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Microplastic Prevalence in Four Oregon Rivers Along a Rural to Urban Gradient Applying a Cost-Effective Validation Technique

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Microplastic prevalence in four Oregon rivers along a rural to urban gradient applying a cost-effective validation technique

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Abstract: Microplastics are ubiquitous in our environment and are found in rivers, streams, oceans, and even tap water. Riverine microplastics are relatively understudied compared to those in marine ecosystems. In Oregon, we sampled eight sites along four freshwater rivers spanning rural to urban areas to quantify microplastics. Plankton tow samples from sites along the Columbia, Willamette, Deschutes, and Rogue Rivers were

analyzed using traditional light microscopy for initial microplastic counts. Application of Nile Red dye to validate microplastics improved microplastic identification, particularly for particles (Wilcox Test; p-value=0.001). Nile Red-corrected microfiber abundance was correlated with human population within five kilometers of the sample site (R^2=0.554), though no such relationship was observed between microparticles and population (R^2=0.183). This study finds plastics present in all samples from all sites, despite the range from undeveloped, remote stretches of river in rural areas to metropolitan sites within Portland, demonstrating the pervasive presence of plastic pollution in freshwater environments.

Key Words: freshwater**,** microfibers, microparticles, Nile Red, population, plankton tow

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Introduction

Plastics, synthetic polymers derived from petroleum, have become a part of daily life in the products that we rely on, with global plastic production estimated at ~330 million metric tons per year in 2016, and projected to double over the next two decades (Lebreton & Andrady 2019). The durability of plastic makes it both appealing as a product and challenging to dispose of properly. Given the limited plastic recycling (Kershaw et al. 2019), a majority of plastic products end up in landfills and experience degradation over time (Lebreton & Andrady 2019). In fact, land-based sources of waste

contribute roughly 80 percent of plastic litter to the marine environment (Sharma & Chatterjee 2017) and up to 12.7 million metric tons of plastic waste generated from coastal countries entered the ocean in 2010 (Jambeck et al. 2015). Despite fairly extensive microplastics research in the marine environment, significant information gaps remain for freshwater systems (Horton et al. 2017).

Microplastics, particles or fibers less than 5 mm in diameter (Erni-Cassola et al. 2017), can be introduced into the environment in many ways. Primary microplastics are manufactured as small particles, while secondary microplastics result from fragmentation of larger plastic debris (Barboza et al. 2018). Microplastics are generally categorized into types including pellets, fragments, fibers, granules, plastic films, and foam (Van Cauwenberghe et al. 2015, Rochman et al. 2019). For this study we grouped observed microplastics into two morphological categories: “fiber” or “particle”.

In marine and freshwater ecosystems, proximity to point and nonpoint sources (e.g., effluent pipes, septic systems, and urban runoff) may affect the amount of plastic found at a given location (Carr et al. 2016). Aquatic microplastics are suspected to originate from wastewater treatment plant (WWTP) facilities and large-scale urban development along freshwater rivers (Eerkes-Medrano et al. 2015, Conley et al. 2019). Microplastics can enter these systems from surface runoff, laundry, and improper waste disposal. Each load of laundry can send hundreds of thousands of microplastics to the WWTP and eventually into aquatic systems (Brodde 2017, Hartline et al. 2016).

There is documented potential for microplastics to cause harm to aquatic organisms and potentially humans that ingest those organisms (Cole 2016, Lebreton &

Andrady 2019, Maes et al. 2017). Microplastics ingested by aquatic species can cause physical and physiological effects, including internal damage to digestive mechanisms, reduced growth rates, and absorption of chemicals bound to microparticles (Cole et al. 2011, Duis & Coors 2016, Lusher et al. 2017). These ingested particles can then accumulate up the food chain as organisms are preyed upon, ultimately bioaccumulating in marine mammals and potentially humans (Rochman et al. 2015, Lebreton & Andrady 2019).

Plankton tows can be used to establish the presence of microplastics in an aquatic environment, as well as to assess microplastic presence within organisms in the food chain. For example, a 2015 Australian study (Hall et al. 2015) used subsurface plankton tows to establish the presence of microplastics in coral reef waters. Hall et al. (2015) found polyurethane, polystyrene, and polyester, which are commonly associated with anthropogenic presence (clothes laundering) and activities like shipping and fishing. Most plastics found were fibrous and less than one mm, suggesting that the microplastics were secondary particles resulting from fragmentation (Hall et al. 2015). Cole's 2016 study also supported the notion that fibers are among the most prevalent microplastic types with synthetic fibers generally manufactured as nylon, polyester, or polypropylene, which are commonly used in the production of textiles and fishing gear. Sources include washing machines, the degradation of cigarette butts, and the fragmentation of nautical equipment like fishing lines and nets (Cole 2016).

To identify microplastics in samples, microscopy coupled with a validation technique is standard (Maes et al. 2017). However, Raman and FTIR validation, the gold standards, are expensive, require trained personnel, and are generally limited to a subset

of samples from the study that are validated, given time and cost constraints. For microplastics research to be more accessible to diverse scientists, including student and non-governmental citizen scientists, a more accessible validation method is required. Nile Red, a lipophilic fluorescent dye able to highlight lipid materials, has gained popularity for the study of microplastics since plastics are petroleum-derived, lipid-containing products. Although more accessible than methods like infrared or Raman spectroscopy, traditional light microscopy can create low data reliability, especially when particles are exceedingly small or clear/white in coloration. As a result, validation is required to confirm that materials counted are, in fact, plastic (Shim et al. 2016; Maes et al. 2017). Nile Red is a cost-effective alternative that is inexpensive to replicate across all samples. Nile Red causes plastics to fluoresce under inexpensive LED light conditions by binding to lipids during the staining process, providing more accurate results than microscopy alone, while reducing validation time and expense.

This project began as a collaboration with Oregon Public Broadcasting (OPB), a local affiliate of the Public Broadcasting System (PBS), to quantify microplastic pollution in Oregon's rivers (Profita & Burns 2019). Collection sites were selected to span rural to urban and undeveloped to developed areas within the Columbia, Willamette, Rogue, and Deschutes River watersheds (Figure 1). The application of Nile Red dye was explored as a low-cost analytical method to improve the accuracy of microplastic identification versus microscopy alone (Maes et al. 2017).

The study objective was to identify patterns of microplastic occurrence in water samples across eight sites on four Oregon rivers testing a cost-effective method to do so. This study sought to answer the questions:

1) Are microplastics present in both rural and urban stretches of rivers in Oregon?

2) Does microplastic abundance correlate with human population?

3) Does the low-cost technique of applying Nile Red dye facilitate microplastic identification of both particles and fibers in freshwater plankton tow samples (e.g., Erni-Cassola et al. 2017)?

We hypothesized that microplastics would be present at all sites; counts would positively correlate with human population density; and that Nile Red would enhance identification over traditional microscopy for all microplastics.

Methods

Study Sites

Eight study sites spanned four rivers in Oregon, ranging from the Columbia River mainstem at the Washington border in the north to the Rogue River in Southern Oregon (Figure 1). Rivers vary in length from the Columbia River spanning 2,010 km to the Deschutes River spanning 280 km (Appendix Table 1). Plastic pollution from point and nonpoint sources is an emerging concern in all four rivers, especially given the cultural and ecological importance of fish species that rely on these habitats for spawning, rearing, and migration.

Samples were collected at eight locations: the Columbia River near St. Helens, the Willamette River near Fall Creek (upstream), Albany (midstream), and Portland (Oregon

Museum of Science and Industry [OMSI] dock; downstream), the Rogue River near Woodruff Bridge (upstream) and in Grants Pass (downstream), and at Big River (upstream) and Tumulo (downstream) on the Deschutes River (Figure 1). Sites were chosen based on a) their proximity to WWTP and urban centers: OMSI in downtown Portland, Albany on the Willamette River, and Grants Pass on the Rogue River or b) their remote locations: Fall Creek on the Willamette, Woodruff Bridge on the Rogue, and Big River on the Deschutes.

Sample Collection

Between September 7th and 14th, 2018, three samples plus a field control were collected from each of the eight sites (n=24) using a General Oceanics plankton tow net with a 0.5m mouth and 200 µm mesh size equipped with a flow meter. Based on previous research studying riverine surface water microplastics using a sample depth of 0.15m (Yonkos et al. 2014), our net was submerged in the river approximately 0.3 to 1m below the river surface (depending on river depth and access conditions) for 15 min to sample subsurface flow (Lenaker et al. 2019). Excess water exited through the mesh netting while debris and plankton were trapped in the cod-end (Vinzant 2016). Samples were poured and the cod-end rinsed with deionized (DI) water into pre-rinsed glass jars for transport back to the Applied Coastal Ecology lab at Portland State University. At each site, a control jar was open during sampling to collect any airborne plastic particles. The total water volume sampled from each river varied greatly, however water volume of each sample collected and rinsed from the net cod-end was approximately 680 mL. Flow was recorded as rotor revolutions (converted to "counts") at each sampling event (see Appendix Table 2), and this value was used to calculate sample volume (m^3) using the

equation: area [(3.14 x (net diameter)²)/4] x distance [(counts x rotor constant)/999999], based on the General Oceanics flow meter user guidelines.

Tissue Dissolution

The plankton tow samples contained a significant amount of biological material making microscope inspection of microplastics difficult. To avoid misidentification, a potassium hydroxide (KOH) digestion was performed to remove naturally-occurring biological material from the samples (Rochman et al. 2015). Each sample was filtered with a 200 µm strainer and the remnants were rinsed into a beaker with 400 mL of filtered DI water and a 10% potassium hydroxide (KOH) solution (Rochman et al. 2015). Covered beakers sat on a 60^{o}C hotplate with a stir bar for 24 hrs before being filtered into a petri dish. Samples that remained murky after the first digestion were split into two petri dishes and were digested a second time to increase clarity. Despite these extra steps, many samples remained muddy so density separation was utilized to effectively isolate the plastics from the biological material (Masura et al. 2015).

Density Separation

Samples were rehydrated, scraped with a shucking tool to loosen the sample from the bottom of the dish, then added to a hypersaline solution with a ratio of 168.4 g of salt (NaCl) to 2 L of water. Jars were sealed and shaken vigorously for 60 sec, then returned to the lab bench for the contents to separate and stratify. Since the hypersaline solution causes heavier sediment particles to sink to the bottom of the jar, while the lighter plastic particles floated to the top (Thompson et al. 2004), heavy plastic particles may have been lost during this step (Crichton et al. 2017). Once the solution had stratified, the liquid was

removed using a vacuum filtration set up: a 2 L glass Erlenmeyer flask connected to the sink faucet by a rubber tube (see Appendix Figure 1) with a glass filter (Whatman 1820-047 Glass Microfiber Binder Free Filter, 1.6 Micron, 4.3 s/100mL Flow Rate, Grade GF/A, 4.7cm Diameter, Amazon) atop. The quart sample jar was then opened and the top layer was poured out to ensure that the plastics were filtered but no sediment was included. Once the water in the beaker was sucked into the Erlenmeyer flask, and the plastic particles were left on the filter paper, it was lifted and transferred to a new petri dish using Excelta 5-SA stainless steel precision tweezers. Petri dish lids were secured with two rubber bands, and the filter papers were stored in a cardboard box for microscope analysis. All glassware in the vacuum setup was rinsed twice with DI water between samples. Nitrile gloves and cotton lab attire were worn during processing to minimize contamination.

Microscope Analysis

Initial microscope analysis (methodology adopted from the Marine & Environmental Research Institute "Guide to Microplastic Identification" nd) differentiated the suspected microplastics by color. Each filter was viewed on a Leica MZ6 light microscope using 40x magnification. Per method protocol, each filter was scanned in its entirety. The physical characteristics of each suspected microplastic were assessed using precision tweezers to test malleability. Parameters including thickness, homogeneity of color, and presence/absence of cellular structures were assessed visually to differentiate plastic from natural materials ("Guide to Microplastic Identification" nd, Masura et al 2015). Each suspected microplastic was photographed and shape and color were recorded. While assessing each filter, a petri dish with DI water sat at the back of

the microscope to collect any potential contamination from the microscope lab room. Each control dish was analyzed under the scope after its corresponding filter paper sample, and contamination was recorded. This procedure was repeated for each filter paper and control pre- and post-Nile Red dye application (April-May, 2019). Field and lab controls were calculated and reported as average microplastic contamination per site (See Appendix Tables 3 and 4).

Nile Red dye preparation, application, and microscope analysis

One mg Nile Red (Santa Cruz Biotechnology, SC-203747C) was mixed with 1 mL acetone to create a stock solution, that was diluted with 100 mL of hexane to create a working solution of 10 µg Nile Red/mL (Wiggin & Holland 2019). After thorough mixing with a stir bar for 3 to 5 hours, the working solution was transferred into an amber dropper bottle, and the solution was applied to each filter paper until coated (about nine drops) and allowed to dry on a 12-hr, 30°C cycle in a drying oven (Wiggin & Holland 2019).

Microscope analysis was repeated for each filter paper and microscope control post Nile Red dye application. To create proper light conditions for fluorescence, the lab room was completely dark and orange safety goggles were taped under the microscope lens to create an orange viewing environment. A 455 nm LED flashlight (Arrowhead Forensics PART NO: A-6994FK) was used to illuminate the samples (Figure 2), causing fluorescence (Wiggin & Holland 2019).

Quality Control

All glassware and lids were rinsed twice with DI water to avoid microplastic contamination. Glassware was inverted or covered if not in use, and controls were used both in the field and lab to quantify contamination. Proper lab attire included nitrile gloves, 100% cotton t-shirt and lab coat to avoid contamination. The following controls were included to account for microplastic contamination during field collection and lab processing: a mason jar was left open during field sampling, and again in lab during the hypersaline procedure; a petri dish was left open during each microscope analysis, and an open dish was left in the oven during the drying cycle (Baechler et al. 2019). Contamination in the above controls was summed and reported per site (Appendix Tables 3, 4).

Data Analysis

All Nile Red statistical analyses were conducted in R Studio version 1.1.453. To test for significant differences, nonparametric t-tests were run between the number of fibers and particles before and after dye application. Shapiro Tests revealed data were abnormal, thus the non-parametric Wilcox Test was used to compare microplastic counts before and after dye application (significance level of <0.05). Tests revealed more microplastics post Nile Red dye than initially counted, so Nile Red "after" counts were used for the site population comparison.

Collection site GPS coordinates were used to determine population estimates within a 5 km radius of each location using Population Estimation Service, a web-based GIS tool developed by NASA's Center for International Earth Science Information

Network (CIESIN) (CIESEN, 2019). Population estimates were derived from the Gridded Population of the World (GPW) v4.11 developed by the Socioeconomic Data and Applications Center (CIESIN, 2019). Population and site information were projected onto a map using ArcGIS Desktop version 15.5.1. (ESRI, 2017).

Aggregated daily data from the collection date based on United States Geological Survey (USGS) or Oregon Water Resources Department (OWRD) flow meters near each sampling location were used to identify flow at or near the sample locations. Microplastics concentration data after Nile Red and the flow data converted into m^3/sec were used to calculate microfibers/sec or particles/sec. We multiplied the per second counts by 3600 (60 sec/min X 60 min/hr) to calculate the number of microfibers/microplastics flowing through the sampling location hourly. Microfiber and microparticle per hour data were regressed onto the 5 km radius human population estimate data using simple linear regression in R Studio. Variables were plotted and fitted with regression lines to explore the strength of linear relationships.

Results

Plastic particles and fibers were found in all samples collected, although the quantity varied significantly. Sizes ranged from 5mm down to 200um as indicated by filters used. Nile Red results revealed a total of 265 fibers ranging from 2 to 30 per sample. Particles totaled 99, ranging from 0 to 23 per sample. This higher occurrence of fibers is consistent with recent literature reporting that microplastic composition in fresh and marine water columns is dominated by fibers at 52%, followed by “fragments” at 29% (Burns & Boxall 2018). For example, the surface water section of the

comprehensive San Francisco Bay microplastic study listed the dominant morphology as fibers followed by “fragments” (Sutton et al. 2019). Similar conclusions have also been demonstrated in estuarine environments (Hitchcock & Mitrovic 2019). Nile Red dye affected microplastic identification, particularly with clear and white fibers and particles. There was minimal difference in the fiber counts before and after Nile Red dye application (Wilcox Test; W statistic =168, p=0.084). However, we positively identified significantly more particles after dye application (Wilcox Test; W statistic = 109, p=0.001). The highest fiber concentrations were found by the OMSI dock (Portland, Oregon), however sample variability within a site was high (Figure 3A, note error bars). The highest particle concentrations were seen at Albany, OMSI, and Tumalo (Figure 3B, Table 1). Sample collection at Albany occurred during a major rainstorm and plastic trash was visible and abundant in the river during sampling.

Microfiber counts per hour (R^2=0.554; F=7.466 on 1 and 6 DF; p=0.034), but not microparticle counts per hour (R^2=0.183; F=1.343 on 1 and 6 DF; p=0.29), correlated with human population density within 5 km (Figure 4A and B).

Discussion

The presence of microplastics in all samples from both urban and rural sites further supports the pervasiveness of microplastics in freshwater systems in Oregon. Specifically, the Oregon river samples indicate a range of contamination, a projected 144 to 2.9 million microfibers per hour, and 48 to 122,000 microparticles per hour passing sample locations, with correlation of microfibers to adjacent human population. The high concentrations of microplastics in Oregon rivers with culturally and ecologically

important fisheries (Myers et al. 2006) highlights the need to better understand how microplastics are entering these rivers and may affect fish populations and the broader ecological communities. In addition, these rivers are of high recreational importance, raising the question of how microplastics may affect human recreational users. In systems with distinct rainy/dry seasons that are not effluent-dominated, first flush periods can be important sources of contaminant loading to downstream systems and tend to have the highest concentrations of contaminants (Hurley et al. 2018). Since samples were collected in early fall before the rainy season, during the lowest flow period of the year (with the exception of the Albany site sampled during an early fall storm), and since we only sampled a small section of the water column, these data likely under-represent the average fiber concentration throughout the water column and annually. As such, these data establish a microplastics baseline representing a snapshot in time.

Our project further supports the value of Nile Red dye as a validation tool, particularly for citizen science-based and student-driven projects that may lack funds to validate microplastics using more expensive techniques (also see Maes et al. 2017; Wiggin & Holland 2019). Nile Red also saved valuable sample processing time by making it easier to isolate plastics. Any sediment obscuring the filter essentially disappeared when the lights were turned out (Figure 2), eliminating background material that originally took significant time to differentiate. Although Nile Red may be an improvement in current practice, it is not a perfect method. Organic debris on the filter paper creates the potential for co-staining of biological material (Helmberger et al. 2020). Thus, it is important to conduct a digestion step and employ knowledge of plastic behavior and visual characteristics to confirm each potential plastic particle or fiber.

Specifically, methods including chemical digestion and density separation are essential for samples to be as "clean" as possible before dyeing.

Several potential sources of error exist in our study. The Columbia River could be an outlier in terms of number of microfibers per population size (Figure 4A) because the samples were collected from a location with a low population within 5km, but the collection site is downstream of dense urban populations including Portland, Oregon and Vancouver, Washington, potential sources of plastics floating downriver. Similarly, Albany microparticle numbers may be an outlier (Figure 4B) since it was the only site sampled during a rain event, possibly skewing the microparticle count relative to the other sites sampled during drier periods. The plankton tow was submerged approximately 0.3 to 1m below the surface at each site to maintain a uniform methodology across samples, but different densities of plastic float in different depths of the water column (Engler 2012, Lenaker et al. 2019); as this variability is not accounted for in our sample design, we expect we under-sampled very light as well as very dense plastics both in the field and during our density separation step during which we may have lost heavier microplastics that sank (Crichton et al. 2017). Additionally, flow was not recorded or incorrectly recorded for one Eugene and one W. Rogue sample, so those site averages were based on two instead of three samples. When USGS or OWRD flow meter data were not in close proximity to the sample sites, daily average flow was calculated by combining flow from the gauge upstream with any discharge that came into the river from tributaries upstream of the sampling site, per guidance of a USGS hydrologist.

In an effort to limit false positives or negatives that could misinform managers and the public, a number of modifications and/or improvements for future studies are

recommended, since study design, quality assurance measures, and microplastic quantification remain non-uniform (Burton 2017). First, rinsing and removing large organic debris (leaves, sticks, algae) prior to KOH digestion would speed up sample processing as presence of large amounts of macro-debris greatly slowed the process. Although the relationship between microplastics and human population utilized radial population surrounding the site, given potential for visitation and recreational use by residents in close proximity, other measures of human population (such as population within the upstream watershed) may yield stronger correlation. It is notable that microplastics were present at sites with low human influence (Table 1). One driver of microplastic presence that we were unable to quantify was the role of improved infrastructure in removal of plastic at WWTPs, an interesting avenue for future study. Further research is also needed to understand finer scale spatial variability in microplastic abundance. Increased understanding of this spatial variability could provide important information for managers and policy makers to more effectively implement measures to reduce contamination in both marine and freshwater environments. Finally, this study supports the existing literature that microplastics are ubiquitous in the natural environment, even in remote locations, and that Nile Red dye aids in citizen-science and GK-16 student-based microplastic research and monitoring.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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at [provided URL]. Learn more about the Open Practices badges from the Center for Open Science: https://pdxscholar.library.pdx.edu.

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Figure 1: The eight sampling locations and their respective populations within a 5 km radius; population density represented by graduated circles.

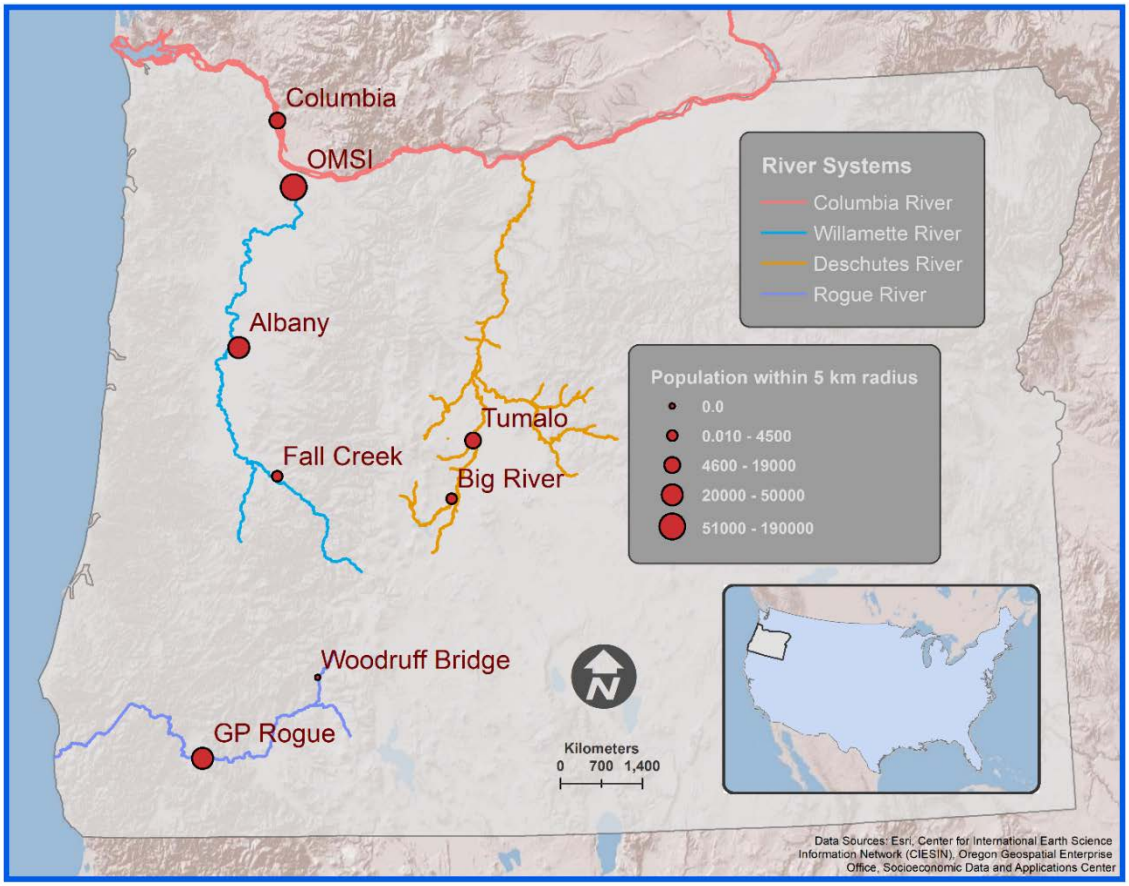


Figure 2: Example of Nile Red fluorescence from the Fall Creek 3 sample.

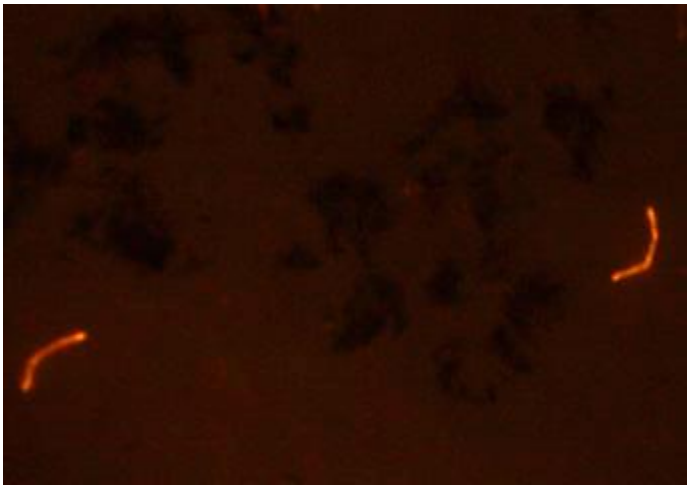


Figure 3: Average number of A) fibers and B) particles per m^3 of water by site. Sites are arranged in descending order from highest to lowest values after Nile Red dye application. Bars represent standard error.

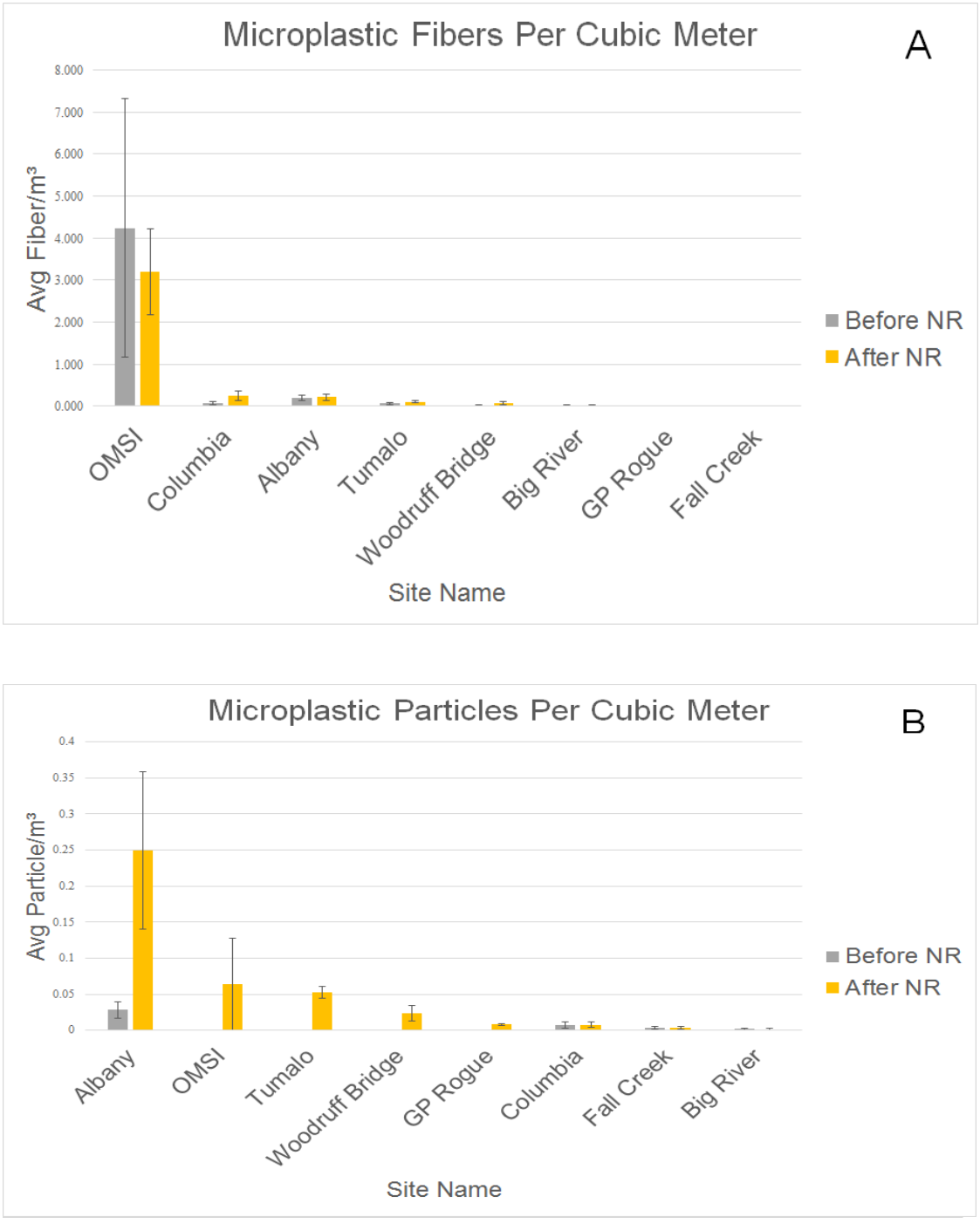
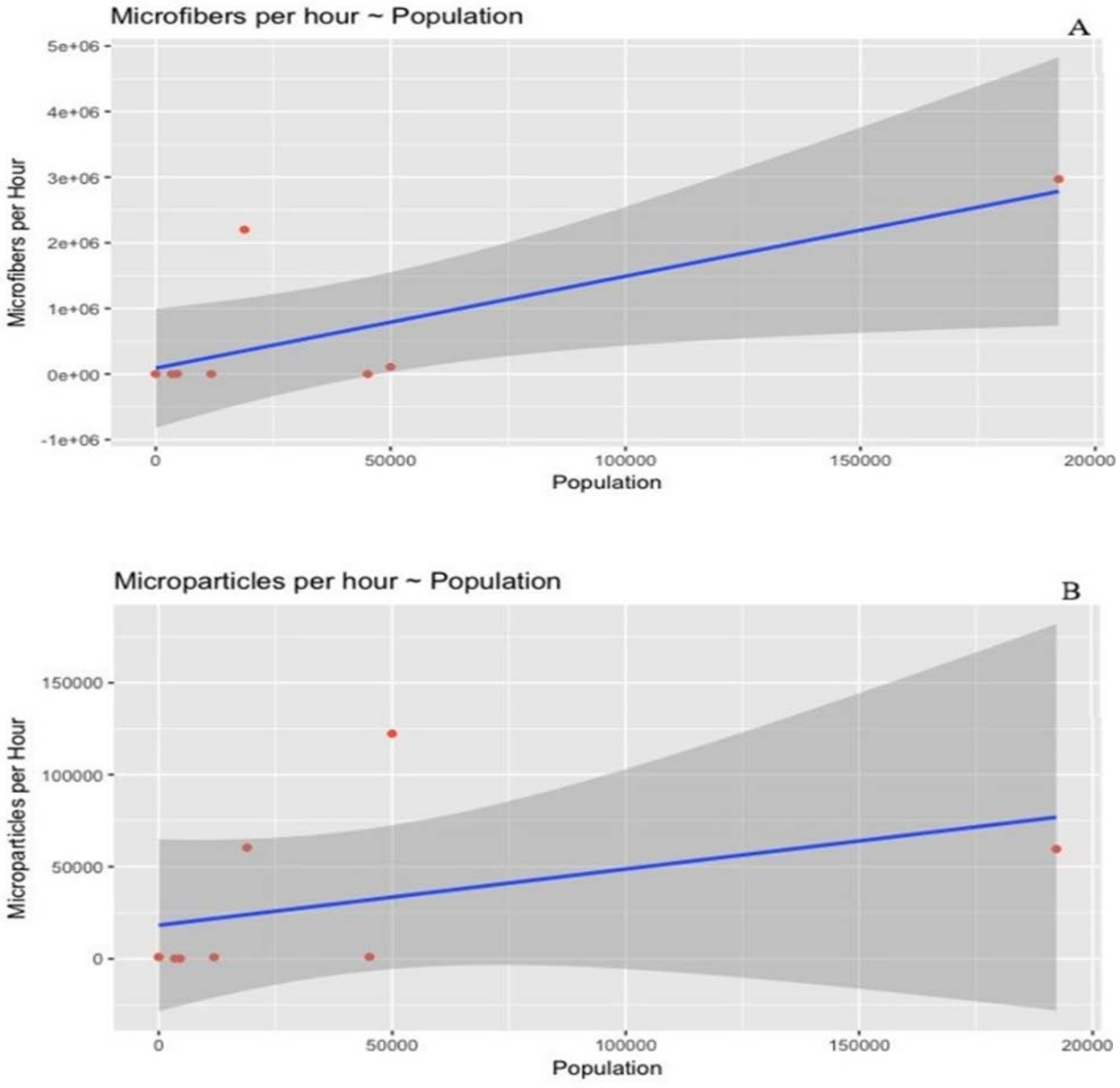


Figure 4: Scatter plots showing the relationship between population within a 5km radius of sampling locations versus the calculated rate per hour of microfibers (A) and microparticles (B) at each location. Standard error is shown in the dark grey shaded areas. Microfiber rates appear to exhibit a marginal positive linear relationship with population, whereas the microparticle rate does not. Note the different scales between A and B.



Graphical Abstract: The eight microplastic sampling locations and their respective populations within a 5 km radius; population represented by graduated circles. Microplastic fibers per cubic meter are also graphed to show differences before (grey) and after (orange) the application of Nile Red dye.

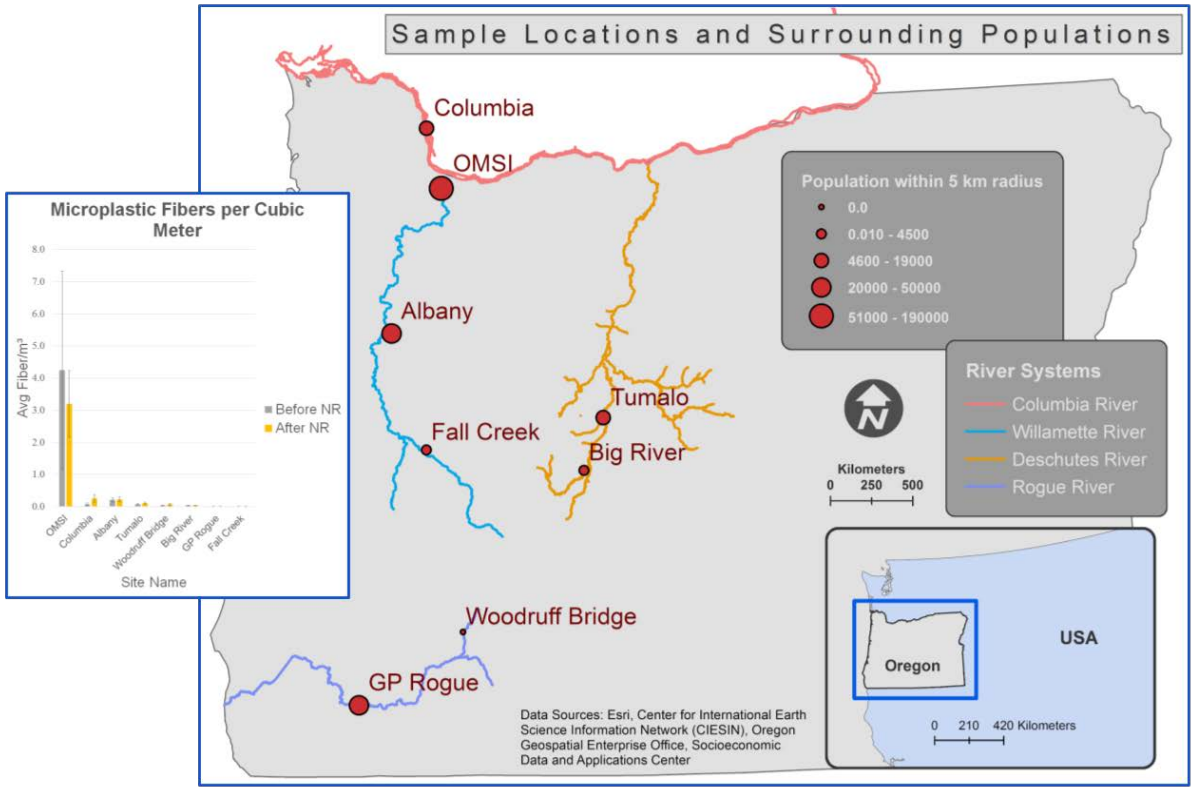


Table 1: Population estimates within a 5km radius and microplastic averages after NR at each site. The far-right column represents the average microplastic percent that could be attributed to contamination.

Site Name	2015 Population Estimate	Avg Fiber/m³	Avg Particle/m³	% of Contamination per Site

Woodruff Bridge	0	0.063	0.023	54.20
Fall Creek	3434	0.009	0.003	0
Big River	4536	0.034	0.001	18.88
Tumalo	11815	0.107	0.052	3.76
Columbia	18887	0.255	0.007	4.89
GP Rogue	45110	0.013	0.007	14.31
Albany	49947	0.22	0.249	8.41
OMSI	192200	3.19	0.064	19.06