

Impact of chitin bioremediation on
wastewater and heavy metal
contaminated soil

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metal contaminated soil



Sandy Hook, Connecticut

Abstract

Chitin is a nitrogenous carbohydrate and the second most abundant organic compound on earth. One major use of chitin is bioremediation and purification on wastewater and contaminated soil. Japanese scientists have found that chitin is effective on the clean up of metals and other organic compounds within water and soil, such as PCBs. With its strong positive charge, chitin can bind to many negatively charged heavy metals and macromolecules. Also, as a polymer it can form long chains which act as natural hooks to catch and coagulate the metals, organic compounds and contaminants from wastewater. Chitin acts as a chelating agent, a polydentate ligand, capable of occupying two or more sites in the coordination sphere (consisting of chitin and a heavy metal, aluminum). Samples of water and soil were contaminated with aluminum chloride. Three trials were conducted, first testing for aluminum in the control samples, and then testing for aluminum after applying the chitin substance. The chitin substance used was in the original natural form of arthropod shells, mainly a crushed form of lobster shells. Water was remediated with chitin over a period of 10 minutes, resulting in a 40-70% reduction of aluminum chloride, and an overnight period, resulting in similar reduction in each of the three trials. Soil testing demonstrates less valuable, preliminary trials indicated only a 40-60% reduction of aluminum chloride. Chitin samples were also combined with different concentrations of shredded polyethylene plastic from used beverage bottles. Since there is such an overabundance of recyclable polyethylene, it was tested to see if the chitin would be more effective if extended. Polyethylene bulking of chitin, while minimally more effective, was not a significantly different remediation strategy. Improvements to soil protocol at an EPA Brownfield Batchelder Site, 44 Swamp Road, Newtown, Connecticut, will be tested to increase the efficiency of site cleanup.

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Biography:

Maricate Conlon is a junior at Newtown High School. She joined the applied science research course to further her interests in environmental and biological sciences. She competitively swims year-round and is a part of the girls varsity swim team. She is also involved in clubs at school; she is a member of the Guidance Honors Association and is her student class council secretary. She has continually been involved with water purification efforts. In July 2005 she swam in the Madison Mile with proceeds going to the Long Island Sound. Also for this swim she collected \$600 in sponsorships to give to the World Help Foundation in order to pay for installation of water purification systems for Sam Sam Kenan, a small village in Ghana. This year she began a club named World Help Youth to join up with the water purification efforts of the World Help Foundation. In the future, she plans to pursue a degree environmental science and/or environmental law.

Introduction:

Chitin is a nitrogen containing polysaccharide consisting of sugar molecules strung together (Dalwo 1998) and is the second most abundant organic compounds in nature behind cellulose. Also, it ranks one of the three most abundant polysaccharides in nature, along with cellulose. Chemically related to glucose, chitin has many versatile properties, making it a good pure material for scientific study. It is natural, non-toxic, non-allergenic, antimicrobial, and biodegradable. This positively-charged substance is able to bond with negatively charged surfaces such as metals, skin and macromolecules, specifically proteins (NOAA Sea Grant 2005).

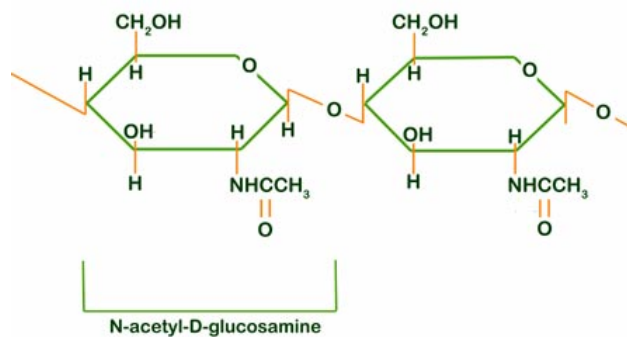


Diagram 1 (NOAA Sea Grant 2005).

Chitin's chemical compound structure is similar to starch and cellulose.

Chitin is a desired substance for many scientists and researchers because it can be processed into derivatives, most readily chitosan. Chitosan is formed when chitin alone is heated within a chemical solution. The main advantage of chitosan is that it is a water-soluble substance and easier to control. Scientific data supports claims that chitosan helps bind fats and cholesterol in the blood stream and initiating clotting of red blood cells (Hoashi 1995). Dr. Sam Hudson of North Carolina State University holds great hope that chitin and its derivatives, especially chitosan, will create valuable new products

which can put America and the scientific world on the verge of new medical, cosmetic, and environmental breakthroughs (Dalwoo 1998).

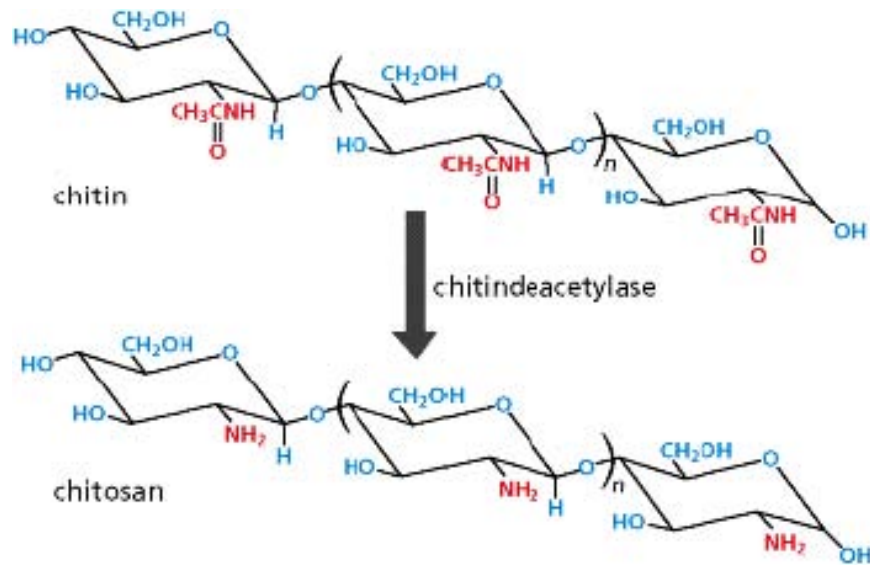


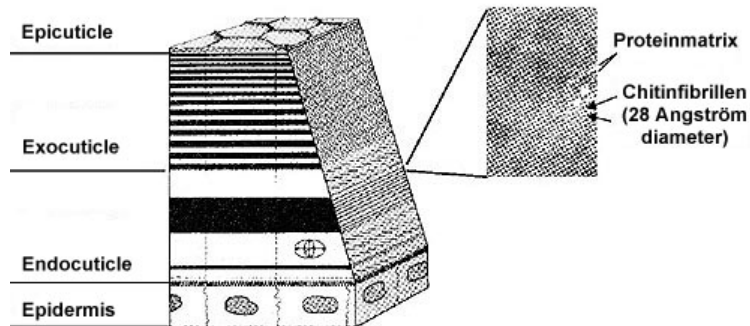
Diagram 2

(Fraunhofer IGB).

Chitin is chemically changed to adapt to the derivative of chitosan.

The pure form of chitin can be found in most arthropods, including insects, scorpions, lobsters, horseshoe crabs, shrimp, etc (Dalwoo 1998). The hard shell or exoskeleton of these creatures is contributed to the millions of tightly interwoven strands of cellulose-like chitin. It is the glue that holds the shell together, flexible enough so the creature can still move about, yet tough enough to serve as a protection mechanism from harmful predators or natural occurrences. Figure 1.3 shows the shell layers of a regular arthropod shell, emphasizing the Chitinbrillen located in the Endocuticle. Chitin is also found in

other marine life, for example the exoskeleton of zooplankton, coral and jellyfish. This natural substance is not only found in animals, but in mushrooms and fungi such as the cell walls of yeast (Roberts 1992). Such ready availability is another appealing aspect of this substance.



Schematical diagram 3 of the arthropod cuticle

(Forschung).

Arthropods exoskeleton is divided into layers, chitin is usually and primarily found in the proteinmatrix of the exoskeleton.

The first investigation of chitin was conducted by Professor Henri Brancott in 1811 in Nancy, France. He discovered the substance in the cell walls of mushrooms as a Professor of Natural History and Director of the Botanical Gardens at the Academy of Sciences (NOAA Sea Grant 2005). The research and development with chitin continued into the 1830's when it was isolated from insects and actually named chitin. The discovery of derivatives of chitin was made in 1859 but Professor C. Rouget leading to a century of fundamental research and preliminary understanding of the substances (NOAA Sea Grant 2005). The interest in the development and potential of chitin and chitosan intensified in the 1930's and early 1940's, with almost 50 patents on chitin products.

However, as the lack of sufficient manufacturing facilities became a pressing issue and the competition for synthetic polymers grew the commercial development of chitin was hindered. For almost 30 years no new discoveries or applications of this substance were researched or exposed to the scientific community, until 1970 when new laws and regulations were passed to limit the amount of contaminated shellfish dumping in coastal waters (Pennisi 1993). Chitin then became the economical way to abide by regulations and dispose of the high level of untreated shellfish waste produced annually. The interest and encouragement of chitin research resumed. A worldwide movement to find and adapt the abilities and applications of chitin began with a specific focus on better utilization of the available source of the substance in shellfish (Chamoy 2000).

Today there are nearly 200 patents in the United States for chitin based products. While there are 15 major chitin processors worldwide the abilities and applications of chitin are still unknown to many in the world, our country and locally (NOAA Sea Grant 2005). Research Universities such as University of Maryland , Delaware, North Carolina and Clemson University have expended time, funding and energy on chitin research. Clemson University is focusing on elimination 100 % of the waste produced when shellfish are discarded around the world (Pennisi 1993).

The uses of chitin are of a wide range of scientific and everyday quality and value. Studies beginning in the 1970's adapted and used chitin and chitosan to aid in nutritional and medicinal purposes. Dietary supplements containing chitin can help to cure or less certain ailment such as high blood cholesterol, high blood pressure, allergies, arthritis and can help with the improvement of skin, hair and nail health (Dalwoo 1998). Medical bandages and antibacterial sponges can be made or contain chitin as a healing

product to control and reduce the seriousness of flesh wounds. First studied by the Japanese, wound healing using chitin is now an industry with patented products. A Japanese firm used chitin to create burn dressings, surface wound dressings, and skin graft donor sites (Dalwoo 1998). These hospital dressings greatly accelerated the healing process and reduced the pain associated with such skin wounds when compared to traditional methods. Other everyday uses and products containing chitin include dental plaque inhibition, sponges, contact lenses, diapers, feminine pads and tampons. These products and many products involving chitin and chitosan have to do with chitin's obvious absorbing qualities (De Campos Takiki 2005).

Another everyday medical aspect of chitin is its proven ability in weight loss techniques. Chitin acts like a fiber and can replace calories in many foods enabling it to increase weight loss margins in tested subjects. The experiment done to test this theory used chickens which consumed a microcrystalline form of chitin. Results show that chickens that had the chitin form in their feed were leaner than the chickens who consumed normal feed (Hoashi 1995).

In addition to the medical uses of chitin, it also is used in the cosmetic industry. Chitin's positive charge enables it to adhere well to the negatively charged skin, teeth, and nails (Roberts 1992). Patented chitin cosmetics include make-up powder, nail polish, moisturizers, and certain toothpastes (Pennisi 1993).

The agricultural industry also has a place for chitin as a protecting barrier or shell to seeds. Chitin is used to treat seeds in order to maximize their resistance to fungal disease. Studies have shown that crops which are grown from chitin coated seeds yields about 50% more crops (Pennisi 1993).

The numerous uses of chitin however seem trivial when compared to the remediation effect it can have on contaminated environmental areas. Chitin is a highly absorptive chelating agent which is used in the biosorption or remediation of wastewater and contaminated soil. As a chelating agent, chitin bonds and attracts the heavy metals around it and thereby decontaminates the surrounding environment. It has been labeled a cost effective way to reduce the amount of contaminated landscapes in the United States. Japan is a leading country in the bio-remediation effect of chitin and has come to observe that chitin can clean up toxic organic compounds, for example, PCBs (Rosner 1995).

This project will primarily focus on the decontamination and remediation of contaminated soil and wastewater from a specified site. The site that the research will be applied to is the Batchelder Site, located on Swamp Rd., Newtown, Fairfield County, Connecticut. The site is an inactive aluminum smelting facility, consisting of two buildings, on about a 30 acre area. The site was in operation from 1947-1987. The land area is surrounded by wetlands to the south, residential areas and homes to the west, woodlands on the east and Housatonic Railroad systems in the north (Batchelder Evaluation 1990).

After a preliminary walk through, in June 1996, conducted by the EPA On Scene Coordinator (OSC), Gary Lipson and Roy F. Weston, and a member of the Superfund Technical Assessment and Response Team (START), Joseph Resca, it was stated there were traces of possible contamination of aluminum, arsenic, lead, nickel and indication of PCBs. Overturned drums containing an unidentified material were dispersed on the property in addition to counted three approximately 20,000-gallon above ground storage tanks set in the northeast sector of the property. Visually the tanks contained only

minimal water and in one tank a petroleum-like substance was observed and noted. The land and area surrounding the tanks was heavily stained and indicative of oil. A former landfill discovered by CT Department of Environmental Protection representative, Thomas O'Connor, was located in the southwest sector of the site and had signs of the liner and earthen cap being breached in different areas. The site remains now as an Environmental Protection Agency (EPA) Brownfield Site (Batchelder Evaluation 1990). Diagram 1.4 is the layout of the contaminated Batchelder site showing each of the areas of contaminated wastewater and most likely contaminated surrounding wetlands.

Additional testing and walk-through dates were conducted in August, September, and October, 1996. During the walk-through 31 samples were attained from different sample locations. Nineteen of the total screened for volatile organic compounds, 21 samples screened for polychlorinated biphenyls (PCBs), and 20 of the 31 screened for selected metal compounds, such as zinc, lead, chromium and silver. All samples were taken within the three surface inches of soil, waste from the tanks and drums, the landfill, and debris from waste piles. For lab confirmation of this data samples were submitted to the New England Regional Laboratory (NERL) for confirmation of the analyses. All soil, water, and waste samples were submitted to confirm the levels of PCB and metal contamination (Batchelder Evaluation 1990).

Due to this contamination of this site it remains on the market owned by the Batchelder trustees. No company has had interest in this site due to the extensive clean up that it would require. The funding and time that would need to be devoted to a chemical or commercial clean up is a disadvantage and problem for companies to take on. The significance of chitin's effect on the Batchelder site would be that if the chitin

remediate the PCBs and metal contamination, then the site could be marketed differently. The new cost effective way to remediate a contaminated site could mean new doors and new revenues for the Trustees and for a new company to use the land (Batchelder Evaluation 1990).

If a two-year test period was allowed, a team of University of Connecticut or even out of state professors and students could work on the remediation this property using the natural source of chitin. After a the period concludes, tests and conclusions could be drawn to assess the group's progress and reduction of contaminating substances. If the contamination level decreases, then the Trustees could market the site as a downgrade from EPA Brownfield Site. Also, if further decontamination, absorption and remediation is needed companies can still pursue the property knowing that there is a cost effective way to clean up and make use of the contaminated land mass.

This project could not only establish remediation with chitin locally, but could encourage the biosorption of other contaminated land masses which are unused and ineffectively marketed.

Problem

Pure clean water is a scarce natural resource. Much of contaminated water in the local Newtown area and around the world is chemically cleaned and purified, yet these techniques are extremely expensive. Soil contamination is also a rapidly growing environmental problem for the United States. Cost effective remediation techniques are scarce, and require much clinical testing before put to use. However, some natural resources can be implemented in such cleanup of wastewater and soil, especially the second most abundant organic c compound, chitin, found in lobster shells. The Batchelder Site located at 44 Swamp Road, Newtown, 06470, is contaminated with heavy metals the most prominent being aluminum and can't be sold due to the extensive necessary clean up. Can chitin in the natural form of lobster shells be used to cost effectively remediate the contaminated wastewater on the site? Can land sites with similar conditions of heavy metal contamination be marketed differently to inquiring companies, which were previously worried about financial investment in chemical remediation?

Materials

Consumables	Supplies	Equipment
Sodium Chloride Lobster Shells Soil Plastic Soda Bottles Distilled Water	Aquarium Separatory Funnel Beakers Cuvetts Filter Paper Funnel PVC plastic tubing Scissors	UV-VIS Spectrophotometer

Procedures

Phase I- Water Remediation Testing

Measure out 100 mL of distilled water into a 200 mL beaker. Using an electric balance measure out 1 gram of Aluminum Chloride. Under a Fume Hood, add the 1 gram of Aluminum Chloride to the 100 mL distilled water. Let this solution sit for 10 minutes to ensure that the Aluminum Chloride dissolves.

Reduce the Aluminum Chloride concentration in the distilled water by using the process depicted in the diagram below. Fill four more 200 mL beakers with 90 mL of distilled water. Then from the first beaker using a pipette, measure 10 mL of the solution into a 100 mL graduated cylinder. Pour the contents of the cylinder into the next beaker.

Continue this process to the fifth cylinder.

1 g Al	→ 10 mL	→ 10 mL	→ 10 mL	→ 10 mL
_____	_____	_____	_____	_____
100 mL H ₂ O	90 mL H ₂ O	90 mL H ₂ O	90 mL H ₂ O	90 mL H ₂ O
1 g Al	10 mL	10 mL	10 mL	10 mL
100 mL H ₂ O	90 mL H ₂ O	90 mL H ₂ O	90 mL H ₂ O	90 mL H ₂ O
359.8 mg	35.98 mg	3.598 mg	.3598 mg	.03598 mg
Al/L	Al/L	Al/L	Al/L	Al/L

Follow the H₂O Manual Aluminum Test. Add 5 Aluminum ECR Reagent packets.

The solution should turn red to indicated aluminum presence. Using a pipette transfer about 3-5 mL of the new red sample to a spectrophotometer sample cell tube. Place this tube along with another tube filled with distilled water into the spectrophotometer. Set the spectrophotometer to "Freerun" method. Set the wavelength to 535 nm. Press start and record the absorption in a data table. Complete this process two more times.

However, the second time, add 2 grams of Aluminum Chloride and the third time start with 3 grams instead of 1 gram. Next, use a blender to crush an ordinary lobster shell into small, fingernail size pieces. Measure out 25 grams of this chitin lobster substance on an electric balance. Transfer the chitin lobster substance to a separatory funnel by means of scoopula. Make certain that the knob on the bottom of the funnel indicates that the dispenser is closed. Pour the first diluted control sample into the separatory funnel. Leave the sample in the funnel for 10 minutes. Turn the knob on the lower part of the funnel to allow the solution to filter out of the solution. Again follow the ECR Aluminum Reagent test and spectrophotometer process. Complete multiple trials for each concentration, .03598 mg Al/L; .07196 mg Al/L; and .10794 mg Al/L, and record in a data table. Create a standard curve graph to analyze data. Repeat the whole procedure except change the process to an overnight remediation period.

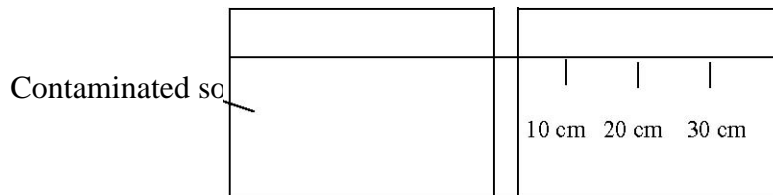
Test effects of addition of shredded polyethylene plastic from used beverage bottles. Add parts (by mass) of chitin and polyethylene in (3 parts chitin/0 parts polyethylene, 2p/1p, 1p/2p, and 0p/3p). Samples were tested at 10 minutes with 0.10794 mg/L of Al solution using the above protocol.

Phase II- Soil Remediation Testing

Using a fish tank or terrarium and PVC tubing, drilled with dime sized holes 3 cm

apart along adjacent side of the cylinder, create a soil testing apparatus depicted in the diagram below.

Mass Out 40 grams of potting soil and add 8 grams of aluminum chloride. Manually work the aluminum chloride into the soil with gloved hands until the chemical is no longer visible on the surface of the soil. Fill the tank with about 1/3 of the soil mixture. Identify the center of the fish tank and insert the PVCC tube (3-6 cm diameter and 20-50 cm in length) into the soil. Make certain there is no soil inside of the tube once it is inserted. When looking down into the tube the bottom of the tank should now be visible. Measure 10, 20, and 30 cm from the center of the tank at a perpendicular angle to the tube. These measurements should be marked with glass marking pencil. See the diagram below.



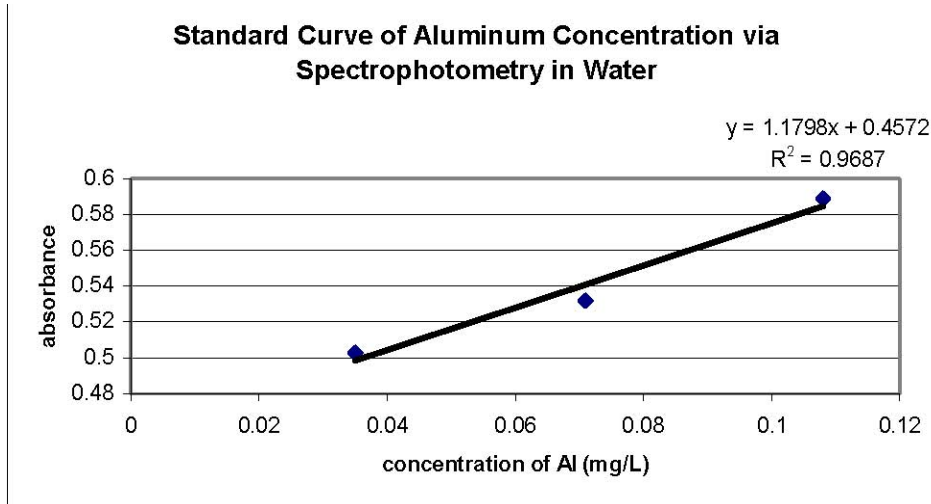
Drilled PVC tubing filled with chitin substance

Take a one gram sample from each of the distances (10, 20 and 30 cm). Place a circular filter paper in a regular plastic funnel. Put the one gram soil sample into the funnel at this time. Using a graduated cylinder, measure out 100 mL of distilled water and pour this water into the funnel to let the water soak in with the soil. Place the beaker below the funnel to catch the filtered water. This water has now absorbed most of the contaminants in the soil, including the controlled contaminant, aluminum chloride. Complete the H₂O Aluminum Water Test as described in step 6 of Phase I procedure. Use a pipette to transport about 3-5 mL of this filtered sample to the spectrophotometer.

Select the "Freerun" method on the machine. Place this test tube along with another small spectrophotometer test tube containing distilled water (the blank) in the machine. Select wavelength 535 nm, press start. Record the absorption read out levels (ABS). Complete this process twice more for a total of 3 trials for each set distance.

Fill the PVC tube with 25 grams of crush/blended lobster shell (chitin substance, blend cleaned shells in blender for 5 minutes). Allow an overnight remediation period for the chitin in the lobster shells to absorb and act as the chelating agent for the aluminum chloride. Afterwards, repeat testing in three trials of the one gram sample from each distance (10, 20, 30 cm from tube) of now remediated soil using the funnel and spectrophotometer process. Record data and create a standard curve.

Results:



Wavelength of Absorbance of Aluminum Contaminated Distilled Water before and After 10 minute Chitin Remediation Period

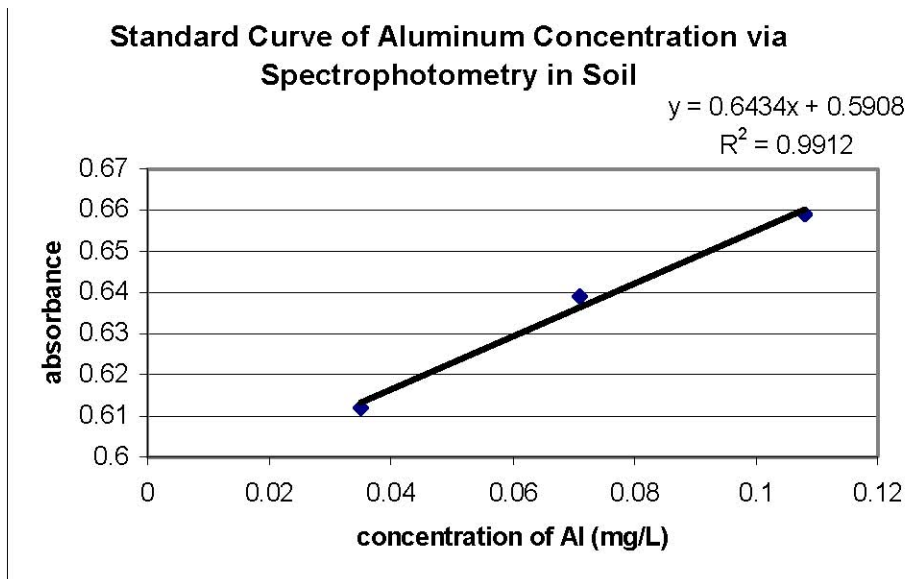
Starting concentrations (mg/L)	Absorption wavelength of control concentrations (nm)	Absorption wavelength after 10 min. remediation trial 3 (nm)	Absorption wavelength after 10 min. remediation trial 2 (nm)	Absorption wavelength after 10 min. remediation trial 2 (nm)	Avg. Percentage of remediation after application of chitin substance
.03598	.503	.473	.466	.482	60.1 %
.07196	.532	.505	.499	.512	42.5 %
.10794	.589	.554	.547	.560	24.3 %

**Wavelength of Absorbance of Aluminum Contaminated Distilled Water
before and After Overnight Chitin Remediation Period**

Starting concentrations (mg/L)	Absorption wavelength of control concentrations (nm)	Absorption wavelength after overnight remediation trial 1 (nm)	Absorption wavelength after overnight remediation trial 2 (nm)	Absorption wavelength after overnight remediation trial 3 (nm)	Avg. Percent of remediation after application of chitin substance
.03598	.503	.449	.455	.459	78.4 %
.07196	.532	.483	.490	.542	42.9 %
.10794	.589	.524	.533	.536	42.1 %

Aluminum Absorbance by Chitin with addition of Polyethylene Plastic

Parts chitin	Parts Polyethylene Plastic	Theoretical Remediation	Actual Remediation	Difference
3	0	62%	62%	n/a
2	1	40.9%	42%	+1.1
1	2	20.4	26.5%	+5.1
0	3	0%	3.2%	+3.2



Wavelength of Absorbance of Aluminum Contaminated Soil Before and After Overnight Chitin Remediation Period

Starting Concentrations (mg/L)	Absorption wavelength of control concentrations (nm)	Absorption wavelength after overnight remediation trial 1 (nm)	Absorption wavelength after overnight remediation trial 2 (nm)	Absorption wavelength after overnight remediation trial 3(nm)	Avg. Percent Remediation after the application of chitin substance
.03598	.659	.626	.620	.621	63.6 %
.07196	.639	.619	.614	.616	44.1 %
.10794	.612	.603	.598	.596	54.6 %

Validity/Analysis

I am confident in the results of my first two phases of experimentation. The precision of my experiments leads me to believe that because there were so few outliers in the data, that the experiment on the whole was very accurate. Yet, there was evidence of error as in all labs and experimentation. The environment that my experiments were placed under was not a controlled lab environment, a factor that could have slightly altered and influenced my results. Also, the H₂O Manual test traced only a small range of concentrated aluminum content in water samples, which limited the number of trials and the different concentrations available for experimentation. Finally, I did not test the soil in Phase I without the PVC tube, which leaves uncertainty on whether PVC may have absorbed the aluminum, and not the chitin.

Conclusions

Chitin is an effective way to remediate wastewater contaminated with heavy metals, such as aluminum. It can reduce a controlled concentration of aluminum in distilled water 40-60%. Chitin in the raw, natural form of lobster shells can be a cost effective way to manipulate the substance and remediate the EPA Brownfield site, Batchelder, Newtown, CT, or other similarly contaminated sites. Other possible variables to pursue in later trials and/or experiments include different heavy metals, such as arsenic, lead, nickel or PCBs. Other trials could use chitosan as the bioremediation technique, then create standard curves to evaluate which substance, chitin or its derivative, was more valuable in bioremediation. Chitin is a useful, absorptive chelating agent when applied to contaminants suspended in soil or wastewater.

This study of the effect of chitin on wastewater is only the start of an in depth study on remediative techniques for wastewater. This project has real life application in many areas. A local site, the Batchelder Site, located at 44 Swamp Road, Newtown, CT is an EPA Brownfield site which this remediating technique could be of value to. This site was originally an aluminum smelting plant, but it now remains closed and owned by the town. It has been on the market for over ten years and the cost to chemically remediate the wastewater on the land is far too much preventing any company from desiring to purchase it. If chitin were used to absorb the aluminum and other heavy metals such as arsenic and PCBs, then it could be a cost effective means of remediating the site and creating a new marketing strategy.

Polyethylene bulking of the chitin was not demonstrated to be significantly more

effective than chitin alone. It was thought the bulking might increase surface area and make the chitin more effective. Since polyethylene is not biodegradable and chitin is, the minimal gains from chitin/polyethylene remediation are outweighed by the negative environmental impact of the plastic.

The soil remediation showed a 40-60% reduction in aluminum for the samples. Water remediation was more successful than soil remediation. Furthermore, the application of phase I is more feasible and realistic than phase II. A major challenge in the remediation of a large piece of property such as the Batchelder Site, is creating a procedure applying the research and methods used in this small scale experiment to a large-scale, real life property.

Due to the need for a different larger-scale procedure, I propose that this site be donated to a research institution with a high level of interest in environmental sciences. This site could become an experimental field work site for bioremediation for a period of two years. After this period of time is complete, the town will evaluate the remediation progress and decide whether to let the research continue until the site is remediated enough to be useful again, therefore creating a new marketing strategy for the town to make money off of this now worthless piece of property.

The final, broad conclusion that can be drawn from these experiments, is that bioremediation with the use of chitin substances, in this case the natural raw form of chitin found in lobster shells, is a feasible option for the remediation of sites containing aluminum contaminated water and soil. To further and expand on this research I would like to team up and correspond with other scientists who are working on similar bioremediation techniques.

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