ABSTRACT

Quantification of Titanium Dioxide Nanoparticles in Environmental Matrices

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A consistent analytical method incorporating sulfuric acid (H_2SO_4) digestion and ICP-MS quantification for titanium dioxide (TiO_2) was validated for 4 environmentally relevant matrices. The method provided Ti recoveries of 97 ± 2.5%, 91 ± 4.0%, 94 ± 1.8%, and 73 ± 2.6% (mean ± standard deviation) from water, fish tissue, periphyton, and sediment, respectively. Lower recoveries from sediment coincided with the percentage of sand in the sediments used for validation suggesting that unrecoverable Ti may be sequestered in the sand matrix.

Our method was then used to quantify Ti in the the four validated matrices along four reaches in the North Bosque River Watershed. Ti concentrations ranged from 0 to 8 $\mu g\ L^{-1}$ in water, 45 to 526 $\mu g\ g^{-1}$ in sediment, 276 to 1520 $\mu g\ g^{-1}$ in periphyton, and 0 to 30 $\mu g\ g^{-1}$ in fish tissue. Ti concentrations in periphyton were significantly higher ($p < 0.001$) than concentrations in other sample types, but were independent of distance from the effluent discharge.

Quantification of Titanium Dioxide Nanoparticles in Environmental Matrices

by

Preston S. Watkins, B.S

A Thesis

Approved by the Department of Environmental Science

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George P. Cobb, Ph. D, Chairperson

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Page bearing signatures is kept on file in the Graduate School.

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DEDICATION

My grandparents, Thomas Preston Edwards and Ollie Ray Edwards, showed me the importance of maintaining our natural environments and that stewardship of the environment was important. They gave this love of nature to all their decedents and left the world a much better place.

CHAPTER ONE

Introduction

The study of currently used metals in industry and how they affect our ecosystems is critical for protection of the environment [1]. Titanium (Ti) is an abundant metal that has been used traditionally as a strong alloy for construction of lightweight parts of aircraft and other mechanisms [2]. The largest growing application of Ti is in the form of nanomaterials [3]. Nanomaterials, as defined by the International Organization for Standardization, are materials with at least one dimension on the nanoscale (1-100nm) [4]. This definition includes metal oxides (TiO_2, ZnO, CuO), metallic (Ag and Au particles), fullerenes, and carbon nanotubes. Nanomaterials in industrial products have seen an exponential increase in production and application in the past 2 decades [5]. Ti is a current research interest with growing nanoscale TiO_2 ($nTiO_2$) applications in consumer products [6-8]. Production estimates of $nTiO_2$ are modeled to comprise the majority of TiO_2 produced and will continue to increase as the form used by industries until at least 2025 [3, 8]. Estimated production of $nTiO_2$, in the United States, is 260,000 metric tonnes per year, as of 2015, and predicted to increase until 2025 [3]. This estimate is not surprising with over 435 metric tonnes per year of $nTiO_2$ being used in Switzerland alone [9]. Consumer products can contain large quantities of $nTiO_2$, with 1 to 10% in sunscreens and varying concentrations in other products such as clothing and food [8, 10]. As nanoparticles are being introduced into more consumer products, research regarding engineered nanoparticles have produced steady increases in

publications since 2006, and the United States was reported to have produced 42% of new publications on engineering nanoparticles from 1999 to 2012 [11]. These publications cover a broad range of topics that is expanding with new nanoparticles being engineered for specific applications, with the largest number of these publications came from the United States [11, 12]. Recent publications include toxicity assessments in fish and transport studies of nanoparticles in aquatic environments, increasing on the limited data available for environmental concentrations of nanoparticles [5, 13-18].

Metals in the Environment

Not only are metals a priority from a toxicological perspective, but unlike many other contaminants, metals do not degrade and can persist within soils and sediments until released when environmental conditions change (e.g. pH, pE, disturbances such as dredging) [19]. Many of these metals are regulated in drinking water because of known toxicological hazards that are associated with metal exposure. Some metals, such as Pb, were known to have toxicity for centuries [20]. The Federal Clean Water Act of 1972 includes 129 priority pollutants including the metals copper, lead, mercury, nickel, silver, zinc, cadmium, beryllium, chromium, and thallium; however, titanium is not included in this regulation [21]. Environmental regulations for these metals are in place to ensure continued ecological and human health, based upon studies that have shown the acceptable and non-acceptable concentrations of these metals within the environment. New industrial application of metals not covered under current environmental regulations expands concerns about environmental exposure to metals and metal oxides. Changes in particle size and structure could alter metal and metal oxide toxicity that results from smaller particles having greater uptake in organisms [16,

22]. Also increased ROS production from a greater surface area and aggregation within cells can damage tissue and DNA [15, 16, 23-26]. There is also evidence that DNA repair can be impaired by $nTiO_2$ and lead to apoptosis by disrupting protein structures [26].

Titanium in the Environment

Ti is the 9th most abundant element in the earth's crust [27]. This abundance makes the element common in the environment where clays are reported to contain Ti concentrations from 0.4 mg/g to 6.6 mg/g and sands ranged from 0.07 mg/g to 3.0 mg/g [28]. These Ti concentrations are consistent with those found in sediments of rivers from around the world: including the Nile River in Africa with 8.32 mg/g, rivers of Brazil with 9.21 to 11.10 mg/g, the Yangtze in China 6.45 mg/g, and North American sediments from Buffalo River with 4.57 mg/g. [29-33]. Ti is found in many compounds within sediments, and is mined in many countries around the world [34]. Although there is an abundance of Ti in sediments, Ti is generally not considered to be soluble in water were Ti concentrations are found at trace levels. Natural surface water concentrations for Ti are found at concentrations below detection limits to 1180 µg/L [29].

TiO_2 Nanoparticles and Nanomaterials

Anatase, rutile, and brookite are the three natural mineral forms of TiO_2. Industrial and research applications most frequently use rutile and anatase, due primarily to the abundance of rutile and the enhanced surface activity of anatase [35, 36]. Bulk TiO_2 is primarily produced using the Kroll process in industries [37, 38]. The white

color and UV properties of $nTiO_2$ improve consumer product performance, longevity, and appeal. Nanomaterials can be found naturally in the environment when formed by base materials found in many soils and sediments. While concentrations of nanomaterials in most natural waterways are seen in low parts per billion (ppb) range, some urban areas have recorded concentrations in the hundreds of ppb for some common nanomaterials [39]. The production of nanomaterials for human use can release nanomaterials from wastewater point sources into the environment [14]. Nanomaterials, prone to aggregation in natural waters, can accumulate in the environment and interact with organic materials [40].

Sunlight is important to the study of metal oxide nanomaterials, due to photocatalytic properties that are activated when exposed to UV light [18]. This activation oxidizes molecules and forms ROS, including hydroxyl radicals, producing nanotoxicity in cells [24]. ROS production can be increased because of the greater surface area on the nanoscale, possibly causing tissue damage [40, 41]. This can lead to an inflammatory response, possibly leading to toxicity [42]. Toxicity studies have also shown mixed results that $nTiO_2$ can be toxic, depending on concentration, when exposed to UV [5, 24, 25, 40].

Methods for Quantification of Titanium and Titanium Dioxide

Commonly Used Methods

Acid decomposition of samples for metals analysis with inductively coupled plasma instruments is necessary with many of the analysis techniques available. Many analytical methods have been reviewed and accepted by the United States

Environmental Protection Agency. The use of hydrofluoric acid (HF) digestion with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis is routinely used in many of laboratories performing $nTiO_2$ quantification [8, 43, 44]. HF is hazardous and expensive requiring increased safety requirements for operators and durability of analytical instruments and equipment [45]. HF is a weak acid, not a mineral acid. HF destabilizes the crystal lattice structures of very stable compounds and liberates the analytes sequestered within the structures. This overestimates the bio accessible fractions of the analytes in environmental samples [46, 47]. Boric acid (H_3BO_3) is often used to remove the free fluoride ions from solution [43]. HF is hazardous and expensive with increased safety requirements for operators and durability of the equipment required to accurately analyze samples digested in HF [45].

Less Common Methods

Methods not containing HF for health or expense reasons opened discussion of potential alternatives that involve many combinations of acids for different lengths of time and even the addition of surfactants to the digestion. Dry ashing samples with ammonium sulfate have been proposed as viable alternatives to HF use and with minor problems with processing noted in the preparation of samples [48]. Research on the addition of Triton-x has also been tested and reported to be successful with ICP-OES systems [5]. These methods both incorporate sample preparation steps that make them more complicated than HF methods but provide similar results as the more hazardous reagents.

Safer alternatives to HF digestion methods have been utilized in cosmetics, foods, and some environmental matrices [8, 49-51] Through multiple types of alternative

methods it has been shown that sulfuric acid (H_2SO_4) at high concentrations may produce similar results to HF in digestions. These methods showed recoveries similar to those of HF digestions in the matrices tested [49]. Recoveries near 100% of spiked TiO_2 in both methods and the newly proposed methods show the potential for transitioning to less hazardous procedures.

Quantification of the nanomaterials with accurate and cost efficient methods is required acquire reliable chemical concentration data from these studies and to assess potential effects from the complex differences between dosing in real world scenarios and those of more controlled lab tests.

Inductively Coupled Plasma Mass Spectrometry

Inductively coupled plasma mass spectrometry (ICP-MS) is an effective method to quantify Ti in a sample that has been digested in acids. Currently, Ti is quantifiable at sub-ppb concentrations accurately with mass spectrometry instruments. ICP-MS systems limit the sample preparation methods that can be used. Organic components must be digested and acid concentrations have to be limited to reduce wear on the systems cones and other internal components. While limitations will be present in any instrument, ICP-MS is capable of accurately quantifying low concentrations of multiple metals and isotopes within the same sample. These benefits make ICP-MS a useful tool in the analysis of environmentally relevant concentrations of metal and metal oxide nanomaterials. Matrix interferences have been reduced considerably with the use of helium chambers that break down many of the polyatomic interferences that complicate the analysis [52]. Reduction in the matrix interferences with reaction chambers reduces interferences, such as polyatomic compounds, SO, making quantification with sulfuric

acid digestion samples possible and accurate at sub-ppb levels [53] The accuracy is improved again with the availability of multi-elemental internal standards that improve the instrument performance throughout sample analysis and limit the variability that comes from older methods of spiking the same amount into all samples. Direct injection of internal standard solution limits changes in the nebulizer and represents a single solution being integrated into the samples at the same rate and concentration throughout the run. The increased abilities of the ICP-MS instrumentation require regular cleaning, calibration tuning and replacement of disposable equipment, however with a well-maintained system, consistent, and reportable data can be generated close to detection limit concentrations.

Mesocosm and Environmental Studies

Mesocosms provide a large amount of control to studies conducted in the environment. Mesocosms can be pools or streams, to better approximate environmental scenarios of concern. These studies can monitor complex physical interactions, such as flow dynamics, that can change the impacts nanomaterials have on organisms [14, 17]. To replicate the environmental impact of nanomaterials in the environment, stream mesocosm protocols have been developed to more closely approximate real-world conditions with continually sampled pH, natural sun exposure, evaporation, and gas exchanges compared with field studies. With the benefit of dosing regularity, trends in data can represent the aqueous fraction of nanoparticles. Organic material in the mesocosm can interact with nanoparticles and help understand aggregation that occurs in the environment. The impacts of moving waters on the uptake seen in both fish gastrointestinal tract and gill organs can be compared to that of natural exposure in

similar environments. The use of different water types as test media for organisms can impact nanoparticle suspension in water and absorption in tissues [41]. Results from these mesocosms can be accurately modeled and extrapolated onto larger water systems. Mesocosm models can provide an understanding into the potential exposure of aquatic organisms to $nTiO_2$ and provide an outlook into the global implications of $nTiO_2$ use in consumer products. Other mesocosm studies provide insight into the growth of organisms in near natural conditions, the toxicity of different dosing regimens and concentrations, and the changes to compounds of interest over time in the stream [15, 17, 54].

Studies on the natural waterways that are impacted by effluent discharges can show the influence effluent has on metal concentrations in the environment. Characterizing Ti in the environment addresses the potential impacts long-term nanoparticle application could have on rivers. Ti is not easily dissolved in water and studies have shown preferences of $nTiO_2$ particles to move into solids as opposed to the water column [55]. Effluent contains many constituents that can complex Ti as they move in the environment [56]. This binding may decrease Ti concentrations in waters because interactions of Ti with a sufficient number of negatively charged ligands can produce neutral species which precipitate from solution. Precipitation incorporates Ti into suspended solids or sediments, within the aquatic environment. Evaluating methods for quantifying $nTiO_2$ concentrations in environmental matrices improves the abilities of laboratories to more accurately address real world impacts of $nTiO_2$ and increases the comparisons that may be made between natural and anthropogenic sources of Ti in aquatic environments.

This thesis discusses the validation and application of a method for the quantification of $nTiO_2$ in four environmental matrices and the comparison of Ti at four reaches of the North Bosque River to understand the impact of a point discharge of effluent has on the river. The four matrices are water, sediment, periphyton, and fish tissue.

CHAPTER TWO

Validation of a Sulfuric Acid Digestion Method for Inductively Coupled Plasma Mass Spectrometry Quantification of TiO_2 Nanoparticles

Abstract

A consistent analytical method incorporating sulfuric acid (H_2SO_4) digestion and ICP-MS quantification has been developed for TiO_2 quantification in biotic and abiotic environmentally relevant matrices. Sample digestion in H_2SO_4 at 110°C provided consistent results without using hydrofluoric acid (HF) or microwave digestion. Analysis of seven replicate samples for four matrices on each of three days produced Ti recoveries of 97 ± 2.5%, 91 ± 4.0%, 94 ± 1.8%, and 73 ± 2.6% (mean ± standard deviation) from water, fish tissue, periphyton, and sediment, respectively. The method demonstrated consistent performance in analysis of water collected over a one month period from four stream mesocosms.

Introduction

Titanium dioxide nanoparticles ($nTiO_2$) are currently used in various consumer products, including cosmetics, food, paint, and plastics [8, 9, 57]. The white color and UV properties of $nTiO_2$ improve consumer product performance, longevity, and appeal. Anatase, rutile, and brookite are the three natural mineral forms of TiO_2. Industrial and research applications most frequently use rutile and anatase, due primarily to the abundance of rutile and the enhanced surface activity of anatase [35, 36]. TiO_2 is primarily produced using the Kroll process in industries, and $nTiO_2$ is produced in

multiple ways including the sol-gel method, solvothermal method, and chemical vapor deposition [37, 38]. Estimated annual production of $nTiO_2$, in the United States, is potentially as high as 260,000 metric tonnes per year, as of 2015, and predicted to increase until 2025 as additional consumer product applications are developed [3, 7]. This estimate is reasonable considering that Switzerland alone uses over 435 metric tonnes of $nTiO_2$ per year [9].

Once in aquatic environments, nanoparticles often aggregate, and aggregation behavior depends upon the nature of the nanoparticles themselves as well as the types and concentrations of organic matter and inorganic constituents, ionic strength and composition, pH, oxidation, and ultraviolet light (UV) exposure [35, 58, 59]. These parameters control surface interactions of nanoparticles and may allow homo- or hetero-agglomeration as well as dissolution [59, 60].

Hydrofluoric acid (HF) digestion with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis is routinely used in laboratories performing $nTiO_2$ quantification [8, 43, 44]. HF cleaves the bonds forming crystal lattice structures of very stable compounds and liberates analytes sequestered within the structures. However, this digestion technique likely overestimates the bio accessible fractions of analytes in environmental samples [46, 47]. In addition, HF is hazardous and expensive with increased safety requirements for operators and durability of the equipment required to accurately analyze samples digested in HF [45].

Sulfuric acid (H_2SO_4) digestion methods can produce high recoveries of $nTiO_2$, but have not been validated in many commonly encountered environmental matrices [40, 50]. Validated methods that reduce risk and cost can expand the number of

laboratories capable of studying the behavior of TiO_2 and other recalcitrant inorganic analytes in the environment. Analytes sequestered in very stable mineral crystal structures are not liberated with H_2SO_4, providing a more relevant bio accessible fraction of titanium from matrices. Safer alternatives to HF digestion methods have been utilized in cosmetics, foods, and some environmental matrices [8, 49-51].

Although ^{48}Ti is the most abundant Ti isotope, ^{47}Ti is the preferred analytical mass because the ^{48}Ca isotope is isobaric with ^{48}Ti [61]. ^{47}Ti has interferences including $^{31}P^{16}O$, ^{46}CaH, $^{35}Cl^{12}C$, $^{32}S^{14}NH$, and $^{33}S^{14}N$ [62], but these polyatomic interferences are most often minimized with the use of reaction chambers, using He as the collision gas, during ICP-MS analysis [52].

With this background in mind, we optimized and validated analytical methods that combine sulfuric acid digestion with ICP-MS analysis to quantify $nTiO_2$ in four environmental relevant matrices. Water, periphyton, fish tissue, and sediment represent a diverse group of matrices that are ecologically important in various aquatic environments. The application of a validated method for matrices common to many natural waterways allows more consistent study of the impact of $nTiO_2$.

Methods and Materials

Reagents

Titanium dioxide - Aldrich Chemistry, titanium (IV) oxide nano powder, 21nm, ≥ 99.5% trace metal basis; PACS-3 marine sediment, Canadian National Research Council Certified Reference Material; Ultrapure H_2O, GenPure 18.2 mΩ; H_2SO_4, Fisher Chemical, Trace Metal Grade; HNO_3, Fisher Chemical, TraceMetal Grade

TiO_2 Addition

For water and tissue samples, $nTiO_2$ was first gravimetrically added to 50 mL screw capped, round-bottom, Teflon (PTFE) digestion vessels (VWR). PACS-3 has known titanium concentrations and no spiking was necessary. $nTiO_2$ addition produced nominal concentrations that ranged from 10 to 38 mg L^{-1} for water and 0.72 to 6.08 mg g^{-1} in biota. These concentrations were constrained by the limits of balance accuracy, expected digestion masses for tissues, and volumes that represent realistic sample preparation procedures.

Water Sample Preparation

After known amounts of $nTiO_2$ were placed into digestion vessels, 5 mL of ultrapure H_2O was added to each vessel. Water samples were then gently vortexed before digestion. Each matrix blank contained a known volume of water without addition of $nTiO_2$. Matrix spikes were made by adding a known volume of titanium standard (998 mg L^{-1} Ti, Sigma-Aldrich) to an aliquot of matrix blank.

Solid Samples Preparation

Fish tissues were obtained from Gulf killifish, *Fundulus grandis*, from established laboratory cultures at Baylor University. Tissues were homogenized and dried for 1 week at 60 °C. Periphyton was collected from limestone rocks in the North Bosque River outside of Valley Mills, Texas, USA (31°40'11.46"N, 97°28'9.58"W). Both tissues were then prepared similarly. Known weights (20 to 90 mg) of individual samples from a given matrix were added to digestion vessels. Solid matrices were hydrated with 4 µl of 2% HNO_3 per mg of dry sample. Samples were then gently vortexed, capped, and

maintained at room temperature for a minimum of 24 h. Each reagent blank contained a known volume of water without addition of $nTiO_2$. Reagent spikes were made by adding a known volume of titanium standard to prepared matrix blank with a known mass. Each matrix blank contained a known mass of matrix without addition of $nTiO_2$. Solid matrix spikes were made by adding a known volume of titanium standard to matrix blank with known mass.

Digestion

Preliminary trials indicated that digestion was optimized when water was digested at a 1:1 ratio with H_2SO_4, and when 2 mL of H_2SO_4 were added to hydrated solid samples with masses ranging from 20 to 100 mg. After acid addition, digestion proceeded in loosely capped vessels for 8 h at 110 °C. Digests were cooled for 15 min, followed by addition of 3 mL of 2% HNO_3. Digests were recapped, digested for 1 h at 110°C, and cooled for 15 min. Digests were volumetrically diluted to 50 mL (Class A flasks) using 2% HNO_3 for rinses and diluent. To achieve concentrations of 10 to 100 µg L^{-1} Ti, appropriate digest aliquots were further diluted with 2% HNO_3 in 25 mL volumetric flasks.

Instrumental Analysis

ICP-MS analysis on an Agilent 7900 utilized Ar (1.10 L min^{-1}) as the carrier gas, and a sample flow rate of 3 mL min^{-1} during sample intake. Helium served as the collision gas (5 mL min^{-1}) (Table 1.1). ^{47}Ti was used for quantification. Four other Ti isotopes (^{46}Ti, ^{48}Ti, ^{49}Ti, and ^{50}Ti) were monitored as well. ^{72}Ge served as the primary internal standard (IS), and digests were analyzed in at least triplicate. Calibration

solutions were made with 2% HNO_3 and ranged from 10 to 100 μg L^{-1} Ti. Calibrations curves contained at least five points and were accepted if $R^2 \geq 0.995$ and responses remained within 10% of expected values. All calibration standards were matrix matched to samples through equivalent addition of H_2SO_4. The response of ^{72}Ge was used as the sole IS for correction of instrument drift. IS responses were accepted if they remained within 80% to 120% of their initial signal while maintaining a relative standard

Table 1.1. ICP-MS Plasma Operating Parameters

RF Power	1550	W
RF Matching	1.8	V
Sample Depth	9	mm
Carrier Gas (Ar)	0.95	L min^{-1}
Dilution Gas (Ar)	0.15	L min^{-1}

deviation below 5% within an individual validation sample analysis. Agilent provides an internal standard mixture (Agilent ICP-MS Internal Std. Mix) that includes many other elements. Although none of these elements were needed for assessing or processing the data reported herein, several of these elements were monitored in case unforeseen interferences were encountered. Calibration standards were analyzed before and after digests to ensure consistency with measurements. Quality control (QC) procedures were implemented, including a continuing calibration check (CCC), and a duplicate analysis of a digest within the last ten samples.

Validation Process

Validation included 3 batches each containing 7 samples, a reagent blank, a spiked reagent blank, a matrix blank, and a matrix spike for each matrix except

sediments, which used the reagent blank and spiked reagent blank for QC. Each validation trial was processed on a different day. The third trial was performed by a second operator using a standard operating procedure for guidance. Operators prepared and digested samples, prepared calibration standards, and calibrated and operated the ICP-MS instrument during the final phase of method validation. If Ti concentrations were detected in reagent blanks, appropriate corrections to recoveries were made.

SEM-EDS Analysis

The remaining solids in digestates from three periphyton samples used during the validation trials were analyzed via scanning electron microscope with energy disruptive X-ray spectroscopy (SEM-EDS). Water was used to dilute and remove further acids and was then decanted. The remaining solids were dried at 70°C for 2 d, and resembled a white sandy powder. A thin layer of powder was collected and mounted onto carbon tape, sputter coated with carbon and analyzed using SEM-EDS (FEI Versa 3D and EDAX). Scanning depth for X-ray spectroscopy was from 2 to 5 µm.

Application of Method to Environmental Samples

This method was used to quantify titanium in water, periphyton, sediment, and fish tissues collected at seven sites from Neils Creek, a tributary of the North Bosque River watershed in Central Texas, USA, and to quantify titanium in periphyton from four stream mesocosms treated with titanium dioxide nanoparticles. All samples were collected into 50-mL HDPE conical vials. Surface water and sediments were collected from the same location at each site. Fish (blacktail shiner**,** *Cyprinella venusta*) were caught using a seine net, euthanized with MS-222 using approved IACUC methods, and

stored at -20°C. Periphyton was scraped from larger limestone rocks at the same site as water and sediment samples. Periphyton was also taken from a mesocosm study involving 4 replicated streams continuously treated with $nTiO_2$ (5 mg L^{-1} nominal). These stream mesocosms recirculated wastewater effluent from the city of Waco, Texas, USA. Periphyton samples were collected from ceramic tiles by scraping with disposable brushes.

Fish were crushed and homogenized in liquid nitrogen using a mortar and pestle. Sediment, fish tissue, and periphyton dry weights were obtained after heating samples to 60-70°C for 1 week.

Statistical Analysis

All data from validation trials were first evaluated utilizing JMP Pro 11 (SAS Institute) to conduct one-way analysis of variance (ANOVA) comparisons. If ANOVA found significant differences, Tukey Kramer HSD was used to compare individual trials. Outliers were identified as having a recovery more than 1.5 times the internal quartile range. Concentrations in mesocosm water were compared throughout the continuous dosing period of the study. Concentrations determined in field and mesocosm samples were log-transformed to normalize data for statistical analyses.

Results

Our method recovered 97 ± 2.5%, 91 ± 4.0%, 94 ± 1.8%, 73 ± 2.6% (mean ± standard deviation) of Ti from water, fish tissue, periphyton, and sediment, respectively (Table 1.2). Standard deviations in all trials were below 11.5%, and the only trial with greater than 10% standard deviation contained one recovery above 110%. Based on the

lowest calibration point, dilutions, and 8x concentration factor for environmental water samples, these recoveries allowed a quantification limit of 1.25 ng mL^{-1} in water and 74.9 ng g^{-1} fish and periphyton.

Water validation showed no inter- or intra-operator differences ($p = 0.0939$) indicating that method performance was consistent among the three trials. No outliers were found in the water trials and recoveries were not biased high or low.

Table 1.2. Mean Recoveries[A] of Titanium in Multiple Environmental Matrices during Validation Trials

Matrix	Operator 1		Operator 2	
	Digest 1[B]	Digest 2[B]	Digest 3[B]	Overall[C]
Water	95.67 ± 2.16	95.65 ± 5.22	99.92 ± 3.88	97.08 ±2.46
Periphyton	92.20 ± 5.00	95.69 ± 3.47	93.20 ± 4.80	93.70 ± 1.79
Fish tissue	92.71 ± 11.21	94.49 ± 2.96	86.76 ± 5.47	91.32 ± 4.04
Sediment	**75.74 ± 0.84**	**70.61 ± 4.67**	74.08 ± 2.34	73.47 ± 2.61

[A] x ± SD, [B] n = 7, [C] n = (7x3),
Statistical difference when compared in bold

Periphyton validation produced no outliers on any of the three validation days, and no inter- or intra-operational differences ($p = 0.3454$). Blank corrected recoveries were ≥ 90% in periphyton. Following periphyton digestion, solid material routinely remained in the bottom of the digestion vessel. SEM-EDS analysis identified SiO_2 as the primary constituent in these solids with no Ti detected (to the X-ray penetration depth).

Fish tissue validation showed no inter- or intra-operator variation, although this tissue type did have the highest standard deviation of all matrices at 4.04% (Table 1.2). This variance is partly because the second validation trial of fish tissues had one high recovery of 114% which exceeded the upper bound (1.5x) of the interquartile range. ANOVA, including the high recovery point, indicated that all three validation trials

were statistically similar ($p = 0.1542$). Dropping the outlier reduced the ANOVA p-value value, but not to the point of significance. The potential outlier was not eliminated for completeness of data reporting and because no operator errors were indicated.

Sediment validations were precise with mean recoveries below 76% and no outliers. ANOVA, followed by Tukeys comparison, showed that trial one and trial two were dissimilar ($p = 0.0015$). The difference was due to the high precision of trial 1 which showed a 1.1% RSD. This allowed the 5% difference in recoveries to be significant. Comparisons with trial three were similar (trial one, $p = 0.1134$; trial two, $p = 0.5776$). Although recovery (73.5%) from sediments was lower than that of the other matrices, low inter- and intra- operator variance demonstrated consistent method performance, so it is likely that there was an inability to liberate Ti from the SiO_2 matrix in sediment.

Throughout validation, reagent blank concentrations were 10 to 100 times lower than the lowest calibration concentrations so no correction was necessary. Blank spikes ($n = 12$ for validation trials) showed recoveries of 101.5% (Table 1.3). Internal standard (^{72}Ge) recovery had a minimum of 82.6% and a maximum of 103.6% during trials and calibration points were rerun to check for instrument drift.

Table 1.3. Titanium Spiked Blank Recovery[A] from Four Environmental Matrices

Water[B]	Periphyton[B]	Fish Tissue[B]	Sediment[B,*]	Overall Average[C]
105.3 ±0.69	99.0 ±4.86	102.3 ±3.61	99.6 ±6.52	101.5 ±4.62

[A] mean ± SD, [B] n = 3, [C] n = 12, *Sediments used reagent blank spike

Ti concentrations from the environmental samples were consistent for individual matrices, with the largest variances found for sediments (Figure 1.1). Mean Ti

concentrations (95% confidence interval) were: 0.47 µg L^{-1} (0.25 - 0.80 µg L^{-1}) in water, 759 µg g^{-1} (672 - 851 µg g^{-1}) in periphyton, 1.58 µg g^{-1} (0.88 - 2.55 µg g^{-1}) in fish tissue, 114 µg g^{-1} (16 - 222 µg g^{-1}) in sediments. Periphyton from the stream mesocosms

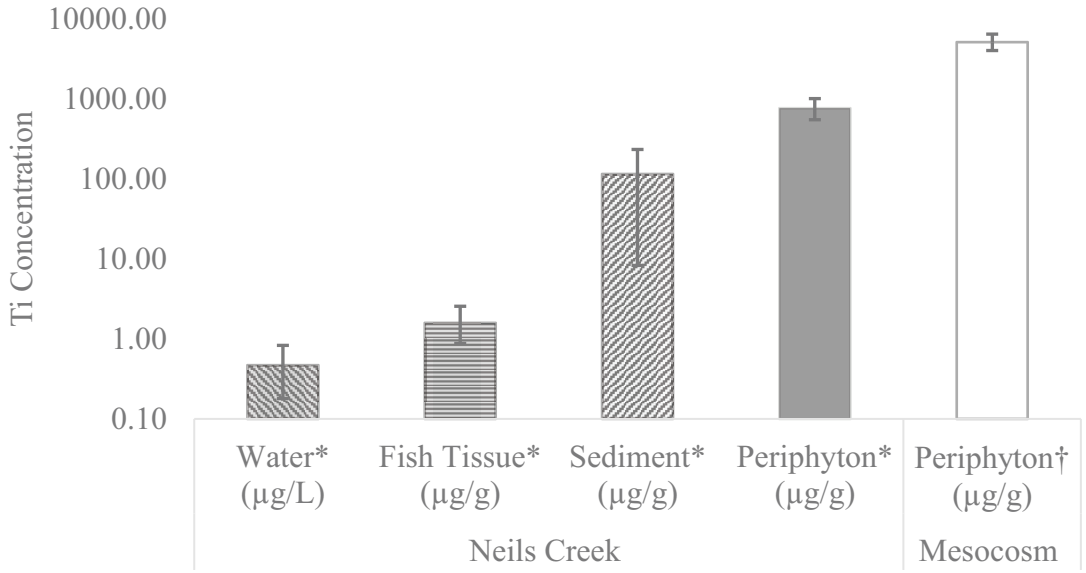

Figure 1.1. Mean titanium concentrations in water, fish tissue, sediments, and periphyton collected from Neils Creek, Valley Mills, Texas, USA, and periphyton collected during a stream mesocosm study with a nominal treatment of 5 mg L^{-1} $nTiO_2$. Error bars indicate 95% confidence interval. $^{*}n = 7$, $^{\dagger}n = 4$

contained Ti concentrations ranging from 3,360 to 6,268 µg g^{-1}, with an average of 5,081 µg g^{-1} which was 7x higher than in periphyton from Neils Creek.

Discussion

TiO_2 concentrations were reliability quantified in four ecologically important environmental matrices. It is interesting to note that this is the first reported method validation for quantification of $nTiO_2$ in periphyton using ICP-MS. Validated methods for quantification of nanoparticles in environmental matrices are increasingly important as nanoparticles are being released into the environment in a myriad of ways [57].

Applications in new consumer products are increasing the consumption and subsequent release of nanoparticles [3, 9, 39]. Concern over the large quantity of nanoparticles being used and potentially released into the environment has prompted modeling and toxicological studies [39, 63]. Recoveries of greater than 95% in water confirmed that method performance at the current temperature and lengths of time were sufficient. Accurate and consistent recoveries encouraged method development for other sample types and allowed application to samples from a mesocosm study.

Fish tissues produced the darkest colored digests after the 8 h digestion step, indicative of incomplete digestion of large organic molecules. However, the color lightened following the final 1 h digestion step with 2% HNO_3, consistent with previous work [40], and the fish tissue validation did not have a recovery lower than the other matrices tested. The first two validations of periphyton and fish tissue had similar values (Table 1.2). The final 1 h digestion step with 2% HNO_3 is known to complete the digestion of organic material by increasing the oxidative strength of the solution.

Periphyton was the only matrix that needed matrix blank correction because quantifiable concentrations were found in the matrix blank. Small portions of sediments and diatoms are naturally part of periphyton assemblages and provide a possible source of Ti in addition to algal and microbial uptake of Ti. Matrix blank correction allowed accurate validation sample Ti quantification.

Solids remained in digestion vessels following sediment digestions. PACS-3 is composed of 26.1±3.4% silicon, very close to the proportion of Ti not accessed by our method (26.53% titanium not recovered in sediments). Thus, it is possible that Ti was sequestered within SiO_2 solids. In this regard, digestion of environmental samples with

H_2SO_4 may likely provide a more realistic representation of bio accessible Ti than does an HF digestion. HF digestion methods cleave SiO_2 bonds in sand and clays, liberating titanium and other inorganic analytes sequestered in the SiO_2 lattice and thereby producing concentrations that exceed bio accessible fractions.

Analysis using ICP-MS allow sub ng mL^{-1} detection. ^{72}Ge did not have any interferences during the validation, and recoveries showed no significant instrumental drift during analysis. Preliminary method development trials indicated that higher concentrations of H_2SO_4 in the digest may increase relative standard deviations (RSDs) for low concentration samples (data not shown). Larger batches of samples from the study indicated instrument analysis was stable with batches up to 30 samples, calibration standard, and QC samples.

Ti concentrations in all water samples collected from Neils Creek were below 1.1 $\mu g\ L^{-1}$, which is in the lower range of known titanium concentrations in surface water (0 to 1180 $\mu g\ L^{-1}$) [29]. Ti in fish tissues spanned a narrow range from 1.46 $\mu g\ g^{-1}$ to 3.68 $\mu g\ g^{-1}$. Conversely, Ti concentrations in sediments varied the most of any matrix 47.14 $\mu g\ g^{-1}$ to 512.50 $\mu g\ g^{-1}$ and had the largest variation with respect to the mean. This variation is not surprising given that sediments are known to be heterogeneous as a result of the dynamic nature of sediment deposition and resuspension as well as the presence of eroded material. Water is far more likely to be homogeneous than sediments.

Environmental waters have many constituents that can form metal complexes and may also precipitate metal salts from solution. These processes stabilize aqueous concentrations of cations in the water column. The homogeneity in fish may be explained by blacktail shiners in the same population having similar foraging habits, uptake, and

elimination processes for Ti. Ti concentrations were higher in periphyton from both Neils Creek and stream mesocosms than in sediments from Neils Creek ($p < 0.001$).

Differences in Ti concentrations in periphyton between the two groups may be explained by the differences in water concentrations. Mesocosms were continuously treated with $nTiO_2$ and waters from the streams had concentrations approximately 150x the concentrations found in Neils Creek water (data not shown).

Conclusions

Our results show that H_2SO_4 digestion of environmentally relevant matrices with ICP-MS analysis accurately measures titanium concentrations. Avoiding more hazardous chemicals (e.g. HF) and complex digestion procedures (e.g. microwave digestion), while still providing consistent and accurate data are major advantages of this method. This method provides an opportunity for labs to analyze $nTiO_2$ in environmental matrices using standard ICP-MS instrumentation without expensive modifications or safety equipment. Nickel coated cones and quartz torches found on basic ICP-MS units are capable of analyzing these samples, streamlining and eliminating addition costs for analysis. This method may be applied to other environmental matrices as well. The ability to routinely quantify $nTiO_2$ from different matrices in both controlled studies and in natural environments provides a more informed evaluation of factors that affect uptake and that control ecological and toxicological risk.

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CHAPTER THREE

Titanium Concentrations in Water, Sediment, Periphyton, and Fish Tissues from the North Bosque River: Influence of an Effluent Discharge on Environmental Concentrations

Abstract

This study addresses possible influences of effluent discharge on titanium (Ti) concentrations in the North Bosque River, Texas, USA. We quantified Ti concentrations in four environmental matrices: water, sediment, periphyton and fish tissues along four reaches of the North Bosque River Watershed. Ti concentrations were consistent in each matrix, ranging from 0 to 8 μg L^{-1} in water, 45 to 526 μg g^{-1} in sediment, 276 to 1520 μg g^{-1} in periphyton, and 0 to 30 μg g^{-1} in fish tissue. Ti concentrations in periphyton were significantly higher ($p < 0.001$) than concentrations in sediment, fish tissues, and water concentrations, but were independent of distance from the effluent discharge. Concentrations of Ti in the North Bosque River are not noticeably changed by effluent discharge throughout the river.

Introduction

Ti and Ti containing compounds are frequently used in industries because their strength and durability, and TiO_2 has applications in a variety of consumer products, including food, cosmetics, and sunscreen [6, 8, 10, 64-66]. Titanium dioxide nanoparticles ($nTiO_2$) are commonly used in consumer products. [8, 10, 28]. In sunscreen products, $nTiO_2$ is added for its ability to act as a UV absorber [67]. However, UV activation of $nTiO_2$ forms reactive oxygen species (ROS), causing a range of toxic

responses. As a consequence of $nTiO_2$ extensive use in commercial products, an estimated 88 tonnes of $nTiO_2$ entered the environment in 2010, potentially contaminating various media [8, 57]. Of that total, 15.6 tonnes are estimated to be released into aquatic systems [57]. $nTiO_2$ enters the aquatic environment through effluent discharge, from the disposal of consumer products, and by direct release from activates including swimming.

Natural waters are reported to contain Ti concentrations from non-detectable to 1180 $\mu g\ L^{-1}$ worldwide, while estimates of $nTiO_2$ concentrations in waste water are between 5 and 15 $\mu g\ L^{-1}$ [29, 57]. Contaminant discharge into river systems prompts concerns for the health of waterways, and the need to reuse water downstream amplifies these concerns. Understanding TiO_2 in river water becomes essential to model the transport of metals in aquatic systems. The Bosque River flows into Lake Waco from four branches. The longest of these branches is the North Bosque River, which flows through four counties: McLennan, Bosque, Hamilton, and Erath. Headwaters of the North Bosque River are northwest of the city of Stephenville, which is the first and largest point source of effluent discharge located on the North Bosque. Along the 185 km of the North Bosque River are seven permitted effluent discharges [68]. These point sources are regulated to discharge 0.2 - 3.0 million gallons of water per day into the North Bosque, depending on the size of the municipality. Wastewater entering these water treatment plants must be treated before it may legally be released into the environment. Currently, municipalities are not required to monitor Ti concentrations released into the environment, allowing the potential for sustained discharges into the environment. Effluent normally contain a mixture of many compounds, and $nTiO_2$ has

been reported in waste water from other locations [57]. Bulk TiO_2 is considered relatively inert, and the potential for toxic effects are diminished by the large particle size and limited routes of exposure [69]. Toxicity differences of $nTiO_2$ versus bulk TiO_2 include a larger surface area of $nTiO_2$ which increases the potential for interaction with UV and subsequent production of ROS species, as mentioned above. The smaller size of $nTiO_2$ facilitates greater $nTiO_2$ accumulation within organisms than does bulk TiO_2, and is reported to damage DNA [70]. This increases concerns for $nTiO_2$ release into the environment.

Within the North Bosque River, various geological formations are present and can be used to understand transport of Ti from effluent discharges into the environment. The geology of central Texas is diverse with many formations, and the North Bosque River flows through multiple geological formations before reaching Lake Waco. Many geological formations from the Cretaceous period produce sediment in the watershed [71]. Upstream areas of the North Bosque River are surrounded by the Glen Rose formation, primarily composed of fine grained limestone and claystone [71]. The next segment of the river is surrounded by the Paluzy formation and the Walnut Clay formation, which consist of fine sand and clay with limestone, respectfully. The river's third segment is surrounded by the Commanche Peak Limestone formation, Denton Clay, Fort Worth Limestone, and Duck Creek Limestone [72]. These formations are primarily composed of limestone of varying hardness. Throughout the Alluvial bed of the river, there are deposits of fluviatile terraces, composed of a gravel, sand, and clay. Clays and sands that contain natural sources of Ti are common within the geological formations. Clays contain Ti at concentrations ranging from 0.4 mg g^{-1} to 6.6 mg g^{-1}

while concentrations in sands range from 0.07 mg g^{-1} to 3.0 mg g^{-1} [28]. These values are consistent with Ti concentrations in sediments found in different water bodies, including the Nile River in Africa with 8.32 mg g^{-1}, rivers of Brazil with 9.21 to 11.10 mg g^{-1}, the Yangtze in China 6.45 mg g^{-1}, and North American sediments from Buffalo River with 4.57 mg g^{-1}. [29-33]

The North Bosque River contains various aquatic organisms that interact with sediments and can indicate organism uptake of Ti, including periphyton and blacktail shiner, *Cyprinella venustus.* Periphyton is an environmental media described as a biomass or community of algae and micro invertebrates [73, 74]. Periphyton grows more readily with increases in nutrients from sources such as effluent [75, 76]. The blacktail shiner is a fish species found throughout the southeastern United States, including multiple Texas rivers with sandy or gravely substrates [77-79]. Blacktails eat a variety of micro invertebrates and algae from the water column [80]. Blacktail shiner is an ideal fish species for our study because many food items that comprise their diet are found in the periphyton matrix, and the broad range in which shiners are found. Also, both periphyton and blacktail shiner are consumed as a food source by species at higher trophic levels. Organisms at high trophic levels are exposed to the metals through their food sources, allowing bioaccumulation of these metals within the food web.

Analysis of biotic and abiotic matrices was used to assess the extent to which effluent point sources release metal constituents into aquatic environments. Comparisons of Ti concentrations in different reaches within a river system were used to determine if effluent discharges affected organisms and humans using the rivers downstream.

Sampling Reaches

Four reaches within the North Bosque River watershed were sampled for sediments, water, periphyton, and blacktail shiner (Figure 2.1).

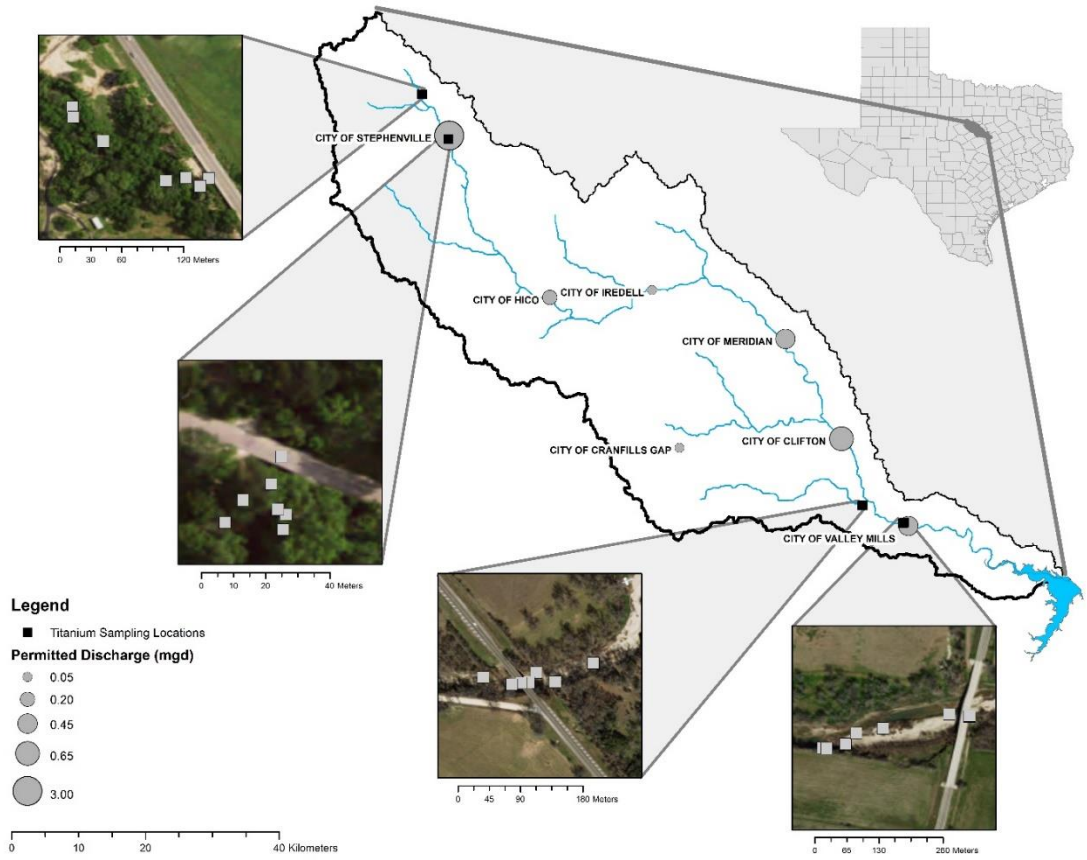

Figure 2.1. Map of North Bosque River Watershed within Texas, USA, Sampling Reaches within the Watershed, and Sampling Locations within the Reaches. (ArcGIS IMAGE)

The first and most upstream reach (PBR) chosen for sampling was northwest of Stephenville (5 km). This location included pools of water separated by riffles of small stones. The second sampling reach (WWS) was southeast of Stephenville and

downstream from the effluent point discharge (0.8 km). This location had a consistent water depth, and pieces of concrete were found throughout the sampling area. The third reach (BR) was a larger portion of the river in Valley Mills, Texas (1 km). The riverbed at this location was wider than at other reaches. More erosion of the river bank was present at the Valley Mills site than at the other locations in the North Bosque River and had a mix of deep pools and riffles. The fourth reach (NC) was on Neil's Creek, a tributary for the North Bosque River northwest of Valley Mills (3 km). Reach 4 was primarily a riffle stream with a few small deep pools and very good visibility. Reach 4 was considered a reference site, as there are no permitted discharges upstream of the sampling location.

Sampling Methods

Sampling was conducted over two days and at two different reaches of the river each day. Each reach was sampled at seven different locations for water, periphyton, and sediment. Each water sample was approximately 50 mL and at least 5 g for sediments. Periphyton was scraped from the rocky substrate at the bottom of the channel. Periphyton abundance varied greatly and sampling was done until a sufficient volume was collected (approx. 5 mL). This gave us periphyton with portions of fine silt embedded into the sample, which was considered part of the matrix. Water was sampled from approximately 2-6 cm below the surface of the water. Sediment composition varied at each of the sampling reaches, ranging from small pebble mixture to coarse sand. Each sample was collected, stored in 50 mL conical vials, and placed into a cooler during field sampling. Samples were stored in a refrigerator after returning to lab and prior to digestion.

Blacktail shiners were collected at each reach with a seine net, with a target of seven samples from each reach. Three reaches produced the targeted seven fish samples, the PBR site provided three fish. Each fish was first identified as a blacktail shiner before being euthanized with MS-222, according to an approved IACUC protocol. Fish were sealed in 50 mL conical vials and kept on ice until they were returned to the lab where they were frozen. All sampling was conducted within a half km at each reach.

Sample Preparation

Water samples were extracted from sampling vials with syringes and filtered through 0.45 µm (PTFE) filters to eliminate sediments. These samples were then concentrated from 10 mL to approximately 1.25 mL in 50 mL cylindrical, Teflon (PTFE) digestion vessels (VWR). Samples were not allowed to boil during this evaporation step and were monitored closely as evaporation occurred at differing rates.

Sediment and periphyton were transferred from 50 mL collection vials to 15 mL conical vials for dry weight determination. If available, 2 g was used for analysis. Sediments and periphyton were dried at 70°C until the dry weight was attained (approximately 1 week).

Fish were removed from freezer storage and allowed to reach room temperature. At this time, the fish were placed into mortars and submerged in liquid nitrogen with forceps until the liquid nitrogen completely evaporated. A pestle was used to grind fish tissues. Homogenized fish tissues were then transferred into weigh boats, weighed and dried at 70°C (approximately 1 week). Once dried, samples were transferred to 50 ml conical vials. A fraction (approx. 100 mg) of these dry tissues were weighed into digestion vessels for analysis.

Digestion Process

Reagents

Research procedures required PACS-3 marine sediment, Canadian National Research Council Certified Reference Material (CRM); H_2O, Genpure 18.2 mΩ; H_2SO_4, Fisher Tracemetal Grade; HNO_3 and Fisher Tracemetal Grade, as described previously [81].

Digestion

Sample masses used for analysis were approximately 250 mg for sediments, 50 mg for periphyton, and 100 mg for fish tissues. Once samples were prepared, digestion of these samples was based on previously published methods [81]. Briefly, H_2SO_4 was added to samples: 1.25 mL in water samples and 2 mL in all other matrices. Digestion proceeded for 8 h at 100°C. After cooling, digests received 3mL of 2% HNO_3 and were heated for another hour at 110°C. Digests were then cooled and volumetrically diluted at least 25 mL. Matrix blanks and matrix spiked blanks were processed along with each batch of samples for each matrix, except sediments for which PACS-3 was used as a CRM. Matrix spikes received a known volume of Ti standard (998mg/L Ti, Sigma-Aldrich) into a known mass of matrix.

Instrumentation

ICP-MS analysis (Agilent 7900) monitored ^{47}Ti for quantification and ^{72}Ge served as the primary internal standard (IS). Each digest was analyzed at least 5 times [81]. Briefly, a minimum of five calibration standards were matrix matched to sample matrices through the addition of H_2SO_4. Responses of the IS were used for correction of

instrument drift. Quality control (QC) samples, including a continuing calibration check (CCC) and a duplicate ICP-MS analysis of one sample was analyzed after every ten samples.

Statistics

Data were first checked for normality and then log transformed. Samples within a given matrix were then grouped together (blocked) by sampling reach and a one-way analysis of variance (ANOVA) followed with a Tukey's Kramer HSD comparison were performed to determine if differences existed. To avoid bias, samples that were below detection were assigned values that were calculated from signals reported by the instrument [81]. Samples that produced values less than zero were assigned a value of zero for statistical analysis. Statistical analysis was performed in JMP (PRO 11) including ANOVA and Tukey Kramer HSD. Microsoft Office Excel (2013) was used for basic calculations including log transformations.

Results

Mean Ti concentrations in water from the four reaches from the North Bosque watershed showed no significant differences ($p = 0.2417$). Reaches 1 and 2 trended above the overall average, while Reaches 3 and 4 trended below the overall average, although no reaches were significantly different (Table 2.1). Variation (RSD) was greatest at Reach 2, and lowest at Reach 1. Average blank spike recoveries were 70% after blank correction. Reach 3 had six samples analyzed.

Table 2.1. Average Ti Concentrations in Four Sample Types from North Bosque River

	Water[A] (μg L^{-1})	Sediment[A, B] (μg g^{-1})	Periphyton[A, B] (μg g^{-1})	Fish Tissue[A, B] (μg g^{-1})
Reach 1	1.14 (0.44–2.16)[*]	333.65 (239.05–435.46)[*]	944.54 (708.80–1212.79)[*]	BDL[‡§]
Reach 2	0.8 (0.22–1.67)[*]	188.36 (104.08–279.09)[*]	758 (544.90–1000.51)[*]	2.51 (0.65–6.47)[*]
Reach 3	0.23 (0.19–0.88)[†]	133.32 (101.77–276.41)[*]	848.62 (624.50–1103.63)[*]	3.24 (0.99–8.02)[*]
Reach 4	0.47 (0.01–1.17)[*]	114.56 (35.50–199.64)[*]	759.78 (546.43–1002.53)[*]	1.58 (0.21–4.49)[*]
Overall Average[¤]	0.66±0.40[Ω]	205.56±91.96[Ω]	827.74±88.62[Ω]	1.33±1.43[Ω]

[A]x (95% confidence interval, lower bound – upper bound: CL 95% based on summary statistics, not after Bonferroni correction), [B]Based on dry weight, [*]n=7, [†]n=6,[‡]n=3, [§]Below Detection limit, [¤]n=4, [Ω] x ± Standard Deviation

Watershed

Ti concentrations in sediments were significantly different between Reach 4 and Reach 1 ($p = 0.0081$). Average concentrations at these two sites were 114 μg g^{-1} and 334 μg g^{-1}, respectively (Table 2.1). Variation (RSD) was greatest Reach 3 and lowest in Reach 2. Average reagent blank spike recovery was 101% and CRM recovery was 77%, which was similar to previously reported recoveries [81].

Periphyton from each location were not significantly different ($p= 0.6290$). Average concentrations trended higher at Reach 1 at 945 μg g^{-1}, and lower at Reach 4 at 758 μg g^{-1} (Table 2.1). Variance (RSD) was the lowest at Reach 3 and greatest Reach 2.

Average reagent blank spike recovery was 96% and matrix spike blank recovery was 98%.

Fish tissues demonstrated no significant differences between reaches ($p = 0.1928$). Ti concentrations ranged from 0 $\mu g\ g^{-1}$ at Reach 1 to 3.24 $\mu g\ g^{-1}$ at Reach 3. Limited samples were found at Reach 1, with only three blacktail shiner sampled. All three samples from Reach 1, three samples from reach 2, and one sample from both Reach 3 and 4 were BDL. Reagent blank spike recovery was 93% and the average matrix blank spike average recovery was 106% (n=2). Reach 1 had the lowest variation (RSD) and Reach 2 had the greatest variation.

Significant differences were found for Ti concentrations determined in different environmental matrices. Periphyton was significantly higher than sediments ($p < 0.0001$), fish tissues ($p < 0.0001$), and water ($p < 0.0001$). Sediments were significantly higher than fish tissues ($p = 0.0007$), and water ($p = 0.0007$). Fish tissues and water were not significantly different ($p = 0.9675$).

Discussion

Within each matrix evaluated, Ti concentrations showed no significant differences throughout the four sampling reaches. Intramatrix similarity of Ti concentrations indicated that effluent from the city of Stephenville was not causing a noticeable change in Ti concentrations at Reach 2 or downstream at Reach 3. Ti concentrations in our study were similar to those found in other studies for Ti in natural waters (0 to 1180 $\mu g\ L^{-1}$) [29]. Ti is not a soluble metal and prefers to either be bound to sediments or biota [57, 58, 82]. This distribution is what we observed (Table 2.1) and is discussed further in the remainder of this section.

Consistency of Ti concentrations in water at these sampling reaches can be expected if no effluent or other point source discharges are influencing Ti in the water column. Complexes of Ti are generally stable in solutions above pH 2, and aggregation factors naturally remove Ti from water [83]. Complexation with both organic and inorganic ligands can produce neutral species which tend to precipitate. The abundance of fulvic acids, amines, and hydroxide ions in the water column provide abundant ligands and available binding sites that normally precipitate Ti species from water. Filtration of samples removed Ti species in large particles or aggregates in the water column and decreases variation within Reaches. Ti remaining in water samples averaged 0.66 μg L^{-1} after filtration (Table 2.1). These were consistent with studies of rivers in other regions including Israel with 1.50 to 2.40 ug/L, North America with 0.0 to 107 μg L^{-1}, and Japan with 0.76 to 1.86 μg L^{-1} [29, 84-86]. Visible observations of water from each site indicated that waters at Reaches 3 and 4 were clearer and Ti concentrations at these sites trended lower than the average concentration, although they were not significantly different than Reaches 1 and 2. Increased suspension of organic materials could prevent higher concentrations of Ti being dissolved in the water column with increased aggregation, limiting the variation in concentrations. Further, sampling the four reaches during periods with adequate flow rates were critical to our study, as Reaches 1 and 4 can diminish to no flow conditions in summer months.

Sediments are known to have mg g^{-1} concentrations of Ti [28, 33, 87, 88]. The changing geology throughout the course of the North Bosque River presents challenges to the characterization of sediments in downstream reaches where sediments are consistently mixed with eroding materials from upstream. Concentration differences

were found in different sub basins of the North Bosque River watershed, but were not dependent on municipal water discharges along the North Bosque River. Variation in concentrations of Ti was higher at the two downstream locations (Reach 3 and 4, 124% and 53% respectively), and a wider array of geological formations lie directly upstream of reaches 3&4 than are found directly upstream of Reaches 1 and 2 (30% and 22% variation in Ti concentrations, respectively). Each geological formations contributes to sediment loading as the river flows downstream and may increase the heterogeneity of sediments downstream, thus increasing the observed variance. Ti concentrations in sediments are expected to be higher than in water because aggregation of Ti species from the water column will likely deposit Ti onto the riverbed, as mentioned above. Sediments are expected to contain greater Ti concentrations than fish because excretion and sequestration factors (liver, kidneys, and lysosomes) limit the accumulation of non-essential metals in fish [89]. Ti concentrations found in the North Bosque River are consistent with or lower than concentrations reported for similar sediments, reported at 4.57 mg g^{-1} in river sediments from North America, 2.74 mg g^{-1} in bay sediments from China, and 0.06 to 3.38 mg g^{-1} in Pacific Ocean sediment and from rivers around the world from 0.1 to 24.6 mg g^{-1} and those discussed earlier [29, 33, 87, 88].

Ti in periphyton was found at concentrations exceeding those for sediments (828 μg g^{-1} and 206 μg g^{-1}, respectfully) and variation (RSD) within reaches was consistently within 35% of the mean. Concentrations were consistent at all four reaches and could not be explained by anthropogenic sources. The constant flow of water through these assemblages allows fine grain sediments to become part of the matrix and contribute to higher Ti concentrations. Similar to suspended materials in the water column, algae in

periphyton provide binding sites for metals in solution as water passes across the periphyton. This increases metal accumulation, potentially resulting from eutrophication in the water body [90, 91]. Variation of Ti concentrations is possibly smaller because of the consistent concentrations found in water. Periphyton are food for fish and a home to many micro invertebrate species in freshwater habitats [73, 80]. Ti concentrations in periphyton are a likely source for Ti in fish tissues.

Consistent Ti concentrations in blacktail shiner between the four reaches indicated no greater exposure to Ti at the studied point source (Reach 2) than the other reaches. Although not statistically significant, the average Ti concentration trended higher further downstream in the river, for Reach 1 to Reach 3. (Table 2.1). Given the limited number of samples from Reach 1 for fish, it is hard to indicate if this is a consistent result. No variation can be reported for Reach 1 because of the consistently low concentrations. Variation was highest at Reach 2, as it was in periphyton. Reach 2 was the point source, and this effluent may be the source of variation in the two biotic matrices. Blacktail shiners consume algae and microorganisms and this could explain the concentrations found in the fish tissues from these reaches, given the hundreds of μg g^{-1} Ti concentrations found in periphyton. Water could be a source for Ti concentrations in fish, however this would require bioaccumulation, and not simply ingestion, given that Ti concentrations in water were 0.23 to 1.14 μg L^{-1}. Metal accumulation in aquatic environments is known to be greatest for organisms that are dependent on sediment for nutrients [92]. Periphyton accumulates metals by inclusion of particulate matter into the matrix of algae, and these suspended particulate are reported to contain 1.0 to 7.5 mg g^{-1} Ti [29, 93]. Fish obtain essential metals from food sources and possess elimination or

sequestration processes, including excretion and homeostasis involving metallothionein within cells (where it binds with metals), that limit the accumulation of excess or non-essential metals coming from ingestion and transport across gills [89, 94].

Conclusions

Ti concentrations in the North Bosque River are not altered by the effluent point discharge in Stephenville, TX. However, a wide range of Ti concentrations were observed in the samples studied, and refinement of these concentration profiles may help explain the movement of Ti within the environment. Ti concentrations in water and sediment were consistent with previous studies and can be explained by abundant organic materials suspended in the water column and geological formations in sediments. Ti concentrations were different among the different sample types, but that difference was not influenced by the wastewater discharge. Periphyton has an ability to sorb metals or incorporate them into the periphyton matrix, which could increase exposure to metals for organisms that consume periphyton. Thus, periphtyon are a possible source of Ti in fish tissues because of ingestion of algae and micro invertebrates.

CHAPTER FOUR

Conclusion

Environmental Ti concentrations can be accurately quantified by H_2SO_4 digestion of environmentally relevant matrices with ICP-MS analysis. In doing so, labs avoid hazardous chemicals (e.g. HF) and complex digestion procedures (e.g. microwave digestion), while still providing consistent and accurate data. Our analytical method provides an opportunity for labs to analyze $nTiO_2$ in a range of environmental matrices using standard ICP-MS instrumentation without expensive modifications or safety equipment. This method also provides the first validated method for quantification of $nTiO_2$ in periphyton.

Application of this method to quantify Ti concentrations in the North Bosque River provided consistent data from multiple reaches for water, sediments, periphyton, and fish. Ti concentrations found in the North Bosque River were consistent with those found in waters and sediments from other studies. The lack of significant differences within a given matrix from background and effluent impacted reaches of the river indicated no effluent dependent changes to Ti concentrations. Comparisons of all four matrices showed a higher Ti concentration in periphyton compared to sediments. These differences may help design studies to improve our understanding of Ti distribution in the environment.

The ability to routinely quantify $nTiO_2$ from different matrices in both controlled studies and in natural environments provides a more informed evaluation of factors that

affect uptake and that control ecological and toxicological risk. Quantification of Ti concentrations in these four matrices broadens our understanding of Ti within biotic and abiotic portions of the environment. Many $nTiO_2$ studies of fish toxicity do not report accumulation of Ti in organisms making comparisons with our field samples difficult. However, concentrations found in waters from this study did not contain Ti concentrations found to have toxic effects on organisms in other studies, while periphyton had higher concentrations, no sources could be found for Ti induced toxicity in periphyton.

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