REDUCING BORON TOXICITY BY MICROBIAL SEQUESTRATION

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ABSTRACT

While electricity is a clean source of energy, methods of electricity-production, such as the use of coal-fired power plants, often result in significant environmental damage. Coal-fired electrical power plants produce air pollution, while contaminating ground water and soils by build-up of boron, which enters surrounding areas through leachate. Increasingly high levels of boron in soils eventually overcome boron tolerance levels in plants and trees, resulting in toxicity. Formation of insoluble boron precipitates, mediated by mineral-precipitating bacteria, may sequester boron into more stable forms that are less available and toxic to vegetation. Results have provided evidence of microbially-facilitated sequestration of boron into insoluble mineral precipitates. Analyses of water samples taken from ponds with high boron concentrations showed that algae present contained 3-5 times more boron than contained in the water in the samples. Boron sequestration may also be facilitated by the incorporation of boron within algal cells. Experiments examining boron sequestration by algae are in progress. In bacterial experiments with added ferric citrate, the reduction of iron by the bacteria resulted in an iron-carbonate precipitate containing boron. An apparent color change showing the reduction of amorphous iron, as well as the precipitation of boron with iron, was more favorable at higher pH. Analysis of precipitates by X-ray diffraction, scanning electron microscopy, and inductively coupled plasma mass spectroscopy revealed mineralogical composition and biologically-mediated accumulation of boron precipitates in test-tube experiments.

INTRODUCTION

Coal-fired power plants have continuously played an important role in the production of electricity for government, economic, and personal use. Although electrical power plants positively influence humanity, their methods of waste disposal often threaten the health of vegetation and wildlife living on adjacent land. The production of energy often indirectly results in environmental degradation of areas such as wetlands, with visible effects that appear only after a significant length of time. For example, the effects of boron toxicity on wetlands surrounding coal-fired electrical power plants may often be seen as a lack of plant growth or inability of the boron-rich soils and groundwater to sustain a variety of plant and animal species.

The use of natural or artificially-constructed wetlands as a method of waste-water reclamation has increased substantially over the past 30 years (Cole, 1998) due to relative effectiveness, low cost of construction, and the passive maintenance approach commonly applied in leachate pond treatments. Coal-fired power plants often employ the use of wetlands as settling basins, which serve to contain coal fly-ash that is discharged from electrical power plants.

The presence of toxic metals in natural or artificial wetlands is often amplified through methods of pollution control, including the discharge of coal fly-ash from coal-fired electrical power plants. Coal that is burned to obtain electricity at power plants often contains naturally-occurring metals, including boron, which remains in the resulting fly-ash and is subsequently discharged into wetland areas surrounding the power plants. At normal levels, boron poses no threat to environmental health, instead serving as an essential nutrient for plant growth. The presence of boron in soils at continuous levels is required for processes of plant growth and seed production (Brown & Shelp, 1997); however, the accumulation of excess boron in soil and groundwater has negative impacts on plant health, due to the increase in adsorption of boron by roots. Boron often occurs in the form of boric acid, which is soluble in water, making the boron more readily available to plants (Nable, Bañuelos, & Paul, 1997). Continuous accumulation of boron within the environment eventually exceeds the tolerance levels of many plants.

The potential use of microorganisms to facilitate in precipitation of boron from the fly-ash ponds associated with coal-fired electrical power plants would provide an environmentally-friendly remediation alternative in returning healthful conditions to a disrupted ecosystem such as a treatment wetland. Naturally-occurring bacteria and algae in a wetland may prove beneficial in reducing the effects of boron toxicity by bacterially-mediated precipitation or incorporation of the mineral within algal cells or precipitates. Sequestration of the excess boron into an unreactive precipitate would potentially decrease boron toxicity by reducing the amount of boron available for plant adsorption.

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METHODS AND MATERIALS

A medium with 52 mg/L boron concentration was created by the addition of boric acid. The medium was then transferred to test tubes in the presence of N_2 gas and autoclaved. Separate solutions of amorphous iron, ferric citrate, calcium chloride, potassium phosphate, and a pH 9.6 buffer, containing HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and Mops, were prepared and autoclaved for approximately one hour. The pH of each tube was adjusted to approximately 7, 8, and 9, in order to show variable chemically- and biologically-mediated precipitation at different alkalinities. Following the adjustment of pH in each tube, different combinations of calcium, phosphate, and either amorphous iron or ferric citrate were added, maintaining tubes with all three of the alkalinities for every specific mineral combination. Sets of tubes containing the various mineral combinations at each alkalinity were inoculated with bacteria from different sample sites, allowing the establishment of nine different culture sets. A set of abiotic controls at the different alkalinities was also established. After 2 weeks of incubation, 5 mL of the supernatant were removed from the test tubes. The precipitate was then filtered from the remaining supernatant onto 0.4-micron Gelman filter discs and rinsed three times with molecular water of pH 11 in order to remove any residue left by the supernatant.

The incorporation of boron from solution into algal cells was tested by the construction of an algae medium to which boric acid was added, resulting in a boron concentration of 73 mg/L. A 9 mL portion of the algae medium was then transferred into test tubes and the alkalinity increased to approximately pH 7, 8, or 9 using a pH 9 buffer solution. The test tubes were then inoculated by addition of one drop, 0.1 mL, 0.3 mL, or 0.5 mL of the algae site sample as well as serial dilutions within each pH for the 2 different algae site samples. The algal cultures were placed in a light incubator and allowed to grow for 2 to 3 weeks. The entire contents of the test tubes were filtered onto 0.4 micron Gelman filter discs, and the filter and contents were then dried and used in later precipitate analyses, with 5 mL of the supernatant remaining after filtration, for each tube, were saved for later analyses.

Table 1. Visual observations of tube contents color change and precipitate formation for bacterial culture sets and abiotic controls.

	Composition	pH 7	pH 8	pH 9
Control				
	Ferric Citrate, Ca, P	no precipitate	no precipitate	no precipitate
	Amorphous Iron	precipitate	precipitate	precipitate
	Amorphous Iron, Ca	precipitate	precipitate	precipitate*
	Amorphous Iron, Ca, P	precipitate	precipitate*	precipitate
Set 1				
	Ferric Citrate, Ca, P	gray precipitate	precipitate	precipitate
	Amorphous Iron	no precipitate	darker	brown precipitate
	Amorphous Iron, Ca	precipitate	slightly darker	brown precipitate
	Amorphous Iron, Ca, P	slightly darker	slightly darker	slightly darker
Set 2				
	Ferric Citrate, Ca, P	darker **	precipitate	precipitate
	Amorphous Iron	no precipitate	slightly darker	darker **
	Amorphous Iron, Ca	no precipitate	slightly darker	darker
	Amorphous Iron, Ca, P	slightly darker	slightly darker	slightly darker
Set 3				
	Ferric Citrate, Ca, P	darker	precipitate	precipitate
	Amorphous Iron	precipitate	darker	brown precipitate
	Amorphous Iron, Ca	slightly darker	darker **	brown precipitate*
	Amorphous Iron, Ca, P	slightly darker	slightly darker	darker
Set 4				
	Ferric Citrate, Ca, P	precipitate	precipitate*	gray precipitate*
	Amorphous Iron	precipitate	slightly darker	dark precipitate
	Amorphous Iron, Ca	precipitate	gray precipitate	gray precipitate
	Amorphous Iron, Ca, P	precipitate*	slightly darker*	darker*

*Samples on which ICP analyses were performed. **Samples analyzed by ICP as well as SEM-EDX.

In order to show existence of boron in the bacterial culture precipitates, as well as contained in the algal cultures, inductively coupled plasma spectroscopic (ICP) analyses of the precipitates were performed. Analyses were conducted using 1 mL of a solution obtained by dissolving the precipitate on a measured one-third section of the filter disc in 5 mL of 10 M hydrochloric acid, which was then diluted with molecular water. The supernatants for the microbial trials were also diluted using molecular water and analyzed by ICP.

The mineralogical structure of the precipitates obtained from the bacterial cultures was determined by analyzing sections of the filtered precipitate from select samples using a Hitachi spectral display S-4700 scanning electron microscope with energy dispersive X-ray (SEM-EDX). The selection of samples that were analyzed was based on the amount of precipitate per one-third section of filter disc, as well as color of precipitate.

Precipitate and supernatant samples obtained from both bacterial and algal cultures were also analyzed using a Hach 2010 direct reading spectrophotometer, as well as a Hewlett Packard 8453 spectrophotometer, following reaction with a Hach test kit specifically for boron detection. Results provided the preliminary data used to determine which samples were most beneficial to analyze by the ICP and SEM-EDX. Selection of samples to be analyzed by the spectrophotometer, as well as the ICP and SEM-EDX, was based primarily on precipitate formation at specific mineral combinations, though coloration and quantity of precipitate were also considered. Analyses of the filtered algae cultures and supernatants were performed on tubes which had a noticeable amount of growth based on change in intensity of color.

RESULTS

The ability of microorganisms to mediate boron sequestration through precipitation of boron into insoluble solids, or isolation of boron within algal cultures, was made evident through data obtained by several different methods of analysis. Visual observation of the amount of precipitation and color of precipitates, light spectroscopy, inductively coupled plasma spectroscopy (ICP), and scanning electron microscopy with energy dispersive X-ray (SEM-EDX), all generated data showing the occurrence of boron sequestration through microbial mediation.

The visible traits of precipitates formed in the bacterial cultures served as one factor in selecting samples that were potentially more beneficial for ICP and SEM-EDX analyses. In all tubes of bacterial medium to which amorphous iron was an added mineral, formation of a precipitate occurred before addition of bacteria. Following inoculation with bacteria, and 2 weeks of incubation, there was a noticeable color change among the precipitates within tubes containing amorphous iron, as well as additional precipitation (see Table 1). In tubes containing the mineral combination of calcium and phosphate with ferric citrate, the controls showed no precipitation, however; there was a substantial amount of precipitation in the biotic tubes containing the same minerals. Precipitates formed in the Set 4 bacterial cultures at pH 8 and 9, with a mineral combination of ferric citrate and calcium, plus phosphate, were a gray color that appeared darker, and in greater quantity than the precipitate in Set 4 at pH 7 with the same mineral combination. The amount of precipitation in tubes at the various alkalinities that contained amorphous iron or ferric citrate, plus calcium and phosphate, was much greater than in tubes containing only amorphous iron. At pH 9 for either amorphous iron or ferric citrate combinations, there was more precipitate formation than in tubes with the same added metal composition cultured at pH 7 or 8.

Visual observations of the algal cultures showed the growth of green as well as red algae. Tubes to which the algae site sample was added in a serial dilution had a lighter initial color than tubes that were inoculated through direct addition of the algae sample.

Data from spectrophotometric analyses showed variations between the amount of boron present in precipitates and supernatants of the bacterial cultures compared to the boron concentration reported in analyses of the abiotic controls, although

Table 2. ICP analyses reporting concentration of boron present in precipitates for select bacterial culture sets and abiotic controls.

Culture Analyzed	Composition	pH	Concentration B (μg)	% B precipitated
Control	Amorphous Iron, Ca	9	10.25	2
Control	Amorphous Iron, Ca, P	8	6.46	1.2
Set 2	Ferric Citrate, Ca, P	7	20.49	3.9
Set 3	Amorphous Iron, Ca	8	18.91	3.6
Set 3	Amorphous Iron, Ca	9	12.53	2.4
Set 4	Ferric Citrate, Ca, P	8	26.57	5.1
Set 4	Ferric Citrate, Ca, P	9	23.73	4.6
Set 4	Amorphous Iron, Ca, P	7	15.59	3
Set 4	Amorphous Iron, Ca, P	8	22.14	4.3
Set 4	Amorphous Iron, Ca, P	9	22.44	4.3

*Composition shows mineral combination specific to each culture. Minerals; present in concentrations 10 mM ferric citrate, 10 mM amorphous iron, 10 mM calcium (Ca), and 20 mM phosphate (P).

Calculated mass of boron in media is 520 μg.

these boron levels varied greatly from the original media. Precipitates from the bacterial cultures contained a greater amount of boron than the precipitates from the controls with the corresponding alkalinities and mineral combinations. Analyses of precipitates and supernatants from cultures containing ferric citrate also proved differing concentrations of boron present at the varying alkalinities and mineral combinations, compared to the amount of boron in the original media, as well as the lack of precipitation in the ferric citrate controls. Results obtained from spectrophotometric analyses served as a guide in selection of samples to further examine by ICP analysis.

Spectrophotometric analyses of the supernatants from the algal cultures showed differing levels of boron present between the cultures containing variable concentrations of the algae site sample. Although spectrophotometric analyses showed considerable contrast between the products from the abiotic and biotic controls, the data was highly variable, therefore no conclusions could be made.

ICP analyses of the precipitates from select bacterial cultures at various alkalinities and mineral combinations detected the presence of boron. The percentage of boron found in the precipitates was calculated from the actual amount of boron reported by ICP analyses (see Table 2), and amount of boron that was added to the original media. There was a noticeable difference in precipitation for the bacterial cultures compared to the controls with the corresponding alkalinities and mineral combinations. The precipitate analyzed from the Set 4 bacterial culture at pH 8 with ferric citrate and calcium, plus phosphate, contained approximately 27 µg of boron from the original 520 µg of boron per tube, (approximately 5%), compared to the ferric citrate control which had no precipitate formation. Inclusion of boron within the precipitates was also evident in the comparison of the Set 4 bacterial culture at pH 8 composed of amorphous iron, calcium, and phosphate, to the control with the same alkalinity and mineral combination. Results showed the rate of boron precipitation was approximately 4% for the bacterial culture, and 1% for the control, suggesting 3% of the boron within the test tube may have been biologically precipitated. Precipitate analyses of the Set 4 bacterial cultures with the mineral combination of amorphous iron and calcium, plus

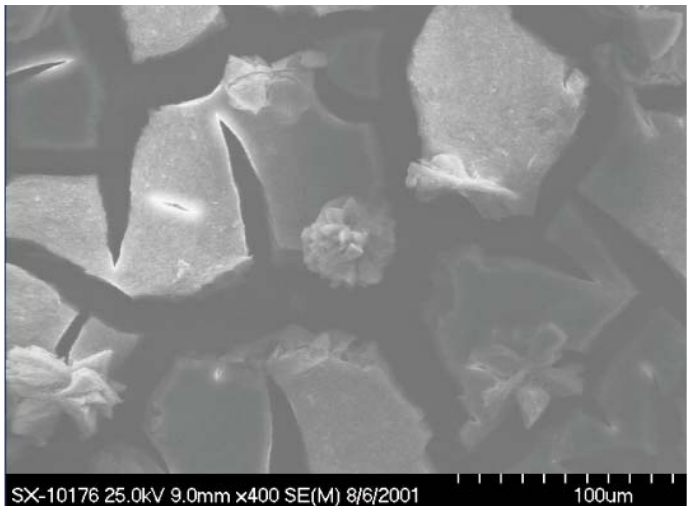

Figure 1A. SEM image showing Bacterial Culture precipitate from tube for Set 2 at pH 7 containing Ferric Citrate, Calcium, and Phosphate.

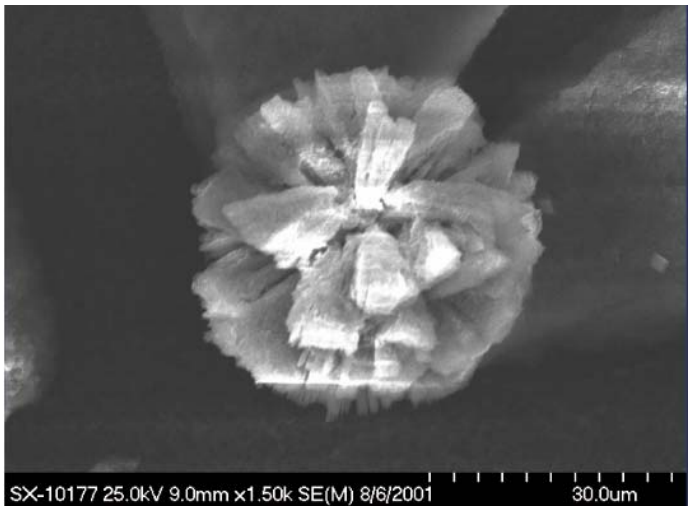

Figure 1B. SEM image showing close-up of select Bacterial Culture precipitate from tube for Set 2 at pH 7 containing Ferric Citrate, Calcium, and Phosphate.

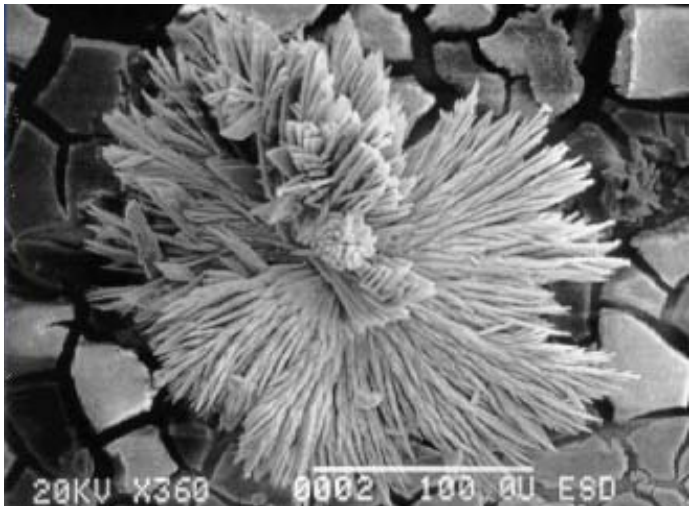

Figure 1C. SEM image showing Bacterial Culture precipitate from tube for Set 2 at pH 7 containing Ferric Citrate, Calcium, and Phosphate.

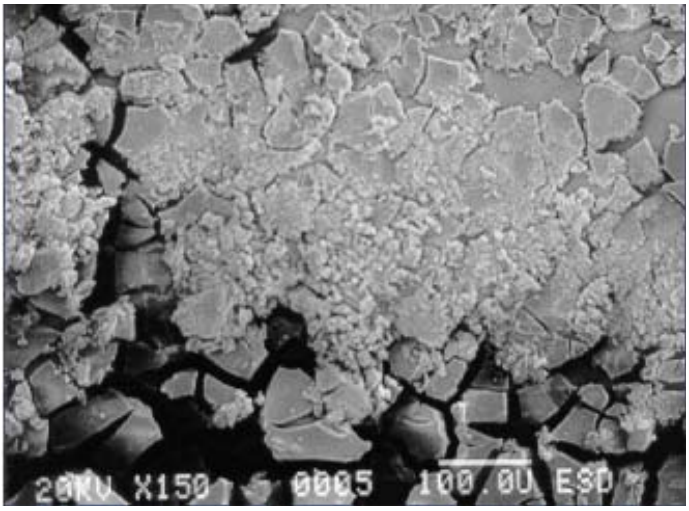

Figure 1D. SEM image showing Bacterial Culture precipitate from tube for Set 2 at pH 7 containing Ferric Citrate, Calcium, and Phosphate.

phosphate, for pH 9 when compared to the pH 7 analysis showed a precipitation rate of approximately 4% to 3%; respectively.

Spectral analyses with the Hitachi S-4700 SEM-EDX, performed on the contents of the tubes for several of the bacterial cultures, showed further evidence of boron precipitation. The precipitates formed at pH 7 in the presence of ferric citrate, calcium, and phosphate, for the Set 2 bacterial culture (see Figures 1A and 1B), were in the shape of spherical rosettes. Formation of various types of precipitates within culture Set 2 was also shown by SEM images (see Figures 1C and 1D), revealing the presence of conical and crystalline precipitates as well. Energy dispersive X-ray analysis (see Figure 1E) of the selected SEM image shown in Figures 1A and 1B, revealed that the precipitate contained large amounts of iron and phosphate, as well as boron. The presence of boron in the precipitate was indicated as a peak located next to carbon on the graph, though due to its close relation with carbon, was subsequently left unlabeled by the graphics program. The SEM images taken of the precipitate formed in the tube for the Set 2 bacterial culture at pH 9 containing amorphous iron, revealed two distinct types of precipitation. The smooth surfaces of the precipitates shown in Figure 2A suggested microbially-mediated precipitation, while the shape of the precipitate in Figure 2B was characteristic of the chemically-mediated precipitate formation of vivianite. Energy dispersive X-ray analysis of the precipitate formed in the tube for the Set 2 bacterial culture (see Figure 2C) showed high levels of carbonate formation in comparison to the presence of iron in the precipitate. The SEM image of the Set 3 bacterial culture at pH 8 containing amorphous iron and calcium (see Figure 3A), also revealed the presence of crystalline precipitates. Formation of additional precipitates over an existing crystalline precipitate (see Figure 3B) was shown in the SEM image of a different section for the same bacterial culture viewed in Figure 3A. The EDX analysis (see Figure 3C) exam-

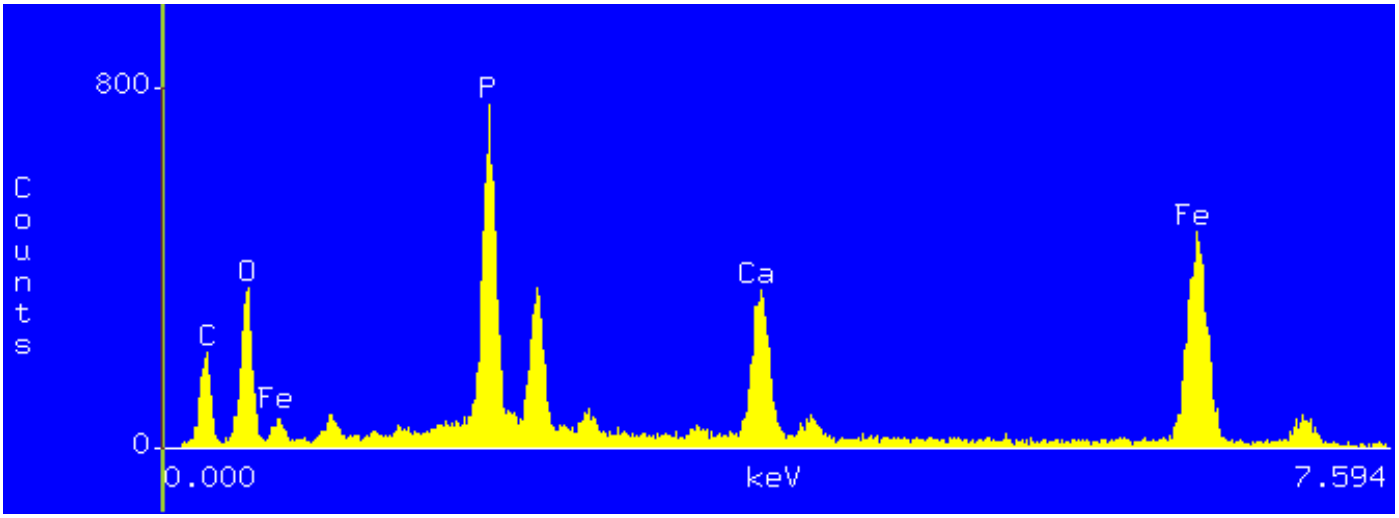

Figure 1E. EDX analysis of bacterial culture precipitate from tubes for Set 2 at pH 7 containing ferric citrate, calcium, and phosphate. Accelerating Voltage: 25 KeV; Take Off Angle: 30°; Live Time: 100 seconds; Dead Time: 15.4

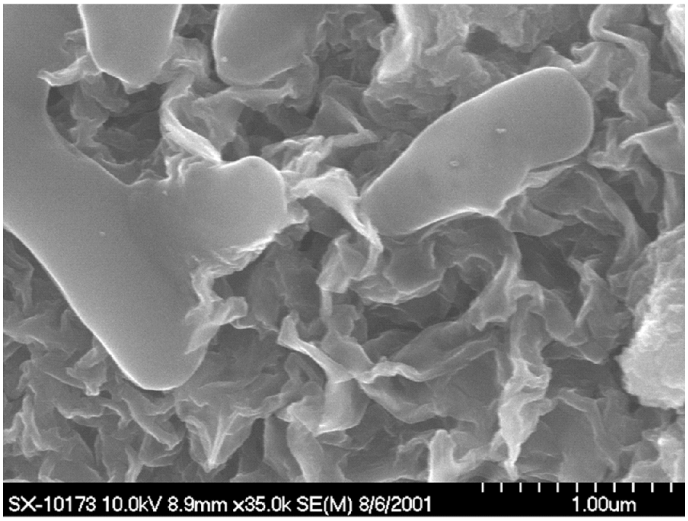

Figure 2A. SEM image showing Bacterial Culture precipitate from tube for Set 2 at pH 9 containing Amorphous Iron.

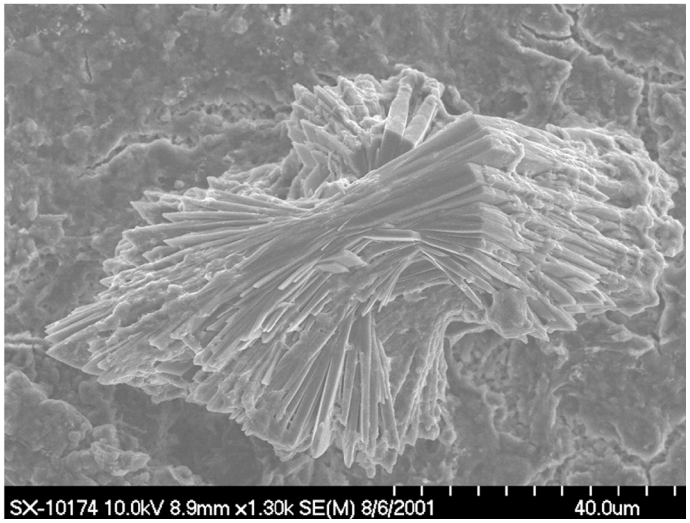

Figure 2B. SEM image showing Bacterial Culture precipitate from tube for Set 2 at pH 9 containing Amorphous Iron.

ining the selected portion of the precipitate shown in the SEM image in Figure 3A for the Set 3 bacterial culture confirmed the existence of boron.

Preliminary analyses performed on the two different algae site samples by Activation Laboratories Limited reported boron concentration of the algae as 52,000 mg/L and 27,400 mg/L, compared to the boron concentration of the water in each sample, which was 2,010 mg/L and 2,680 mg/L, respectively (data not shown). The high levels of boron within the algae compared to the water, suggested the ability of algae to incorporate boron into cells. Inclusion of boron within algal cultures was partially verified by ICP analyses, revealing the presence of boron in the contents of the cultures that remained on the filter disc after filtration, although it was not determined whether the boron was present within the cells or in algae precipitates.

DISCUSSION AND CONCLUSIONS

The microbially-mediated sequestration of boron was demonstrated by the precipitation of insoluble solids in the presence of bacteria, and the incorporation of boron from solution within algal cultures. The method by which bacteria facilitated the precipitation of boron was unclear, although data gathered by SEM-EDX analyses showing high levels of iron in the precipitates, suggested it might have been related to the reduction of iron.

Visual observations of the bacterial cultures provided evidence of biologically-mediated precipitation, shown by the formation of precipitates in tubes containing mineral combinations with ferric citrate, when compared to abiotic controls with identical compositions, which had no visible precipitation. A noticeable change of color from brown to dark gray, which occurred to the precipitates in a majority of the tubes contain-

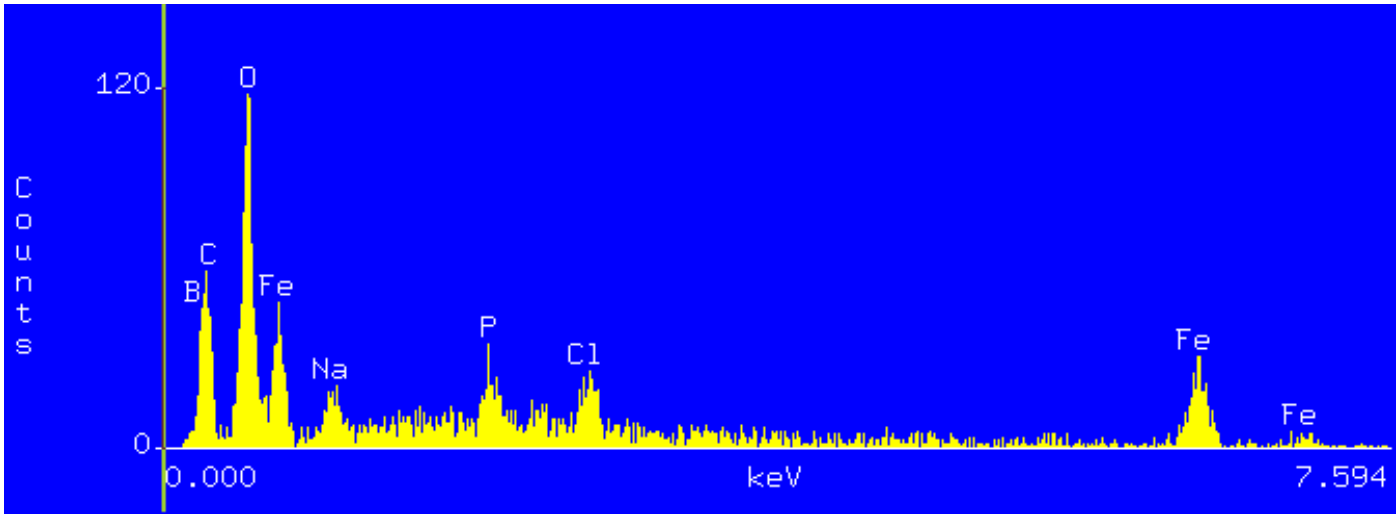

Figure 2C. EDX analysis of bacterial culture precipitate from tube for Set 2 at pH 9 containing amorphous iron. Accelerating Voltage: 25 KeV; Take Off Angle: 30°; Live Time: 100 seconds; Dead Time: 7.5

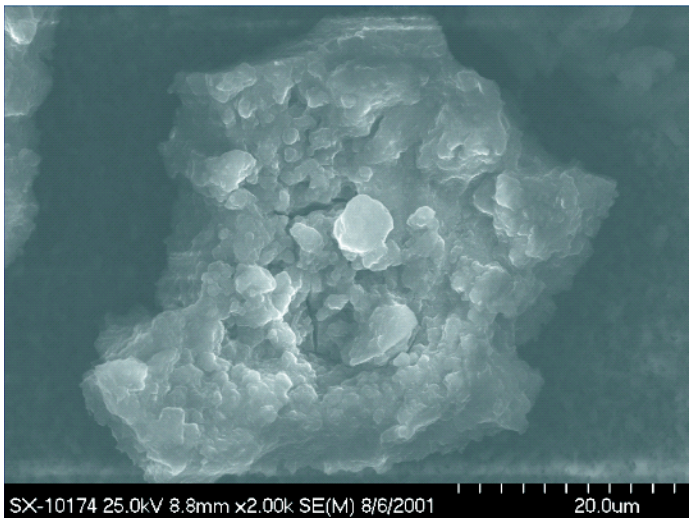

Figure 3A. SEM image showing Bacterial Culture precipitate from tube for Set 3 at pH 8 containing Amorphous Iron and Calcium.

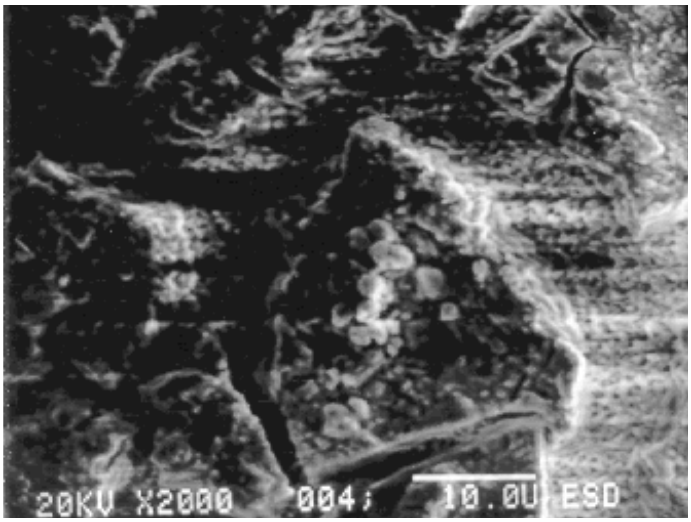

Figure 3B. SEM image showing Bacterial Culture precipitate from tube for Set 3 at pH 8 containing Amorphous Iron.

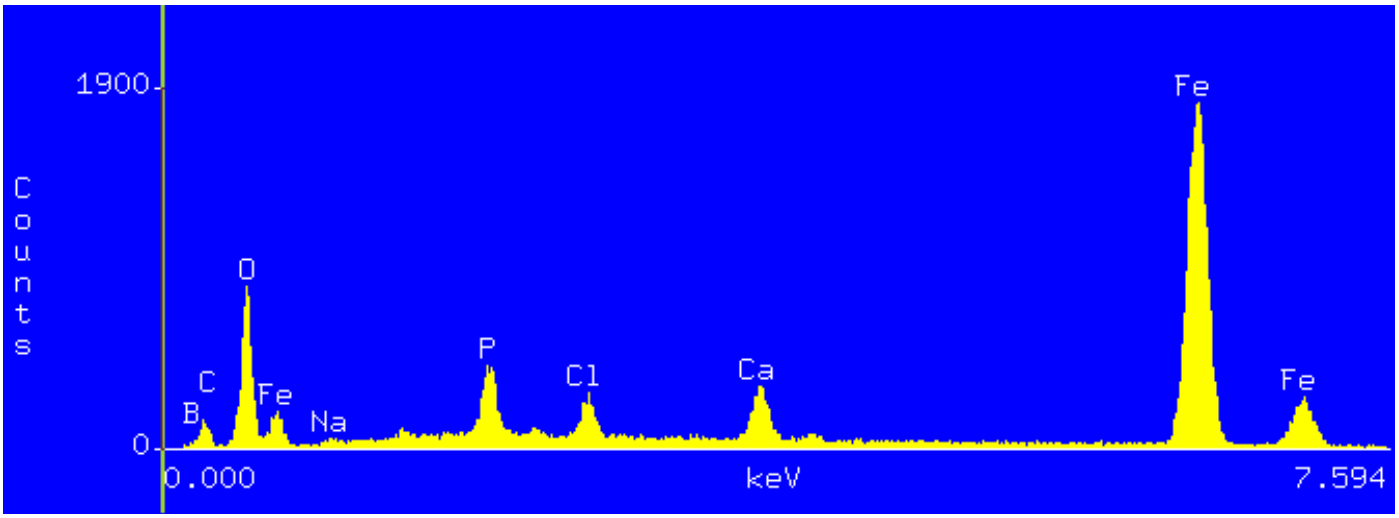

Figure 3C. EDX analysis of bacterial culture precipitate from tube for Set 3 at pH 8 containing amorphous iron and calcium. Accelerating Voltage: 35 KeV; Take Off Angle: 30°; Live Time: 100 seconds; Dead Time: 21.3

ing amorphous iron, after addition of bacteria, suggested that the added bacteria facilitated the reduction of iron. The change in reactivity of amorphous iron following its reduction may have resulted in the formation of additional precipitates containing iron and several of the other added minerals. High levels of iron in select portions of the precipitates were reported by SEM-EDX analyses. Precipitation occurred in a greater number of bacterial cultures containing ferric citrate under pH 9 conditions when compared to tubes at pH 7, suggesting that conditions with higher alkalinity were preferential. In several of the bacterial culture sets, formation of precipitates in tubes containing ferric citrate occurred only at pH 7, suggesting the existence of several different types of bacteria mediating precipitate formation. A greater amount of precipitation in tubes that contained either ferric citrate or amorphous iron, with calcium and phosphate, compared to tubes containing only iron, suggested that calcium and phosphate, as well as iron, were incorporated into the precipitates, as shown by SEM-EDX analyses.

Further evidence of boron precipitation through mediation by bacteria was provided by ICP analyses. Comparison of analyses for bacterial culture precipitates to controls of identical alkalinity and mineral composition show that bacteria served a significant role in increasing the amount of boron precipitation. Data from ICP analyses of the precipitates also revealed that a greater amount of boron precipitation occurred at higher alkalinity, as well as with additional mineral constituents, coinciding with the visual observation that more precipitates had formed.

Mineralogical analyses performed on select sections of several precipitates with SEM-EDX verified the presence of boron, shown in relation to levels of additional minerals present in specific combinations within the various tubes. Energy dispersive X-ray analyses on sections of the bacterial culture precipitates revealed the presence of high levels of iron and phosphate for cultures containing amorphous iron as well as ones with ferric citrate. Although precipitates were formed in the controls containing amorphous iron, the lack of precipitation that occurred in the ferric citrate controls suggested the inclusion of iron and phosphate within the precipitates was facilitated by bacteria. Formation of carbonates through incorporation of CO_2 within the precipitates was shown in the EDX analysis of the precipitate for the Set 2 bacterial culture at pH 9 containing amorphous iron.

The potential application of technologies incorporating principles of environmentally friendly pollution control methods, such as microbially-mediated sequestration of boron into a form that is less reactive and toxic to plants and animals, may provide a way to remediate the effects of environmental degradation while encouraging biological growth and preserving wetland health.

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