Water Resources Research Institute
of The University of North Carolina

Report No. 473

DECONTAMINATION OF HEAVY METALS IN NORTH CAROLINA GROUNDWATER USING MANGANESE OXIDE NANOFIBERS

By
[1]Ericka Ford, [2]Terrence Gardner and [1]Yaewon Park

[1]Department of Textuile Engineering, Chemistry and Science
College of Textiles
[2]Department of Soil Science
College of Agricultural and Life Science
North Carolina State University
Raleigh, NC

UNC-WRRI-473

The research on which this report is based was supported by funds provided by the North Carolina General Assembly and/or the National Oceanic and Atmospheric Administration through NC Sea Grant in response to a joint call for funding issued by NC Sea Grant and the NC Water Resources Research Institute.

WRRI Project No. 17-02-SG
Sea Grant Project No. R/MG-1708
April 2018

1. Summary

We have conducted interdisciplinary research on functional nanofibers for mitigating water contamination with manganese (Mn). Our work resonates with the mission and goal of WRRI and NCSG in that we explore novel materials to provide clean water to North Carolina residents who has limited access to quality water. Our nanofibers will help provide clean water to Piedmont, NC residents relying on contaminated well water with Mn. Filamentous fungi-nanofiber hybrids and Mn oxide coated nanofibers removed more than 90% of manganese ions and 60% of copper ions from water within 24 hours. Nanofibers are deemed ideal substrates to hold fungi and particles to prevent secondary contamination. To use our developed fibers in purification of drinking water, oxidative enzymes were embedded within nanofibers to mimic Mn oxidizing fungi. Manganese peroxidase containing fibers removed manganese ions and iron ions from contaminated well water samples provided by the Wake County government. Undergraduate students, Aaron Keeler and Drake Johnson, provided data on heavy metal adsorption of the fungi-nanofiber hybrids from the well water sample. Interestingly, the fungi-nanofiber hybrids reduced the level of Fe, Pb, Zn but increased the level of Mn in the well water after 24 h of exposure. There might be an ion exchange on the manganese oxide surfaces or manganese ions leaching from the nanofibers. Submerging fungi-nanofiber hybrids in pure water for some time before use prevented Mn(II) leaching. For the cost-effectiveness of the fiber production, laccase was attempted to substitute manganese peroxidase to develop nanofiber-based water filters. We expect our fibers will further find application in coastal water clean-up and prevention. This work is a collaborative work among Textile Engineers, Soil Scientists and Chemists. Our educational partnership with Durham Technological Community College informed undergraduate students about the use of engineered fibers to mitigate contamination by waste sites. We have disseminated our results to the public through journal articles, conference presentations, press releases through University communication and social media.

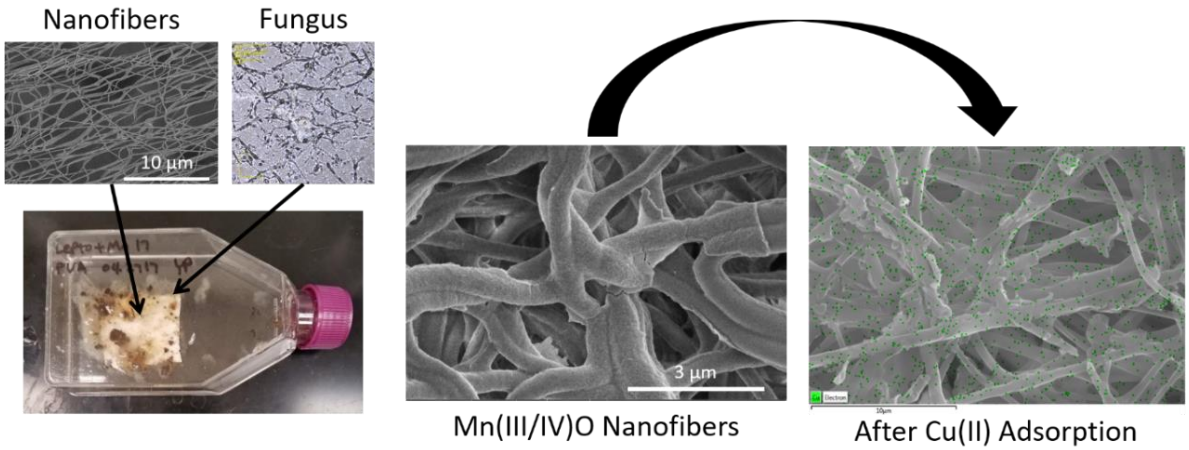
2. Fungi-Nanofiber Hybrid Filters for Heavy Metal Removal

2.1. Abstract

Heavy metals are removed from water through chemical reactions, ion exchange or adsorption media, and bioremediation. Manganese, Mn(II), oxidizing fungi support bioremediation through the conversion of Mn(II) ions into manganese oxide (Mn(III/IV)O) deposits that in turn adsorb Mn(II) and other heavy metal ions from the environment. In this study, two fungal isolates, *Coniothyrium* sp. and *Coprinellus* sp., from a Superfund site (Lot 86, Farm Unit #1) water treatment system were incubated in the presence of nanofibers. Upon fungal attachment to manganese chloride seeded nanofibers, *Coniothyrium* sp. catalyzed the conformal deposition of Mn(III/IV)O along hyphae and nanofibers, but *Coprinellus* sp. catalyzed Mn(III/IV)O only along its hyphae. Mn(III/IV)O coated nanofibers (with and without *Coniothyrium* sp.) were most effective against (Mn(II)) and copper (Cu(II)) metal ions removal than lead (Pb(II)).

Keywords: soil fungi, heavy metal ions, lead, copper, nanofibers, manganese oxide, water purification

Graphical Abstract



2.2. Introduction

The release of heavy metal contaminants into groundwater poses a serious threat to human and environmental health.[1,2] Techniques used for heavy metal removal from water include chemical precipitation reactions, ion exchange, adsorption, and bioremediation.[3,4] Chemical precipitation is

most widely used industrially, because the reactions are simple, and its cost is relatively low. Heavy metal cations react with hydroxide or sulfide anions to form water-insoluble salts. However, toxic by-products such as low-density sludge and hydrogen sulfide (H_2S) fumes can persist upon reaction.[4] Ion-exchange is a highly efficient, high capacity heavy metal removal technique. Heavy metal ions attach to polymeric beads or fibrous filter media as they replace protons belonging to surficial sulfonic acid (-SO_3H) or carboxylic acid (-COOH) groups.[5]

Metal carbonates, metal oxides, and carbon allotropes are commonly used inorganic adsorbents. Heavy metal removal is influenced by the surface area of adsorbents. Thus, nanoscale particles are expected to have greater adsorption efficiency per mass than microscale adsorbents that are 100-10,000 times their size.[2] Nanoparticle separation and contamination is a major challenge to its use in water purification.[6] Therefore, high surface area nanofibers are presumed the best platforms for particle confinement and next-generation hybrid materials.[7]

The electrospinning technique produces nanofibers from polymeric solutions that are placed under high voltage. Nanofibrous adsorbents are formed by electrospinning dopes containing nanosize particles[8] or dopes containing sol-gel precursors for their synthesis.[9,10] Also, inorganic particles are synthesized at the surface of nanofibers hydrothermally[11] or by the sol-gel approach for particle synthesis along nanofibers[12]. For example, Park et al. seeded electrospinning dopes with polymerized micelles to nucleate the hydrothermal growth of $CaCO_3$ spheres- which threaded the nanofibers.[13] The $CaCO_3$ coated nanofibers adsorbed anthraquinone, acid blue dye from water. Wu et al. fabricated composite nanofibers of SiO_2, functionalized with thiol groups, that were capable of >400 mg/g of copper ion (Cu(II)) adsorption.[10]

Bioremediation is most frequently used by the US Environmental Protection Agency (EPA) to treat groundwater *in situ*. Its popularity has grown since the 1990's because the cost-benefit ratios for heavy metal and radionuclide removal by chemical treatments are deemed unacceptable.[3] Bioremediation is a time-consuming strategy because it may take several months or years to identify the appropriate organisms and conditions for effective remediation.[14,15] Nevertheless, the

ubiquitous nature of microorganisms that can autonomously attack pollutants through oxidation and reduction makes bioremediation an attractive strategy for remediation long term.[16]

Manganese, Mn(II), oxidizing bacteria and fungi are used to decontaminate polluted coal mine drainage sites.[17,18] Limestone beds inoculated with Mn(II) oxidizing bacteria precipitate dark brown deposits of Mn(III/IV) oxide (i.e. Mn(III/IV)O), which adsorb other heavy metal ions from groundwater.[19] Since Mn(II) oxidizing bacteria and fungi are found throughout aquatic and terrestrial environments, biogenic Mn(III/IV)O naturally participate in environmental remediation.[20,21,22,23]

Bacillus sp. SG-1, *Leptothrix discophora* SS-1, *Pseudomonas putida* strains MnB1, and GB-1 are oxidizers of bacteriogenic Mn(III/IV)O.[24] [25] These bacteria secrete Mn(II) oxidative enzymes that catalyze the Mn(II) conversion to Mn(III/IV)O.[24] Often times, the bacteria are found encrusted in Mn(III/IV)O deposits.[24] Multicopper oxidase enzymes (like laccase and manganese peroxidase) have a similar effect, in that copper ions tend to contribute to the oxidization of various organic and inorganic substrates.[24]

The formative and adsorptive behaviors of mycogenic Mn(III/IV)O are less understood than of bacteriogenic Mn(III/IV)O. Fungal activity is less susceptible to changes in pH, heavy metal ion concentrations, and temperature than bacteria.[14] In practice, fungal Mn(II) oxidizers were more effective than bacterial Mn(II) oxidizers during the remediation of coal mine drainage.[18] These ascomycetes were found to oxidize Mn(II)[25] -*Acremonium*, *Alternaria*, *Cladosporium*, *Coniothyrium*, *Curvularia*, *Penicillium*, *Phoma*, *Verticillium*- along with wood-rotting basidiomycetes (e.g. *Coprinellus*).[26] Fungi may also produce multicopper oxidases to aid Mn(II) oxidation,[25] but the genetic sequence of multicopper oxidase from bacteria and fungi have little resemblance.[24]

In general, biogenic Mn(III/IV)O have superior adsorption properties compared to synthetically produced Mn oxides. Unit cell forms of MnO_6 octahedra reassemble layered (e.g. birnessite) or tunneled structures (e.g. todorokite).[21] Most biogenic birnessite contains a significant

amount of Mn(III) in the octahedral layers.[21] Mn(III/IV)O has an abundance of vacancies, which lends to its highly adsorptive nature.[20,24] Dissolved salts are often found throughout the interstices of layered Mn(III/IV)O.[21]

Biogenic Mn(III/IV)O[27] and fungal cells[28] are well-known bioadsorbents for heavy metals. Thus, hybrids of Mn(III/IV)O yielding fungi and nanofibers are posed as environmentally sound approaches for passive heavy metal removal from water. This novel approach immobilizes mycogenic adsorbents along the surface of nanofibers. The implications of this innovative approach are to support the scalable use of nanotechnologies towards groundwater treatment. Mn(III/IV)O morphologies, as catalyzed by *Coprinellus* species (sp.) and *Coniothyrium* sp., were investigated. *Coprinellus* sp. and *Coniothyrium* sp. are plant pathogens, but neither of them poses known threats to human health. The adsorptive capacity of *Coniothyrium* sp. hybrids was tested against Mn(II), Cu(II), and lead (Pb(II)). The EPA secondary maximum contaminant levels for Mn(II) is 0.05 mg/L.[29] EPA action levels are 1.3 mg of Cu(II)/L and 0.015 mg of Pb(II)/L in drinking water. If a tenth of tap water exceeds either action level, then additional treatments must be used to amend water systems.[30]

2.3. Materials and Methods

2.3.1. Materials

*S*trains designated C1CAP-d1AYA and ASB2-d1KA, classified as *Coprinellus* sp. and *Coniothyrium* sp., respectively, were isolated from the activated carbon column (CC) and air-stripper (AS) of Superfund site, Lot 86, Farm Unit #1 remediation system in Raleigh, NC.[31,32] Dextrose, yeast extract, casamino acids, HEPES acid, agar, $CaCl_2$, $MgSO_4$, $MnCl_2$, $FeCl_3$, $CuSO_4{\cdot}5H_2O$, $ZnSO_4{\cdot}7\ H_2O$, $CoCl_2{\cdot}6H_2O$, $Na_2MoO_4{\cdot}2H_2O$ and 88% hydrolyzed poly(vinyl alcohol) (PVA, having a molecular weight of ~130 kDa), 70% glutaraldehyde (GA) in water, and 1 N sulfuric acid (H_2SO_4) were purchased from Sigma Aldrich.

2.3.2. Methods

Nanofiber Preparation

Neat PVA and PVA/$MnCl_2$ nanofibers were electrospun from aqueous dopes. 11 wt% of PVA was dissolved in 100 mL of water under constant stirring for 1 h at 60 °C. PVA/$MnCl_2$ was prepared from a 100 mL solution of 11 wt% PVA and 0.1 g of $MnCl_2$.

The laboratory scale electrospinning set-up is shown in Figure 1. PVA and PVA/$MnCl_2$ nanofibers were electrospun at 15 kV, horizontal tip to collector distance of 8 cm, and feed rate of 0.8 mL/h using an 18-gauge needle. Nanofibers were collected onto aluminum mesh at room temperature and 40-50% relative humidity.

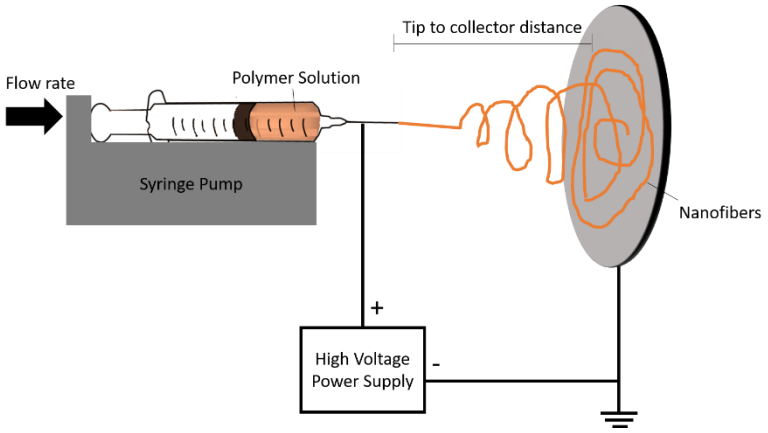


Figure 1. The schematic shows the laboratory-scale electrospinning set-up.

Crosslinking Nanofibers

PVA and PVA/$MnCl_2$ nanofibers were crosslinked in GA solution. 300 mM GA in water/acetone was prepared from 70 % aqueous GA stock and 50 mL of acetone. One drop (~50 µL) of sulfuric acid catalyst was added. Nanofibers of 4 x 5 cm^2 were immersed into GA solution for 24 h at room temperature. Afterwards, nanofibers were air dried at room temperature for at least 24 h before use.

Media Preparation

Liquid Leptothrix media, with and without Mn(II) ions, were prepared in 3 steps. In Step (1), 1 g of dextrose, 0.5 g of yeast extract, 0.5 g of casamino acids and 2.38 g of HEPES acid were

added to 1 L of distilled water. Afterwards in Step (2), this solution was autoclaved for 30 minutes and cooled to 60 °C using a room temperature water bath. Finally in Step (3), 0.5 mM of $CaCl_2$, 0.83 mM of $MgSO_4$, 1 mM of $MnCl_2$, 3.7 mM of $FeCl_3$, 1 mL of trace metal mix were added to prepare Leptothrix media having Mn(II) ions. Leptothrix media without Mn(II) did not have 1 mM of $MnCl_2$ added in Step 3.

For solid leptothrix media preparation, 15 g of agar was added in Step 1 with the same steps. Trace metal mix was prepared by adding 10 mg of $CuSO_4 \cdot 5H_2O$, 44 mg of $ZnSO_4 \cdot 7\ H_2O$, 20 mg of $CoCl_2 \cdot 6H_2O$, and 13 mg of $Na_2MoO_4 \cdot 2H_2O$ in solution. Afterwards, the trace metal mix was filtered through sterile 0.2 μm porous filters.

Fungal Treatment of Nanofibers

Leptothrix culture media was inoculated with *Coprinellus* sp. and *Coniothyrium* sp. in the presence of nanofibrous sheets (of 1x1 cm^2 or 4x5 cm^2). Nanofibers and fungal plugs were simultaneously added to liquid media. On solid plates, fungal plugs were placed on top of nanofibers, under sterile conditions. PVA and PVA/$MnCl_2$ nanofibers were immersed in 20-30 mL of Leptothrix culture media in 75 mL Erlenmeyer flasks. Fungal growth over 4 weeks occurred at room temperature in the dark.

The fungi were removed from the fungi-nanofiber hybrids by immersion in 20 mL of deionized water, stirring at 500 rpm for 30 min, or sonication in 20 mL of water or ethanol for 2 h. Table 1 summarizes the samples used throughout this study and their descriptions.

Table 1. Summary of Nanofibers with and without Fungal Treatment

Sample Name (Optional)	Description
PVA	Neat electrospun PVA nanofibers
PVA/$MnCl_2$	PVA nanofibers having 0.1 wt% $MnCl_2$
Mn(III/IV)O-#17	*Coniothyrium* sp. incubated in Mn(II)-containing media and have Mn(III/IV)O
-	*Coniothyrium* sp. incubated in Mn(II)-containing media with PVA nanofibers
Mn(III/IV)O-#17-PVA/$MnCl_2$	*Coniothyrium* sp. incubated in Mn(II)-containing media with PVA/$MnCl_2$ nanofibers
Mn(III/IV)O-PVA/$MnCl_2$ (-#17)	Mn(III/IV)O-PVA/$MnCl_2$ after removing *Coniothyrium* sp. hyphae with 2h of ethanol sonication
-	Mn(III/IV)O-PVA after removing *Coprinellus* sp. hyphae with 2h of ethanol sonication
-	Mn(III/IV)O-PVA/$MnCl_2$ after removing *Coprinellus* sp. hyphae with 2h of ethanol sonication

Microscopic Analysis

Confocal micrographs of fungi incubated on Leptothrix solid plates and in the presence of nanofibers were imaged on the LEXT OSL4000 3D. The morphology of fungi-nanofibers and nanofibers were observed using field emission scanning electron microscopy (FE-SEM, FEI, Verios 460L) at an operating voltage of 2 kV. Specimens were sputter coated with gold and

palladium before SEM analysis. Image analysis of SEM micrographs was used to calculate the average fiber diameter from at least 50 nanofibers.

Spectroscopic Analysis of Mn(III/IV)O

The surface chemistry of nanofibers and their mycogenic hybrids were characterized by energy dispersive spectroscopy (EDS, attached to the Verios 460L). The operating voltage was 20 kV. EDS spectra were used to confirm the locale of metal oxides and absorbed heavy metals.

The Nomadic^{TM} Raman Microscope by BaySpec was used to analyze Mn(III/IV)O nanofibers. Raman spectra were collected using the 532 nm laser at 150 mW power, 10X objective lens, 20 scans, and 1 sec exposure time.

Heavy Metal Adsorption

Heavy metal sorption by PVA/$MnCl_2$, Mn(III/IV)O-#17, Mn(III/IV)O-#17-PVA/$MnCl_2$, and Mn(III/IV)O-PVA/$MnCl_2$(-#17) were measured. 3 mg of each sample was added to 20 mL of the metal ion solution.

Specimen were immersed in heavy metal solutions (of 0.55 mg Mn(II)/L, 2.63 mg Cu(II)/L, and 0.029 mg of Pb(II)/L) at pH 7 for 24 h at room temperature. Inductively-Coupled Plasma-Optical Emission Spectrometer (ICP-OES, Perkin Elmer 8000) was used to measure unabsorbed concentrations of heavy metal ions. Adsorption q (mg/L) was determined by Equation 1:

$$q = V\frac{(C_i - C)}{S} \quad (1)$$

V is the volume (L) of solution in contact with sorbent; C_i and C are initial and final concentrations of the sorbate (mg/L), respectively; S is the dry weight of sorbent (g). Heavy metal removal (%) was determined by Equation 2:

$$Removal\ (\%) = \frac{(C_i - C)}{C_i} \times 100 \quad (2)$$

2.4. Results and Discussion

Fungal Growth and Mn(III/IV)O Deposition

Photographs of fungi grown in Leptothrix media revealed white, cotton ball-like mycelia (Figure 2a,b). Dark brown deposits, indicative of Mn(III/IV)O,[22,33] formed after 1 week of *Coprinellus* sp. and *Coniothyrium* sp. incubation in Mn(II)-containing media (Figure 2a,b). Adding PVA/$MnCl_2$ nanofibers into media without Mn(II) did not yield the Mn(III/IV)O deposits (Figure 2a,b). The average diameter of PVA nanofibers without $MnCl_2$ was 186 ± 116 nm, and PVA/$MnCl_2$ nanofibers was 126 ± 46 nm (Figure 2c,d respectively).

Interestingly, PVA/$MnCl_2$ nanofibers yielded more mycelia growth, and hyphal structures tended to adhere more strongly to nanofibers in comparison to neat PVA nanofibers that were immersed in Mn(II)-containing media (Figure 2a,b). Likewise, more mycelia and Mn(III/IV)O grew on PVA/$MnCl_2$ nanofibers when grown on Leptothrix solid plates (Figure S1). Confocal microscopy images (Figure S2) showed Mn(III/IV)O deposits only on PVA/$MnCl_2$ fibers cultured in Mn(II)-containing media.

Fungal growth in the presence of nanofibers and Mn(II) was monitored over 4 weeks (Figure 2e). Incubation beyond 2 weeks did not enhance the spread of Mn(III/IV)O deposits along nanofibers. Nevertheless, nanofibers were maintained in culture media for more than 4 weeks to obtain fully grown fungi.[22] PVA/$MnCl_2$ nanofibers were more densely covered with mycelia. Mn(II) ions within nanofibers were assumed to promote fungal growth and Mn(III/IV)O formation. While studying the effect of manganese ions at varying concentrations on select fungi isolates, Jaworska et al. reported that the responses (e.g., influence fungi growth, conidial germination, and antagonistic activities) of *Trichoderma spp*. to manganese ions depended on both the metal concentration and the fungal isolate.[34]

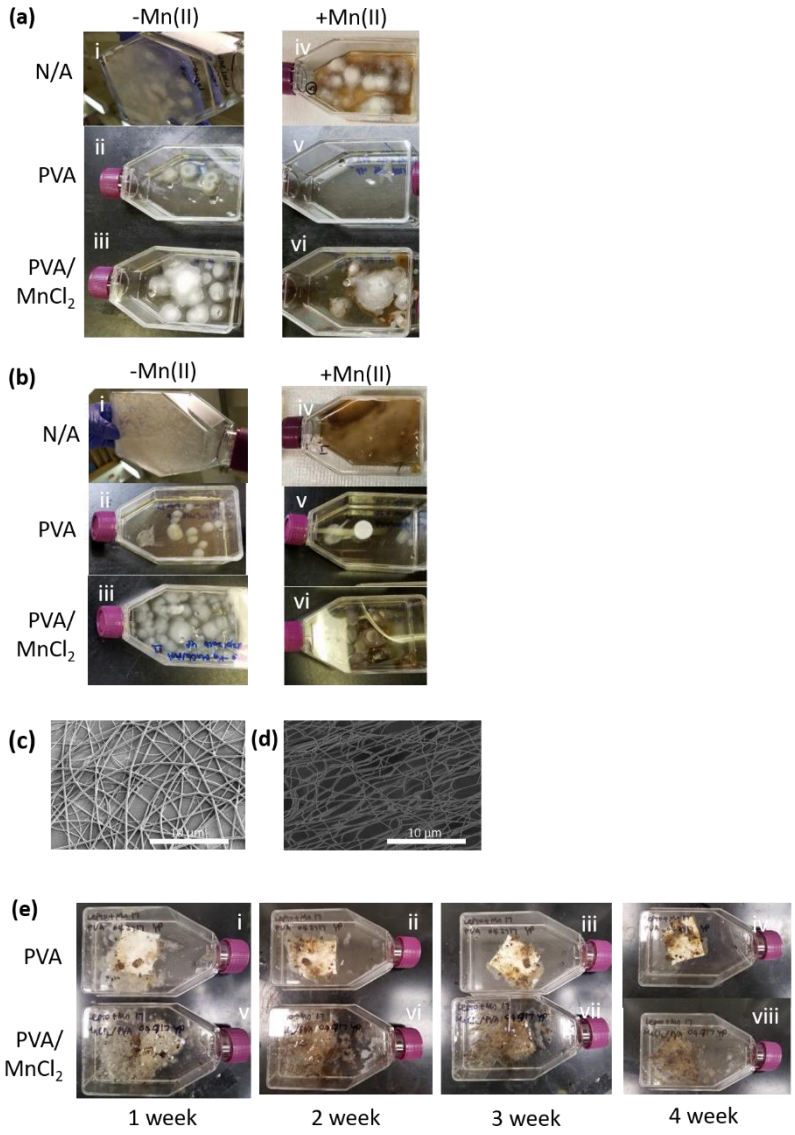


Figure 2. Fungi (a) *Coprinellus* sp. and (b) *Coniothyrium* sp. were grown for 1 week in Leptothrix media with Mn(II) or without Mn(II) (i.e. -Mn(II)). Fungi (a and b) were incubated without nanofibers (of 1x1 cm^2) or with nanofibers. Nanofibers were either of neat PVA or PVA/$MnCl_2$. SEM images of (c) PVA and (d) $MnCl_2$/PVA nanofibers are shown. Photographs show (e) *Coniothyrium* sp. grown for up to 4 weeks in Mn(II)-containing media 4x5 cm^2 sheets of nanofiber (either PVA or PVA/$MnCl_2$). This growth pattern was also observed for *Coprinellus* sp.

The attachment of *Coprinellus* sp. and *Coniothyrium* sp. mycelia to nanofibers was confirmed by inspection of SEM images (Figure 3). Mn(III/IV)O morphology can differ between each fungal species.[22] *Coprinellus* sp. catalyzed the growth of spherical Mn(III/IV)O particles when cultured in Mn(II)-containing media. Spherical Mn(III/IV)O particles were more prominent along the hyphae of PVA/$MnCl_2$ nanofibers than on neat PVA nanofibers. *Coniothyrium* sp. catalyzed the growth of sheet-like Mn(III/IV)O in Mn(II)-containing media. Extracellular matrix appeared at the interface of nanofibers and *Coniothyrium* sp. hyphae. Round spores were observed as seen in Figure 3bi. Fruiting bodies were also visible in photographs (Figure S2). Thus, nanofibers did not inhibit fungal reproduction by spores.

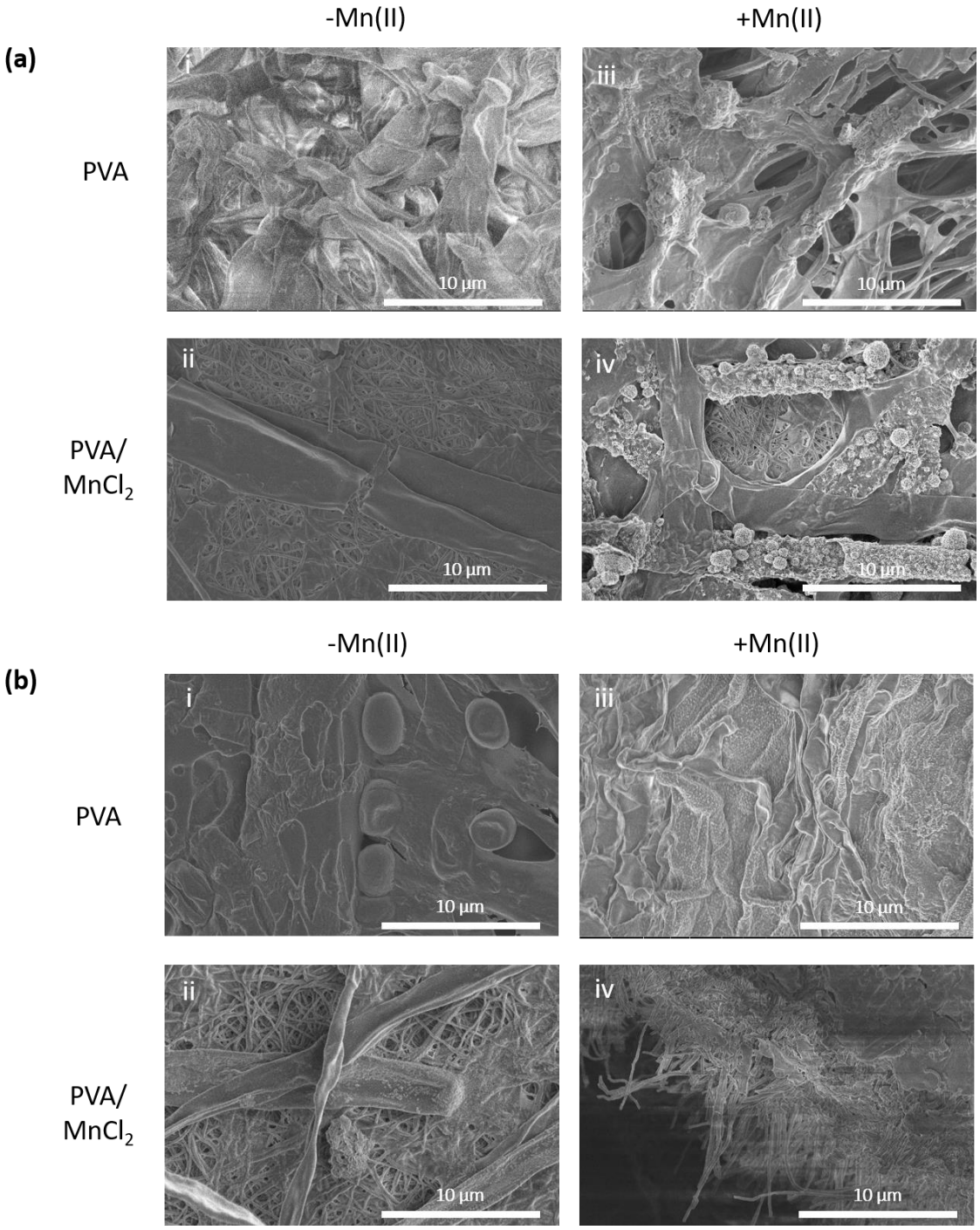


Figure 3. Micrographs show (a) *Coprinellus* sp. (b) *Coniothyrium* sp. grown on PVA and PVA/$MnCl_2$ nanofibers immersed in -Mn(II) or +Mn(II) liquid media.

Elemental Analysis of Fungal Mn(III/IV)O Hybrids

Mn(II) within PVA/$MnCl_2$ fibers were not detected by EDS, because Mn(II) at the surface of PVA/$MnCl_2$ nanofibers was below the EDS detection limit, i.e. 0.1 wt%. Therefore, any manganese detection must result from the oxidation of Mn(II) in solution. With both fungal types, manganese content as measured by EDS was qualitatively higher along PVA/$MnCl_2$ than neat PVA nanofibers (Figure 4). EDS spectra showed impurities of Fe, S, Mg, and Ca within Mn(III/IV)O. As expected, Mn(III/IV)O can adsorb other metal ions from Leptothrix media. Ivarsson et al. detected non-Mn(II), metal ions within the vacancies of biogenic Mn(III/IV)O by EDS.[33] *Coniothyrium* sp. can release enzymes coupled with P, S, K, and Ca.

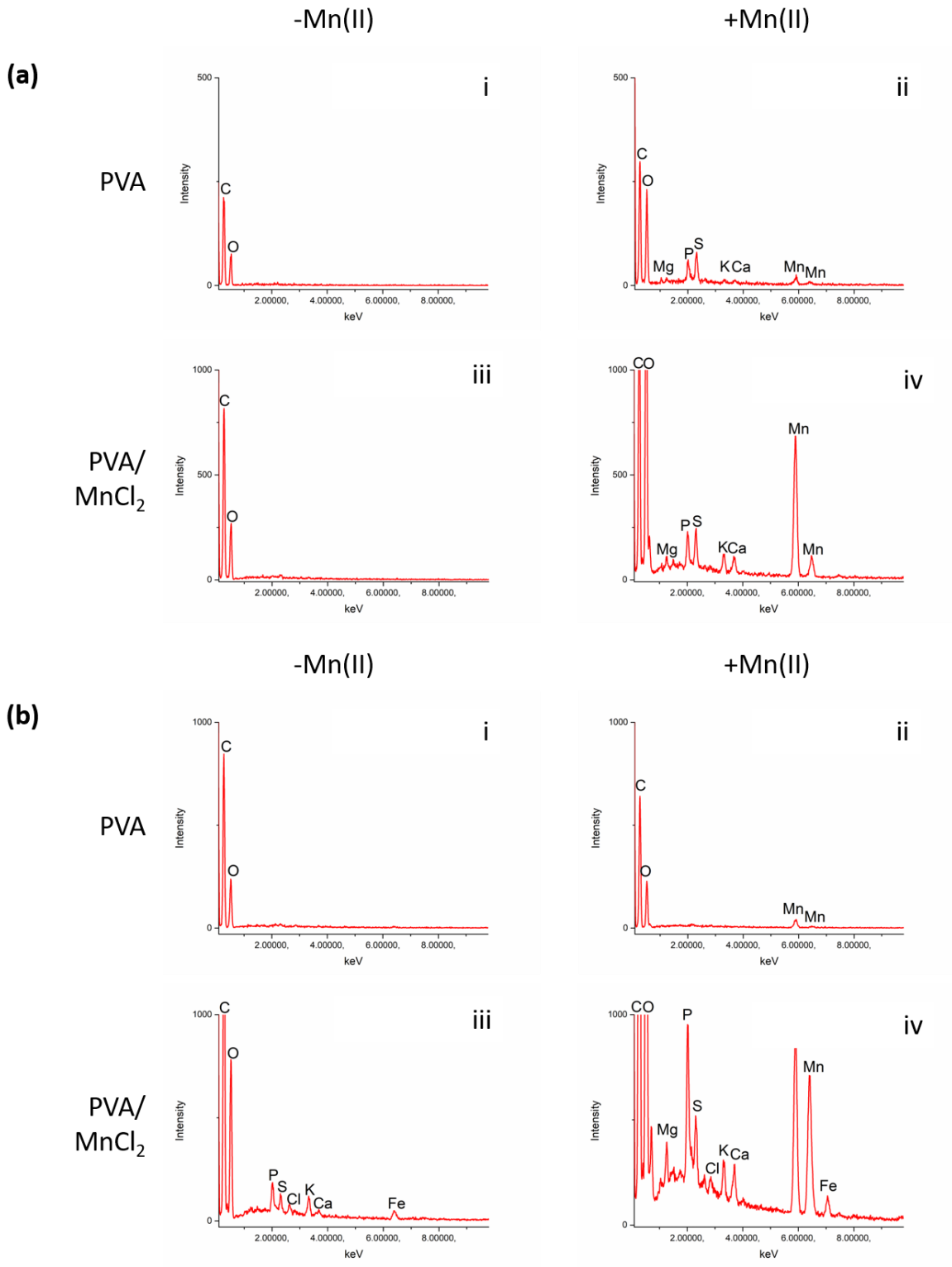


Figure 4. EDS graphs of (a) *Coprinellus* sp. and (b) *Coniothyrium* sp. on PVA (i, ii) or PVA/$MnCl_2$ nanofibers (iii, iv) in liquid media -Mn(II) or with +Mn(II).

Mn(III/IV)O Deposition on Nanofibers

A layer of fungal hyphae intermeshed with nanofibers is illustrated in Figure 5: Mn(III/IV)O-#17-PVA/$MnCl_2$. Cracks formed in the topical layer of *Coniothyrium* sp. after stirring hybrids immersed in water (Figure 5a). The cross-sectional view shows hyphae attached to both sides of nanofibers in a sandwich structure (Figure 5b). After sonicating the sample for 1 h in water, some fungal hyphae remained on the nanofibers (Figure 5c). But sonicating the fungi-nanofiber hybrids for 2 h in ethanol removed most of the fungal hyphae (Figure 5d). This latter technique was used to remove fungus from hybrids that were incubated in Mn(II)-containing media.

EDS was used to investigate the deposition of Mn(III/IV)O onto nanofibers as a result of their adherence to Mn(II) oxidizing fungus. Mn was not detected on the surface of PVA and PVA/$MnCl_2$ nanofibers that were incubated with *Coprinellus* sp. (Figure 6a,b). Also, Mn was not identified on the surface of neat PVA nanofibers that were incubated with *Coniothyrium* sp. (Figure 6c). Mn(III/IV)O coating on PVA/$MnCl_2$ nanofibers, as catalyzed by *Coniothyrium* sp., was durable to withstand 2 h of sonication in water. Mn was detected on the surface of Mn(III/IV)O-PVA/$MnCl_2$(-#17) nanofibers. A conformal Mn(III/IV)O coating was found on the fiber surface after 2 h of ethanol sonication (Figure 6d). Figure 7 is an EDS chemical map of manganese, oxygen, and carbon distributions along mineralized nanofibers.

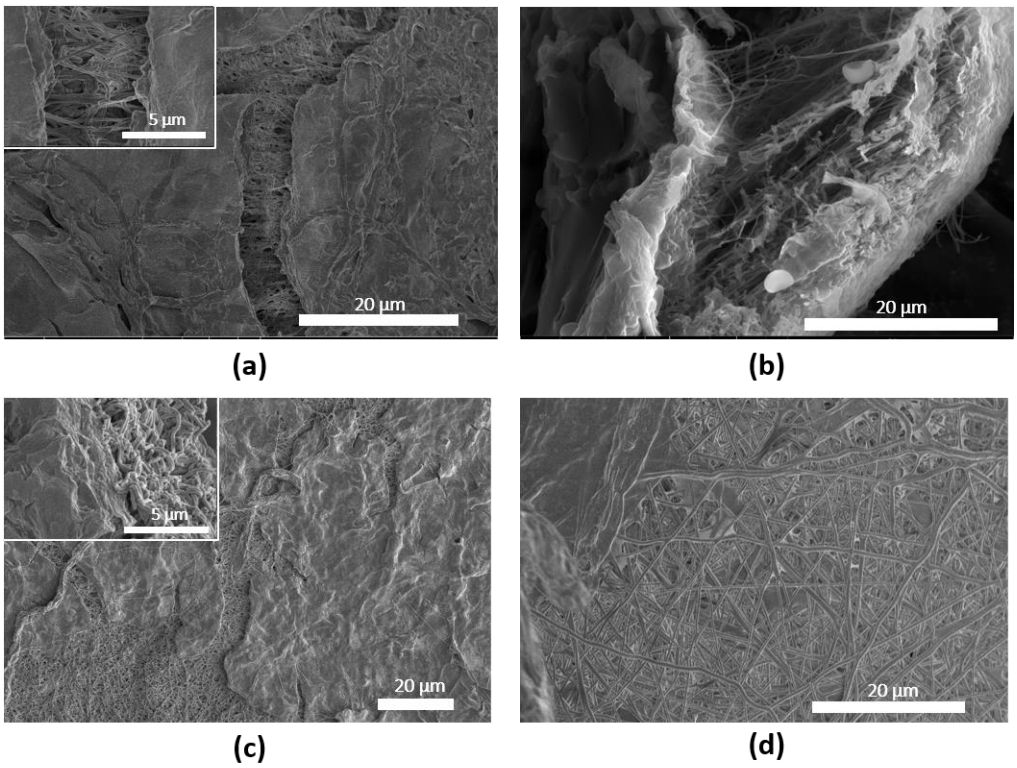


Figure 5. SEM images of Mn(III/IV)O-#17-PVA/$MnCl_2$ after water immersion and magnetic stirring for 30 min at 500 rpm; both (a) longitudinal and (b) cross-sectional views are shown. Hybrids were further treated with (c) 1 h of sonication in water or (d) 2 h of sonication in ethanol.

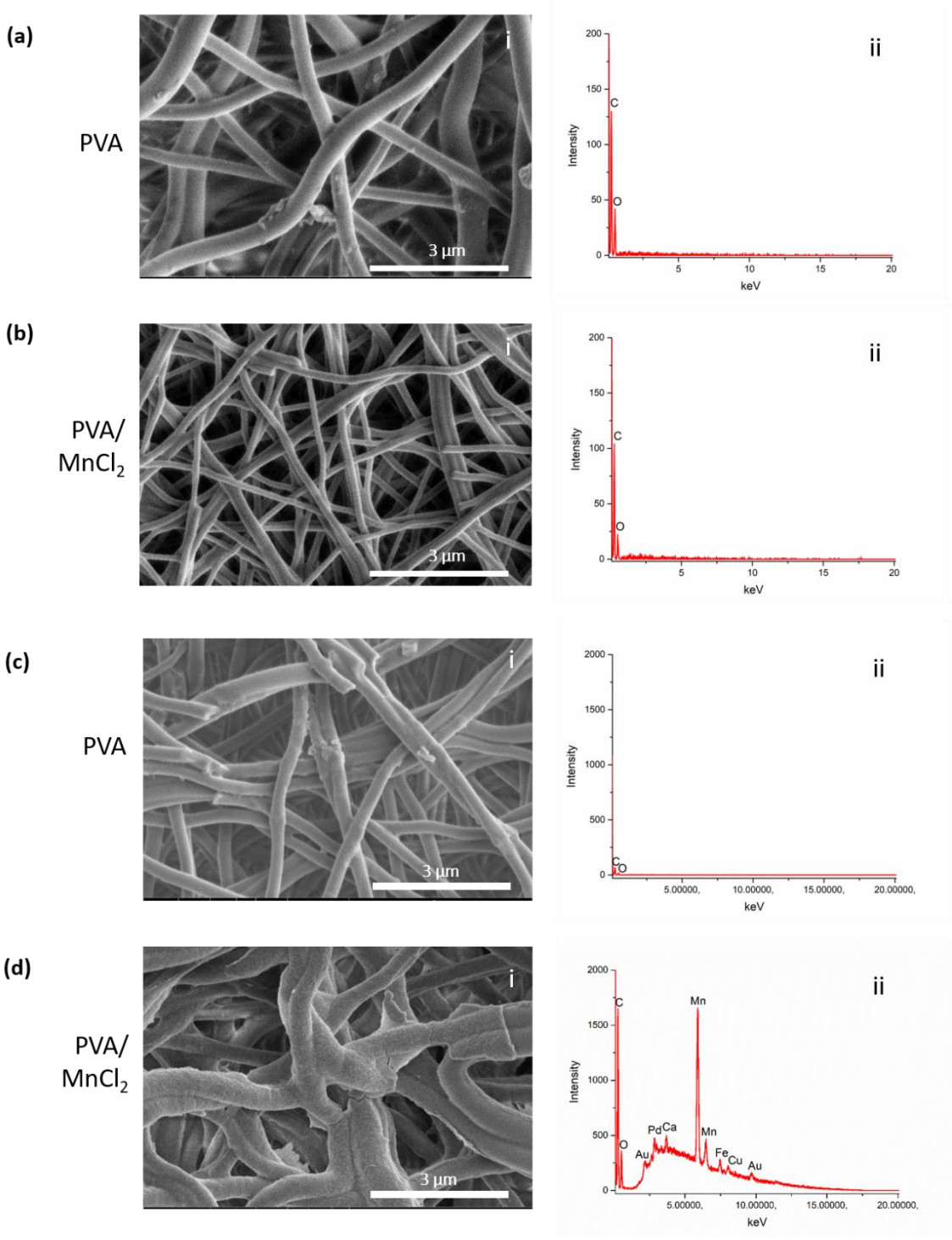


Figure 6. (i) SEM images and (ii) EDS spectra from *Coprinellus* sp. incubated with (a) PVA and (b) PVA/$MnCl_2$ nanofibers are shown. (i) SEM images and (ii) EDS spectra of *Coniothyrium* sp. incubated with (c) PVA and (d) PVA/$MnCl_2$ nanofibers are shown.

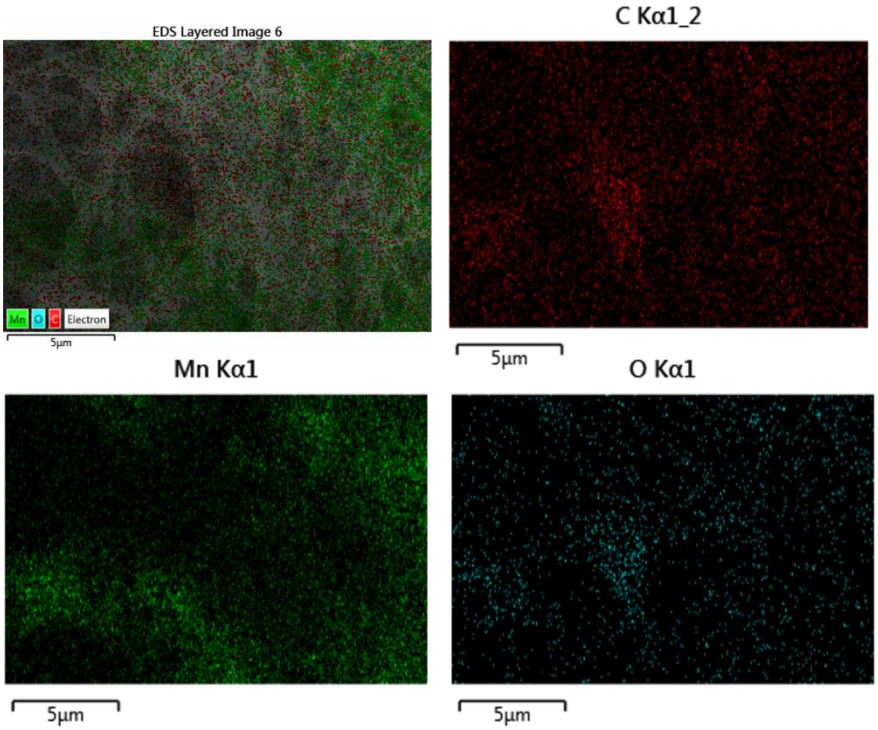


Figure 7. EDS map of Mn(III/IV)O-PVA/$MnCl_2$ (-#17) shows C in red, Mn in green, and O in blue.

Fungi can secrete various oxidative enzymes as their hyphae continue to lengthen. This results in the cycling of nutrients throughout the environment.[14] Manganese oxidizing fungi express extracellular oxidative enzymes such as manganese peroxidase. This group of proteins are capable of oxidizing Mn(II) to Mn(IV) at accelerated rates of Mn biomineralization: Several orders of magnitude faster than either abiotic catalysis on mineral surfaces or homogeneous oxygenation in aqueous solution.[26,35] *Coprinellus* sp. and *Coniothyrium* sp. apparently have different modes of Mn(II) oxidation. Manganese-oxidizing enzymes associated with the hyphae cells of *Coprinellus* sp. In contrast, enzymes were secreted from *Coniothyrium* sp. hyphae. Oxidizing enzymes were captured by the extracellular matrix which exuded from *Coniothyrium* sp. and onto the surrounding nanofibers (Figure 3b). Santelli et al. reported that locale of Mn(III/IV)O deposition (i.e. on hyphal surfaces or extracellular polymer adjacent to cells) varies among different Ascomycete fungi probably due to primary placement of enzymes.[22]

Oxidative enzymes released from *Coniothyrium* sp. migrated to PVA/$MnCl_2$ nanofibers, as Mn(II) diffused to the surface of water-swellable nanofibers. In turn, Mn(III/IV)O formed a conformal coating along nanofibers. Herein, a novel approach to mineralization was observed- where oxidative enzymes external to the nanofibers catalyzed the nucleation and growth of Mn(III/IV)O along the nanofibers when Mn(II) was in the media. For synthetic mineralization, nanofibers are typically seeded with polyelectrolytes to nucleate the growth of inorganic minerals hydrothermally, as observed in Park et al[13] and Yang et al[36] work on $CaCO_3$ nanofibers.

Raman spectra in Figure 8 show characteristic bands of Mn(III/IV)O[33] in the range of 480-700 cm^{-1} for Mn(III/IV)O-PVA/$MnCl_2$(-#17). Low Raman intensities were observed for Mn(III/IV)O, which resembled birnessite, as reported by Julien et al.[37] Yu et al. reported Mn(III/IV)O catalyzed by *Paraconiothyrium* sp. (a *Coniothyrium*-like ascomycete) is birnessite.[38] The layered birnessite structure can adsorb metal ions between atomic layers and vacancies found throughout its imperfect crystal structure.[24] In the next section, heavy metal adsorption by *Coniothyrium* sp. derived Mn(III/IV)O hybrids were tested.

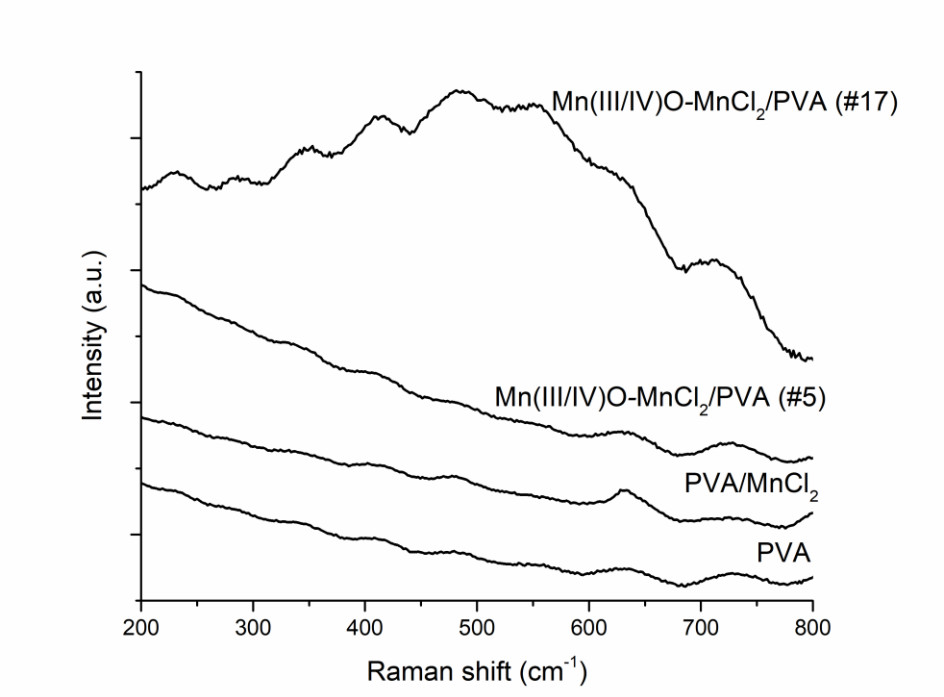


Figure 8. Raman spectra of nanofibers treated with *Coprinellus* sp. and *Coniothyrium* sp.

Heavy Metal Adsorption by Coniothyrium* sp. *Hybrids

It is noteworthy that hyphae-nanofiber derivatives removed heavy metals as well if not better than Mn(III/IV)O coated hyphae alone. (Table 2). Hybrid nanofibers removed heavy metals from solution in order from Mn(II) > Cu(II) >> Pb(II). The molecular size of heavy metal ions may have been size excluded from vacancies within the Mn(III/IV)O coating. Laus and Favere also associated smaller radius of Cu(II) than that of Cd(II) to better adsorption by chitosan.[39] After adsorption experiments, the surface chemistry of PVA/$MnCl_2$ and Mn(III/IV)O-PVA/$MnCl_2$(-#17) nanofibers were mapped by EDS. Figure 9 shows the distribution of Cu(II) and Mn(II) by both types of nanofibers. As expected from Table 2 results, Cu(II) detected along Mn(III/IV)O coated nanofibers was more intense than along PVA/$MnCl_2$.

Both Mn(III/IV)O-#17-PVA/$MnCl_2$ and Mn(III/IV)O-PVA/$MnCl_2$(-#17) removed more than 90 % of the Mn(II) from the water which then became well below the EPA limit- in 24 hours. The nanoscale dimensions of Mn(III/IV)O coated nanofibers appear to have enhanced Mn(II) sorption capacities. Taffarel and Rubio[40] reported 4 g/L of manganese oxide coated zeolite achieved 90 % removal of Mn(II) from the water after 2 h, whereas 150 mg/L of Mn(II) was more efficiently removed from water by *Coniothyrium* sp.-nanofiber derivatives Mn(II). EXAFS spectra examinations reported by Duckworth et al., also indicate that mycogenic manganese oxides from Lot 86 biofilm containing these particular isolates are similar in both the structure of Mn(III/IV)O and the binding of Ba, Co, and Zn to those observed with laboratory-grown bacteriogenic Mn(III/IV)O.[32]

Pb(II) has an atomic radius that is significantly larger than Mn(II) and Cu(II). Thus, its size can hinder Pb(II) adsorption into the vacancies of Mn(III/IV)O and its coordination with surficial atoms through nonspecific bonding. Nelson et al. reported Pb(II) adsorption by bacteriogenic Mn(III/IV)O and synthetic $\boldsymbol{\beta}$-MnO_2 were 1344.67 mg/g and 0.078 mg/g, respectively.[41] Droz et al. noted that bacteriogenic Mn(III/IV)O (from *Pseudoonas putida* GB-1) had more structural defects than mycogenic Mn(III/IV)O (from *Coprinellus* sp.).[26]

Table 2. Comparing Aqueous Heavy Metal Adsorption by *Coniothyrium* sp. Derived Mn(III/IV)O Coatings and Nanofibers After 24 h

	Mn(III/IV)O-#17	PVA/$MnCl_2$	Mn(III/IV)O-#17-PVA/$MnCl_2$	Mn(III/IV)O-PVA/$MnCl_2$ (-#17)
Mn(II) *Removal* (%)	67 ± 0	18 ± 0	93 ± 0	99 ± 0*
Mn(II) *q* (mg/g)	2.69 ± 0.01	0.66 ± 0.01	3.41 ± 0.00	3.65 ± 0.00*
Cu(II) *Removal* (%)	58 ± 0	38 ± 0	66 ± 0	57 ± 0
Cu(II) *q* (mg/g)	10.22 ± 0.08	6.62 ± 0.04	11.64 ± 0.03	10.04 ± 0.03
Pb(II) *Removal* (%)	52 ± 8	43 ± 4	30 ± 5	18 ± 5
Pb(II) q (mg/g)	0.10 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.04 ± 0.01

* Mn(II) concentration after Mn(III/IV)O-PVA/$MnCl_2$ immersion was less than the detection limit of 0.005 mg/L.

** Original concentrations of each heavy metal solutions were: 0.55 mg/L Mn(II), 2.63 mg/L Cu(II), and 0.029 mg/L Pb(II) at pH 7 as measured by ICP-OES.

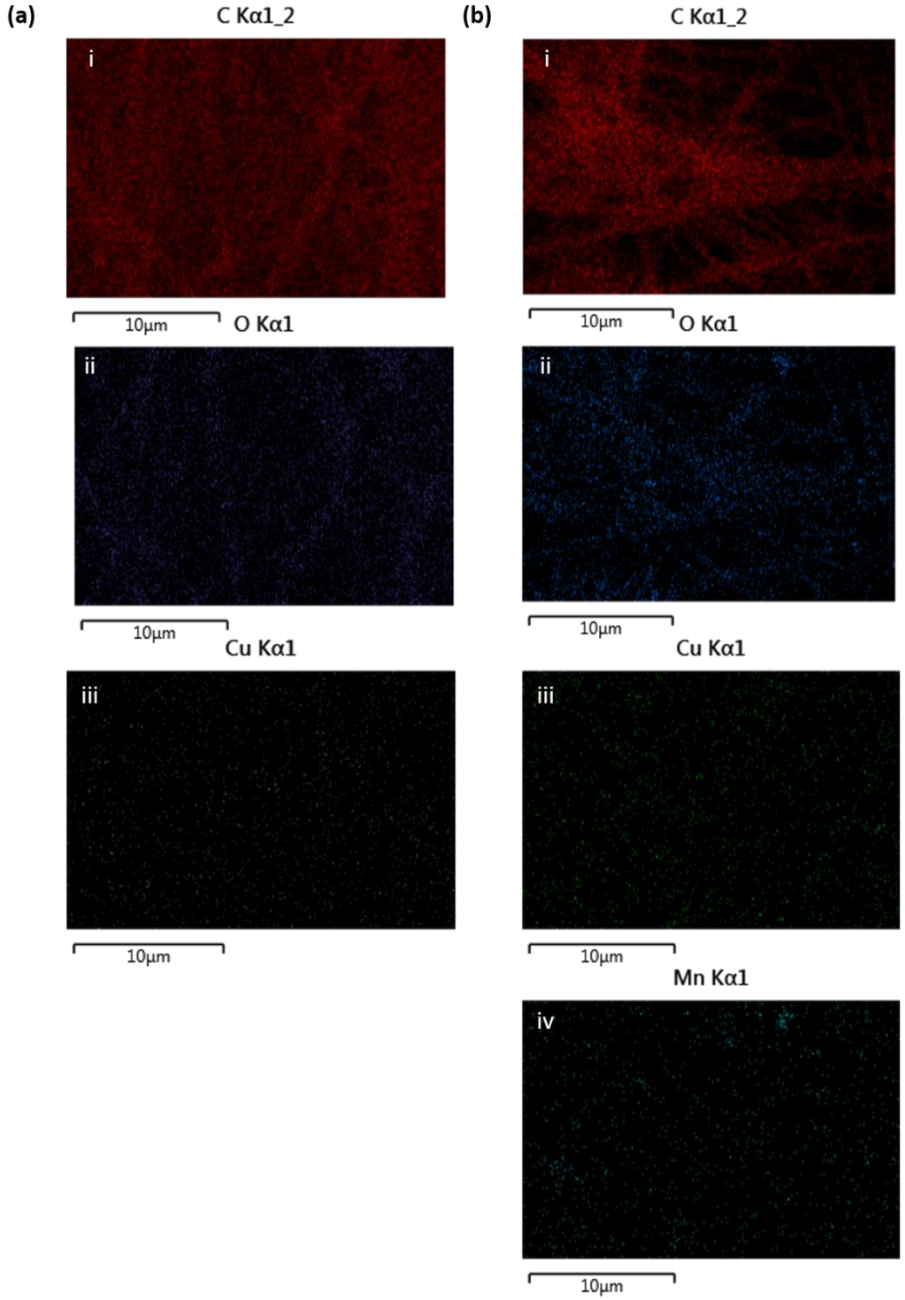


Figure 9. EDS chemical mapping of (a) PVA/$MnCl_2$ and (b) Mn(III/IV)O-PVA/$MnCl_2$(-#17) nanofibers after Cu(II) adsorption: (i) C in red, (ii) O in blue, (iii) Cu in green, and (iv) Mn(II) in cyan.

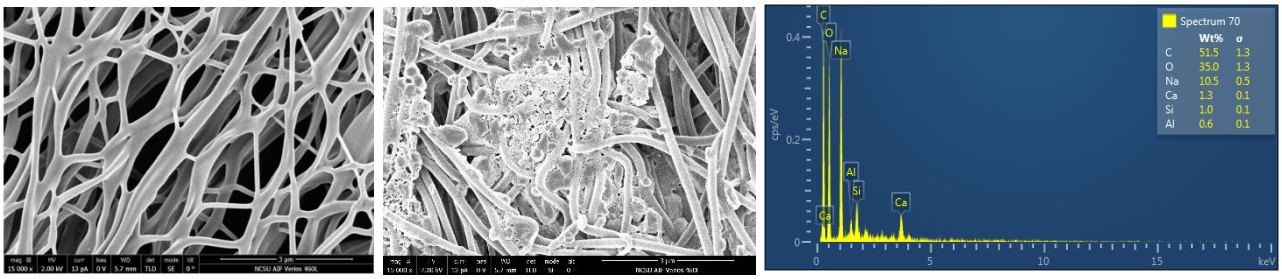
3. Oxidative Enzyme Containing Fibers

To mimic the manganese oxidizing fungi, we aimed to embed manganese peroxidase (MnP) into nanofibers. MnPs oxidized Mn(II) in acetate buffer (pH 5.5) at 30 °C after 24 h of incubation as indicated by dark brown deposits and decrease in Mn(II) concentration. Even with very small

amount (0.1 wt % of MnP relative to PVA mass) of MnPs within the PVA fibers oxidized Mn(II) (Table 3). Interestingly, coating was formed and blocked pores of MnP/PVA fibers after 24 h of incubation (Figure 10). The coating mostly comprises C, O, Na, and Ca. Na might come from acetate buffer and Ca is one of the elements within MnP enzymes. Mn(II) ions were diffused within the fibers, but MnPs seem to oxidize other elements to form membrane like coating along the fiber surfaces.

Table 3. Mn(II) Concentration After 24 h Incubation

Sample	Mn(II) concentration before incubation (mg/L)	Mn(II) concentration after incubation (mg/L)
MnP	286	191
PVA + MnP		220
MnP + H_2O_2		177
MnP + H_2O_2 + PVA		153
MnP/PVA fiber		150



MnP/PVA
183 ± 93 nm

(a) **(b)** **(c)**

Figure 10. Microscopic image of MnP/PVA fibers (a) before and (b) after incubation in Mn(II) added acetate buffer for 24 h. (c) EDS elemental analysis of coating on MnP/PVA fibers after the incubation.

The well water collected from a farm in Raleigh was provided by the Wake County government. The composition of well water is shown in Table 4. Fe and Mn within the well water exceeds the EPA limit which is 0.3 mg/L and 0.05 mg/L for Fe and Mn respectively. The fibers we prepared were tested against Fe and Mn within the well water for 24 h to see if they can remove any of heavy metal contaminants (Table 5). Mn(II) within the PVA/$MnCl_2$ fibers were leached out to the well water. This phenomenon was not observed with Mn(II) charged water that was prepared in laboratory. Within 3 mg of PVA/$MnCl_2$ fiber samples, 0.002 mg of Mn(II) was contained. The Mn(II) concentration of well water was 0.13 mg/L and that of laboratory charged water was 0.55 mg/L. Mn(II) leaching from the PVA/$MnCl_2$ fibers were pronounced within well water samples. After 24 h of immersion of PVA fibers did not change Mn(II) and Fe(II) concentration of well water significantly. MnP/PVA fibers slightly reduced both Mn(II) and Fe(II) concentrations. Higher amount of MnP within the fibers would promote heavy metal removal from well water. Foreign materials were coated on MnP/PVA fiber surfaces and the composition was mostly C, O and Ca. Ca of well water or MnP could have been detected. Mn(II) and Fe(II) from well water seem to be diffused into the MnP/PVA fibers. Manganese oxidizing fungi-nanofiber hybrids more effectively removed Fe(II) compared to nanofibers or MnP/PVA fibers. However, Mn(II) concentration of the well water increased. It is noteworthy that such increase of Mn(II) by the manganese oxidizing fungi-nanofiber hybrids were not observed with 0.55 mg/L Mn(II) aq. solution. Ion exchange between Fe(II) and Mn(II) within the Mn(III/IV)O coating is possible and need further investigation.

Table 4. Well Water Composition (provided by Wake County)

Pb flushed (mg/L)	**NO_3 (mg/L)**	**NO_2 (mg/L)**	**Alkalinity (mg/L)**	**Ag (mg/L)**	**As (mg/L)**	**Ba (mg/L)**
<0.003	<0.5	<0.1	44	<0.01	<0.005	<0.1
Ca (mg/L)	**Cd (mg/L)**	**Cr (mg/L)**	**Cu (mg/L)**	**F (mg/L)**	**Fe (mg/L)**	**Hardness (mg equivalent $CaCO_3$/L)**
8.6	<0.001	<0.01	<0.05	0.17	1.21	33
Mg (mg/L)	**Mn (mg/L)**	**pH**	**Se (mg/L)**	**Na (mg/L)**	**Zn (mg/L)**	**Hg (mg/L)**
2.8	0.11	6.96	<0.005	7.1	<0.50	<0.0005
Total coliform (Pre/Abs)	**E. coli (Pre/Abs)**					
Absent	Absent					

Table 5. Mn And Fe Concentration after 24 h of Exposure to the Developed Fibers Measured by ICP-OES

Sample	**Mn(II) (mg/L)**	**Fe (II) (mg/L)**
Well water (control)	0.126 ± 0.001	0.905 ± 0.023
PVA fibers	0.130 ± 0.005	0.884 ± 0.017
PVA/$MnCl_2$ fibers	0.183 ± 0.001	0.696 ± 0.002
MnP/PVA fibers	0.119 ± 0.000	0.738 ± 0.003
Mn(III/IV)O- #17	0.325 ± 0.001	0.344 ± 0.003
Mn(III/IV)O- #17-PVA/$MnCl_2$	0.311 ± 0.001	0.608 ± 0.006
Mn(III/IV)O- PVA/$MnCl_2$ (-#17)	0.565 ± 0.002	0.552 ± 0.002

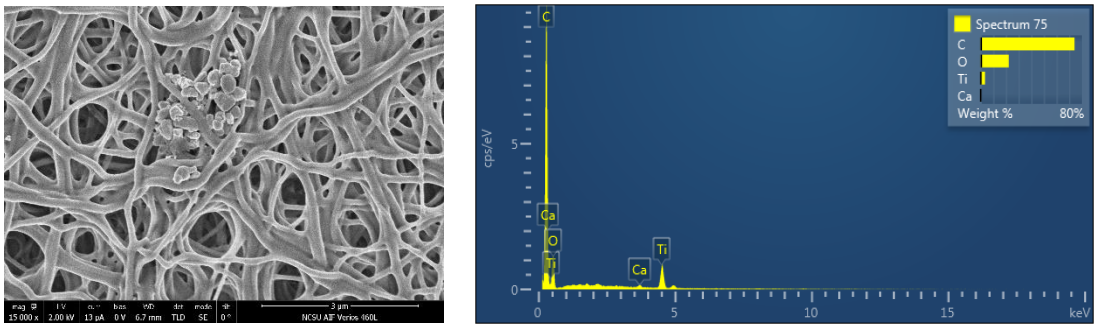


Figure 11. MnP/PVA fiber morphology and chemical analysis after 24 h immersion within well water.

4. Outreach Activities

We did outreach activities to introduce local community college students on our manganese oxide coated nanofibers and bioremediation of fungi in collaboration with Dr. Kathy Zarilla, Science Chair at Durham Technological Community College. On January 11th and 23rd, I gave a talk about my research on using fungi and nanofibers in water remediation to freshmen students. Students had a chance to do experiments on dye removal by fungi-nanofiber hybrids. Handouts and slides that were used in the outreach class can be found in Supporting Information.

Dr. Ghada Rabah, teaching assistant professor in the Department of Chemistry at NC State University and our team are preparing to make a collaborative research laboratory class in Fall 2018. From this partnership, undergraduate students at NC State have had a chance to participate in research and generate publishable data. Dr. Rabah provided us with preliminary results of Mn oxide phase developed by fungi. Especially, undergraduate students Drake Johnson and Aaron Keeler are using the ICP-OES to test the heavy metal removal kinetics of the fungi-nanofiber composites from the contaminated well water that was provided by the Wake County government. They were instrumental to identify possible Mn(II) leaching from the PVA/$MnCl_2$ fibers. Students suggested to submerge fungi-nanofiber composite in water for some time to prevent Mn(II) leaching and Mn(III/IV)O coated nanofibers had less Mn(II) leaching problem with better heavy metal removal properties. See attached slides made by the students.

5. Phosphate Removal by Nanofiber-based Filters

Phosphate removal was not observed with the manganese oxidizing fungi-nanofiber hybrids. Therefore, we sought to develop new nanofiber-based filters for phosphate. Lanthanum carbonate ($La_2(CO_3)_3$) is widely used in medicine to reduce P level in patients with kidney disease.[42] Lanthanum (La) is one of the rare earth metals, but unlike its name the element is moderately abundant in earth's crust.[43] Surprisingly, only Ji et al. attempted to apply $La_2(CO_3)_3$ in water treatment contaminated with phosphate. They incorporated $La_2(CO_3)_3$ in sodium alginate beads.

As the beads were swollen in water, phosphate was captured onto $La_2(CO_3)_3$ inside of the bead.[44] However, phosphate removal when $La_2(CO_3)_3$ is on the surface of soft materials is unknown. Mechanisms for phosphate removal by mineral is still unclear, but many researchers consider surface complexation as the primary mechanism.[45] Therefore, high surface area nanofibers are presumed to be the best platforms for $La_2(CO_3)_3$ confinement and subsequent phosphate removal.[7,46]

The alternating dipping method, which was used to successfully coat nanofibers with spherical calcium carbonate particles,[13] is presented as a synthetic approach for $La_2(CO_3)_3$. Crosslinked poly(vinyl alcohol) nanofibers were immersed in alternating solutions of aqueous lanthanum acetate and sodium carbonate for up to 10 times. Thin flower-like petals of $La_2(CO_3)_3$ crystals aggregated within the nanofibrous mesh. Where the fibers are densely packed, flower-like petals aggregate to form almost film-like structures after 10 cycles of coating. Na_2CO_3 yielded flower-like particles intermittently along the sparse nanofibrous mesh (Figure 12a). Whereas, K_2CO_3 and Cs_2CO_3 formed dense aggregate films regardless of the fiber density with sharper edges (Figure 12b,c). Our hypothesis is that cations of carbonate sources adsorbed onto $La_2(CO_3)_3$ crystals and affected crystal structure and morphology. Since Na has smaller ionic size than K and Cs, more amount of Na would be adsorbed onto $La_2(CO_3)_3$ crystals. $La_2(CO_3)_3$ mineralized fibers from K_2CO_3 and Cs_2CO_3 showed much better phosphate removal than the fibers from Na_2CO_3 (Table 6). Phosphate removal by $La_2(CO_3)_3$ mineralized fibers are better than most of the previously reported values by adsorbent materials.[47]

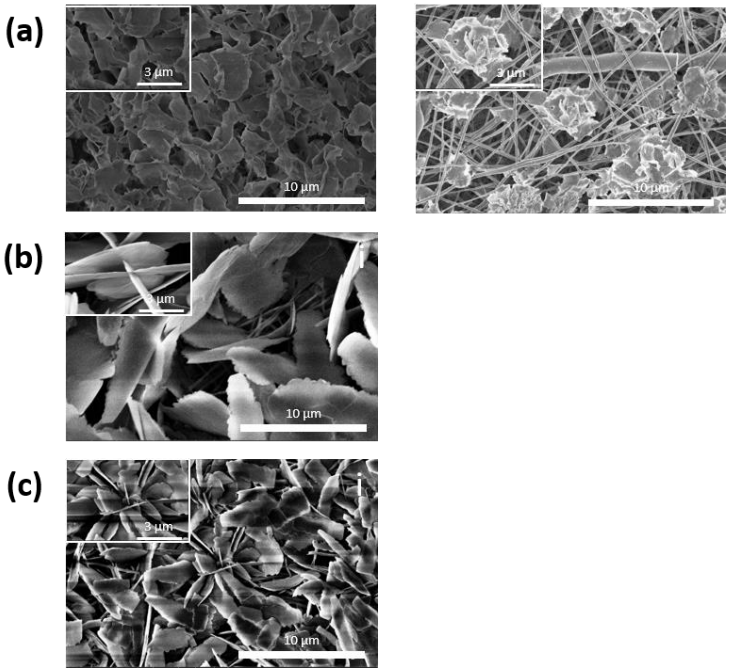


Figure 12. $La_2(CO_3)_3$ mineralized nanofibers after 10 cycles with (a) Na_2CO_3, (b) K_2CO_3 and (c) Cs_2CO_3.

Table 6. Phosphate Removal by Lanthanum Carbonate Mineralized Nanofibers

	$La_2(CO_3)_3$ x10-Na	$La_2(CO_3)_3$ x10-K	$La_2(CO_3)_3$ x10-Cs
P adsorption (mg/g)	42.00	103.56	93.78
Mineralization Degree (%)	41	52	67

6. Achievements

6.1. Publications

- **Park Y**, Ford E, Titanium Oxide Sol-Gel Induced Wrinkling of Electrospun Nanofibers, Macromolecular Chemistry and Physics (peer-reviewed), accepted.
- **Park Y,** Ford E, Liu S, Gardner T, Mycogenic Manganese Oxide Nanofibers for Heavy Metal Ions Removal, Submitted
- **Park Y,** Ford E, Lanthanum Carbonate Nanofibers for Phosphate Removal, In preparation.

6.2. University Press Releases

- Featured on the College of Textiles news: https://textiles.ncsu.edu/news/2017/11/yaewon-park-sea-grant/

6.3. Presentations

Briefing to the North Carolina State University's Chancellor

- Park Y, Graduate Research: Decontamination of Heavy Metals in North Carolina Groundwater Using Manganese Oxide Nanofibers, Feb 19 2018

Conference Presentations

- Park Y*, Ford E, Lanthanum Carbonate Nanofibers for Phosphate Removal from Water, Oral presentation at Textile Research Open House, Raleigh, NC, 2018.
- Park Y*, Ford E, Biomineralization-inspired Mineral Coating for Nanofiber Adsorbents Development, Oral presentation at Excellence in Graduate Polymer Research Symposium, ACS National Meeting, New Orleans, LA, 2018. **(Invited as a representative for the Department of Textile Engineering, Chemistry and Science)**
- Park Y*, Shuang L, Gardner T, Ford E, Heavy Metal Removal by Mycogenic Manganese Oxide Nanofibers, Poster Presentation, SciMix, ACS National Meeting, New Orleans, LA, 2018.
- Park Y*, Rawat P, Ford E, Wrinkling of TiO2 Sol-Gel Coated Electrospun Fibers, Poster Presentation at NWI Spring IAB Meeting, North Carolina State University, Raleigh, NC, 2017.
- Park Y*, Ford E, Effect of Surface Chemistry of Nanofibers on Mineral Coating, Invited Oral Presentation at The Fiber Society 2017 Fall Conference, The University of Georgia, Athens, GA, 2017. **(Invited and given Graduate Student Association (GSA) Award for Conferences)**

- Park Y*, Ford E, Bioinspired Mineral Coating on Nanofibers, Oral Presentation at NWI Spring IAB Meeting, North Carolina State University, Raleigh, NC, 2017.
- Park Y*, Liu S, Gardner T, Ford E, Nanofiber Surface Functionalization by Manganese Oxidizing Fungi, Poster presentation at 9th Annual Triangle Soft Matter Workshop, UNC Chapel Hill, Chapel Hill, NC, 2017.
- Park Y*, Liu S, Gardner T, Ford E, Biogenic Nanofiber Mineralization by Manganese Oxidizing Fungi, Poster presentation at The Research Day, College of Textiles, North Carolina State University, Raleigh, NC, 2017.

7. Conclusions

We have successfully developed manganese oxide nanofiber filters for removing Mn and other toxic heavy metals from water. The fungi-nanofiber hybrid filters removed more than 90% of Mn from water within 24 h in laboratory settings. Mn(II) on the nanofiber surfaces promoted Mn oxide coating by enzymes secreted from the *Coniothyrium* sp. fungi. In our recent publication, pilot scale electrospinning machine was shown to affect fiber surface chemistry and microstructure due to high applied voltage.[12] Thus, scaling-up of nanofiber production with high applied voltage would conducive for the fungi-nanofiber hybrids coated with Mn oxides. As we share our results at various academic conferences, we have attracted interests from academia and textiles/nonwovens industry. Wake county government kindly provided us with the contaminated well water collected from rural Raleigh. We have tested our fungi-nanofiber hybrids and oxidative enzyme containing fibers in well water containing high concentrations of Mn, Fe, Ca, etc. Interestingly, the fungi-nanofiber hybrids reduced the level of Fe, Pb, Zn but increased the level of Mn in the well water after 24 h of exposure. There might be an ion exchange on the manganese oxide surfaces or manganese ions leaching from the nanofibers. Therefore, fungi-nanofiber hybrids are recommended to be submerged in pure water for some time before use to prevent Mn(II) leaching. On the other hand, enzyme nanofibers removed all the ions from the well water. We suggest using

higher amounts of oxidative enzymes with low cost instead of manganese peroxidase to further develop water filters for well water. With our results, we have done outreach activities to introduce local community college students to the use of nanofibers and fungi for water remediation. Undergraduate students, Aaron Keeler and Drake Johnson, actively participated in this research through project-based course CH452 Measurements and provided data on heavy metal adsorption of the fungi-nanofiber hybrids.

Supporting Information

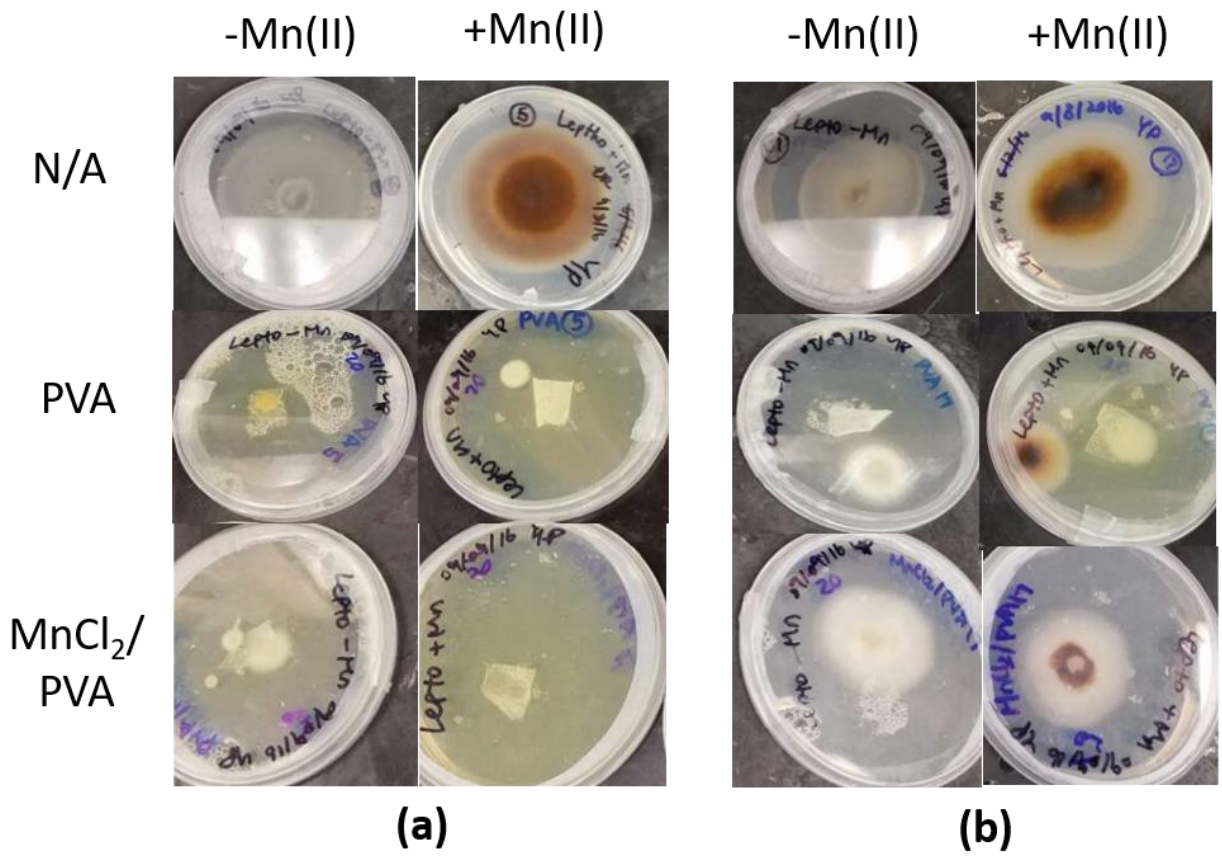


Figure S1. *Coprinellus* sp. (a) and *Coniothyrium* sp. (b) were grown in the dark at room temperature for 1 week on solid plates without and with manganese ions: -Mn(II) and +Mn(II), respectively. Neat PVA and PVA/$MnCl_2$ nanofibers were added to Leptothrix solid media.

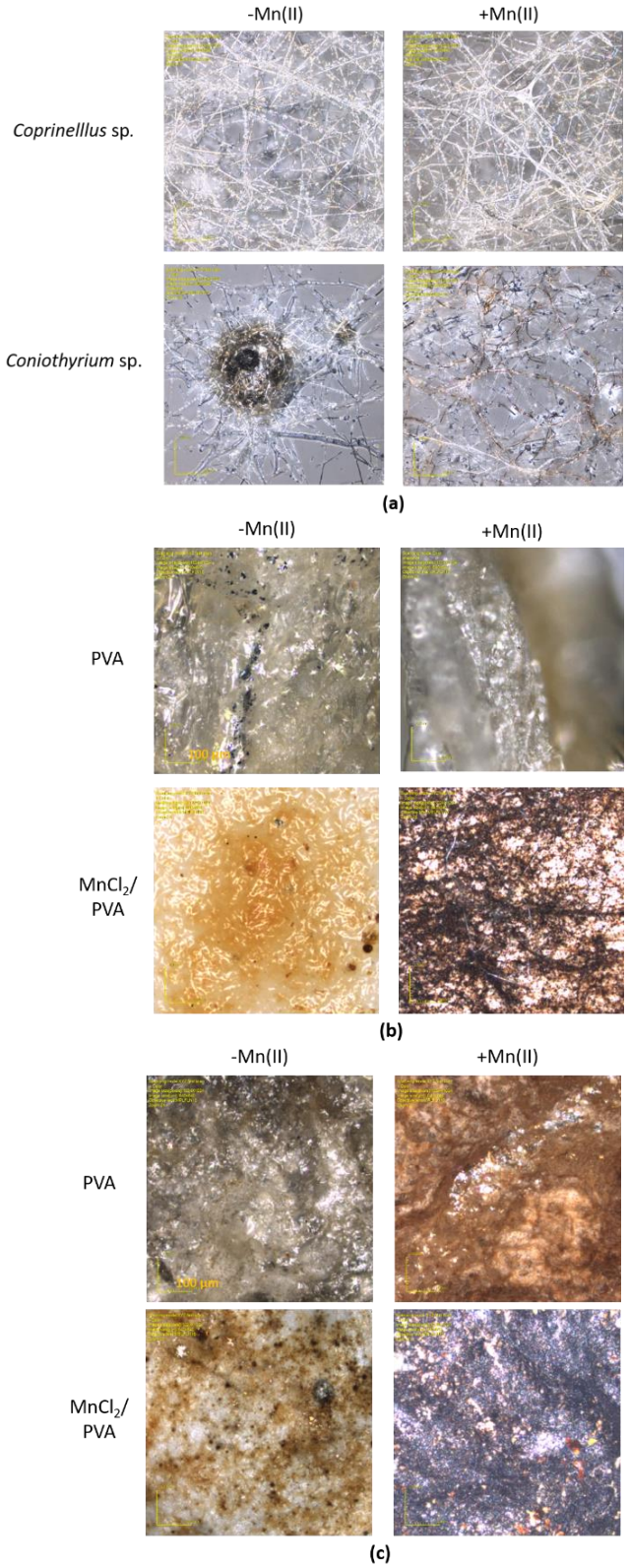


Figure S2. Confocal microscope images of (a) *Coprinellus* sp. and *Coniothyrium* sp. on -Mn(II) and +Mn(II) solid plates. (b) *Coprinellus* sp. and (c) *Coniothyrium* sp. were grown on PVA and $MnCl_2$/PVA nanofibers in Leptothrix liquid media -Mn(II) and +Mn(II).

Handouts for Outreach Activities at DTCC

Lab 1 - The Metric System and Measurements

Objectives:

1. To make measurements using the metric system and to convert those values to other metric units.
2. To choose the correct equipment to measure lab materials.
3. To learn how to make temperature conversions.

Introduction:

The metric system is the system of measurement used in most disciplines of science. You must have a working knowledge of the metric system. The system is actually simpler than the English system because it is based on units of ten (much like our monetary system). This lab is designed to introduce you to using the metric system for measurements and provides a simple method for conversions.

The three types of measurements we will be considering are mass (weight), volume, and length.

In the metric system, the basic unit of mass is the **gram**. This is abbreviated as g.

One gram is approximately equal to 1/30 of an ounce.

The basic unit in the metric system for volume is the **liter**. This is abbreviated as L.

One liter is approximately equal to one quart.

The basic unit in the metric system for length is the **meter**. This is abbreviated as m.

One meter is approximately equal to one yard.

Larger measurements in the metric system are made with larger units in multiples of ten of the base unit. This is similar to our monetary system. For example, we say that a new iPod costs 300 dollars instead of 30,000 cents. In the metric system, instead of saying that you weigh 70,000 grams, you could say that you weigh 70 kilograms. All larger and smaller units are given names and abbreviations based on the basic unit. These are listed in the table below.

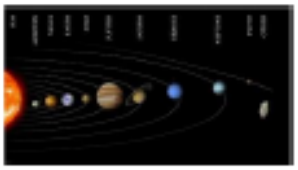
Prefix & symbol	Number of units in 1 of these:	Exponential form
Tera- (T)	1,000,000,000,000	10^{12}
Giga- (G)	1,000,000,000	10^{9}
Mega- (M)	1,000,000	10^{6}
kilo- (k)	1,000	10^{3}
hecto- (h)	100	10^{2}
deca- (da)	10	10^{1}

UNIT	1	10^{0}
deci- (d)	0.1	10^{-1}
centi- (c)	0.01	10^{-2}
milli- (m)	0.001	10^{-3}
micro- (μ)	0.000001	10^{-6}
nano- (n)	0.000000001	10^{-9}
pico- (p)	0.000000000001	10^{-12}

The metric units we will be using in this class are in the following table. Conversion between these units can be done by moving the decimal point.

k-	h-	da-	u	d-	c-	m-	-	-	μ-	-	-	n-	Å
kilo-	hecto-	deca-	(unit) meter gram liter	deci-	centi-	milli-	blank	blank	micro	blank	blank	nano-	angstrom

Relative Distances in Metric (SI) Units

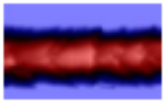


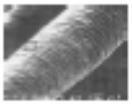
Inter-planet Distances

Distances Across the

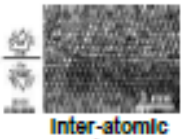
Lab Bench

Microscope Slide





Diameter of Human



Inter-atomic Distances

Carbon Forms

Carbon Nanotube

Intra-atomic Distances

- **Gigameters (Gm) = 1,000,0000,000 m = 1 billion**
- **Megameters (Mm) = 1,000,000 m = 1 million meters**
- **Kilometers (km) = 1,000 m**
- **Meters (m) = 1 m**
- **Centimeters (cm) = 100^{th} of a m = 1 hundredth of a**
- **Millimeters (mm) = $1,000^{th}$ of a m = 1 thousandth of a meter**
- **Micrometers (μm) = $1,000,000,000^{th}$ of m = 1 millionth of a meter**
- **nanometers (nm) = $1,000,000,000^{th}$ of m = 1 billionth of a meter**
- **Angstroms (Å) = $10,000,000,000^{th}$ of a meter**
- **Picometers (pm) = $1,000,000,000,000^{th}$ of m = 1 trillionth of a meter**

Exercise 1: Making Conversions

To make conversions within the metric system, count the number of spaces to move from the original prefix to the new prefix. Move the decimal point the same number of spaces and in the same direction. Remember, if no decimal appears in a number, it is understood that the decimal is after the last numeral. It is also convention to place a zero before the decimal point in numbers less than one.

To convert milliliters to liters, find the milli- prefix (for mL) in the table above. The liter unit is three spaces to the left of milli-, so you need to move the decimal three spaces to the left to convert from mL to L:

700. mL = 0.700 L

To convert liters to microliters, find the "unit" (for L) in the table above. The micro- prefix (for μL) is six spaces to the right of "unit," so you need to move the decimal six spaces to the right to convert from L to μL:

5 L = 5,000,000 μL

Practice doing metric conversions by converting the following:

50 L = _____ mL

62.8 g = _____ kg

200 kL = _____ L

5 mg = _____ g

1 cm = _____ μm

2.73 nm = _____ m

10 μm = _____ mm

2 m = _____ nm

500 μm = _____ Å

5 kg = _____ g

24 nL = _____ mL

27.6 km = _____ m

0.005 mm = _____ cm

0.06 mL = _____ nL

Lab 2 – Spectrophotometry

Determination of Concentration Using Spectrophotometry

Objectives:

1. To define solution, solvent, and solute.
2. To understand the concept of concentration and to prepare solutions by serial dilution.
3. To define and understand wavelength, absorbance, transmittance, and reflection.
4. To learn to use the spectrophotometer.
5. To draw and interpret line graphs.

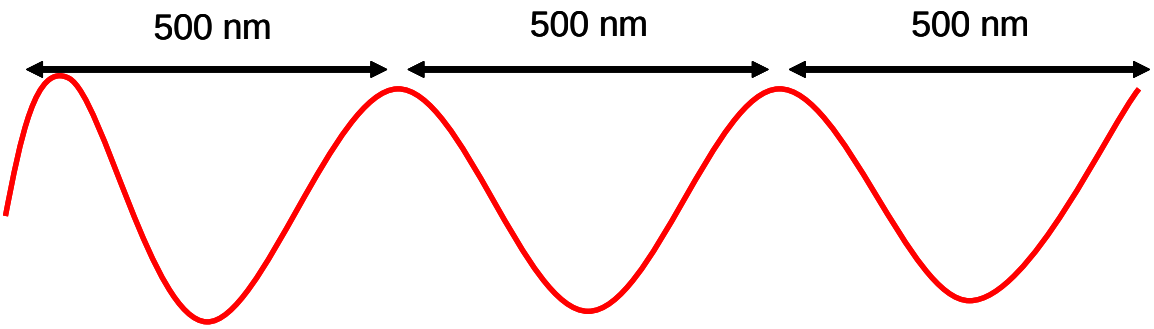
A **solution** is a homogeneous mixture consisting of a **solute** (a solid, liquid or gas) dissolved in a **solvent** (a solid, liquid, or gas). In biological systems, the solvent is usually water. The blood in our veins, the sap in a tree, the extracellular fluid that bathes all cells, and the cytoplasm that fills each cell are all solutions.

The amount of solute dissolved in a given volume of solution is the **concentration** of that solution. A strong or concentrated solution has more solute per given volume of solute than a weak or dilute solution. There are many different ways to express solution concentration. It may be expressed as a percentage of solute per volume, or as mass per volume (for example, grams per milliliter, or g/mL).

There are many different methods used to determine solute concentration. We will focus on a method that is useful for solutes that contain pigment (have color). Pigments are substances that

absorb light. If the solute is a pigment, solutions with high solute concentration will be visibly darker than solutions with low solute concentrations. In order to quantify the degree of darkness, we will use a method to measure the amount of light absorbed by the pigment. We will see that more concentrated solutions will absorb more light than dilute ones.

Visible light is a type of **electromagnetic energy**. Electromagnetic energy travels in the form of waves. Electromagnetic waves are measured from crest to crest in nanometers (nm), and this distance is called the **wavelength**.

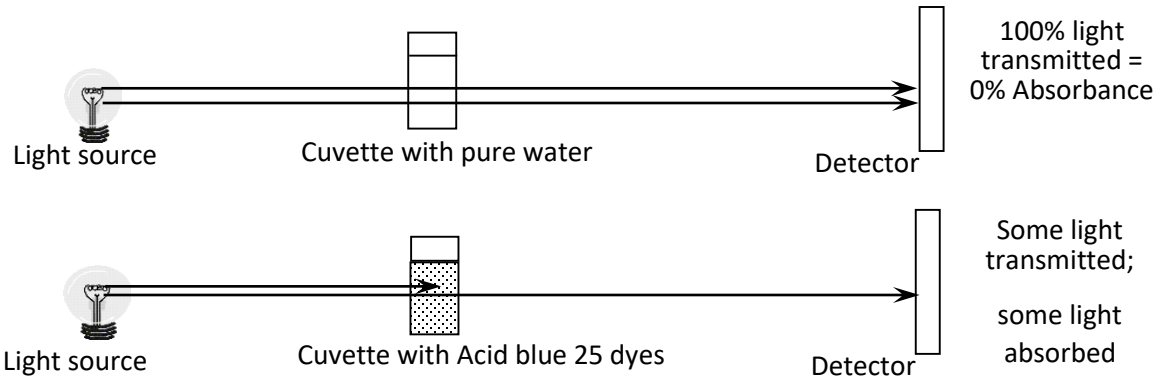


A wave of electromagnetic energy with a wavelength of 500 nm.

The wavelengths of visible light waves are between 380 and 750 nm. All of these waves *combined* produce what appears to our eyes as white light. However, individual wavelengths have particular colors. For example, light waves between 380 and 450 nm in wavelength are violet, and light waves between 620 – 750 nm in length are red. You can see all of the colors when you pass light through a prism, which separates the wavelengths. A rainbow is produced in the sky when rain droplets serve as tiny prisms to separate white light into its component colors.

How the wavelengths of light interact with pigments determines how we see color. Different wavelengths of light can be either **absorbed**, **transmitted** (passing straight thorough), or **reflected** (bouncing off) by pigments. If all of the wavelengths of light combined (all colors) are absorbed by a pigment, it appears black to our eyes. In contrast, if all of the wavelengths of light combined are reflected by a pigment, it appears white to our eyes. If you observe that an object is red, that is because the red wavelengths of light are being reflected by the pigments in that object back to your eyes. That means that the wavelengths of light *other* than red are either being absorbed or transmitted by the pigments in the object.

In this lab, you will be determining the amount of light (wavelength 500 nm) that is absorbed by several different concentrations of Acid blue 25 dyes. The more concentrated the pigment solution is, the more light it can absorb. We will accurately measure the amount of light absorbed by each solution with a device called a **spectrophotometer**. A spectrophotometer ("spec" for short) is a device that shines light, set to a specific wavelength, through a sample solution. The light shines through the sample and hits a detector on the other side. The detector reads how much of the light was absorbed by the sample solution. The figure below illustrates how a spectrophotometer works.



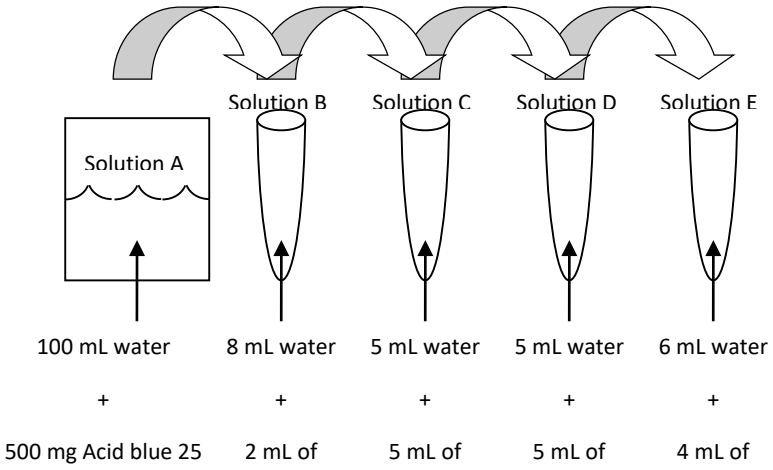
For pure water (top), none of the light has been absorbed and all of it passed straight through. For the dye solution (bottom), some light was absorbed and some was transmitted.

Exercise 1: Serial dilution and calculating concentrations of dye solutions

In this exercise, you will carry out **serial dilution** of dye solution according to the procedure and the diagram below. In a serial dilution, an initial solution is made and used to make a second solution. Then the second solution is used to make a third solution, and so on.

Procedure:

1. Place a small square of wax weighing paper on the digital balance and press the button labeled "tare" or "auto-zero".
2. Use a metal spatula to weigh 500 mg (0.50 g) of Acid blue 25 dyes.
3. Transfer the Acid blue 25 dyes to a 250 milliliter (mL) beaker and add 100mL water. Stir with a glass rod until all of the solute dissolves. This is <u>solution A.</u>
4. Label 4 test tubes B, C, D, and E.
5. Use a 5mL pipette and a green pipette pump to add the correct volume of water to each of the four tubes as listed below and on the diagram:
    a. Test tube B: 8 mL
    b. Test tube C: 5 mL
    c. Test tube D: 5 mL
    d. Test tube E: 6 mL
6. Solution B will be prepared by diluting a portion of solution A. To do this, measure 2mL of solution A and transfer to tube B which should already contain 8mL of water. Mix by pipetting up and down.
7. Draw 5 mL from tube B and add to the 5 mL of water in tube C. Mix as before. Draw 5 mL from tube C and add to the 5 mL of water in tube D. Mix as before. Finally, draw 4 mL from tube D and add to the 6 mL of water in tube E. Mix as before.



Procedure for serial dilution of Acid Blue 25 dye solutions.

Now you will calculate the concentration of each solution you have made in units of mg/mL.

First, calculate the concentration of solution A:

Concentration of solution A = $\frac{500\ mg}{100\ mL}$ = _____ mg/mL

Because we are not sure exactly how many mg of dye has been added to solutions A, B, C, D, and E, we must use a different method to calculate their concentrations. To calculate the concentration of a solution that has been made from another solution, as in serial dilutions, you can use the following equation.

$$C_1V_1=C_2V_2$$

Where:

C_1 is the concentration of the first solution you are using to make the second (new) solution,

V_1 is the volume you add of the first solution to make the second (new) solution,

C_2 is the final concentration of the second (new) solution, and

V_2 is the final volume of the second (new) solution.

We will typically be trying to calculate the value for C_2, so we can rearrange the above equation to solve for C_2 and calculate the concentration of each solution using the equation:

$$C_2 = \frac{C_1 V_1}{V_2}$$

Remember that the identity of C_1 and C_2 changes with each step!

Calculate and fill in the column for concentrations in following table. We will fill in the absorbance values later.

Recall: Concentration of solution A = _____ mg/mL

Identity of Solution	Volume and identity of dye added (V_1)	Volume of water	Total volume of solution (V_2)	Concentration (mg/mL)	Absorbance
Solution B	2 mL of A	8 mL	10 mL		
Solution C	5 mL of B	5 mL	10 mL		
Solution D	5 mL of C	5 mL	10 mL		
Solution E	4 mL of D	6 mL	10 mL		

Exercise 2: Measuring Absorbance at 500 nanometers (nm)

Acid blue 25 absorbs light at 602 nanometers (nm). Therefore, you will be measuring the absorbance of your samples with the wavelength set at 602 nm on the spectrophotometer. Check to be certain the spectrophotometer is set for the correct wavelength and for absorbance (not transmittance).

Procedure:

1. Follow your lab instructor's directions to set the blank (water) to zero absorbance on the spec.
2. Continue to follow your lab instructor's directions to read the absorbance of samples B through E, starting with the most dilute first (E). Record results in the table above.
3. Be sure to pour all wastes in the beaker provided.
4. Measure the absorbance of the Acid blue 25 dye sample labeled "UNKNOWN" and record below:

Absorbance of the unknown dye sample: _____

Exercise 3. Graphing the relationship between concentration and absorbance and using it to determine an unknown sample concentration

Now that you have measured the absorbance for solutions A-E, you will graph your data. Your graph will show the mathematical relationship between concentration and absorbance for the dye. To create the graph, use the following procedure.

1. Using the graph paper provided on the last page of this exercise, label the X-axis "Concentration in mg/mL." Number the X-axis from 0 – 1 mg/mL, using the darkest lines to represent 0.1 mg/mL intervals.

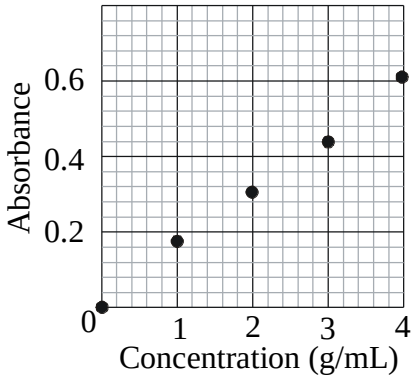
2. Label the Y-axis “Absorbance.” Number the Y-axis from 0 – 1, using the darkest lines to represent 0.1 intervals.
3. Plot and label the values from the above table. The “Concentration” and “Absorbance” columns are your X and Y values for each plotted point, respectively.
4. Once each point is placed on the graph, note that the points appear to form a line. Use a ruler to draw a straight line that is the best fit with most of the points. This line is not exact. The straight line gives you a rough estimate of the linear relationship between concentration (the independent variable) and absorbance of the dye solutions (the dependent variable). This is called a **standard curve**.

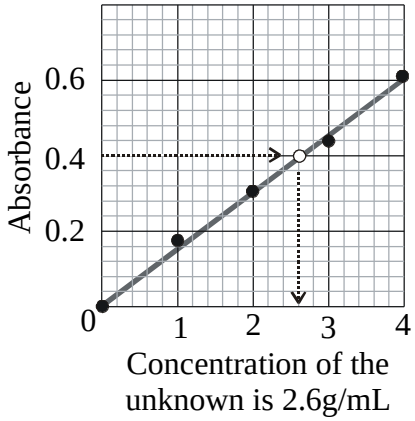
When the linear relationship between concentration and absorbance (the standard curve) is known, determination of an unknown concentration, when the absorbance is known, can be done in two ways: visually or mathematically.

Visual determination

To visually determine the concentration of an unknown, locate its absorbance value on the Y-axis of the graph and draw a horizontal line to intersect your best-fit line. Then draw a vertical line downward from that intersection to the X-axis. Where this vertical line hits the X-axis is the value of X, or the concentration of the unknown solution. An example is shown below.

Conc.	Abs
0	0.00
1	0.19
2	0.25
3	0.42
4	0.61
Unk	0.40





Visual determination of the concentration of an unknown solution.

Use the visual method to determine the concentration of your unknown sample.

Concentration of the unknown dye solution sample: _____

Mathematical determination

Calculate the slope (m) of your line by selecting 2 points on your best-fit line. These points should be spaced well apart and CANNOT be data points that you have already plotted. Next, calculate the slope (m) of the line by using the following the equation:

$$m = \frac{y_2 - y_1}{x_2 - x_1} \qquad \frac{Y_2 - Y_1}{\quad} \qquad m = \_\_\_\_\_$$

Using the equation for a straight line y = mx + b, you can determine the concentration or absorbance of any dye solution, even beyond the limits of your graph.

y = absorbance, m = slope, x = concentration, b = y intercept (zero in this case, because pure water of 0 mg/mL concentration has zero absorbance)

Use the equation for a straight line to calculate the concentration of your unknown sample:

Concentration of the unknown dye sample: _____

Using the equation for a straight line and your slope value, calculate:

1) the absorbance you would expect from a solution with a concentration of 46 mg/ml:

2) the concentration of a solution with an absorbance of 2.10:

Exercise 4. Determine how much of dyes were removed by fungi.

1. Immerse fungi mat (fungi + nanofibers) in dye solution with known concentration (C_o) . Fungi mat can remove dyes from water by the process called biosorption (adsorption + absorption).
2. Measure UV absorbance of filtered dye solution over different time (30, 60, 90 min)
3. Using standard curve, estimate the concentration of dyes after filtration (C_f)
4. Calculate dye removal over time according to the equation below:

$$\text{dye removal (\%)} = \frac{C_o - C_f}{C_o} \times 100$$

Time (min)	Absorbance	Concentration	Dye Removal (%)
0			
30			
60			
90			

Why do we care about concentrations and spectrophotometry?

In biological systems, the maintenance of certain solution concentrations is critical. The oxygen (O_2) concentration in the blood is an example of this. The blood oxygen concentration of a patient undergoing surgery is continuously measured by a device called a Pulse-Ox monitor. Oxygen is transported through the blood bound to a protein called hemoglobin. This protein is red when oxygen is bound to it and a slightly different color in its deoxygenated form. The monitor measures the amount of red, oxygenated hemoglobin. This measurement is done using a variation of spectrophotometry.

The measurement of glucose concentration in blood or "blood sugar" is done using spectrophotometry. A diabetic patient places a drop of blood on a sample strip containing an enzyme. The enzyme reacts with the glucose in the blood, producing a colored product that is then measured in a home spec. These instruments are called blood sugar monitors or blood sugar meters.

Questions:

1. What is a solution? What is a solute? What is a solvent?

2. If one solution is darker than the other, which is more concentrated?

3. What does it mean to say that one solution is more concentrated than another one?

4. Why is it necessary to draw a best-fit line to determine the concentration of the unknown solution?
5. What are the two ways in which concentration of the unknown solution determined in this experiment?

6. Give an example of metric units that may be used to describe concentration.

Check with your instructor for instructions before completing the following calculations.

7. A 1M (molar) solution is the molecular weight of the solute in grams (a mole) dissolved in 1 liter of solvent

Note the molecular weights of the elements shown:

Na = 23

K = 39

Mg = 24

Cl = 35.5

O = 16

Br = 80

H = 1

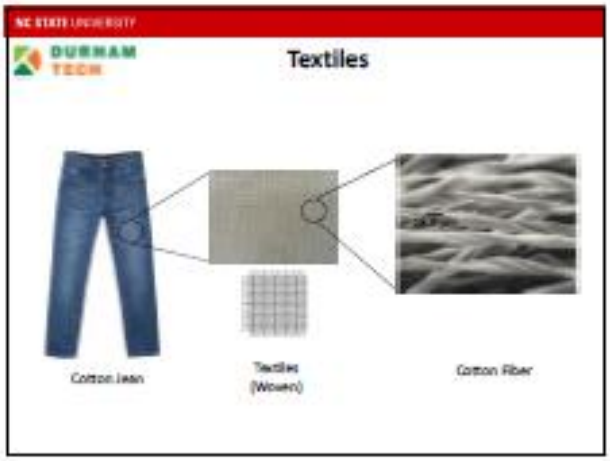
Answer the following questions:

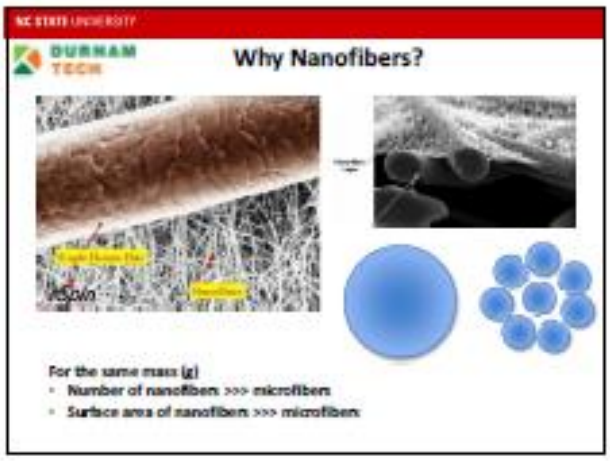
How many grams of potassium hydroxide (KOH) would you need to make up a 5M solution in 1 liter of water?

How many grams of sodium chloride (NaCl) would you need to make a 2M solution in 500 ml of water?

How many grams of magnesium bromide ($MgBr_2$) would you need to prepare a 0.5M solution in 100 ml of water?

Slides for Outreach Activities at DTCC





NC STATE UNIVERSITY

DURHAM TECH

How to Do Metric Conversion -2

According to National Institute of Standards and Technology (NIST)
https://www.nist.gov/pml/weights-and-measures/metric-si/unit-conversion

4 Steps when do not have single conversion e.g. mm → km

1. What do you have? Starting Units
2. What do you want? Desired Units
3. Identify Conversion Factors (=Relationship between starting unit & desired unit)
4. Cancel units where you can and do the math

$$Starting\ unit \times \frac{linking\ unit}{starting\ unit} \times \frac{desired\ unit}{linking\ unit} = desired\ unit$$

e.g. convert 5 mm to km

1. What do you have? mm
2. What do you want? km
3. Identify Conversion Factors: $1\ mm = 10^{-3}\ m$ → $\frac{1\ mm}{10^{-3}\ m} = 1$; $1\ km = 10^{3}\ m$ → $\frac{1\ km}{10^{3}\ m} = 1$
4. Cancel units wher you can and do the math

$$5\ \cancel{mm} \times \frac{10^{-3}\ \cancel{m}}{1\ \cancel{mm}} \times \frac{1\ km}{10^{3}\ \cancel{m}}$$

NC STATE UNIVERSITY

DURHAM TECH

Kingdom Fungi

Yeast (single celled fungi)	Mold (multi-celled, filamentuous)	Mushroom (fungi that form large fruiting bodies/mushrooms)
		

- **Hyphae:** fungal hair
- **Mycelia:** network of hyphae
- **Enzymes:** secreted from the tip of hyphae that can break down various things
- Spore: similar to seed, it enables fungi to reproduce
- **Fruiting body:** a special structure that hold spores

NC STATE UNIVERSITY

DURHAM TECH

Our Soil Fungi Came From...

Superfund Site: North Carolina State University (Lot 86, Farm Unit #1), Raleigh, NC

- Superfund sites: EPA designated sites as highly contaminated with organic pollutants, heavy metals, etc.
- North Carolina State University (NCSU) disposed of waste from science laboratories and agricultural facilities in Lot 86 from 1969 to 1980.
- Several microorganisms act to 'clean up' heavy metals, i.e. Mn^{2+} ions removal by precipitation

NC STATE UNIVERSITY

DURHAM TECH

Manganese Oxide Producing Soil Fungi

$Mn^{2+} \xrightarrow{\text{Enzyme}} MnO_2$

Colorless, soluble in water — Dark brown, insoluble (solid)

Mn Oxides Produced by Different Fungus

Biogenic Mn Oxides Captures Pb^{2+}

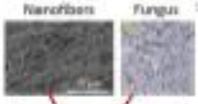
- Several microorganisms remove dissolved Mn^{2+} ions by precipitation
- Mn oxidizing soil fungi were isolated from (Lot 86, Farm Unit #1) superfund site
- Some regions of NC groundwater are contaminated with Mn^{2+} ions

NC STATE UNIVERSITY

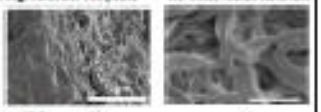
DURHAM TECH

Fungi-Nanofiber Filters

Nanofibers — Fungus



Fungi-nanofiber composite — Mn Oxide-Coated Nanofiber



Pb(II) removal [%]	80	58
Cu(II) removal [%]	66	57
Mn(II) removal [%]	28	90+*

NC groundwater

- Fungi grew on the top of nanofibers and formed manganese oxides
- *Coniothyrium* sp. formed sheet of manganese oxides on hyphae and nanofiber surfaces
- Strong interaction with hyphae-nanofiber-Mn oxides makes the nanofibers great water filters

NC STATE UNIVERSITY

DURHAM TECH

What is Solution?

- **Solution:** homogeneous mixture of **solute** dissolved in **solvent**
- **Solute:** the substance that dissolves in a solvent
- **Solvent:** the substance in which a solute dissolves

Today we will focus on dye solution
How can we measure concentration of dye solution?

$$\text{solution concentration}\left(\frac{mg}{L}\right) = \frac{\text{mass of solute (mg)}}{\text{volume of solution (L)}}$$

Solute: Acid Blue 25 Dye — Solvent: water

NC STATE UNIVERSITY

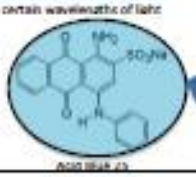
DURHAM TECH

Color of Dye

- White light is a combination of all different colors

If a substance absorbs here...

- Dyes absorb a certain wavelength of light



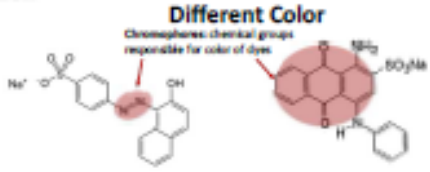
It appears as this colour

NC STATE UNIVERSITY

DURHAM TECH

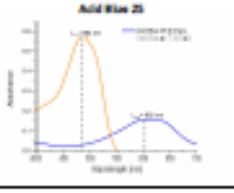
Different Dye Structures → Different Color

Chromophores: chemical groups responsible for color of dyes



Acid Orange 7

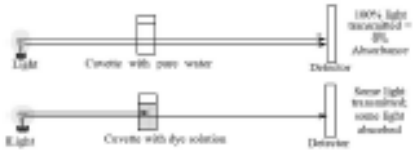
Acid Blue 25



NC STATE UNIVERSITY

DURHAM TECH

Ultra Violet Visible Spectrophotometer



- UV-Vis spectrophotometer measures light absorbed by dye in solution
- As more dyes is added to solution, more light is absorbed
- You can estimate **dye concentration in an unknown solution** once **the relationship between dye concentration and absorbance of UV-Vis light is known**

NC STATE UNIVERSITY

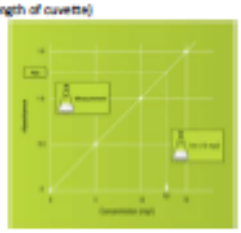
DURHAM TECH

Beer Lambert Law

- **Absorbance** is proportional to the **concentration** of solution

$$A = \varepsilon c l$$

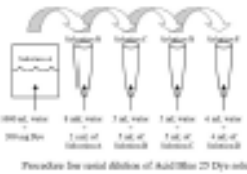
A: absorbance
ε: constant
c: concentration of solution
l: optical path length (length of cuvette)



NC STATE UNIVERSITY

DURHAM TECH

Serial Dilution-1



Procedure for serial dilution of Acid Blue 25 Dye solutions.

- What is concentration of solution A? **200 mg/L**
- What is concentration of solution B then?

$$C_1V_1 = C_2V_2$$

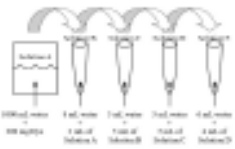
C_1 is the concentration of the first solution you are using to make the second (new) solution,
V_1 is the volume you add of the first solution to make the second (new) solution,
C_2 is the final concentration of the second (new) solution, and
V_2 is the final volume of the second (new) solution.

$$200\ \frac{mg}{L} \times 2\ mL = C_2 \times 10\ mL \qquad \therefore C_2 = 40\ mg/L$$

NC STATE UNIVERSITY

DURHAM TECH

Serial Dilution-2



Procedure for serial dilution of Acid Blue 25 Dye solutions.

- What is concentration of solution A? **200 mg/L**
- What is concentration of solution C, D, & E?

$$C_1V_1 = C_2V_2$$

Identity of Solution	Volume and Identity of Kool-aid added (V_1)	Volume of water	Total volume of solution (V_2)	Concentration (mg/L)	Absorbance
Solution B	2 mL of A	8 mL	10 mL	40 mg/L	
Solution C	5 mL of B	5 mL	10 mL		
Solution D	5 mL of C	5 mL	10 mL		
Solution E	4 mL of D	6 mL	10 mL		

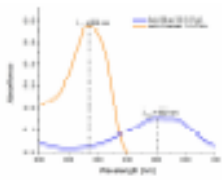
NC STATE UNIVERSITY

DURHAM TECH

Standard Curve

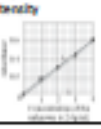
Relationship between dye concentration – absorbance

Identity of Solution	Volume and Identity of Kool-aid added (V_1)	Volume of water	Total volume of solution (V_2)	Concentration (mg/L)	Absorbance
Solution B	2 mL of A	8 mL	10 mL	40 mg/L	
Solution C	5 mL of B	5 mL	10 mL		
Solution D	5 mL of C	5 mL	10 mL		
Solution E	4 mL of D	6 mL	10 mL		



- Measure absorbance at 602 nm (where the dye absorbs the light most)
- Fill out the table
- Draw graph where x-axis is dye concentration and y-axis is absorbance intensity

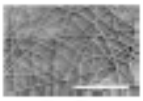
e.g.)



NC STATE UNIVERSITY

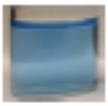
DURHAM TECH

Dye Removal by Fibers

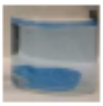


Nanofibers

Fungi + Nanofibers



Original dye concentration (C_o)



dye concentration after immersing fibers for certain amount of time(C_{time})

Time (min)	Absorbance	Concentration (mg/L)	Dye Removal (%)
0			
30			
60			
90			

Undergraduate Students' Slides

NC STATE UNIVERSITY

Metal Ion Removal from Wake County Well Water

Drake Johnson, Aaron Keeler

NC STATE UNIVERSITY

Motivation

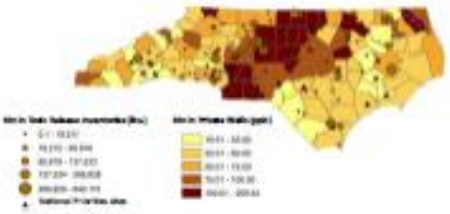
- The presence of heavy-metal ions in drinking water is a risk factor for many disorders, according to the EPA.
- Short-term problems include:
  - Headache
  - Nausea
  - Skin discoloration
- Long-term exposure results in:
  - Cardiomyopathy
  - Liver/kidney damage
  - Possible neurodegenerative disorders

United States Environmental Protection Agency

NC STATE UNIVERSITY

Motivation

- Metal ions are most common in well water; a prominent pollutant in North Carolina is manganese



NC STATE UNIVERSITY

Statistics

- A well-water sample from Raleigh, NC:

Metal Ion	EPA Recommended (mg/L)	Raleigh Well Sample (mg/L)
Manganese	0.05	0.11
Iron	0.3	1.21

- Nearly 3 million North Carolinians rely on well water, a third of the total population

North Carolina Health and Human Services

NC STATE UNIVERSITY

Approach

- Dr. Ericka Ford and Dr. Yaewon Park developed a fiber which adsorbs metal ions from aqueous solutions.

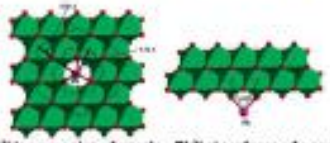
Dr. Ericka Ford

- A poly(vinyl alcohol) fiber matrix containing manganese(II) chloride is subjected to the fungi *Coniothyrium sp.*
  - This $MnCl_2$ is utilized by the fungi to form biogenic manganese oxides.
  - These oxides adsorb other metal ions due to their electronic structure.

Dr. Yaewon Park

NC STATE UNIVERSITY

Ion Uptake Mechanism



Pb^{2+} in vacancies of poorly crystalline Mn oxides

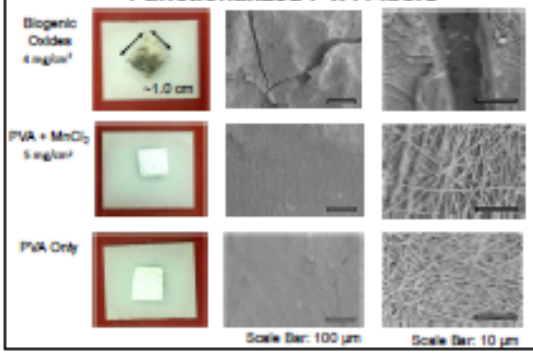
Pb^{2+} at surfaces of poorly crystalline Mn oxides

- Biogenic oxides formed by the fungi have a greater potential for adsorption due to imperfections in the crystalline matrix.

Villalobos, M. et al. *Environ. Sci. Technol.* **2003**, *37*, 649-76.
Tebo, B.M. et al. *Annu. Rev. Earth Planet Sci.* **2004**, *32*, 287-328.

NC STATE UNIVERSITY

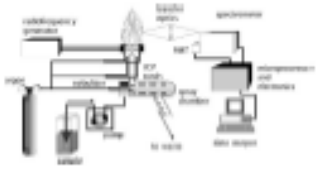
Functionalized PVA Fibers



NC STATE UNIVERSITY

Instrumentation

- We proposed the use of Inductively Coupled Plasma-Atomic Emission (ICP-AE) spectroscopy.
  - Samples are aspirated into Argon plasma torch
  - Detector scans specified wavelengths for atomic emissions



NC STATE UNIVERSITY

Why ICP-AE?

- Other instrumentation such as NMR, Raman, and IR can be used to determine metal ion concentration; however, ICP-AE is especially attractive:
  - High sensitivity to many analytes
  - Low run-time, scanning for multiple species simultaneously
  - Low Limit of Detection, usually within parts-per-billion
  - Fewer interferences with plasma torch

NC STATE UNIVERSITY

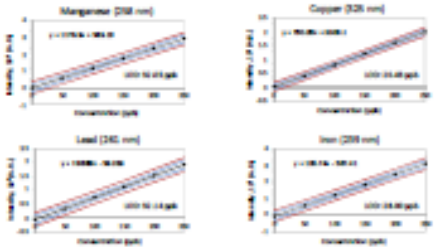
Quantification of Analytes

- Calibration curves were generated for metals of interest using a concentrated metal standard containing various species.
- Using an Excel macro created by the CH452 instructors, error analysis was conducted.
  - Generates the 95% confidence and prediction limits for a series of data
  - Assists in determination of outliers and the limit of detection.

NC STATE UNIVERSITY

Metals Studied

- Scope was limited to metals common in well drinking water, as well as heavy metals previously tested in the Ford lab.



NC STATE UNIVERSITY

Well Water Analysis

- Each fiber was submerged in a Raleigh well water sample for 24 hrs
- Significant decrease in all ions except Mn

Fe^{2+}	Conc. (ppb)	Difference (ppb)	Percent Change
Well Water	113.8 (0.9)	-	-
Fungi Fiber	11.8 (0.7)	-102. (1.6)	-89.6 (1.5)
MnO_2 Fiber	13.4 (0.7)	-100. (1.6)	-88. (1.2)

Zn^{2+}	Conc. (ppb)	Difference (ppb)	Percent Change
Well Water	570. (3.1)	-	-
Fungi Fiber	446. (1.6)	-123. (3.7)	-21.6 (0.6)
MnO_2 Fiber	482. (1.9)	-88. (4.0)	-15.6 (0.9)

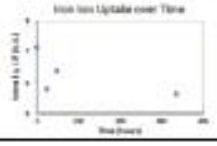
Fe^{3+}	Conc. (ppb)	Difference (ppb)	Percent Change
Well Water	825. (6.3)	-	-
Fungi Fiber	675. (5.1)	-150. (7.4)	-18. (1.2)
MnO_2 Fiber	670. (2.6)	-155. (6.8)	-18.8 (0.8)

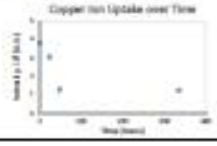
Mn^{2+}	Conc. (ppb)	Difference (ppb)	Percent Change
Well Water	145. (3.6)	-	-
Fungi Fiber	1275. (6.7)	+550. (8.3)	+443 (5.2)
MnO_2 Fiber	121. (1.8)	-24. (4.4)	-16.6 (2.7)

Metals Studied

- Scope was limited to metals common in well drinking water, as well as heavy metals previously tested in the Ford lab.

Ion	Percent Uptake	
	24 hrs	48 hrs
Fe^{2+}	4. (1.6)	20. (1.3)
Cu^{2+}	23.7 (0.7)	66. (3.4)
Mn^{2+}	-6.1 (0.2)	-169 (1.1)



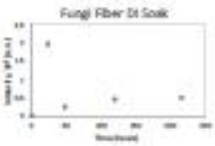


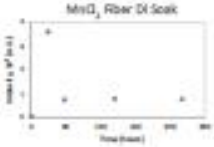
Manganese issue

- Analysis of standard 1 ppm manganese solutions after subjection to the fiber for 24 hours revealed an increase in metal concentration.
  - Also observed in all other metal solutions
- It was hypothesized that this was due to the dissolution of manganese chloride that had not been converted to oxides
  - Investigation involved soaking a fungi-treated fiber in DI water, checked over the course of a week

Leeching Investigation

- Results from soaking fibers in DI water over several days revealed:
  - Initial increase in manganese conc. of solutions
  - Subsequent decrease to a somewhat constant conc.





Fiber Compositions

- Sought to quantify how much of the total manganese in the fibers was being leeched out
- Each fiber was dissolved in concentrated nitric acid; the solutions were diluted, then aspirated

Fiber Type	Total Mn Mass (μg)	% Mn by mass
$MnCl_2$	154	3.1
Fungi-treated	11	0.3

- Additionally, the fungi oxidize approximately 93% of available $MnCl_2$ within the fiber

Fungi Removal

- Fungi were not removed before testing; possible origin of leeching or adsorption interference.

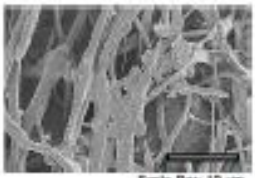
- Fibers were sonicated in ethanol for 2 hours, followed by a rinse in DI water
  - Placed back into 1 ppm Mn solution

Procedure	Initial Mass Mn in Sol. (μg)	Mass Mn in Sol. after 48 hrs (μg)	Total Mn Uptake (μg/mg fiber)
No Sonication	20.5	30.1 (0.09)	+1.92
Sonicated	20.5	11.6 (0.3)	-1.78

Improving Membrane Performance

- Sonication in ethanol for 2 hours, followed by DI rinse

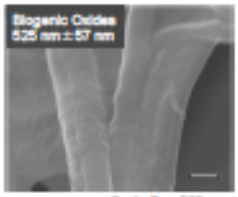

Scale Bar: 100 μm


Scale Bar: 10 μm

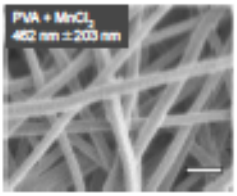
NC STATE UNIVERSITY

Improving Membrane Performance

- PVA+$MnCl_2$ vs Biogenic Oxides
- Morphological changes on surface of individual fibers
- Slight increase of diameter of individual fiber



Scale Bar: 500 nm



Scale Bar: 500 nm

NC STATE UNIVERSITY

Conclusion

- The fiber is effective in the removal of several ions from standard 1 ppm solutions and well water samples
- Prior to sonication, a significant increase in manganese concentration was observed in both DI water and 1 ppm solutions of metals
- Sonication has been observed to eliminate the leeching issues associated with manganese
  - Additionally removes fungi from surface to promote adsorption

NC STATE UNIVERSITY

Future Work

- Further test sonicated fibers in metal solutions and well water solutions
- Continue to investigate time dependence of ion uptake from these solutions
- Explore concentration dependence of adsorption
  - Possible saturation limits

NC STATE UNIVERSITY

Acknowledgements

Sea Grant North Carolina WRRI

Dr. Ericka Ford
Dr. Yaewon Park

Dr. Nathalia Ortiz

Dr. Ghada Rabah
Dr. Stefan Franzen

References

(1) Theron, J.; Walker, J. A.; Cloete, T. E. Nanotechnology and Water Treatment: Applications and Emerging Opportunities. *Crit. Rev. Microbiol.* **2008**, *34* (1), 43–69.

(2) Hua, M.; Zhang, S.; Pan, B.; Zhang, W.; Lv, L.; Zhang, Q. Heavy Metal Removal from Water/wastewater by Nanosized Metal Oxides: A Review. *J. Hazard. Mater.* **2012**, *211–212*, 317–331.

(3) Environmental Protection Agency (EPA). *Superfund Remedy Report*; 2013; Vol. 14.

(4) Fu, F.; Wang, Q. Removal of Heavy Metal Ions from Wastewaters: A Review. *J. Environ. Manage.* **2011**, *92* (3), 407–418.

(5) Kurniawan, T. A.; Chan, G. Y. S.; Lo, W. H.; Babel, S. Physico-Chemical Treatment Techniques for Wastewater Laden with Heavy Metals. *Chem. Eng. J.* **2006**, *118* (1–2), 83–98.

(6) Ray, P. Z.; Shipley, H. J. Inorganic Nano-Adsorbents for the Removal of Heavy Metals and Arsenic: A Review. *RSC Adv.* **2015**, *5* (38), 29885–29907.

(7) Sawhney, A. P. S.; Condon, B.; Singh, K. V.; Pang, S. S.; Li, G.; Hui, D. Modern Applications of Nanotechnology in Textiles. *Text. Res. J.* **2008**, *78* (8), 731–739.

(8) Kim, Y. J.; Ahn, C. H.; Lee, M. B.; Choi, M. S. Characteristics of Electrospun PVDF/SiO2 Composite Nanofiber Membranes as Polymer Electrolyte. *Mater. Chem. Phys.* **2011**, *127* (1–2), 137–142.

(9) Jin, S.; Park, Y.; Park, C. H. Preparation of Breathable and Superhydrophobic Polyurethane Electrospun Webs with Silica Nanoparticles. *Text. Res. J.* **2016**, *86* (17), 1816–1827.

(10) Wu, S.; Li, F.; Wang, H.; Fu, L.; Zhang, B.; Li, G. Effects of Poly (Vinyl Alcohol) (PVA)

Content on Preparation of Novel Thiol-Functionalized Mesoporous PVA/SiO2 Composite Nanofiber Membranes and Their Application for Adsorption of Heavy Metal Ions from Aqueous Solution. *Polymer (Guildf).* **2010**, *51* (26), 6203–6211.

(11) Dong, H.; Wang, D.; Sun, G.; Hinestroza, J. P. Assembly of Metal Nanoparticles on Electrospun Nylon 6 Nanofibers by Control of Interfacial Hydrogen-Bonding Interactions. *Chem. Mater.* **2008**, *20* (21), 6627–6632.

(12) Park, Y.; Ford, E. Titanium Oxide Sol-Gel Induced Wrinkling of Electrospun Nanofibers. *submitted* **2017**.

(13) Park, Y.; Rawat, P.; Ford, E. Role of Polymerized Micelles on the Calcium Carbonate Mineralization of Nanofibers. *Ind. Eng. Chem. Res.* **2017**, *56* (29), 8241–8250.

(14) Harms, H.; Schlosser, D.; Wick, L. Y. Untapped Potential: Exploiting Fungi in Bioremediation of Hazardous Chemicals. *Nat. Rev. Microbiol.* **2011**, *9* (3), 177–192.

(15) Environmental Protection Agency (EPA). *A Citizen's Guide to Bioremediation*; 2012.

(16) Environmental Protection Agency (EPA). *Introduction To in Situ Bioremediation of Groundwater*; 2013.

(17) Santelli, C. M.; Pfister, D. H.; Lazarus, D.; Sun, L.; Burgos, W. D.; Hansel, C. M. Promotion of Mn(II) Oxidation and Remediation of Coal Mine Drainage in Passive Treatment Systems by Diverse Fungal and Bacterial Communities. *Appl. Environ. Microbiol.* **2010**, *76* (14), 4871–4875.

(18) Burgos, W. D.; Tan, H.; Santelli, C. M.; Hansel, C. M. Importance of Fungi in Biologial Mn(II) Oxidation in Limestone Treatment Beds. *J. Am. Soc. Min. Reclam.* **2010**, *2010* (1), 71–88.

(19) Vail, W.; Riley, R. Process for Removing Manganese from Solutions Including Aqueous

Industrial Waste. US5441641A, 1995.

(20) Villalobos, M.; Bargar, J.; Sposito, G. Mechanisms of Pb(II) Sorption on a Biogenic Manganese Oxide. *Environ. Sci. Technol.* **2005**, *39* (2), 569–576.

(21) Remucal, C. K.; Ginder-Vogel, M. A Critical Review of the Reactivity of Manganese Oxides with Organic Contaminants. *Environ. Sci. Process. Impacts* **2014**, *16* (6), 1247.

(22) Santelli, C. M.; Webb, S. M.; Dohnalkova, A. C.; Hansel, C. M. Diversity of Mn Oxides Produced by Mn(II)-Oxidizing Fungi. *Geochim. Cosmochim. Acta* **2011**, *75* (10), 2762–2776.

(23) Toner, B.; Fakra, S.; Villalobos, M.; Warwick, T.; Sposito, G. Spatially Resolved Characterization of Biogenic Manganese Oxide Production within a Bacterial Biofilm. *Appl. Environ. Microbiol.* **2005**, *71* (3), 1300–1310.

(24) Tebo, B. M.; Bargar, J. R.; Clement, B. G.; Dick, G. J.; Murray, K. J.; Parker, D.; Verity, R.; Webb, S. M. Biogenic Manganese Oxides: Properties and Mechanisms of Formation. *Annu. Rev. Earth Planet. Sci.* **2004**, *32* (1), 287–328.

(25) Miyata, N.; Tani, Y.; Sakata, M.; Iwahori, K. Microbial Manganese Oxide Formation and Interaction with Toxic Metal Ions. *J. Biosci. Bioeng.* **2007**, *104* (1), 1–8.

(26) Droz, B.; Dumas, N.; Duckworth, O. W.; Pen, J. A Comparison of the Sorption Reactivity of Bacteriogenic and Mycogenic Mn Oxide Nanoparticles. *Environ. Sci. Technol.* **2015**, *49*, 4200–4208.

(27) Ouvrard, S.; Simonnot, M.-O.; Sardin, M. Reactive Behavior of Natural Manganese Oxides toward the Adsorption of Phosphate and Arsenate. *Ind. Eng. Chem. Res.* **2002**, *41* (11), 2785–2791.

(28) Gadd, G. M. Biosorption: Critical Review of Scientific Rationale, Environmental

Importance and Significance for Pollution Treatment. *J. Chem. Technol. Biotechnol.* **2009**, *84* (1), 13–28.

(29) Environmental Protection Agency (EPA). Secondary Drinking Water Standards: Guidance for Nuisance Chemicals https://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals.

(30) Environmental Protection Agency (EPA). National Primary Drinking Water Regulations https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#seven.

(31) Gardner, T.; Rivera, N.; Andrews, M.; Santelli, C.; Duckworth, O. Diversity and Abundance of Microbial Community in NC Superfund Site Remediation System Biofilm, and Their Role in Mn Oxidation. In *Synergy in Science: Partnering for Solutions*; American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 2015.

(32) Duckworth, O. W.; Rivera, N. A.; Gardner, T. G.; Andrews, M. Y.; Santelli, C. M.; Polizzotto, M. L. Morphology, Structure, and Metal Binding Mechanisms of Biogenic Manganese Oxides in a Superfund Site Treatment System. *Environ. Sci. Process. Impacts* **2017**, *19* (1), 50–58.

(33) Ivarsson, M.; Broman, C.; Gustafsson, H.; Holm, N. G. Biogenic Mn-Oxides in Subseafloor Basalts. *PLoS One* **2015**, *10* (6), 1–13.

(34) Jaworska, M.; Dłuzniewska, J. The Effect of Manganese Ions on Development and Antagonism of Trichoderma Isolates. *Polish J. Environ. Stud.* **2007**, *16* (4), 549–553.

(35) Tebo, B. M.; Johnson, H. A.; McCarthy, J. K.; Templeton, A. S. Geomicrobiology of manganese(II) Oxidation. *Trends Microbiol.* **2005**, *13* (9), 421–428.

(36) Yang, D.; Yu, K.; Ai, Y.; Zhen, H.; Nie, J.; Kennedy, J. F. The Mineralization of Electrospun Chitosan/poly(vinyl Alcohol) Nanofibrous Membranes. *Carbohydr. Polym.* **2011**, *84* (3), 990–996.

(37) Julien, C.; Massot, M.; Baddour-Hadjean, R.; Franger, S.; Bach, S.; Pereira-Ramos, J. P. Raman Spectra of Birnessite Manganese Dioxides. *Solid State Ionics*. 2003, pp 345–356.

(38) Sasaki, K.; Matsuda, M.; Urata, T.; Hirajima, T.; Konno, H. Sorption of Co2+ Ions on the Biogenic Mn Oxide Produced by a Mn-Oxidizing Fungus, Paraconiothyrium Sp. WL-2. *Mater. Trans.* **2008**, *49* (3), 605–611.

(39) Laus, R.; De Fávere, V. T. Competitive Adsorption of Cu(II) and Cd(II) Ions by Chitosan Crosslinked with Epichlorohydrin-Triphosphate. *Bioresour. Technol.* **2011**, *102* (19), 8769–8776.

(40) Taffarel, S. R.; Rubio, J. Removal of Mn2+from Aqueous Solution by Manganese Oxide Coated Zeolite. *Miner. Eng.* **2010**, *23* (14), 1131–1138.

(41) Nelson, Y. M.; Lion, L. W.; Shuler, M. L.; Ghiorse, W. C. Effect of Oxide Formation Mechanisms on Lead Adsorption by Biogenic Manganese (Hydr)oxides, Iron (Hydr)oxides, and Their Mixtures. *Environ. Sci. Technol.* **2002**, *36* (3), 421–425.

(42) Drüeke, T. B. Lanthanum Carbonate as a First-Line Phosphate Binder: The “Cons.” *Semin. Dial.* **2007**, *20* (4), 329–332.

(43) Voncken, J. H. L. *The Rare Earth Elements: An Introduction*; 2016.

(44) Ji, X.; Wu, D.; Wang, Y.; Ge, L.; Hong, W.; Xue, R.; Wang, B. Fabrication of Lanthanum-Based Phosphate Binder Using Cross-Linked Alginate as a Carrier. *RSC Adv.* **2015**, *5* (68), 55191–55200.

(45) Goldberg, S.; Sposito, G. On The Mechanism Of Specific Phosphate Adsorption By

HyDroxylated Mineral Surfaces: A Review. *Commun. Soil Sci. Plant Anal.* **1985**, *16* (8), 801–821.

(46) Yetisen, A. K.; Qu, H.; Manbachi, A.; Butt, H.; Dokmeci, M. R.; Hinestroza, J. P.; Skorobogatiy, M.; Khademhosseini, A.; Yun, S. H. Nanotechnology in Textiles. *ACS Nano* **2016**, *10* (3), 3042–3068.

(47) Yang, B.; Liu, D.; Lu, J.; Meng, X.; Sun, Y. Phosphate Uptake Behavior and Mechanism Analysis of Facilely Synthesized Nanocrystalline Zn-Fe Layered Double Hydroxide with Chloride Intercalation. *Surf. Interface Anal.* **2018**, *50* (3), 378–392.

(1) Theron, J.; Walker, J. A.; Cloete, T. E. Nanotechnology and Water Treatment: Applications and Emerging Opportunities. *Crit. Rev. Microbiol.* **2008**, *34* (1), 43–69.

(2) Hua, M.; Zhang, S.; Pan, B.; Zhang, W.; Lv, L.; Zhang, Q. Heavy Metal Removal from Water/wastewater by Nanosized Metal Oxides: A Review. *J. Hazard. Mater.* **2012**, *211–212*, 317–331.

(3) Environmental Protection Agency (EPA). *Superfund Remedy Report*; 2013; Vol. 14.

(4) Fu, F.; Wang, Q. Removal of Heavy Metal Ions from Wastewaters: A Review. *J. Environ. Manage.* **2011**, *92* (3), 407–418.

(5) Kurniawan, T. A.; Chan, G. Y. S.; Lo, W. H.; Babel, S. Physico-Chemical Treatment Techniques for Wastewater Laden with Heavy Metals. *Chem. Eng. J.* **2006**, *118* (1–2), 83–98.

(6) Ray, P. Z.; Shipley, H. J. Inorganic Nano-Adsorbents for the Removal of Heavy Metals and Arsenic: A Review. *RSC Adv.* **2015**, *5* (38), 29885–29907.

(7) Sawhney, A. P. S.; Condon, B.; Singh, K. V.; Pang, S. S.; Li, G.; Hui, D. Modern Applications of Nanotechnology in Textiles. *Text. Res. J.* **2008**, *78* (8), 731–739.

(8) Kim, Y. J.; Ahn, C. H.; Lee, M. B.; Choi, M. S. Characteristics of Electrospun PVDF/SiO2 Composite Nanofiber Membranes as Polymer Electrolyte. *Mater. Chem. Phys.* **2011**, *127* (1–2), 137–142.

(9) Jin, S.; Park, Y.; Park, C. H. Preparation of Breathable and Superhydrophobic Polyurethane Electrospun Webs with Silica Nanoparticles. *Text. Res. J.* **2016**, *86* (17), 1816–1827.

(10) Wu, S.; Li, F.; Wang, H.; Fu, L.; Zhang, B.; Li, G. Effects of Poly (Vinyl Alcohol) (PVA) Content on Preparation of Novel Thiol-Functionalized Mesoporous PVA/SiO2 Composite Nanofiber Membranes and Their Application for Adsorption of Heavy Metal Ions from Aqueous Solution. *Polymer (Guildf).* **2010**, *51* (26), 6203–6211.

(11) Dong, H.; Wang, D.; Sun, G.; Hinestroza, J. P. Assembly of Metal Nanoparticles on Electrospun Nylon 6 Nanofibers by Control of Interfacial Hydrogen-Bonding Interactions. *Chem. Mater.* **2008**, *20* (21), 6627–6632.

(12) Park, Y.; Ford, E. Titanium Oxide Sol-Gel Induced Wrinkling of Electrospun Nanofibers. *submitted* **2017**.

(13) Park, Y.; Rawat, P.; Ford, E. Role of Polymerized Micelles on the Calcium Carbonate Mineralization of Nanofibers. *Ind. Eng. Chem. Res.* **2017**, *56* (29), 8241–8250.

(14) Harms, H.; Schlosser, D.; Wick, L. Y. Untapped Potential: Exploiting Fungi in Bioremediation of Hazardous Chemicals. *Nat. Rev. Microbiol.* **2011**, *9* (3), 177–192.

(15) Environmental Protection Agency (EPA). *A Citizen's Guide to Bioremediation*; 2012.

(16) Environmental Protection Agency (EPA). *Introduction To in Situ Bioremediation of Groundwater*; 2013.

(17) Santelli, C. M.; Pfister, D. H.; Lazarus, D.; Sun, L.; Burgos, W. D.; Hansel, C. M.

Promotion of Mn(II) Oxidation and Remediation of Coal Mine Drainage in Passive Treatment Systems by Diverse Fungal and Bacterial Communities. *Appl. Environ. Microbiol.* **2010**, *76* (14), 4871–4875.

(18) Burgos, W. D.; Tan, H.; Santelli, C. M.; Hansel, C. M. Importance of Fungi in Biologial Mn(II) Oxidation in Limestone Treatment Beds. *J. Am. Soc. Min. Reclam.* **2010**, *2010* (1), 71–88.

(19) Vail, W.; Riley, R. Process for Removing Manganese from Solutions Including Aqueous Industrial Waste. US5441641A, 1995.

(20) Villalobos, M.; Bargar, J.; Sposito, G. Mechanisms of Pb(II) Sorption on a Biogenic Manganese Oxide. *Environ. Sci. Technol.* **2005**, *39* (2), 569–576.

(21) Remucal, C. K.; Ginder-Vogel, M. A Critical Review of the Reactivity of Manganese Oxides with Organic Contaminants. *Environ. Sci. Process. Impacts* **2014**, *16* (6), 1247.

(22) Santelli, C. M.; Webb, S. M.; Dohnalkova, A. C.; Hansel, C. M. Diversity of Mn Oxides Produced by Mn(II)-Oxidizing Fungi. *Geochim. Cosmochim. Acta* **2011**, *75* (10), 2762–2776.

(23) Toner, B.; Fakra, S.; Villalobos, M.; Warwick, T.; Sposito, G. Spatially Resolved Characterization of Biogenic Manganese Oxide Production within a Bacterial Biofilm. *Appl. Environ. Microbiol.* **2005**, *71* (3), 1300–1310.

(24) Tebo, B. M.; Bargar, J. R.; Clement, B. G.; Dick, G. J.; Murray, K. J.; Parker, D.; Verity, R.; Webb, S. M. Biogenic Manganese Oxides: Properties and Mechanisms of Formation. *Annu. Rev. Earth Planet. Sci.* **2004**, *32* (1), 287–328.

(25) Miyata, N.; Tani, Y.; Sakata, M.; Iwahori, K. Microbial Manganese Oxide Formation and Interaction with Toxic Metal Ions. *J. Biosci. Bioeng.* **2007**, *104* (1), 1–8.

(26) Droz, B.; Dumas, N.; Duckworth, O. W.; Pen, J. A Comparison of the Sorption Reactivity of Bacteriogenic and Mycogenic Mn Oxide Nanoparticles. *Environ. Sci. Technol.* **2015**, *49*, 4200–4208.

(27) Ouvrard, S.; Simonnot, M.-O.; Sardin, M. Reactive Behavior of Natural Manganese Oxides toward the Adsorption of Phosphate and Arsenate. *Ind. Eng. Chem. Res.* **2002**, *41* (11), 2785–2791.

(28) Gadd, G. M. Biosorption: Critical Review of Scientific Rationale, Environmental Importance and Significance for Pollution Treatment. *J. Chem. Technol. Biotechnol.* **2009**, *84* (1), 13–28.

(29) Environmental Protection Agency (EPA). Secondary Drinking Water Standards: Guidance for Nuisance Chemicals https://www.epa.gov/dwstandardsregulations/secondary-drinking-water-standards-guidance-nuisance-chemicals.

(30) Environmental Protection Agency (EPA). National Primary Drinking Water Regulations https://www.epa.gov/ground-water-and-drinking-water/national-primary-drinking-water-regulations#seven.

(31) Gardner, T.; Rivera, N.; Andrews, M.; Santelli, C.; Duckworth, O. Diversity and Abundance of Microbial Community in NC Superfund Site Remediation System Biofilm, and Their Role in Mn Oxidation. In *Synergy in Science: Partnering for Solutions*; American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 2015.

(32) Duckworth, O. W.; Rivera, N. A.; Gardner, T. G.; Andrews, M. Y.; Santelli, C. M.; Polizzotto, M. L. Morphology, Structure, and Metal Binding Mechanisms of Biogenic Manganese Oxides in a Superfund Site Treatment System. *Environ. Sci. Process. Impacts*

2017, *19* (1), 50–58.

(33) Ivarsson, M.; Broman, C.; Gustafsson, H.; Holm, N. G. Biogenic Mn-Oxides in Subseafloor Basalts. *PLoS One* **2015**, *10* (6), 1–13.

(34) Jaworska, M.; Dłużniewska, J. The Effect of Manganese Ions on Development and Antagonism of Trichoderma Isolates. *Polish J. Environ. Stud.* **2007**, *16* (4), 549–553.

(35) Tebo, B. M.; Johnson, H. A.; McCarthy, J. K.; Templeton, A. S. Geomicrobiology of manganese(II) Oxidation. *Trends Microbiol.* **2005**, *13* (9), 421–428.

(36) Yang, D.; Yu, K.; Ai, Y.; Zhen, H.; Nie, J.; Kennedy, J. F. The Mineralization of Electrospun Chitosan/poly(vinyl Alcohol) Nanofibrous Membranes. *Carbohydr. Polym.* **2011**, *84* (3), 990–996.

(37) Julien, C.; Massot, M.; Baddour-Hadjean, R.; Franger, S.; Bach, S.; Pereira-Ramos, J. P. Raman Spectra of Birnessite Manganese Dioxides. *Solid State Ionics*. 2003, pp 345–356.

(38) Sasaki, K.; Matsuda, M.; Urata, T.; Hirajima, T.; Konno, H. Sorption of Co2+ Ions on the Biogenic Mn Oxide Produced by a Mn-Oxidizing Fungus, Paraconiothyrium Sp. WL-2. *Mater. Trans.* **2008**, *49* (3), 605–611.

(39) Laus, R.; De Fávere, V. T. Competitive Adsorption of Cu(II) and Cd(II) Ions by Chitosan Crosslinked with Epichlorohydrin-Triphosphate. *Bioresour. Technol.* **2011**, *102* (19), 8769–8776.

(40) Taffarel, S. R.; Rubio, J. Removal of Mn2+from Aqueous Solution by Manganese Oxide Coated Zeolite. *Miner. Eng.* **2010**, *23* (14), 1131–1138.

(41) Nelson, Y. M.; Lion, L. W.; Shuler, M. L.; Ghiorse, W. C. Effect of Oxide Formation Mechanisms on Lead Adsorption by Biogenic Manganese (Hydr)oxides, Iron (Hydr)oxides, and Their Mixtures. *Environ. Sci. Technol.* **2002**, *36* (3), 421–425.

(42) Drüeke, T. B. Lanthanum Carbonate as a First-Line Phosphate Binder: The “Cons.” *Semin. Dial.* **2007**, *20* (4), 329–332.

(43) Voncken, J. H. L. *The Rare Earth Elements: An Introduction*; 2016.

(44) Ji, X.; Wu, D.; Wang, Y.; Ge, L.; Hong, W.; Xue, R.; Wang, B. Fabrication of Lanthanum-Based Phosphate Binder Using Cross-Linked Alginate as a Carrier. *RSC Adv.* **2015**, *5* (68), 55191–55200.

(45) Goldberg, S.; Sposito, G. On The Mechanism Of Specific Phosphate Adsorption By HyDroxylated Mineral Surfaces: A Review. *Commun. Soil Sci. Plant Anal.* **1985**, *16* (8), 801–821.

(46) Yetisen, A. K.; Qu, H.; Manbachi, A.; Butt, H.; Dokmeci, M. R.; Hinestroza, J. P.; Skorobogatiy, M.; Khademhosseini, A.; Yun, S. H. Nanotechnology in Textiles. *ACS Nano* **2016**, *10* (3), 3042–3068.

(47) Yang, B.; Liu, D.; Lu, J.; Meng, X.; Sun, Y. Phosphate Uptake Behavior and Mechanism Analysis of Facilely Synthesized Nanocrystalline Zn-Fe Layered Double Hydroxide with Chloride Intercalation. *Surf. Interface Anal.* **2018**, *50* (3), 378–392.