediation of stream water impacted by acid precipitation. Actives are duplicate averages; controls are singlet; error bars represent one standard deviation.

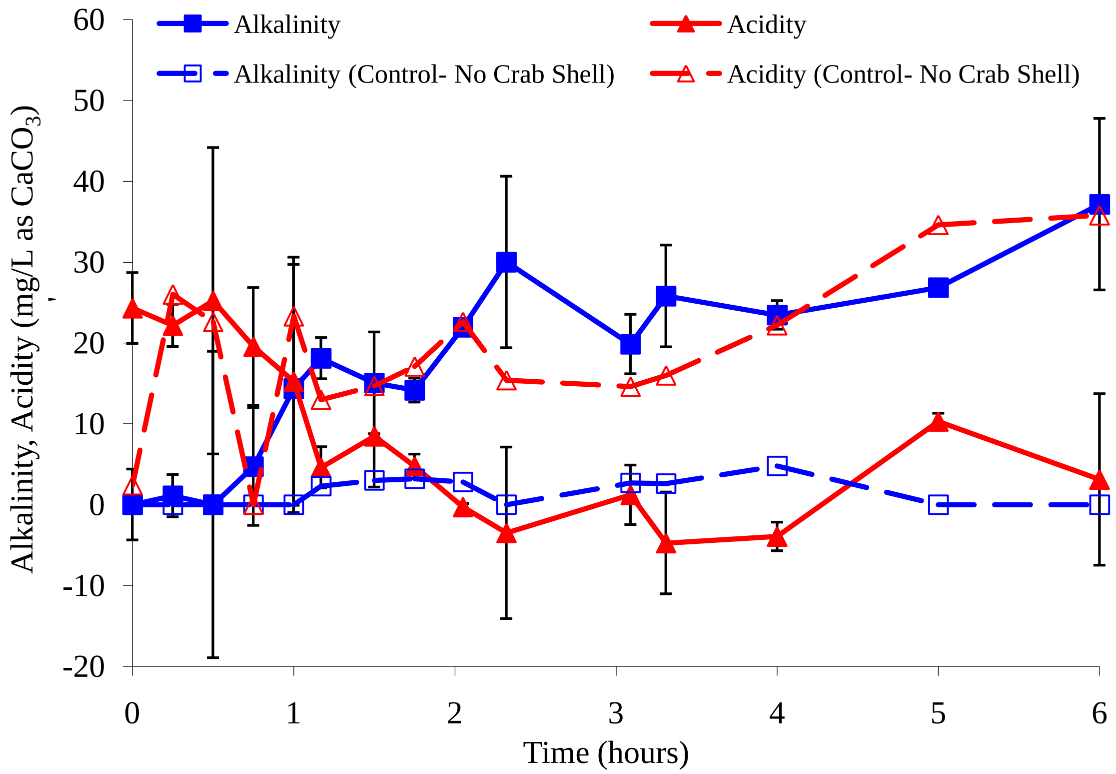


Figure B-2.2. Alkalinity and acidity measurements for the 6-hour microcosm experiment testing the effectiveness of crab shell for the remediation of stream water impacted by acid precipitation. Actives are duplicate averages; controls are singlet; error bars represent one standard deviation.

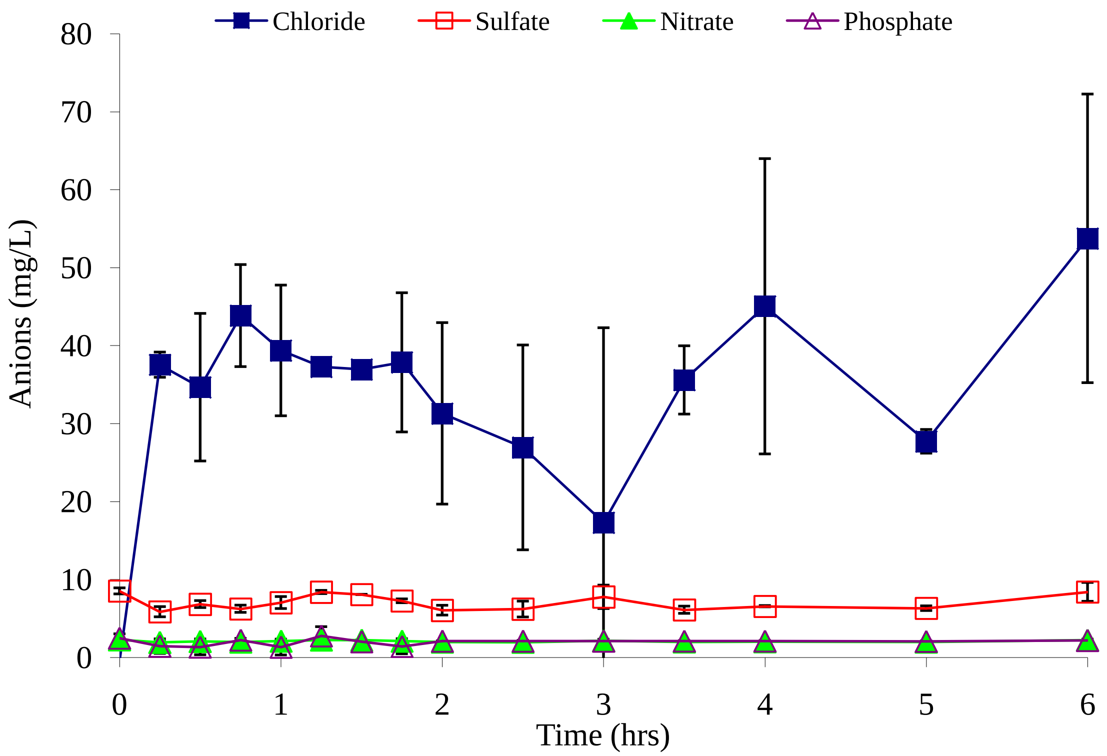
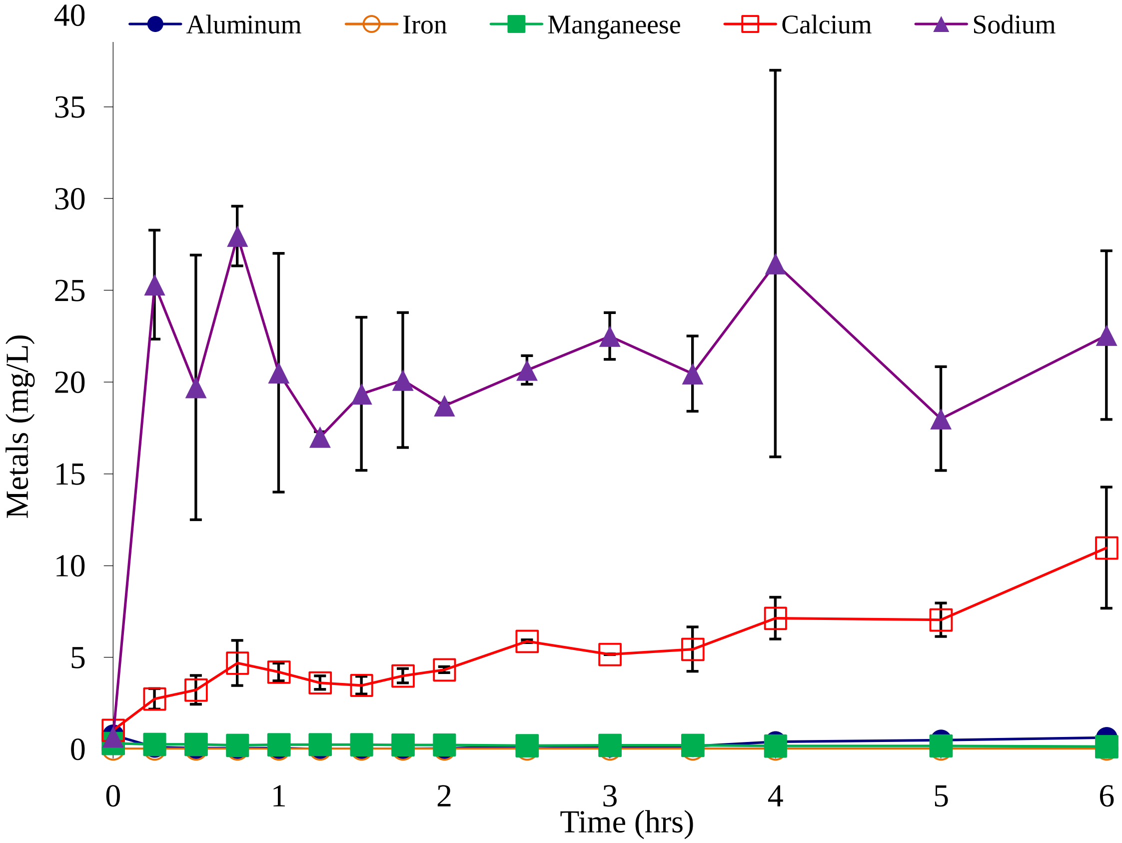


Figure B-2.3. Anion results for the 6-hour microcosm experiment testing the effectiveness of crab shell for the remediation of stream water impacted by acid precipitation. Values are duplicate averages; error bars represent one standard deviation.



FigureB-2.4. Metals Analysis of 6-hour microcosm experiment testing the effectiveness of crab shell for the remediation of stream water impacted by acid precipitation. Values are duplicate averages; error bars represent one standard deviation.

APPENDIX C

C-1. TRACER TEST CALCULATIONS

The HRT, variance, and dispersion number were calculated from the tracer test data using Equations C-1.1 through C-1.4 (Metcalf & Eddy 2003). The dispersion number is the ratio of mass transport due to dispersion and advection. The calculated HRT (t) was used as the estimated retention time (τ) to calculate the dispersion number. The HRT and measured flow rate were then used to calculate pore volume.

$$\bar{t} = \frac{\sum t_i C_i \Delta t_i}{\sum C_i \Delta t_i} \qquad \textbf{Eqn C-1.1}$$

$$\sigma^2 = \frac{\sum {t_i}^2 C_i \Delta t_i}{\sum C_i \Delta t_i} - (\bar{t})^2 \qquad \textbf{Eqn C-1.2}$$

$$\frac{\sigma^2}{\tau} = 2d + 8d^2 \qquad \textbf{Eqn C-1.3}$$

$$PV = \bar{t} * C \qquad \textbf{Eqn C-1.4}$$

Where:

$\bar{t}$ = HRT
t_i= time at ith measurement
C_i= tracer concentration at ith measurement
$\Delta\ t_i$= time increment about C_i
σ^2= variance
τ= estimated HRT
d= dispersion number
PV= column pore volume
Q= flow rate

C-2. TRACER TEST RESPONSE CURVES

A sodium chloride tracer solution was used to determine the HRT, effective pore volume, and dispersion coefficient for the columns. The nominal flow rate was 0.5 mL/min throughout the tracer tests and entirety of the 55 day continuous-flow column experiment, although the measured flow rate varied slightly throughout the experiment for each column. Calculations for HRT, dispersion number, and effective pore volume are described Appendix C-1. The tracer response curves are shown below.

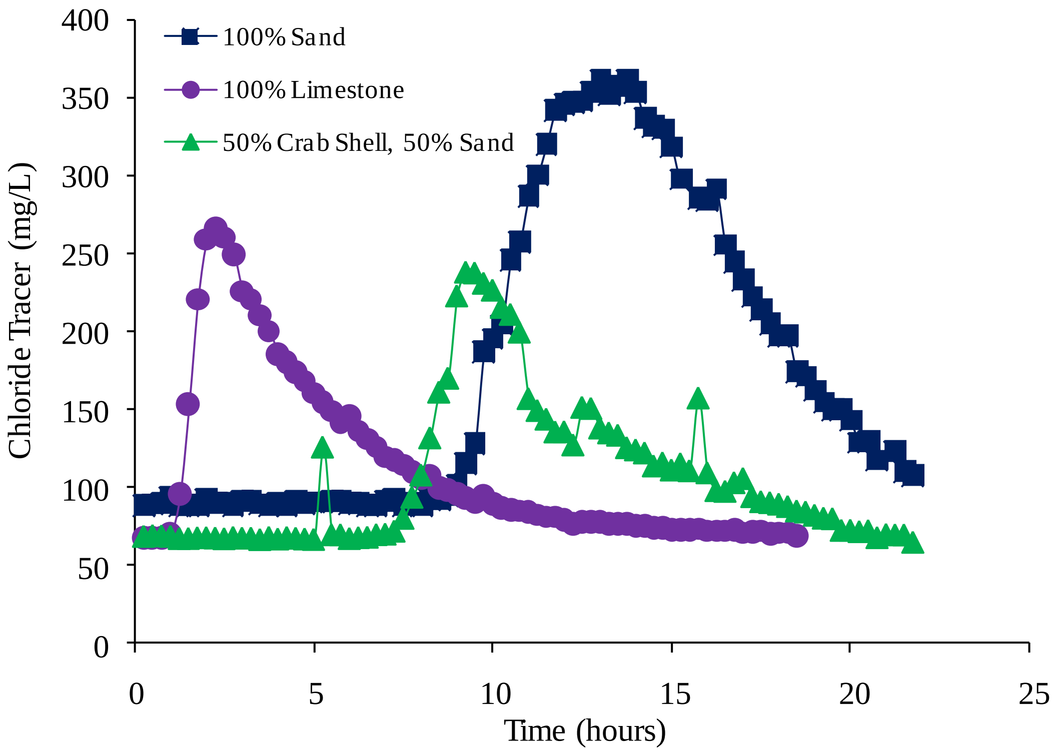


Figure C-2.1. Tracer Test Response Curve for 100% Sand, 100% Limestone, and 50% Crab Shell, 50% Sand continuous-flow columns.

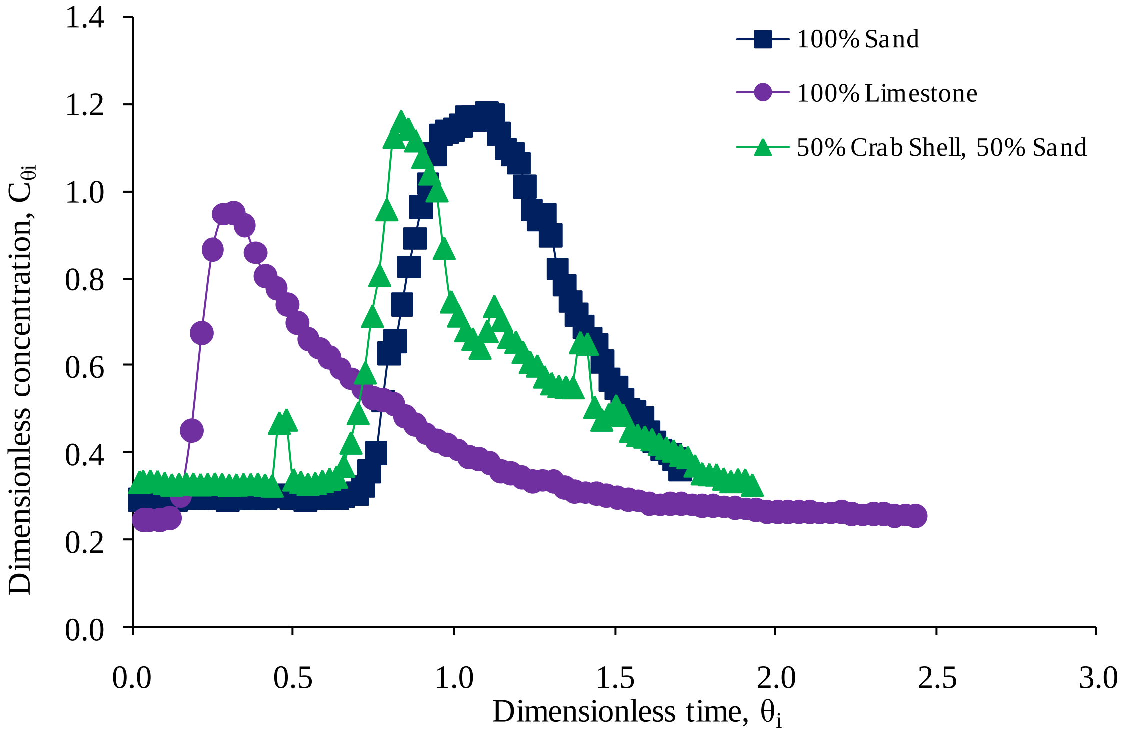


Figure C-2.2. Tracer Test Response Curves for 100% Sand, 100% Limestone, and 50% Crab Shell, 50% Sand continuous-flow columns in dimensionless time and concentration