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AQUATIC TOXICITY AND FATE OF NICKEL COATED GRAPHITE FIBERS, WITH COMPARISONS TO IRON AND ALUMINUM COATED GLASS FIBERS

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RESEARCH AND TECHNOLOGY DIRECTORATE

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The toxicity of nickel coated graphite was examined using *Daphnia magna*, the water flea (48 hr assay), and *Cyprinodon variegatus*, the sheepshead minnow (96 hr assay). Daphnia were the most sensitive test organism having a 48 hr EC_{50} of 1.0 mg/L soluble nickel. The sheepshead minnow showed no toxic signs up to 1000 mg/L nickel fibers. Fate studies (21 days) were conducted using various water hardness and salt water media.

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PREFACE

The work described in this report was authorized under Sales Order No. 1J2L. This work was started in June 1991 and completed in December 1991.

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CONTENTS

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AQUATIC TOXICITY AND FATE OF NICKEL COATED GRAPHITE FIBERS, WITH COMPARISONS TO IRON AND ALUMINUM COATED GLASS FIBERS

1. INTRODUCTION

There is a general interest of the military services in various candidate obscurant materials. The effects of the materials on the environment are critical for acceptance. One material of concern is nickel coated graphite, of which aquatic toxicity data are lacking.

The fate and toxicity of metals in the aquatic environment is an extremely important issue due to the fact that metals can accumulate in the tissues of aquatic organisms. Since man relies on the sea as a vital food source, the consumption of metal contaminated seafood is of great concern.

Most open literature reports examine the toxicity and fate of metal salts, overlooking the fact that metals can be introduced into the environment as particles or filings. Through maneuvers and field testing / training the possibility exists that the nickel coated graphite will be released into an aquatic ecosystem.

This report examines the toxicity of nickel coated graphite fibers to *Daphnia magna* (cladocera, a fresh water organism) and *Cyprinodon variegatus* (sheepshead minnow, a marine organism). Daphnia were exposed to the fiber leachate for a 48 hr period, while the minnows were exposed directly to the fibers for 96 hrs.

The fate of the metal coating was also examined in 21 day studies using marine water (30 ppt) and fresh waters of varying hardness. The results are compared to similar studies done on iron and aluminium coated glass fibers.

2. METHODS AND MATERIALS

All testing conformed to Environmental Protection Agency (EPA) (1,2) and American Society for Testing and Material (ASTM) (3) guidelines.

2.1 Daphnia Assays

Daphnia less than 24 hrs old are used in these assays. Their body size is approximately 0.5 mm in length and would be extremely difficult to find in a test chamber of fibrous materials. Moving the fibers around to locate the organisms at the monitoring intervals would caused physical injury that could skew test results. To avoid this possibility, the fibers were soaked in the appropriate media for 96 hrs. The media was poured through a single layer of cheese cloth to filter out the bulk of the fibers, and the supernatant was then serially diluted.

Daphnia magna were obtained from Dr. Freida Taub at the University of Washington in Seattle and reared for the past eight years in this laboratory using methods described by Goulden, *et al.* [4]. Daphnia stock cultures were fed a mixture of vitamin enriched *Ankistrodesmus falcatus, Selenastrum capricornutum* and *Chlamydomonas reinhardi* 90. Daphnia culture media was derived from well water which was passed through a treatment system containing limestone pH adjustment, iron removal, carbon filtration and UV sterilization. The well water was monitored for 92 commonly found ground water pollutants every four months by Watercheck National Testing Laboratories, Inc..

The test beakers were placed into a temperature controlled room at 20 °C with a light/dark cycle of 16:8 hrs with 315 ft candles of light. Two replicates per concentration containing 10 daphnia less than 24 hrs old, in a total of 100 mls of test solution was used. The pH and hardness measurements were taken at the start of each test. At 24 and 48 hrs, daphnia were checked for mortality. If the daphnia were not actively swimming, they were touched with a pasture pipet. If there was no response or the daphnia could not swim actively for 15 seconds it was considered immobilized. The EC_{50} (the effective concentration at which 50 percent of the organisms are immobilized) values were computed using the probit analysis as prepared by Kessler [5]. The EC_{50}s were also tabulated graphically using a least square regression analysis verifying all probit analyses.

2.2 Fish Assays

There are a number of aquatic marine fish available for testing. We have chosen *Cyprinodon variegatus* (sheepshead minnow, .5 inches) obtained from SP Engineering Technology. The sheepshead is easy to rear and can be obtained throughout the year from various suppliers. They are capable of being bred in the laboratory, ensuring disease and parasite free specimens. These minnows are used nation-wide as a test organism, therefore data comparisons are available.

The fish were acclimated to Forty Fathoms synthetic sea water prepared in well water with a final salinity of 30 ppt. Fish were observed for stress and disease for a minimum of 14 days prior to testing. If the fish appeared stressed, developed any diseases, or if 5 % of the stock died within 48 hrs of testing, stocks were replaced. The temperature was maintained at 22 °C ± 1°, with a light / dark cycle of 16/8 hrs.

The feeding regime consisted of one feeding of freshly hatched brine shrimp (Columbian strain) in the mornings and one feeding of Tetramine flake food in the afternoons. The amount of food served was equal to the amount the fish could eat within one minute, without food build up on the bottom of the holding tanks. Feeding was stopped 24 hrs before testing.

Acid washed gallon glass bottles were used as test chambers. The fibers (3000, 1500, 750, 375 and 18.9 mg) were placed in each jar and filled to the 3 L level using salt water media, yielding concentrations of 1000, 500, 250, 125, and 6.3 mg/L respectively. There were two replicates per concentration containing 10 fish each. Loading did not exceed 0.5 g total fish/L. Fish were exposed for 96 hrs with observations taken every 24 hrs. If the fish had no gill movement, it was considered dead and removed from the test chamber. Statistical analysis was conducted as described above in the daphnia assays.

2.3 Fate Determinations

The ionization of the nickel coating was monitored for 21 days in fresh water of varying hardness and in synthetic sea water (30 ppt).

The fresh water studies used distilled water and reagent grade salts to bring the hardness up to desired levels (see Table 1 for hardness, pH and amount of salts used). The synthetic sea water was prepared as described in the fish assays. The hardness of the salt water could not be measured using current in-house methods, due to the salt water ingredients having adverse reactions with the indicators.

Fibers (200.0 mg) and 200 mls of media were placed into 250 ml polycarbonate screw top flasks. There were six replicate flasks prepared for each water type. Each flask was randomly assigned to a sampling day (Day 0, 1, 3, 7, 14, or 21). The flasks were allowed to set at room temperature (22 °C), and agitated once a day. Two 10 ml samples were removed from the vessels and filtered through a .45 micron filter and analyzed for nickel with atomic absorption (AA) spectroscopy. Blanks of each water

* SP Engineering Technology, 29 Congress Street, Salem, MA

Table 1. Quantities of Chemicals used to harden the water to desired hardness.

Water Type	$NaHCO_3$	$CaSO_4(2H_2O)$	$MgSO_4$	KCl	pH*	Hardness* (mg/L $CaCO_3$)
		(Required Salts, mg/L)				
Very Soft	[illegible].0	7.5	7.5	0.5	7.2	20.0
Soft	48.0	30.0	30.0	2.0	7.7	40.0
Hard	192.0	120.0	120.0	8.0	8.3	130.0
Very Hard	384.0	240.0	240.0	16.0	8.3	200.0

* The pH and hardness were measured at time 0 before the addition of fibers.

type were also run to monitor background concentrations. Results were plotted against a standard curve and subjected to regression analysis to determine the levels of material in solution. For instrument settings see appendix A.

3. RESULTS

The nickel coated graphite was non-toxic to sheepshead minnows but toxic to daphnia. The minnows were exposed directly to 1000 mg/L nickel coated fibers and had no visible adverse effects. The total dissolved nickel concentration after 96 hours of the fibers being placed in salt water (30 ppt) was 5.4 mg/L (Table 2).

To prevent possible physical damage to the daphnia when conducting mortality counts, the nickel coated fibers were soaked in daphnia media for 96 hrs and filtered out with a single layer of cheese cloth. The supernatant was then diluted to test concentrations. The concentration of soluble nickel in the supernatant at 100 % was 1.8 mg/L and the resulting 48 hr EC_{50} was 1.0 mg/L nickel. Using the chemical scoring system for hazardous and exposure assessment, the toxicity of nickel coated graphite to daphnia is ranked 6 (on a scale of 1 - 9, 9 being the most toxic) (6).

In 21 day fate studies, the water hardness ranged from 21 ppm ($CaCO_3$) in the very soft water to 200 ppm ($CaC0_3$) in the very hard water with a pH range of 7.2 - 8.3 respectively (Table 1). The hardness of the salt water could not be determined using current in house methods. The ingredients of the salt water did not react properly with the water hardness indicators. As the water hardness increased the concentration of dissolved nickel increased (Figure 1). In fresh water, the highest dissolved nickel concentration was 5 mg/L (very hard water), and 7.4 mg/L in salt water. Significant differences in dissolved nickel concentration resulted between the salt water, soft and very soft waters.

There was no visual evidence that the nickel coating was flaking off the fiber as in the case of iron coated glass fibers (7). In figure 2, a picture of the nickel coated graphite, before the addition to water, shows the nickel coating riddled with holes which increase the surface area exposure for water contact.

4. DISCUSSION

While extensive literature exists on the toxicity of metal salts in the aquatic environment, few studies have been published concerning the effects of metal particles. Often overlooked is the fact that many metals are introduced into the environment as alloys such as brass, solder, and even stainless steel. Metals may leach/dissolve at different rates, depending on water parameters (pH, hardness, clay and organic content). These parameters also influence the toxicity of metals to organisms. Lower pH will dissolve metals more rapidly while clay and dissolved organics will bind with and precipitate the metals [8,9]. Water hardness also plays a roll in reducing the toxic effects of metals to organisms. The toxicity of metals is reduced in water of high hardness, due to competition between hardness metals (Ca^{++} and Mg^{++}) and heavy metals for the active sites on cell membranes [10].

Nickel is naturally occurring world wide in low concentrations. In soils, the world average is 75 µg/g. In fresh and salt waters, the average nickel concentrations are 0.3 µg/L and 2 µg/L respectively [11]. Nickel is transported by runoff and through atmospheric deposition due to smoke stack emissions. In comparison, iron and aluminium are also naturally occurring in soils and the world oceans, however the concentrations, except in sea water, are several orders of magnitude higher than nickel ($5.6X10^4$ µg Fe/g and $8.2X10^4$ µg Al/g in soil 670 µg Fe/L and 400 µg Al/L in fresh water, 3 µg Fe/L and 5 µg Al/L in sea water).

Table 2. Toxicity comparison of nickel to several other metal coated fibers.

Test Material	Test Species	EC_{50} (mg/L) Total Fibers	EC50 (mg/L) Soluble Metal
Nickel Coated Graphite	Daphnia magna	> 1000.0	1.0
	Sheepshead Minnow	> 1000.0	>5.4 *
Iron Coated Glass	Daphnia magna	> 1000.0	BDL**
	Sheepshead Minnow	> 1000.0	BDL
Aluminium Coated Glass	Daphnia magna	> 1000.0	BDL
	Sheepshead Minnow	> 1000.0	BDL

* Highest concentration of soluble Ni at 1000 mg/L of fibers.
** BDL - Below detectable limits, No dissolved metals from the fibers were detected.

AQUATIC FATE OF NICKEL COATED GRAPHITE

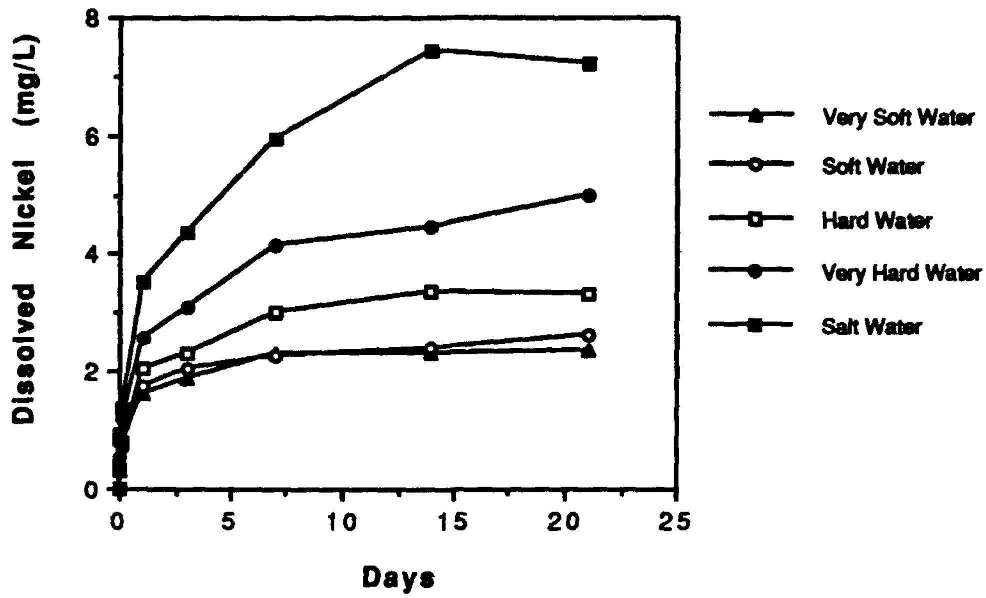

Figure 1. As water hardness increases, the concentration of total dissolved nickel increases. Equilibrium is reached in 7 days in fresh water and 14 days in salt water.

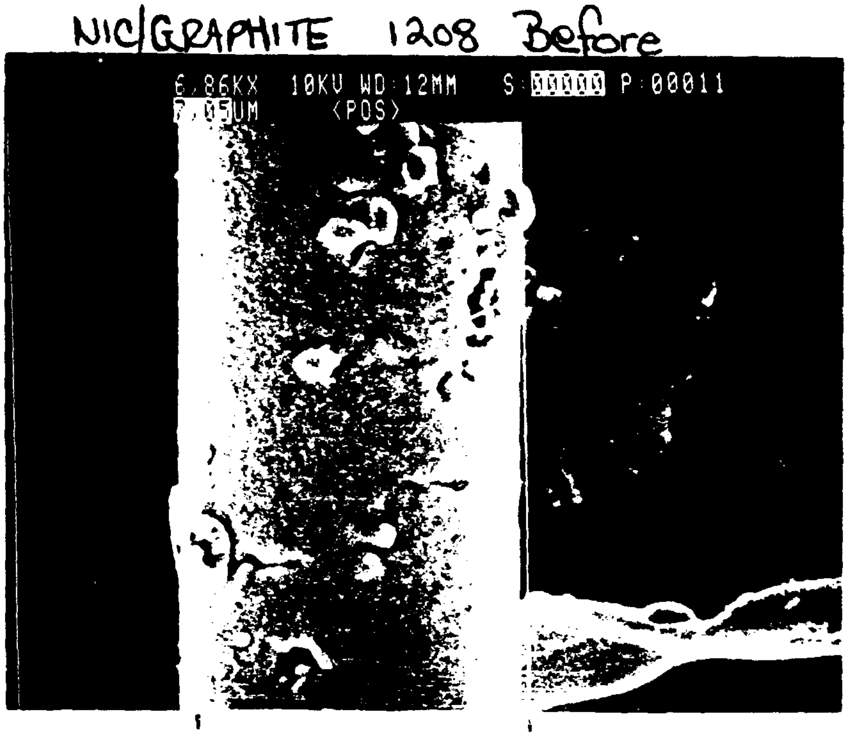

Figure 2. Scanning electron micrograph of nickel coating on the graphite fiber. Notice the holes and crevasses in the coating.

The fate and toxicity of metals in the aquatic environment is an extremely important issue due to the fact that metals can accumulate in the tissues of aquatic organisms. Since man relies on the sea as a vital food source, the consumption of metal contaminated seafood is of great concern.

Open literature suggests that nickel does not bioaccumulate (tissue concentrations of nickel higher than the environmental levels) in significant amounts [12,13]. Nickel is listed among the 23 essential minerals required (in trace amounts) in the function of aquatic organisms [14,15]. Therefore, trace amounts of nickel will always be found is fish tissue. On the average, edible seafood (shell fish, marine and fresh water fish, and crustaceans) contain nickel levels below 0.75 µg/g [16].

In fate studies, after 21 days of the nickel coated graphite fibers being suspended in the various water types, the soluble nickel levels did not get high enough to cause acute effects to sheepshead minnows (Figure 1). Nickel in salt water had a maximum soluble concentration of 7.2 mg/L after 21 days.

Similar studies subjecting iron coated and aluminium coated glass fibers to 21 day fate studies using the same methods as described in this report [7]. The coating on the iron coated glass did not dissolve into solution, and was therefore not bio-available, which resulted in this particulate not being toxic to fish or daphnia. The coating from the aluminium coated glass did dissolve into solution, however it was not toxic to the fish and daphnia. Aluminium is one of the least toxic metals found in nature because it does not present itself in an available form [14]. In an aquatic environment dissolved aluminium (Al^{+++}) forms $Al(OH)_3$ and precipitates out of solution.

In figure 3, the dissolved metal from the nickel, iron and aluminium coatings were plotted according to water types. Data from day 7 analysis were used because in most instances the metal concentration was approaching equilibrium. When water hardness increased, the amount of dissolved metal also increased producing a wide range of soluble nickel concentrations. The pH range between the various waters was 7.2 to 8.3. The pH difference alone is not enough to create such dissolved metal differences. For example (in the nickel studies) the pH of the hard and very hard waters was 8.3 for both. Yet the very hard water had 28 % more dissolved nickel. Similar phenomenon occurred with aluminium. This reaction is not explained in this paper and should be examined further.

Soluble nickel was toxic to daphnia at 1 mg/L and non-toxic to sheepshead minnows up to 5.9 mg/L (maximum soluble nickel after 96 hrs). Open literature reports nickel to be toxic (96 hr EC_{50}) to fathead minnows at 32.2 mg/L soluble nickel. After 21 days, there was not enough soluble nickel in solution to produce acute toxic effects to the minnows. Nickel concentrations of 0.73 mg/L have been reported to have an effect on the reproduction rate (egg production) of fathead minnows, with a no effect levels of .38 mg/L soluble nickel [17]. Also, concentration of 0.09 mg/L dissolved nickel will adversely effect the reproduction rate of daphnia. In the studies reported in this paper, after 5 days of soaking the nickel coated fibers in water, there was not enough soluble nickel detected in any of the water types to produce acute effects to sheepshead minnows. However, the concentrations are high enough to produce chronic reproductive effects in fathead minnows (data dealing with chronic effects of nickel to sheepshead minnows was unavailable in open literature).

Nickel is reported as being one of the least toxic metals in solution. Using data generated from fathead minnow exposures (open literature), the 96 hr EC_{50} for copper, zinc, cadmium and nickel were 0.43, 7.2, 9.2 and 32.2 mg/L respectively [18,19,20].

Nickel has not been reported to bioaccumulate in significant amounts, therefore it is not considered to be an immediate human health factor. Nickel has not

DISSOLVED METALS IN VARYING WATER TYPES

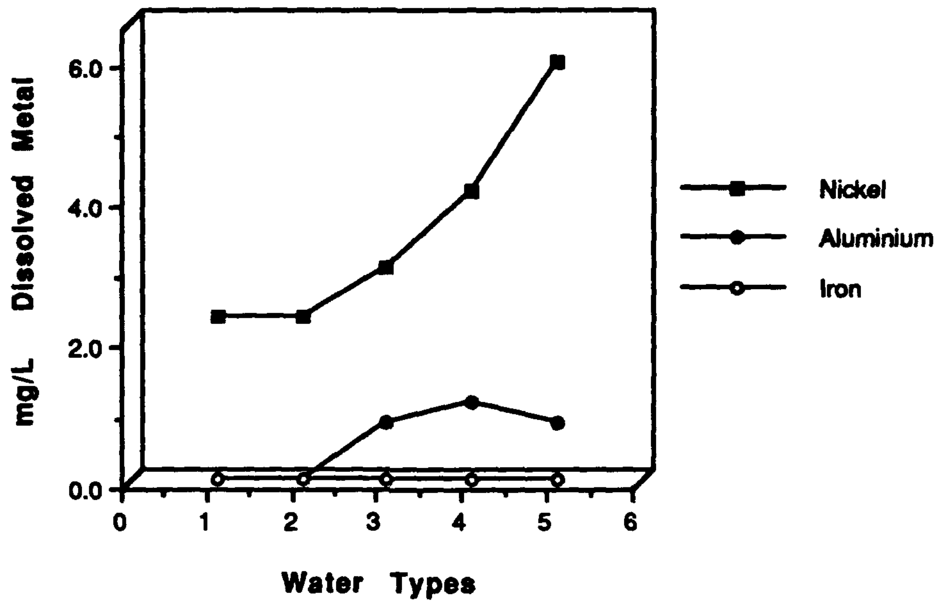

Figure 3. The above graph compares the concentration of dissolved metal in the various water types. Day 7 data was used for this comparison because the majority of the metals were approaching equilibrium. The water types are labeled as follows; 1 - very soft water, 2 - soft water, 3 - hard water, 4 - very hard water, and 5 - salt water.

been regulated by the National Primary Drinking Water regulations [21]. However, long term effects of elevated levels of nickel are impossible to determine using short term studies. The long term ramifications can only be seen by studying growth rates, reproduction rates or behavior changes. If a training site is being subjected to continued insult to nickel coated graphite fibers, possible long-term effects may impact the ecological balance. The effects will be dependant upon the concentration and duration of each exposure. Environmental factors such as wind, tides and run off, will also play a critical roll in determining the degree of impact nickel coated graphite will have on the environment.

5. CONCLUSIONS

- Nickel coated graphite fibers had no effects on the marine minnows at 1000 mg/L of fibers. The soluble nickel concentrations only reached a maximum of 5.4 mg/L in 96 hrs in sea water. Soluble nickel was toxic to daphnia at an EC_{50} = 1.0 mg/L soluble nickel.

- Iron coatings were not soluble in fresh or marine waters and was non-toxic to the test organisms. The aluminium coatings were slightly soluble in fresh and marine media.

- As the water hardness increased the amount of dissolved metal increased. In most cases the salt water media yielded the highest concentrations of soluble metals.

- Nickel is reported not to bioaccumulate and is one of the least toxic metals, based on fathead minnow data (nickel toxicity < Cu, Zn, & Cd).

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APPENDIX

PERKIN ELMER 460 INSTRUMENT SETTINGS FOR NICKEL ANALYSIS

Wave Length	232 nm
Slit Size	0.2 nm
Flame	Air/Acetylene
Lamp Power	30 ma
Signal	AA
Integration Time	2.0 sec.