Analysis of Organic and Heavy Metal Pollutants in Marine Planktonic Organisms from Bamfield, British Columbia by ICP-OES and GC-MS

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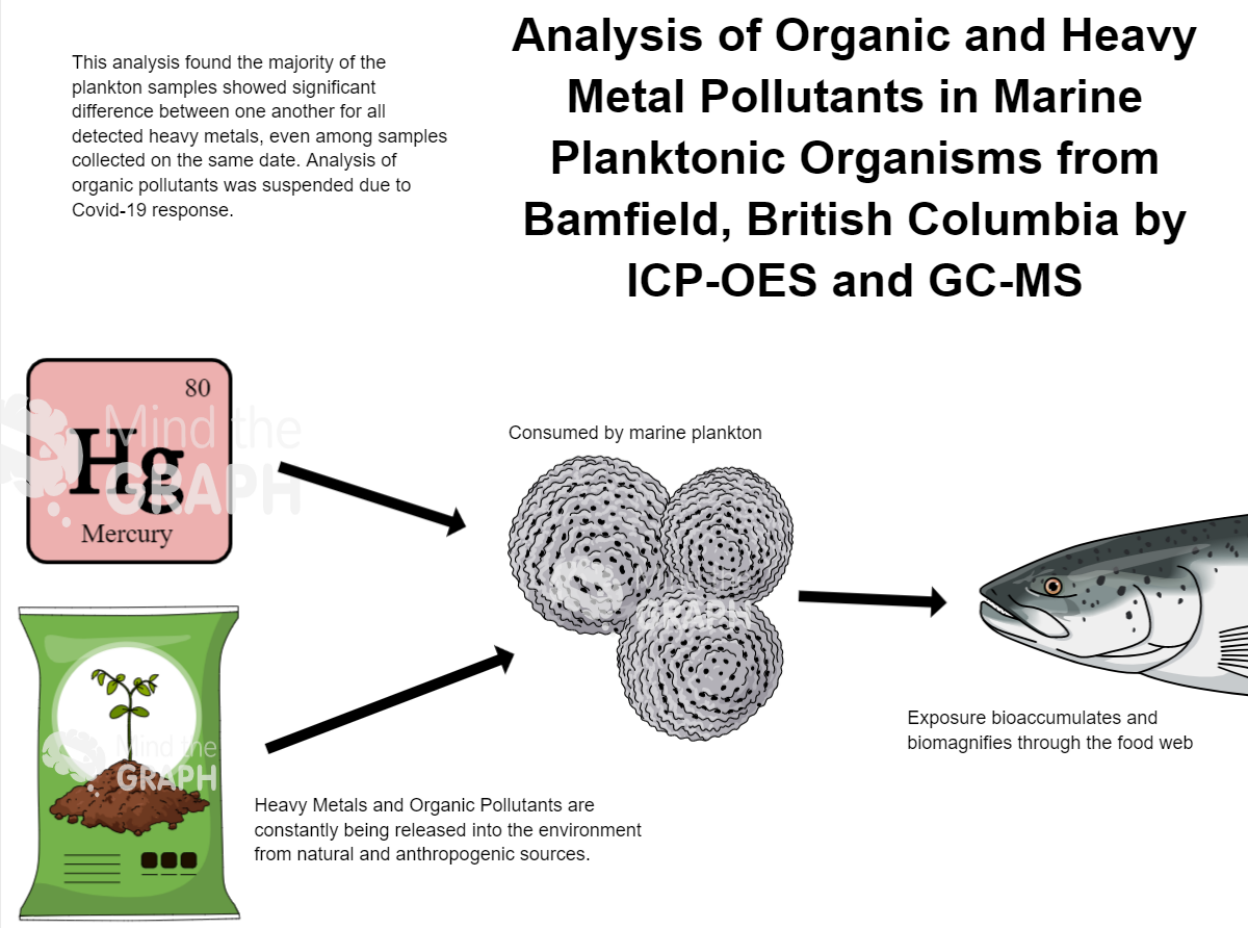
Abstract:



Plankton form the base of the marine ecosystem (Levinton, J.S., 2017). It is for this reason it is important to understand the interactions between these critical organisms and the pollutants that they encounter. Heavy metals occur naturally in trace amounts and are continuously being released into the atmosphere, hydrosphere, lithosphere, and biosphere of the Earth (Atici et al., 2010; Burada et al., 2014; Callow, P., 1993). The increase in sea water temperatures is increasing the solubility of heavy metals in the water (Levinton, J.S., 2017). Organic pollutants such as pesticides are hydrophobic and lipophilic (Chiuchiolo et al., 2004).

Previous results obtained by Kathryn Farmer in 2015 found that an increase in some heavy metals and not others in the four year difference in the Bamfield samples. Analysis of

organic pollutants by GC-MS was unable to determine if peaks were the result of pesticides or not and was not able to identify any specific pesticides through the NIST library (Farmer, 2015).

This analysis found the majority of the plankton samples showed significant difference between one another for all detected heavy metals. Analysis of organic pollutants was suspended sue to Covid-19 response.

Key Words: Plankton, Heavy Metals, Pesticides, ICP-OES, GC-MS

Introduction:

Plankton form the base of the marine ecosystem (Levinton, J.S., 2017). It is for this reason it is important to understand the interactions between these critical organisms and the pollutants that they encounter. With a high surface area to volume ratio, planktonic organisms have many reception sites with which to sorb nutrients (Jurik et al., 2011; Yigiterhan et al., 2011). Electrostatic and covalent bonds are formed with pollutants and they are drawn into the plankton, concentrating them into a much smaller volume (Gonzalez-Davila, M., 1995; Yigiterhan et al., 2011).

Heavy metals occur naturally in trace amounts and are continuously being released into the atmosphere, hydrosphere, lithosphere, and biosphere of the Earth (Atici et al., 2010; Burada et al., 2014; Callow, P., 1993). There are natural sources, such as volcanoes, natural weathering, and thermal vents, anthropogenic sources, such as mining, the extraction of gold and silver, and the combustion of fossil fuels, and natural sources being accelerated by anthropogenic activity, such as the thawing of permafrost being accelerated by anthropogenic climate change (Baird & Cann, 2012; Burada et al., 2004; Calow, P., 1993; Stern et al., 2012; Government of Canada, 2010). Mercury, lead, cadmium, chromium, and arsenic become more

toxic as a cation and highly toxic when bonded to a short carbon chain (Baird & Cann, 2012). The increase in sea water temperatures is increasing the solubility of heavy metals in the water (Levinton, J.S., 2017).

Organic pollutants such as pesticides are hydrophobic and lipophilic (Chiuchiolo et al., 2004). Chlorinated pesticides in sea water photolyze and can have a half-life as small as 55 days (Lartiges, S.B., & Garrigues, P.P., 1995). Herbicides such as Atrazine, the most commonly used herbicide in America at the time, prevent photosynthesis and restrict phytoplankton growth (DeNoyelles et al., 1982). Other pesticides like Cypermethrin, commonly used in UK fish farming operations, alter biodiversity by restricting some plankton more than others (Medina et al., 2004).

Previous results obtained by Kathryn Farmer in 2015 were based on two samples collected from Bamfield, British Columbia in 2011 and 2015 and one from Hilo Bay, Hawai'i in 2015 for a comparison to determine if heavy metals and organic pesticides are being circulated by the ocean global conveyer system. Plankton identification was only conducted on samples from 2015 (Farmer, 2015). Both samples predominantly contained diatoms with Skeletonema being the most abundant species (Farmer, 2015). The Bamfield sample was rich in arthropods, cnidarians, and larval chordates while the Hawai'i sample had much more phytoplankton, no cnidarians, and very few chordates (Farmer, 2015). Samples were not large enough to accurately determine species richness and diversity at either site (Farmer, 2015).

Analysis of heavy metals found an increase in some heavy metals and not others in the four year difference in the Bamfield samples (Farmer, 2015). Mercury, arsenic, cadmium, and cobalt concentrations increased, chromium concentrations decreased, and lead concentrations

remained approximately constant (Farmer, 2015). A comparison of the samples collected in 2015 from Bamfield, British Columbia and Hilo Bay, Hawai'i found lower concentrations of arsenic, cadmium, cobalt, mercury, and lead in Hawai'i but higher concentrations of chromium (Farmer, 2015). Additionally a comparison between the Hawai'i plankton sample and a sample of sea water collected at the same site found that the plankton selects for specific heavy metals (Farmer, 2015).

Analysis of organic pollutants by GC-MS was unable to determine if peaks were the result of pesticides or not and was not able to identify any specific pesticides through the NIST library (Farmer, 2015). The control plankton, while helpful, was not able to account for the diversity of biota in the marine samples (Farmer, 2015).

These curious results merit further investigation and experimentation. I hypothesize that: with increasing ocean temperatures, and the subsequent increase in solubility, concentrations of heavy metals in marine plankton will be higher than previous results in 2011 and 2015; with the continued use of organic pesticides, the concentrations of organic pollutants in marine plankton will be increased relative to concentrations in 2015.

Materials & Methods:

Methods were modified from Farmer's work in 2015 to accommodate small sample sizes.

Standards of Calcium, Iron, and Selenium were donated to MacEwan University, originally produced by SPEX CertiPrep. Standards of Cobalt and Manganese were donated to MacEwan University, originally produced by SPC Science. Standards for Arsenic, Cadmium, and Mercury were purchased from Fluka Analytical. Standards of Copper, Lead, and Zinc were

purchased from Fisher Chemical. The standard for Chromium was produced at MacEwan University.

Restek organonitrogen pesticide mix #1 and chlorinated pesticide #2 standards and fatty acid methyl ester (F.A.M.E.) C8-C24 standard from Sigma Aldrich.

Sample Collection and Preparation:

Twenty samples were collected from Bamfield, British Columbia by the Bamfield Marine Sciences Centre between the dates of October 10, 2018, and February 19, 2019. Plankton samples were collected at the head of the Grappler Inlet at 48° 50.867" N and 125° 6.475" W with a 150 µm mesh net. The samples were then preserved in 70 % ethanol at 5 °C until analysis. Preserved samples were placed in a laminar flow hood to allow the ethanol to evaporate off. Remaining ethanol was removed using a LABCONCO FreeZone 6 freeze drier.

Plankton Taxonomy:

Plankton specimen were identified to Order and Family using a dissecting microscope and counted based on their respective group order. Larger plankton specimen required the use of a compound microscope.

Heavy Metals by ICP-OES:

Procedure was modified from Jaric et al. (2011). 50 mg of freeze dried plankton was weighed into a 100 mL beaker using a Mettler Toledo XS204 analytical balance. 10 mL of concentrated hydrochloric acid (36-38 %) was slowly added to samples followed by 2 mL of concentrated nitric acid (68-70 %) and a magnetic stir bar. Samples were placed on a stir plate set to 200 °C and 60 rpm in a laminar flow hood until approximately 10 mL of sample remained. After digestion the sample was cooled to room temperature and vacuum filtered using a

sintered glass filter and rinsed with deionized water. Samples were then qualitatively transferred into a 25 mL volumetric flask and diluted to volume with deionized water until analysis. Samples were analyzed using a Thermoscientific iCAP 6000 series ICP spectrometer using iTEVA control center software. Standards of arsenic, cadmium, cobalt, copper, chromium, mercury, lead, selenium, and iron will be made from stock 1000 ppm solutions to obtain a calibration curve at concentrations of 0.1 ppm, 1.0 ppm, 5.0 ppm, and 25 ppm. A t-test was used to determine the statistical significance of differences in metal concentrations across the samples.

Organic Pollutants by GC-MS:

Procedure was modified from Santhi et al. (2012). 50 mg of freeze dried plankton was weighed into a 10 mL glass vial using a Mettler Toledo XS204 analytical balance. 3 mL of dichloromethane was added to the vial and sonicated for 200 minutes at room temperature (23 °C). After extraction, samples were vacuum filtered with 75 µm filter paper and resuspended in 1 mL of dichloromethane to be transferred into a GC vial before being dried under nitrogen using a small aluminium manifold, and resuspended in 0.5 mL dichloromethane. Samples were analyzed on an Agilent Technologies 5975C VL MSD GC-MS with triple-axis detector controlled by ChemStation software and will be separated using a HP-5 5 % Phenyl Methyl Siloxane GC column coupled with a quadrupole mass spectrometer with helium carrier gas. Chemometrics was used to accentuate relevant data from the chromatogram and better identify relevant peaks. These peaks were then compared to the NIST library. A control plankton was not used because it could not adequately account for the variety of plankton within each sample.

Results:

Figure 1. Analysis of Low Concentration Heavy Metals in Marine Plankton

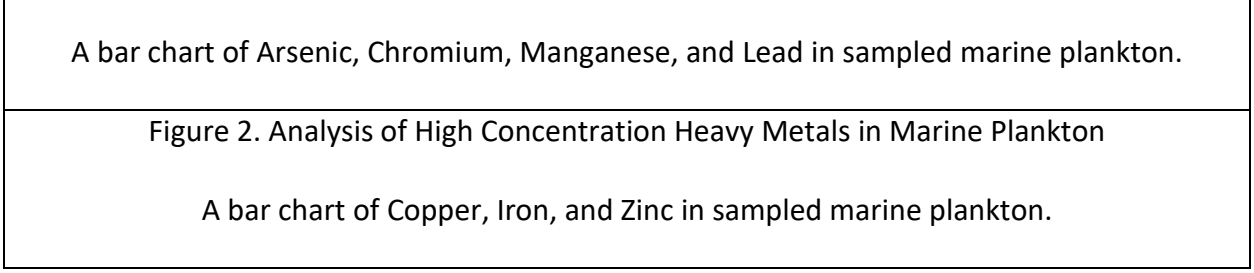
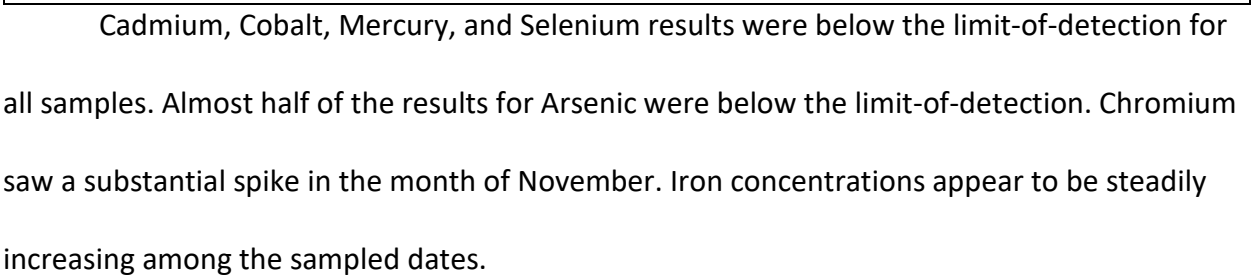
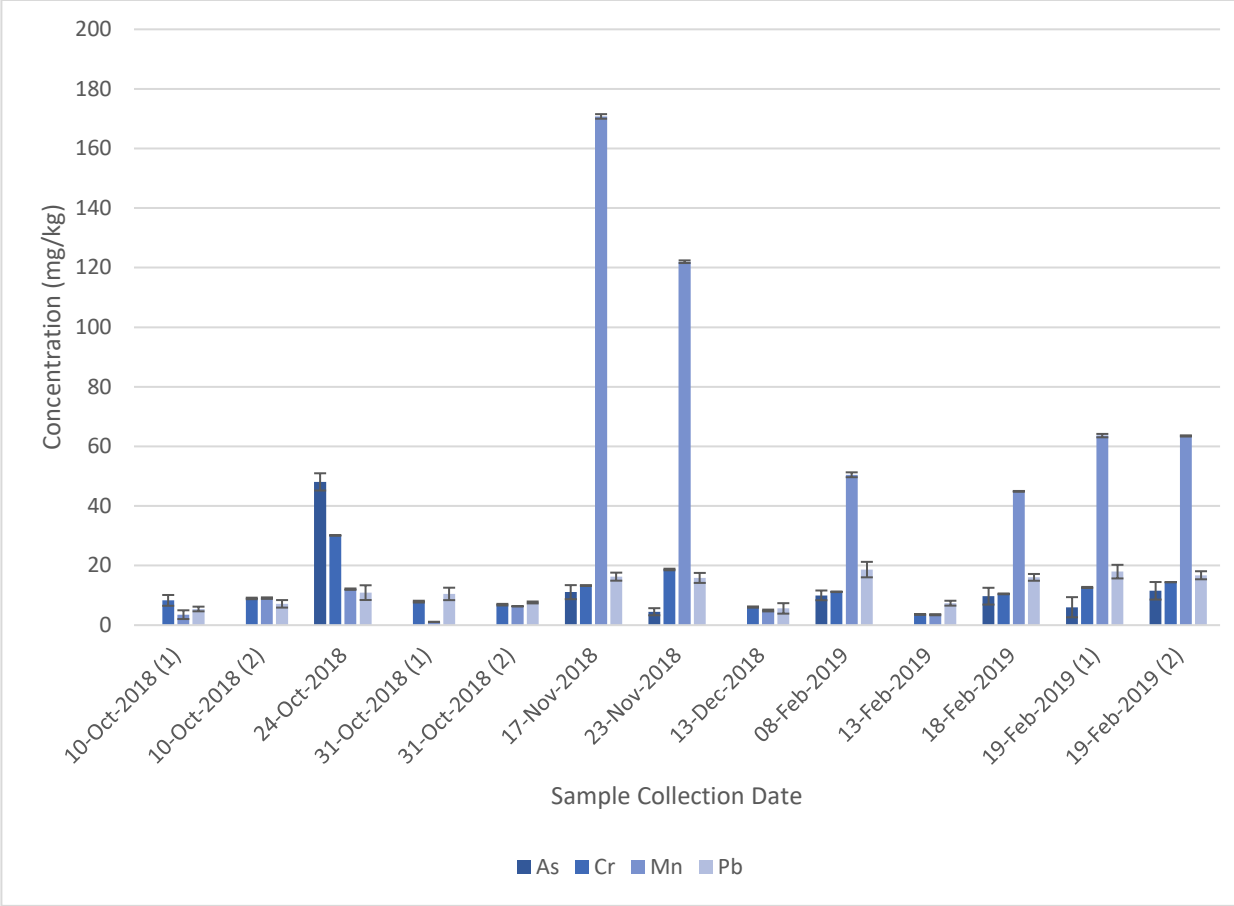


Figure 2. Analysis of High Concentration Heavy Metals in Marine Plankton



Cadmium, Cobalt, Mercury, and Selenium results were below the limit-of-detection for all samples. Almost half of the results for Arsenic were below the limit-of-detection. Chromium saw a substantial spike in the month of November. Iron concentrations appear to be steadily increasing among the sampled dates.

An array of t-tests found that at the 95 % confidence interval (t-crit = 2.132) the majority of samples were significantly different from one another, even among samples collected on the same day.



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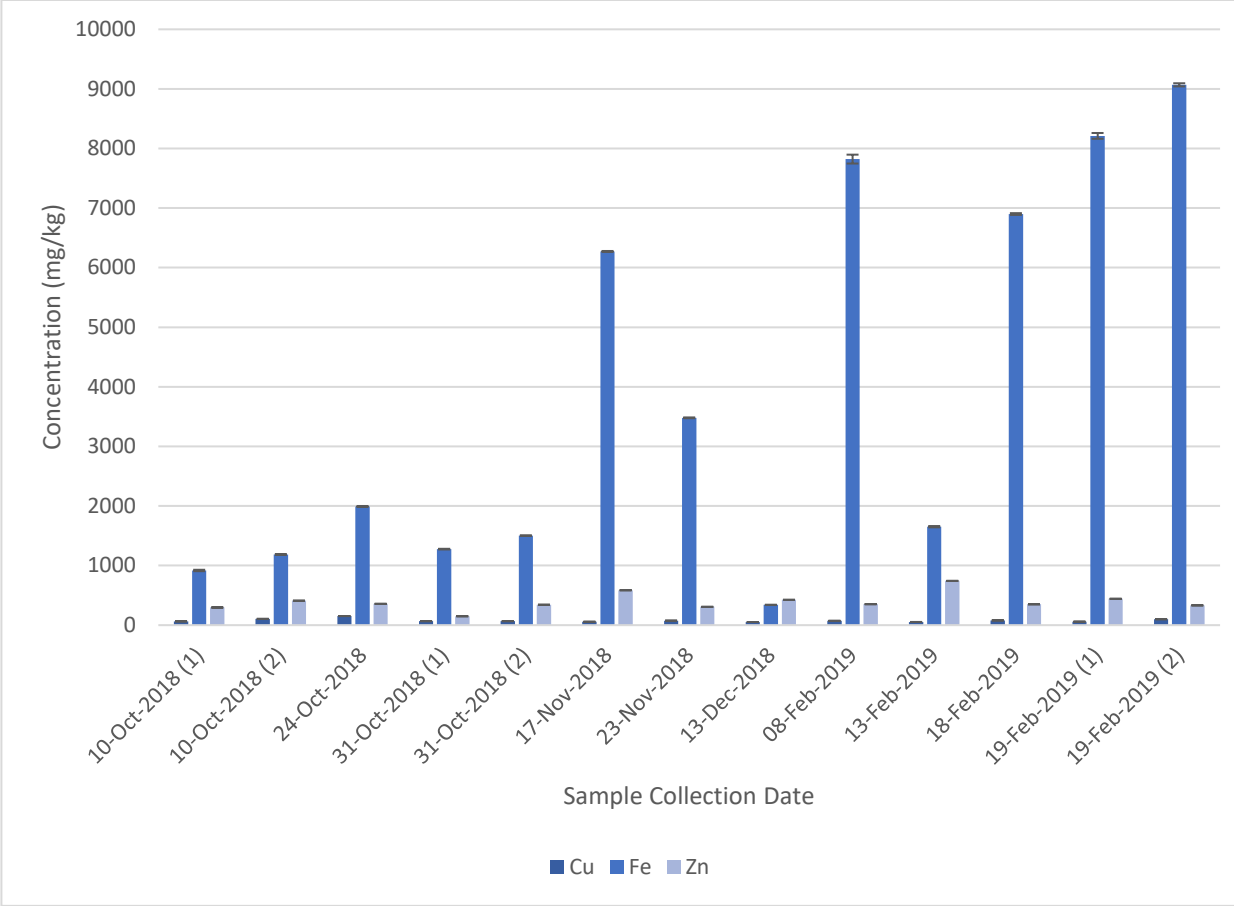

Figure 2. Analysis of High Concentration Heavy Metals in Marine Plankton

Discussion:

The majority of the samples showed significant difference between one another for all detected heavy metals. Increased sample frequency and longer collection timelines would assist in identifying any present trends. Collecting larger samples would also help quantify the elements that were below the limit-of-detection. Some of the values for heavy metal concentrations were significantly higher than the values collected by Farmer in 2011 and 2015 while others were significantly lower.

Analysis of organic pollutants and plankton taxonomy was suspended due to Covid-19 response.

Future avenues of research include but aren't limited to: determining if there are any seasonal increases or decreases in plankton pollution throughout the year which may help identify more specific sources to these pollutants; development of an artificial intelligence capable of identifying and quantifying organic pollutants regardless of sample medium; a comparison of the heavy metal and organic pesticide pollution in the Pacific, Atlantic, and Arctic Oceans in Canada; and Investigating why plankton select for some heavy metals and not others as described in the Hawai'i samples in Kathryn Farmer's original work (2015).

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