Aluminum Oxide, Soluble Aluminum, and Coral Toxicity

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Abstract

Aluminum oxide is a widely used phosphate remover. The question arises as to whether aluminum oxide provides soluble aluminum in the aquarium environment, and whether soluble aluminum is toxic to aquarium life. This paper addresses both the question of aluminum solubility and aluminum toxicity. A series of controlled experiments, in both fresh water and salt water, examines the aluminum solubility of aluminum oxide used for phosphate removal. Results of the study indicate that aluminum oxide is so insoluble as to render the material completely safe.

Aluminum toxicity depends on the solubility of aluminum and the presence of biologically active forms of aluminum. Effects of aluminum in an environment are highly dependent on the form of aluminum. The toxicity portion of this paper will review the effects of aluminum oxide use for phosphate removal on corals.

Introduction

Aluminum, both in the form of salts and in the form of solid alumina, removes phosphate from aqueous solutions. (See, for example, the papers by Chen *et al.*, Fytianos *et al.*, Huang, Tanada *et al.*, and Xie *et al.*) For this reason, it has found widespread use in aquarium systems. Researchers have noted the toxicity of aluminum to fish (studies have focused primarily on fresh water fish), but have also pointed out the pH dependence of this phenomenon (Sparling *et al.*). The solubility and speciation of aluminum is pH dependent, as well as concentration and temperature dependent. This paper examines the contribution of soluble aluminum from alumina, and the effect of this ionic aluminum on corals.

Part I. Chemistry

Discussion

Aluminum oxide, or alumina, is listed as being insoluble in water, and only very slightly soluble in acid and alkali (Weast). In fact, the solubility of aluminum compounds in general is pH dependent. As Figure 1 indicates, aluminum can exist in at least five forms, depending upon pH (Hayden and Rubin). Of these five forms, the completely soluble form is the Al^{3+} form that exists at a low pH (generally below pH = 4.5). Figure 2 examines just the lower pH portion of the curve, and indicates that the soluble fraction reaches its maximum at a pH below 4. Note that these curves may be shifted left or right depending upon changes in aluminum concentration, temperature, etc. (Batten). Note also that, for the conditions shown in these two figures, the

trivalent soluble fraction has disappeared above a pH of approximately 5.3, and the monovalent anionic soluble fraction does not become appreciable until above a pH of 9.

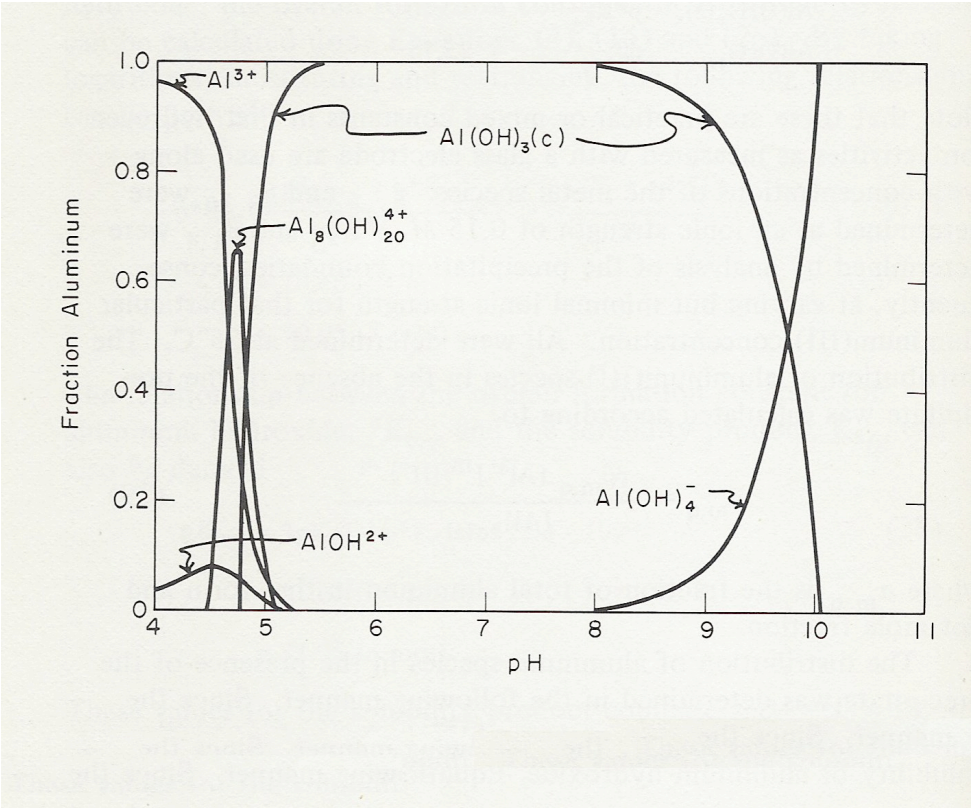

Figure 1. Distribution of 5.0 x 10^{-4} M hydrolyzed aluminum (III) as a function of pH, from Hayden and Rubin

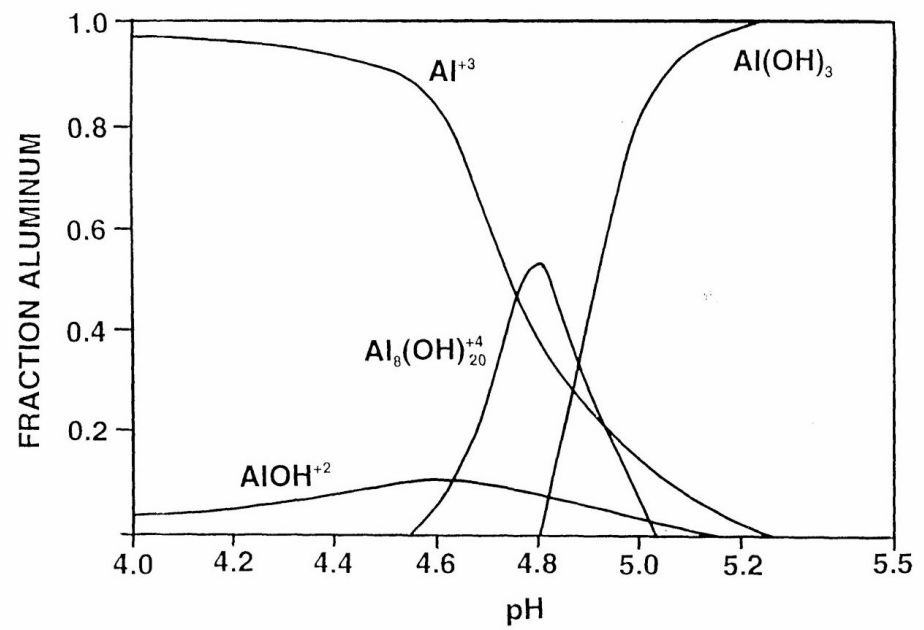

Figure 2. Distribution of hydrolyzed aluminum (III) as a function of pH, redrawn to concentrate on lower pH values, from Batten

These figures indicate that a low pH provides the greatest opportunity to solubilize aluminum from alumina. Accordingly, we initiated a series of experiments using acidified deionized water, and acidified deionized water in which a commercially available reef salt was dissolved, according to label instructions. The details are given in the experimental section below. Note that, even with a low initial pH, the commercially available reef salt buffered the pH to between 8.2 and 8.3.

We used two doses of aluminum oxide: the label (recommended) dose, and triple the label dose. Given the cost of aluminum oxide, and the volume displaced by the product, triple the label dose represents the highest usage rate that one would expect to encounter.

Results

The results from both sets of experiments are listed in the table below:

Sample	pH	Aluminum, mg/L
Freshwater Blank	5.33	Non-detect
Freshwater Control	5.95	Non-detect
Aluminum Oxide Standard Dose	5.33	Non-detect
Aluminum Oxide Triple Dose	5.34	0.2
Saltwater Blank	8.25	Non-detect
Saltwater Control	8.24	Non-detect
Aluminum Oxide Standard Dose	8.26	Non-detect
Aluminum Oxide Triple Dose	8.26	Non-detect

The detection limit for this analysis (inductively coupled plasma-atomic emission spectrometry) was 0.2 mg/L. In only one case, that of fresh water at a low pH with triple the label dose of alumina, was the detection limit reached. In all other instances, aluminum was non-detectable.

Conclusion

From this portion of the work we conclude that under reef conditions (pH near 8) there is no detectable soluble aluminum released from alumina. Under conditions of low pH and high dosage levels, soluble aluminum can be released from alumina. At three times the label dosage rate, we detected 0.2 mg/L aluminum at a pH of 5.3

Experimental

The water employed in this study was obtained from the city of Madison, Georgia water treatment plant, and run through ion exchange columns and acidified. This water was used in the freshwater study and as make-up water for the saltwater study. This water is also the blank for the freshwater part of the study.

The saltwater was made with Reef Salt™ from Seachem Laboratories, using 34 grams of Reef Salt™ per liter of water, resulting in a specific gravity of 1.021. Even with the low initial pH of the make-up water, the buffering from the Reef Salt™ resulted in a pH that was only very slightly lower than ideal, but entirely acceptable. This water is also the blank for the saltwater part of the study.

The control for the freshwater part of the study is blank water containing 0.565 g of Gray Coast™ calcite from Seachem Laboratories per liter of water. For the saltwater portion of the

study, the control is saltwater prepared as described above containing 0.565 g of Gray Coast™ calcite per liter of water.

The aluminum oxide used in this study was Phosguard™ from Seachem Laboratories. The standard dose for this product, from the label, is 250 ml of Phosguard™ per 300 liters of water, or 0.833 ml of Phosguard™ per liter of water. This is the same as 0.565 g of Phosguard™ per liter of water. This is the amount added as "standard dose." The "triple dose" is three times this amount, or 1.695 g of Phosguard™ per liter, which is the same as 750 ml of Phosguard™ per 300 liters of water.

Neither the Gray Coast™ control nor the Phosguard™ was washed before use in the experiment. This represents a "worst-case" situation, as small particle size dust would be expected to contribute aluminum more easily to the solution than bulk material, due to the large specific surface area of the dust, compared with that of the bulk material.

The study proceeded for 60 days prior to sampling for analysis.

Analytical work was performed by Analytical Services, Inc. (ASI), an environmental monitoring and analytical laboratory in Norcross, Georgia. ASI was chosen because it is a laboratory that is certified by the National Environmental Laboratory Accreditation Program (NELAP), the United States Department of Agriculture, and by 14 states. The analytical procedure used was EPA Method 200.7, "Trace Elements in Water, Solids, and Biosolids by Inductively Coupled Plasma-Atomic Emission Spectrometry." The detection limit for aluminum at this laboratory using this technique is 0.2 mg/L.

The samples were not filtered prior to analysis.

Part II. Toxicology

Aluminum Oxide Toxicity

Toxicity is defined by a dose-response relationship. If there is no exposure, there can be no dose, no absorption, and no response. The key to understanding toxicity is the knowledge that all elements pose toxicity issues under the right circumstances.

Cellular up take/absorption

Cells take in compounds from the surrounding environment using a variety of mechanisms. Smaller uncharged particles may enter the cell via passive transport. Other mechanisms, such as endocytosis or active transport, bring materials into the cell. Cellular absorption is a normal occurring process and very efficient. Cellular absorption may introduce other materials into a cell that are not necessarily needed, given that the materials are available for absorption. Absorption into the cell constitutes exposure. Once absorption happens, normal function of the cell must be disrupted to note a negative impact. The resulting impact could be acute or chronic.

Aluminum

Aluminum is the third most abundant element and the most abundant element found in the Earth's crust. It does not exist in nature without being bound to other compounds. It is not soluble in water under normal conditions. Aluminum can typically be found in oxide or silicate states. Aluminum oxide is so abundant in its natural form that even the aragonite in a tank contains some aluminum oxide. Aluminum may be found in parts per billion even in various foods consumed by people.

As described previously, pH impacts the solubility and state of aluminum. The very first factor that determines the bioavailability of aluminum is pH. In acidic conditions, it is possible for aluminum to form complex insoluble polymers that exhibit very low bioavailability. Other factors that influence bioavailability (in succession after pH) include the following:

2. Counter ions
3. Dietary factors
4. Formation of Al-lipid complexes
5. Damage to membrane barriers
6. Multiple chemical states of Al salts
7. Route of exposure and mechanism

In Vitro

In vitro methods reduce the need for animal studies. While it is a developing field of toxicology, relevant data may still be gathered from in vitro studies. In vivo studies of aluminum prove very complex in nature. Many factors must be monitored to elicit effect such as: pH, counter ions, method of presentation, route of exposure, duration of exposure, dose, and more. All of the parameters must be monitored to yield a dose-response relationship for aluminum that must exist in the hydrated salt form. Since aluminum does not exist in nature in a soluble hydrate form, manipulation of conditions and the state of aluminum must occur for observations in vivo/ in vitro studies. In vitro studies of aluminum also require direct monitoring of the conditions for production and maintenance of soluble aluminum species. In vitro work helps establish dose-response relationships as concentrations begin in elevated states and are slowly lowered, thus establishing physiological responses.

Cellular response to change in environment on average leans towards universal reactions. Osmotic regulation of cells may cause a cell to shrink or swell to adjust to the environment. This is an observed function in many types of cells. Cell death is another observed reaction due to cell differentiation or response to the environment. This is important to note because responses to the environment can be due to many underlying unknown factors. Of course, in toxicity, agents at particular levels can be noted to cause death.

Experiment

Aluminum oxide was compared to aluminum chloride by simply observing the two for negative impacts. Bacteria were isolated via serial dilution of 1:10 and 1:100 in DI water. Colonies were plated on nutrient agar. The first nutrient agar consisted of glucose, NaCl, yeast extract, agar, and DI water. The broth was mixed and autoclaved for 30 minutes. The broth was allowed to cool and poured into sterile Petri dishes. The second broth consisted of tryptone, NaCl, yeast extract, and agar. Two forms of broth were used to ensure the best growth of bacteria. On the plates, mostly gram-negative rod and cocci bacteria were cultured. Two colonies were isolated and grown individually for genetically similar bacteria.

Zone inhibition tests constituted the first type of testing. This is a simple means of observing limitations in growth and motility of bacteria in response to a relative agent. Sterile Petri dishes received a uniform 5 ml of nutrient agar broth. Cellulose fibers of 2-3 cm discs were soaked in solutions of aluminum chloride and aluminum oxide, one each at 100 ppm and 200 ppm. Full lawn growth of bacteria was observed with each form of aluminum. This procedure was duplicated producing the same results. Bacteria from the plates were viewed under the microscope alive and later killed via heat-sealing for staining. Results observed found normal viable bacteria on each plate surrounding the fibers soaked in either form of aluminum.

The second part of the test used test tubes filled with LB broth and varying concentrations of either aluminum chloride or aluminum oxide. The pH of the broth was the only other variable. The pH was adjusted with a tris buffer in order to raise it.

*It should be mentioned that as the pH increased, aluminum chloride began to precipitate. Aluminum oxide suspensions were used due to the insoluble nature of the chloride form and a combination of the higher pH.

Full growth of media throughout the broth was observed, given that the varying layers of the broth support more facultative aerobic and anaerobic bacteria. Microscopic review of bacteria revealed fully functional viable bacteria with the ability to exhibit normal culture life cycle behavior. Normal behavior was noted in samples with as higher as 400 ppm of Aluminum chloride and aluminum oxide.

Agar plates were then used with varying levels of each source of aluminum. Agar plates consisting of 100, 200, 300, 400, 500, 600, and 800 ppm each of aluminum chloride and aluminum oxide were swabbed for lawn growth. At a concentration of 800 ppm of aluminum chloride is where the plates noticeably did not have full lawn growth. Other plates of aluminum oxide still exhibited full lawn growth. This does not mean that these numbers transfer over to other organisms, but it does give a clue to the physiological response to aluminum at such a high level. These numbers in turn give a fairly good indication that insoluble aluminum can be used within a great range of safety. Applying these results from in vitro studies, PhosGuard can be examined with corals thus reducing the need for hundreds of corals.

Thirty genetically similar Sarcophyton were obtained from Scientific Coral. The corals were placed in a larger system to allow them to recover from shipping. After a week of recovery, the

corals were moved to individual tanks with 2.5 liters of salt water with matching parameters. The holding tanks initially included a filter pump, biological media (Matrix), and filter floss. The corals were again allowed to recover from the transit. Corals recovered notably well by observing full extension of the coral body. Twenty-two corals were placed each in a separate tank. Six corals received the suggested dose, six corals received two times the suggested dose, and six corals received three times the suggested dose. Four corals received no exposure for control purposes. Without rinsing the filtration media, it was placed centrally in the filtration set up allowing no water to bypass the media. Upon initial addition, no coral irritation was observed. Two weeks later, all corals survived with these results, noting a loss due to two corals not exhibiting full extension when compared to other corals.

	Control (no PhosGuard)	Normal Dose	2X	3X
Sarcophyton before PhosGuard	4	6	6	6
Sarcophyton Initial PhosGuard	4	6	6	6
Sarcophyton 2 weeks after PhosGuard	3	6	5	6

Notably, one coral from the control was somewhat stressed. Seventeen of eighteen corals remained healthy and fully extended.

Conclusion

Aluminum oxide is an insoluble form of aluminum. At the pH most hobbyist keep reef tanks, release of aluminum is not an issue for corals. Aluminum oxide is not easily absorbed into the cell to cause negative reactions. Even at three times the dose of PhosGuard, soft-bodied corals such as Sarcophyton, do very well with more than the recommended dosage of PhosGuard.

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