ANALYSIS OF PUGET SOUND NEARSHORE SEDIMENT AND GHOST SHRIMP (*Neotrypaea californiensis*) SPECIMENS IN GRAY WHALE (*Eschrichtius robustus*) FEEDING SITES

by

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ABSTRACT

Analysis of Puget Sound nearshore sediment and ghost shrimp (*Neotrypaea californiensis*) specimens in gray whale (*Eschrichtius robustus*) feeding sites.

Megan Bungum

The purpose of this study is to evaluate the environmental health of migratory gray whale (*Eschrichtius robustus*) feeding sites and their preferred Puget Sound prey, ghost shrimp (*Neotrypaea californiensis*). This was determined by examining the presence and/or concentration of known biologically adverse contaminants. The concentrations of Polychlorinated biphenyls (PCBs), Mercury (Hg), and Aluminum (Al) were evaluated from near-shore sediment and ghost shrimp specimen samples in two known gray whale feeding sites located in the Whidbey Island Basin of Puget Sound. An additional sampling site, located in Port Susan, was also evaluated for near-shore sediment samples. This sampling site lies ~4.89 nautical miles NW from observed feeding sites and was chosen for its ghost shrimp population and public accessibility. Samples (n = 10) of near-shore sediment were collected from area sediment (n = 5) in the three sites during an active feeding period of the migratory Puget Sound gray whales, February – April 2021. Samples (n = 2) of ghost shrimp specimens (n = 4) were also collected from several gray whale feeding pits in an active feeding site during this period. Hg was not detected in any of the samples analyzed. Al concentrations were detected in all sediment and animal samples analyzed (Sample ID #3 = 6,720 mg/kg, Sample ID #4 = 6,170mg/kg, Sample ID #5 = 6,610 mg/kg, Sample ID #6 = 692 mg/kg) from the second survey period, in April 2021. The Al concentrations were noted as elevated according to the Washington Department of Ecology’s Clarc table of soil method A unrestricted land use. PCBs were detected in a single sediment sample taken from a gray whale feeding pit (Sample ID #5) as the Aroclor 1016 and Aroclor 1260. The value of Aroclor 1016 (Sample ID #5 = 1.7mg/kg) was notably higher than the recommended parameters set by the Washington Department of Ecology’s Clarc table of soil protective of groundwater saturated (0.12 mg/kg). The value of Aroclor 1260 (Sample ID #5 = 2.1mg/kg) was also substantially higher than the recommended parameters set by the Washington Department of Ecology’s Clarc table of soil method B noncancer (0.5mg/kg) and soil protective of groundwater saturated (0.036mg/kg).

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Chapter One: Migratory Gray Whales

Introduction

Gray whales (*Eschrichtius robustus*) are an iconic mysticeti (baleen) species to the western coast of the United States of America, which are famous for having the longest migratory journey (~22,000 km; NOAA 2021) of known mammals. The gray whale spends its breeding and calf rearing months in the waters of Baja, Mexico, and travels to arctic waters near Alaska to feed on the highly productive blooms of benthic sea life (Usha et al. 1993). As gray whales migrate along the western coast of the United States, they travel past or through Puget Sound, Washington between the months of December-February (south-bound migration) and February-May (north-bound migration) (Usha et al. 1993). These whales have been observed entering this urban coastline in search of benthic prey since the 1980's.

Although the vast majority of gray whales on the migratory path do not enter Puget Sound to feed, some do and they are referred to as "Sounders", "Saratoga Grays", or "Puget Sound Regulars" (Usha et al. 1993). These whales have been shown to recruit others to enter Puget Sound and an overall increase in gray whales entering this area has risen yearly (Usha et al. 1993). This rise in individuals entering Puget Sound in search of prey has also been linked to poor body conditions and emancipated whales (Varanasi et al. 1993). This raises the question of a possible lack of food resources for migratory whales, which may force them off their pathways in search of prey in this region.

The prey which these whales seek in Puget Sound is presumed to be the ghost shrimp (*Neotrypaea californiensis*) (Usha et al. 1993). Gray whales consume their prey by sucking in large quantities of water and sediment and then filtering out the excess materials to trap the prey in their baleen (Weitkamp et al. 1992). The consumption of urban coastal sediment by this species raises questions about the possible contamination of sediment by heavy metals, mercury, and anthropogenic compounds, such as PCBs, which may have negative health effects.

Population History & Unusual Mortality Event (UME)

Gray whales were once commonly found throughout the Northern Hemisphere, with robust populations residing in both the Northern Atlantic and Northern Pacific Oceans (NOAA Fisheries 2020). The Northern Atlantic populations were eradicated in the 18th century by what is presumed to be commercial whaling practices, environmental stressors, or a combination of both (Bruniche-Olsen et al. 2018). The remains of these populations are now limited in their geographic distribution to the Northern Pacific Ocean, with two distinct subpopulations, "stocks", inhabiting the western coasts of North America (Eastern Pacific Gray Whale) and the eastern coasts of Asia (Western Pacific Gray Whale) (NOAA Fisheries 2020) (Figure 1; NOAA Fisheries 2021).

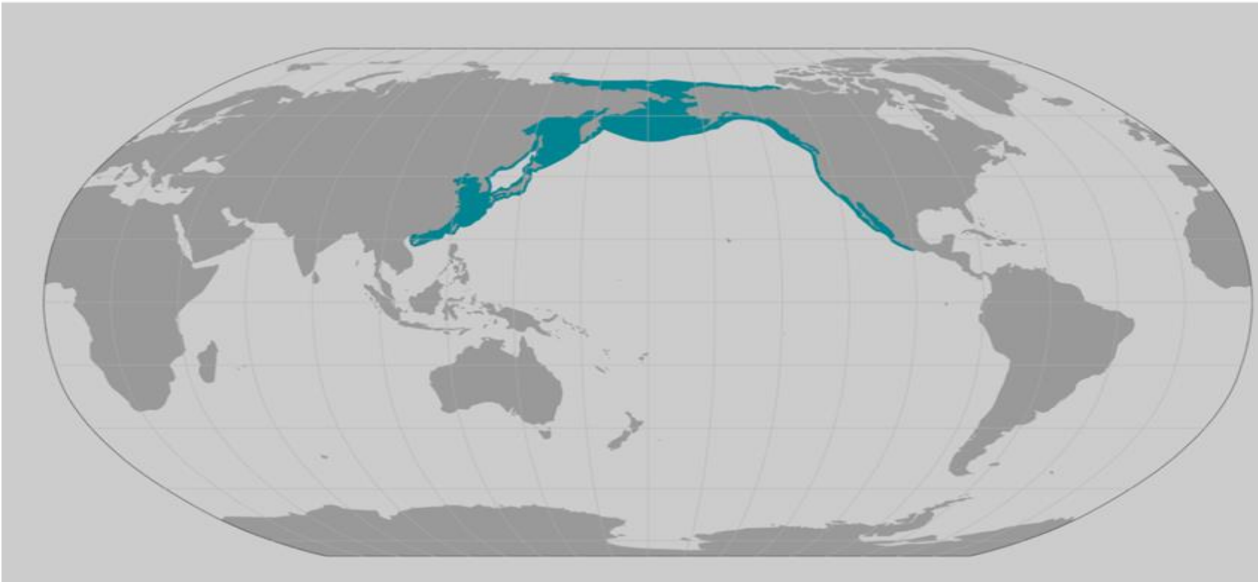

Figure 1. Total gray whale (Eastern and Western Pacific gray whale) distribution range in the Northern Pacific Ocean, highlighted in blue. (NOAA Fisheries 2020).

The Eastern Pacific stock gray whales migrate annually along the western coast of North America, where strandings and Unusual Mortality Events (UME) have increased over the past 20 years (Stimmelmayr & Gulland 2020). The first documented UME took place in 1999-2000, where a total of 651 gray whales stranded (Raverty et al. 2020). This UME accounted for an overall net loss of nearly 20% of the Eastern Pacific stock population (Laake et al. 2012). For comparison, the previous mean annual stranding rate was estimated to be only 41 individuals between the years of 1995-1998 (Raverty et al 2020). This figure remained at or below 28 individuals until 2019 (Raverty et al 2020). In 2019 another UME took place, in which a total of 215 total gray whale stranded (Figure 2; NOAA Fisheries 2020) (Raverty et al. 2020).

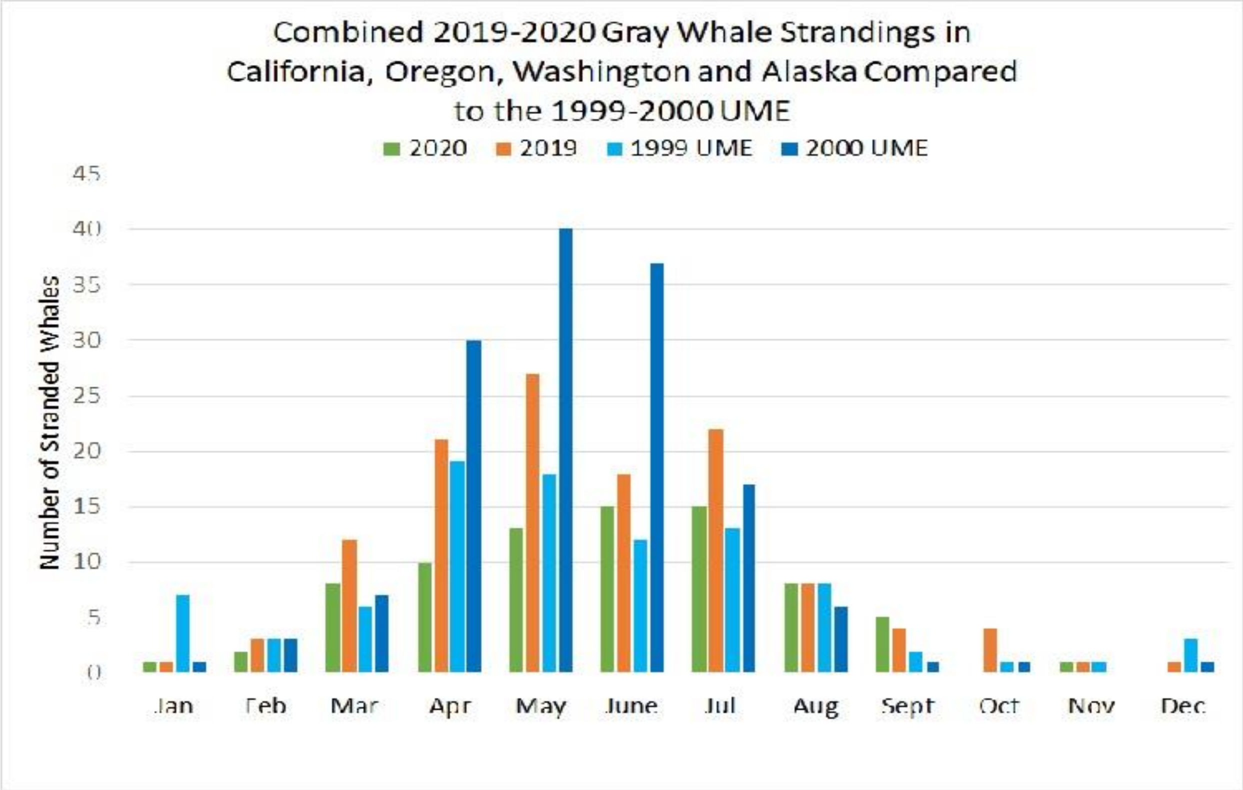

Figure 2. Comparison of 2019-2020 (2019 = Orange, 2020 = Green) and 1999-2000 (1999 = Light Blue, 2000 = Dark Blue) UME Eastern Gray Whale stranding frequencies (NOAA Fisheries 2020).

A single identifiable cause of these Eastern stock strandings and UMEs is currently unknown, but several research studies have explored hypotheses of potential toxins and contaminants as a source of mortality. Gray whales may be especially predisposed to contaminants and toxins due to their near shore feeding habitats and sediment filter feeding mechanism. The consumption of sediment, especially from heavily developed urban coastal regions like the Puget Sound, raises questions about possible ingestion of foreign materials, toxins, and contaminants. Heavily urbanized coasts provide chronic exposure to environmental chemicals (Wise et al. 2019) and some of these compounds are associated with negative health effects through the process of bioaccumulation and storage in tissues.

A further exploration of the sediment quality in known feeding sites, with concentrations of potential contaminants, such as aluminum, mercury, and PCBs are needed to address these questions and concerns. Along with an assessment of sediment quality data, it is also essential to examine literature on the toxicity, biological pathways, and source of these contaminant materials.

Chapter Two: Literature Review: Toxicity of Contaminant Materials

Introduction: Overview of Essential & Non-essential Metals

There are two general forms of metals which effect the biological functioning of large pelagic marine mammals, and these are separated into the groups of essential metals and non-essential metals. Essential metals are those which are required for proper biological functioning and biochemical processes, and these include; copper, zinc, iron, and selenium (Bowles 1999). These essential metals are generally required in low doses to sustain biogeochemical processes and an imbalance in concentrations can produce disease or other deleterious effects (Bowles 1999). The non-essential metals are those which are not known to provide biological or biochemical function to marine mammals and are instead often toxic at low levels (Bowles 1999). These non-essential metals include mercury, lead, aluminum, and cadmium (Bowles 1999).

These two types of metals can enter the pelagic environment in a variety of ways and have the propensity to bioaccumulate in cells. These metals can exist in the water column and benthic sediment by both natural and anthropogenic routes. The natural existence of metals can be sourced from erosion, volcanic activity, and inputs from freshwater sources (Bowles 1999). The anthropogenic sources of metals can be attributed to waste disposal, mining operations, industrial inputs, fossil fuels, and the use and disposal of chemicals (Bowles 1999). These metals have the ability to bio magnify or bioaccumulate in cells by becoming stored in soft and hard tissues (Martinez-Finley et al. 2012). Both types of metals are presumed to use the same cellular transport pathways to enter cells and can be taken into the body through pulmonary (inhalation), cutaneous (skin), or oral ingestion routes (Martinez-Finley et al. 2012).

The bioaccumulation and biomagnification of non-essential metals affects members of the cetacean family differently, and this is due to both the physiological manner in which they ingest prey and the trophic level of prey items (Kershaw & Hall 2019). The taxonomic family of cetacean is split into the two suborders of Odontocetes (toothed whales) and the Mysticeti (baleen whales). The Odontocetes use their toothed mouths to feed primarily higher in the trophic food chain and ingest a variety of fish, birds, squid, shark, and other cetaceans (Barros & Clarke 2009). The Mysticeti, which include the gray whale species, are filter feeders which ingest water and sediment which is then filtered out to trap prey which is much lower on the trophic food chain. A few examples of these prey items include a variety of small fish, zooplankton, shrimp, and krill (Barros & Clarke 2009). Due to the bioaccumulation and biomagnification properties of non-essential metals, the ingestion of higher trophic level prey leads to greater levels of toxicity in the suborder Odontocetes (Kershaw & Hall 2019).

Toxicity of Non-Essential Metals

Aluminum

Aluminum exists in nature, primarily in rocks and minerals, as the most naturally abundant metal on Earth's surface (Perrollaz et al. 1990). Aluminum is rarely found in its elemental form and is instead most prevalent as a trivalent Al^{3+} metal (Perrollaz et al. 1990). The toxicity of aluminum is associated with its bioaccumulation in skeletal and soft tissues and is a controversial neurotoxin (Perrollaz et al. 1990). Since Al is a non-essential metal, with no known biological function in mammalian bodies, it is presumed that excess amounts detected in tissues are adverse.

The controversial neurotoxic effects of Al stem from studies of Alzheimer's Disease (AD), multiple sclerosis, Parkinson's Disease, and dementia in humans. Al is known to be present in human brain tissue and is shown to accumulate with age (Exley 2014). This accumulation of Al and other metals in the human brain are linked to the pathogenesis of AD (Percy et al. 2011). AD can be characterized by an unusual distribution of metal ions, such as iron, aluminum, copper, and zinc in the human brain (Percy et al. 2011). The free metal cation of Al^{3+} is both highly biologically reactive and biologically available to neurological cells (Exley 2014). It is proposed that the ease of oxidation of Al^{3+} may lead to a chemical reactive pathway (Figure 3; Percy et al. 2011.), which creates reactive oxygen species (O^{2-}, H_2O_2, HO) and prooxidants (AlO_2^{2+}) harmful to biological processes (Percy at al. 2011).

1. $2{}^{\cdot}O_{2^-} + 2H^+ \rightarrow H_2O_2 + O_2$
2. $Fe^{2+} + H_2O_2 \rightarrow HO^{\cdot} + OH^- + Fe^{3+}$ (Fenton reaction)
3. ${}^{\cdot}O_{2^-} + Fe^{3+} \rightarrow O2 + Fe2+$
4. ${}^{\cdot}O_{2^-} + H_2O_2 \rightarrow HO\cdot + OH^- + O_2$ (Haber Weiss reaction)

Figure 3. Metal-ion reaction series depicting iron (Fe^{3+}) and suggesting a pathway for Al^{3+}, which can produce prooxidant and superoxide (Percy et al. 2011).

In human and lab animal trials, Al^{3+} is also shown to be deposited in skeletal, liver, and kidney cells. These studies further revealed links between Al toxicity and renal (kidney) failure in rats (Martinez-Finley et al. 2012). The effects of Al on cetaceans are currently unknown, but a study of juvenile gray whale necropsies discovered Al concentrations which were in relatively high concentrations in liver (4,200 ng/g wet weight), kidney (2,800 ng/g wet weight), and brain tissues (1,000 ng/g wet weight) (Tilbury et al. 2002). The highest overall levels of Al observed in this study came from the stomach contents (3,900,000 ng/g wet weight) of the juvenile gray whales, and it was suggested that this may be due to the bioavailability of Al in sediment, along with the diet, feeding behavior, and feeding location preferences (Tilbury et al. 2002).

Mercury

Mercury (Hg) exists in nature in two distinct physical forms, as vapor (Hg^0) and methylmercury compounds (CH_3Hg^+) (Clarkson 1997). The main source of Hg on earth occurs naturally, in a global Hg cycle, through geological activity (volcanoes and geothermal vents), erosion, plant growth, degassing from aquatic and terrestrial environments, and burning of biomass (Gwoerk et al. 2016). Hg can become water soluble and enter marine aquatic systems through surface gas exchanges, or as input from land run-off and freshwater sources (Clarkson 1997). Hg accumulates in marine sediments, which become a substantial sink for Hg (Gwoerk et al. 2016), and is known to undergo many transformations, including methylation (Clarkson 1997). The methylation of Hg by benthic bacteria (Clarkson 1997) causes the binding of Hg to biological proteins (Qiying et al. 2021). These Hg containing proteins then bioaccumulate up the trophic food chain through predator/prey interactions (Clarkson 1997).

The toxicity of Hg is attributed to its bioaccumulation effects in soft tissues and is a well-known neurotoxin, genotoxin, and immunotoxin (Kershaw & Hall 2019). The toxicity of Hg in cetaceans is linked to hepatic (liver) and renal damage (Kershaw & Hall 2019). Hg is known to cross the blood barrier *in utero* and is suggested to effect juvenile and adult cetaceans differently (Bolea-Fernandez et al. 2019). In a study of long-finned pilot whales, it was shown that Hg levels were higher in the muscle tissues of juvenile whales and lower in the liver tissues (Bolea-Fernandez et al. 2019). The opposite of this was shown in adult whale tissue, with higher levels of Hg observed in the liver tissues and lower levels observed in muscle tissues (Bolea-Fernandez et al. 2019). It was suggested that this difference in Hg levels identified in tissues may be due to changes in Hg metabolism and detoxification mechanisms (Bolea-Fernandez et al. 2019). The movement of Hg from liver to muscle tissue, may be a detoxification technique to protect sensitive organs (Bolea-Fernandez et al. 2019).

It has also been hypothesized that Hg contamination may affect members of the cetacean family differently, with high contaminant levels reported in some members of cetaceans who show no signs of toxicity (Kershaw & Hall 2019). The demethylation process and the interaction of selenium (Se) with methyl mercury (MeHg) may account for the high levels of Hg in cetacean tissues with no outward signs of toxicity (Kershaw & Hall 2019). However, the unique feeding mechanism of the gray whale, which involves the continuous filtering of benthic sediment material, may especially expose this cetacean to the more toxic form of MeHg (Gui D et al. 2014). The methylation of Hg occurs in benthic sediments, where the less toxic form of aqueous Hg is converted to the highly neurotoxic MeHg by sulfate and iron reducing bacteria (Gui D et al 2014). MeHg can both biomagnify and bioaccumulate in soft tissues, and this creates health concerns to the individual and greater trophic food chain (Gui D et al. 2014).

Toxicity of PCBs

Polychlorinated biphenyls (PCBs) are anthropogenic compounds which do not occur naturally in the environment. The toxicity of PCBs is attributed to their lipophilic nature and ability to both biomagnify and bioaccumulate in soft tissues, such as, fat and blubber (Jones & de Voogt 1999). PCBs are a part of a broader group of synthetic substances, known as persistent organic pollutants (POPs), which are known to cause neurotoxicity, reproductive failure, cancer, and immunotoxicity (Mongillo et al. 2012). The affinity of PCBs and other POPs to accumulate and magnify in fat and blubber tissue creates a specific threat to cetacean species, and this is due to its release from blubber tissue into the bloodstream and its transference to offspring during gestation and lactation. The migratory Eastern Pacific stock of gray whales depend on the breakdown of their blubber storage to make a successful round-trip journey, and this may cause toxic levels of PCBs to enter sensitive tissues and the bloodstream.

A blubber biopsy study of PCB concentrations in killer whales (n = 47) which inhabit the greater Salish Sea, has revealed concerning levels of PCBs (Ross et al. 2000). These biopsies were taken from three separate communities of killer whales, which are the northern residents, southern residents, and the transients (Ross et al. 2000). The transient killer whales, which are migratory and feed on marine mammals, had the highest levels of PCB contaminants (males: 251 ± 54.7 mg/kg, females: 58.8 ± 20.6 mg/kg) (Ross et al. 2000). The southern residents, which reside year-round in Puget Sound and feed on salmon, had the second highest levels of PCB contaminants (males: 146.3 ± 32.7 mg/kg, females: 55.4 ± 19.3mg/kg) (Ross et al. 2000). The northern residents, which reside in Canadian waters and feed on salmon, had the lowest levels of PCB contaminants (males: 37.4 ± 6.1 mg/kg, females: 9.3 ± 2.8 mg/kg) (Ross et al. 2000). It is suggested that the higher concentrations of PCBs observed in the southern resident killer whales

(SRKW), which consume similar prey to the northern residents, may be due to localized contaminated prey or deposits of PCBs (Ross et al. 2000).

Further research into predicted levels of PCB and PBDE accumulations in SRKW tissue revealed that calves were projected to contain the highest levels of total PCBs and PBDEs (polybrominated diphenyl ethers) of any age class, followed by adult males, then post-reproductive females (Mongillo et al. 2012). It is noted that PCBs have caused behavioral changes in mice, and calf mortality in bottlenose dolphins, but no such conclusive links have been reached with the SRKW (Mongillo et al. 2012). This study did note that the SRKW is one of the most PCB contaminated cetacean species in the world and that PBDEs were shown to increase exponentially with age class (Mongillo et al. 2012).

Although the SRKW may be especially contaminated with PCBs and PBDEs, in waters which it shares with migratory gray whales, those contaminant levels have been declining in other local species. In a 2013 study of blubber biopsies taken from juvenile harbor seals, it was revealed that PCBs, PBDEs, PCDEs (polychlorinated diphenyl ethers), and PCNs (polychlorinated naphthalene) have declined in Salish Sea harbor seals (Ross et al. 2013). This study observed an overall decrease (71% - 98%) of PCBs, PCDEs, and PCNs in harbor seal tissue from 1983 to 2013 (Ross et al 2013). It is noted, however, that the highest levels of PCBs and PCDEs observed in this study were taken from harbor seal populations which reside in southern Puget Sound waters (Ross et al. 2013).

Chapter Three: Methods

Site Selection

Three sites were chosen for field sampling, based on previous literature and observations (Figure 4; CRC 2014) of heavily concentrated gray whale feeding pits and known ghost shrimp population sites. Accessibility to sites was also taken into consideration.

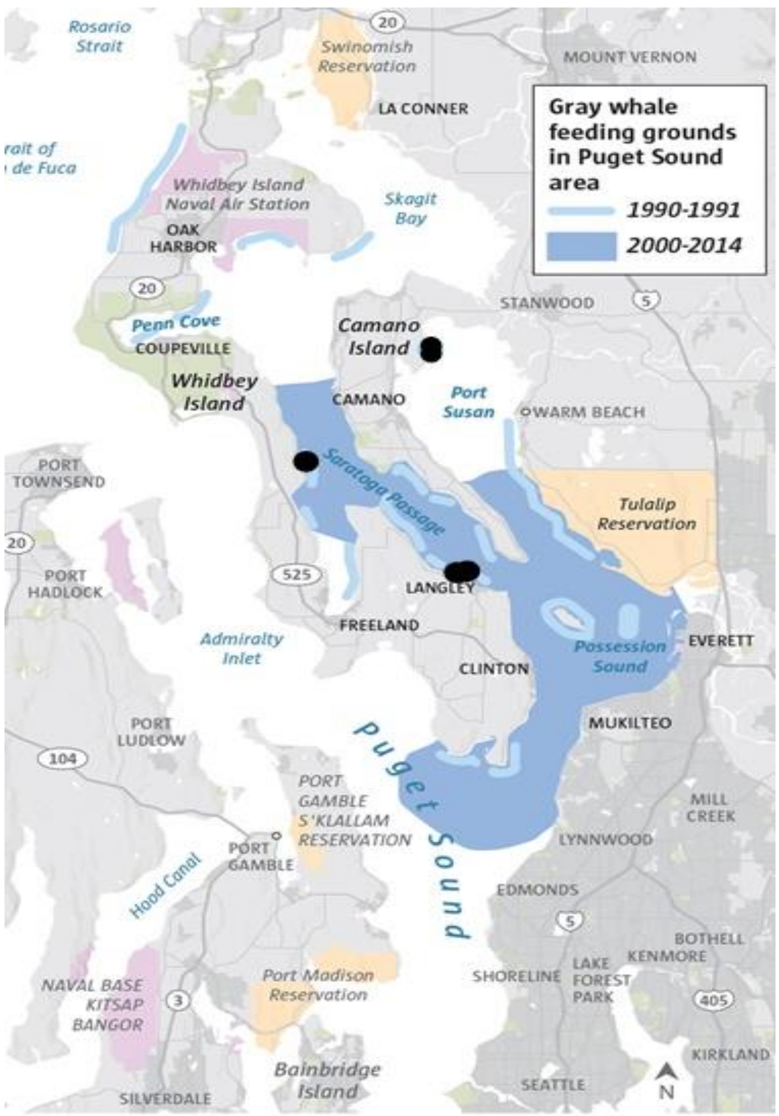

Figure 4. Gray whale feeding site locations (1990-2014) of the Whidbey Basin in Puget Sound (Cascadia Mammal Research Center 2014). Sampling locations (2021) added by the author, in black circles.

The sites used for field sampling in this study are located in the Whidbey Island Basin and Saratoga Passage of Puget Sound, on the islands of Camano and Whidbey (Figure 5.). The sites selected on Whidbey Island are located on its eastern portion and include the Langley Waterfront in Langley, WA, and Hidden Beach in Greenbank, WA. Both sites have been recorded as annual feeding grounds for migratory gray whales. The site selected on Camano Island is located on the northeastern portion of the island and includes the Iverson Trail Preserve Beach. This site is not known as a gray whale feeding ground and lies ~ 4 to 5 nautical miles NW of annually observed feeding grounds. This site was chosen for its known ghost shrimp population, proximity to observed feeding grounds, and public accessibility.

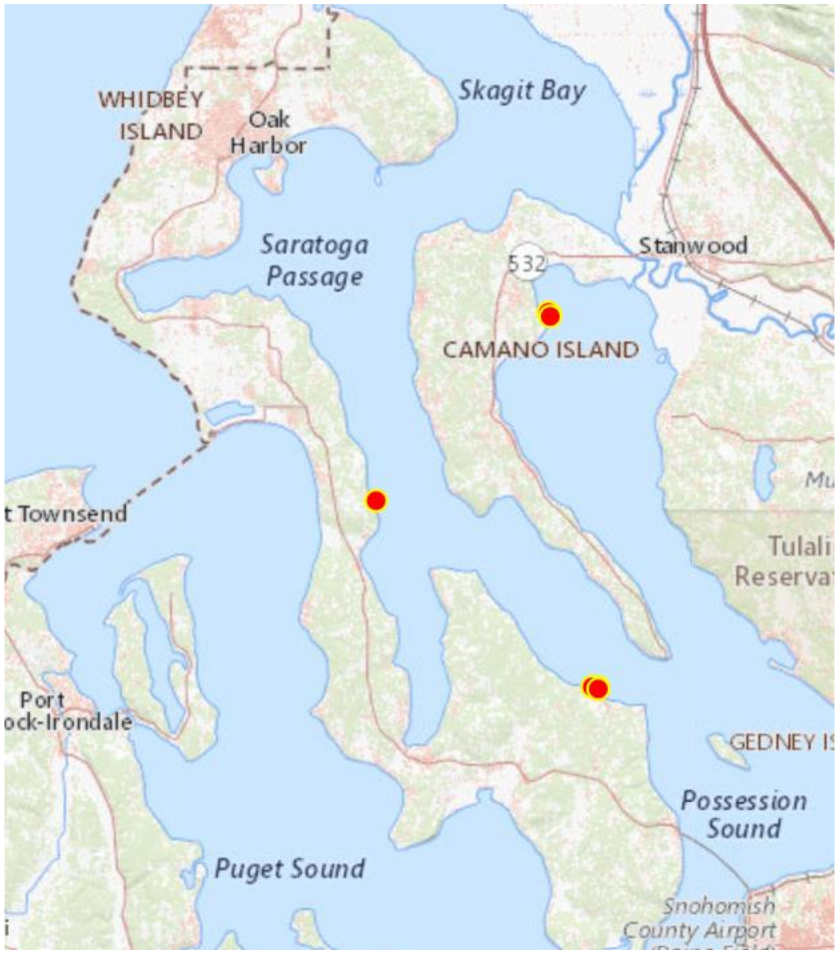

Figure 5. Field collection sites for two survey periods (Feb-April 2021) submitted using the ArcGIS Survey123 application.

Sample Collection

Near-shore sediment sample collection (Sample ID# = 1-5) was performed using a suction device, "clam-gun", to retrieve sediment cores during two low-tide (tide < 1ft) survey periods (February & April 2021) (Table 1). Appropriate measures were taken to avoid cross-contamination of samples during the sample collection phase.

Table 1. Near-shore sampling location ID, location, type, and estimated tidal level (feet) during two field sampling periods (February-April 2021).

Sample ID	Location Name	Longitude	Latitude	Date	Time	Sample Type	Tide (ft) estimate
1	Langley Waterfront	-122.408	48.0423	2/5/2021	5:12PM	sediment	0.5
2	Iverson Trail Preserve	-122.44	48.2125	2/6/2021	6:35PM	sediment	-0.38
3	Iverson Trail Preserve	-122.44	48.2114	4/17/2021	5:48PM	sediment	-0.058
4	Hidden Beach	-122.56	48.1277	4/18/2021	3:17PM	sediment	0.7
5	Langley Waterfront	-122.408	48.0417	4/18/2021	4:08PM	sediment	0.2
6	Langley Waterfront	-122.408	48.0417	4/18/2021	4:11PM	animal	0.2

Upon collection, the sediment samples were placed in clean glass test tubes, with wooden cork stoppers, and labeled with location, estimated tidal level, depth, and dominant sediment texture. Duplicate samples (n = 2) were taken from each collection site, and the GPS coordinates were recorded with ArcGIS Survey 123, to provide a total of 10 sample collections for potential laboratory analysis. Gray whales were observed to be actively feeding, via reported sightings and feeding observations from trained observers of the Orca Network group, during both survey periods. The presence of ghost shrimp tunnel boring was noted at all collection sites, and a high level of sandy substrate was noted.

Ghost shrimp specimen collection was performed during one survey period (April) on the Langley waterfront site, in Langley, WA (Sample ID# = 6). Several gray whale feeding pits (4) were observed during the low tide and collection was taken from both live and dead ghost shrimp within these pits (Figure 6.). Duplicate samples (n = 2) of ghost shrimp specimens (4) were collected to submit for total laboratory analysis of this site.

Figure 6. Gray whale feeding pit with deceased ghost shrimp in center, Langley waterfront, Langley, WA (Sample ID# = 6) (April 2021).

Laboratory Analysis

Sediment and ghost shrimp samples were analyzed for Mercury (Hg) and Polychlorinated biphenyls (PCBs) concentrations at Libby Environmental, INC (3322 South Bay Road NE Olympia, WA 98506). These were near-shore sediment (Sample ID# = 1-5) and ghost shrimp specimen samples (Sample ID# = 6). Libby Environmental, INC standard operating procedures were used to analyze the samples, as described below.

The method used for Hg analysis is a cold-vapor atomic absorption procedure (EPA Method 7471), which measures the Resource Conservation and Recovery Act (RCRA) analyte in soils, sediments, bottom deposits, and sludge type materials. The Hg is reduced to its elemental vapor state and its absorption is measured by an atomic absorption spectrophotometer. All samples were prepared by utilizing a well homogenized sample, which was then submitted to one of two digestion methods involving potassium permanganate ($KMnO_4$) solution. The samples were then analyzed by constructing a calibration curve, which determines the peak height and Hg values. The typical detection limit for this method is 0.000.2 mg/L, and the Practical Quantitation Limit (PQL) is 0.5 mg/kg. Quality control was performed using a lab-controlled sample of Hg matrix spike values, duplicate collection samples, and a Relative Percent Difference (RPD). All samples were within the acceptable recovery limits for matrix spikes (75% - 125%) and acceptable RPD limits (20%). There were no detected levels of Hg present in any of the submitted samples (Sample ID# 1-6) (Appendix 1.).

The method used for PCBs analysis was gas chromatography (EPA Method 8082) to determine the concentration of PCBs as Aroclors or individual PCB congeners. The samples were well homogenized and prepared for extraction with methylene chloride and acetone. The samples were then analyzed by gas chromatography for the Aroclors 1016, 1260, 1221, 1232, 1242, and 1254. The sample peaks were then compared to the known Aroclor standards. The PQL for this analysis is 0.1mg/kg. Quality control was performed using a lab-controlled sample, a blank sample, and duplicate collection samples. All samples were within the acceptable recovery limits for surrogate (65% -135%), acceptable recovery limits for matrix spikes (75% -

125%), and acceptable RPD limits (20%) (Appendix 1.). There were no detectable PCBs present in sample IDs# 1,2,3,4, and 6. There were PCBs detected in one collection sample (Sample ID# = 5) in the form of PCB congener Aroclor 1016 (1.7mg/kg) and Aroclor 1260 (2.1mg/kg) (Table 2.). These values are >1 order of magnitude above the PQL.

Table 2. Libby Environmental, INC total Aroclor 1016 and Aroclor 1260 concentrations (mg/kg) (nd = none detected) of near-shore sediment and ghost shrimp specimens from two collection periods (February-April 2021)(* indicates detected value).

Sample ID	Location Name	Aroclor 1016 Concentration (mg/kg)	Aroclor 1260 Concentration (mg/kg)	Collection Date	Date Analyzed	Matrix
1	Langley Waterfront	nd	nd	2/6/2021	2/10/2021	sediment
2	Iverson Trail Preserve	nd	nd	2/7/2021	2/10/2021	sediment
3	Iverson Trail Preserve	nd	nd	4/17/2021	4/21/2021	sediment
4	Hidden Beach	nd	nd	4/18/2021	4/21/2021	sediment
5	Langley Waterfront	1.7*	2.1*	4/18/2021	4/21/2021	sediment
6	Langley Waterfront	nd	nd	4/18/2021	4/21/2021	animal

Fremont Analytical (3600 Fremont Ave N, Seattle, WA, 98103) analyzed near-shore sediment (Sample ID# = 3,4,5) and ghost shrimp specimen samples (Sample ID# = 6) for total aluminum (Al) concentrations. Fremont Analytical standard operating procedures were used to analyze the samples.

The method used for Total Al analysis is the EPA Method 6020B, which utilizes inductively coupled plasma-mass spectrometry (ICP-MS) to determine heavy metal concentrations. The samples were well homogenized and digested with an acid solution. The samples were then filtered and analyzed by mass spectrometry. The method blank for this analysis reported no value of Al detected below a reporting limit of 7.87 mg/kg. Quality control was performed using a lab-controlled sample of Al matrix spike values, duplicate collection samples, and a Relative Percent Difference (RPD). The PQL for this analysis is 0.05 mg/L. All samples were within the acceptable recovery limits for matrix spikes (75% - 125%) and acceptable RPD limits (20%) (Appendix 1.). Total Al concentrations were detected in all the submitted samples (Sample ID# 3,4,5, & 6) (Table 3.). All measurements were >1 order of magnitude above the PQL and often >2.

Table 3. Fremont, INC total Aluminum concentrations (mg/kg) of field sampling 2 sites (Sample ID# 3,4,5, & 6) (April 2021).

Sample ID	Location Name	Aluminum (mg/kg)	Return Limit (RL)	Collection Date	Date Analyzed	Matrix
3 (April 1A)	Iverson Trail Preserve	6720	159	4/17/2021	4/27/2021	sediment
4 (April 2A)	Hidden Beach	6170	157	4/18/2021	4/27/2021	sediment
5 (April 3A)	Langley Waterfront	6610	153	4/18/2021	4/27/2021	sediment
6 (April 4B)	Langley Waterfront	692	7.87	4/18/2021	4/22/2021	animal

Chapter Four: Results

There were no detected levels of Hg from any samples taken during the two survey periods (February – April 2021). There were no detected levels of PCBs from any samples taken during the first survey period, in February 2021. Total Al concentration levels were not tested on any samples during the first survey period.

There were two detected PCB contaminant concentration results reported, and both findings were from the second sampling period (April 2021). The laboratory analysis revealed elevated Al and PCB congener concentrations in one sample (Sample ID# =5). The elevated PCBs were of the congeners Aroclor 1016 and Aroclor 1260 (Figure 7.).

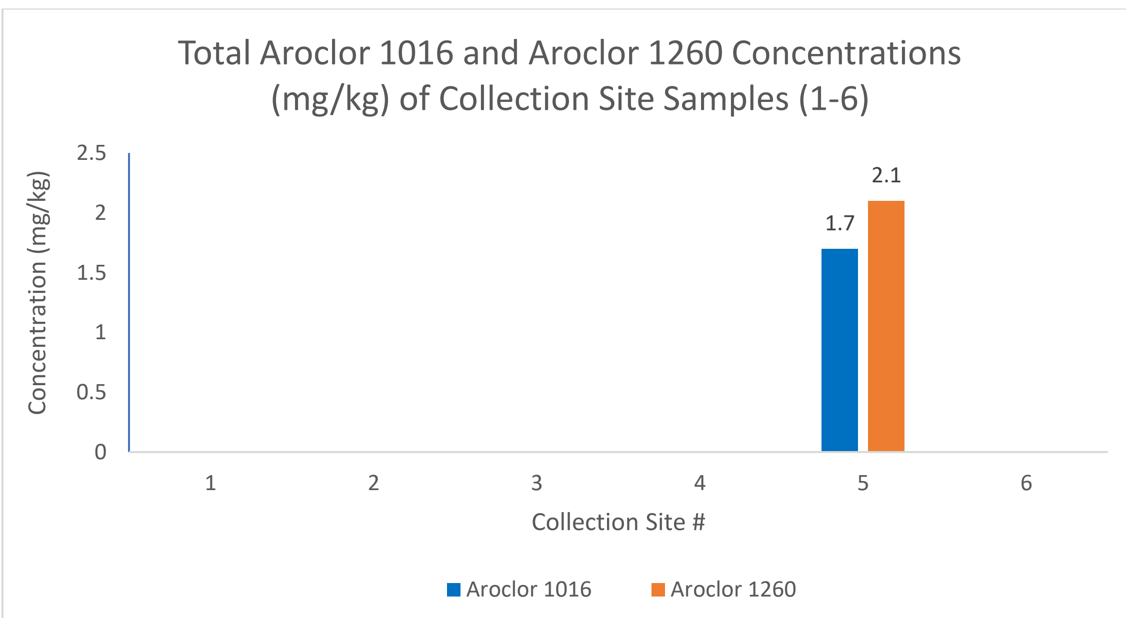

Figure 7. Concentrations (mg/kg) of PCBs Aroclor 1016 and Aroclor 120 detected in collection site samples (1-6) during the second survey sampling period (April 2021).

Notable PCBs were detected in one near-shore collection sample (Sample ID# = 5), which was taken from an observed gray whale feeding pit, located at the Langley Waterfront, in Langley, WA, on April 18th, 2021. The resulting PCB values detected were of Aroclor 1016 (1.7mg/kg) and Aroclor 1260 (2.1mg/kg) (Table 3.). The detected Aroclor 1016 concentration (Sample ID# 5 = 1.7mg/kg) was markedly above the threshold values defined by the Washington Department of Ecology's Clarc table (Table 5.) of soil protective of groundwater saturated (0.12 mg/kg). The detected Aroclor 1260 concentration (Sample ID# 5 = 2.1mg/kg) was also substantially higher than the Washington Department of Ecology's Clarc table (Table 5.) value of soil method B noncancer (0.5mg/kg) and soil protective of groundwater saturated (0.036mg/kg). A comparison of the observed Aroclor congener values to the Washington Department of Ecology's Clarc table values are represented in Figures 8 & 9.

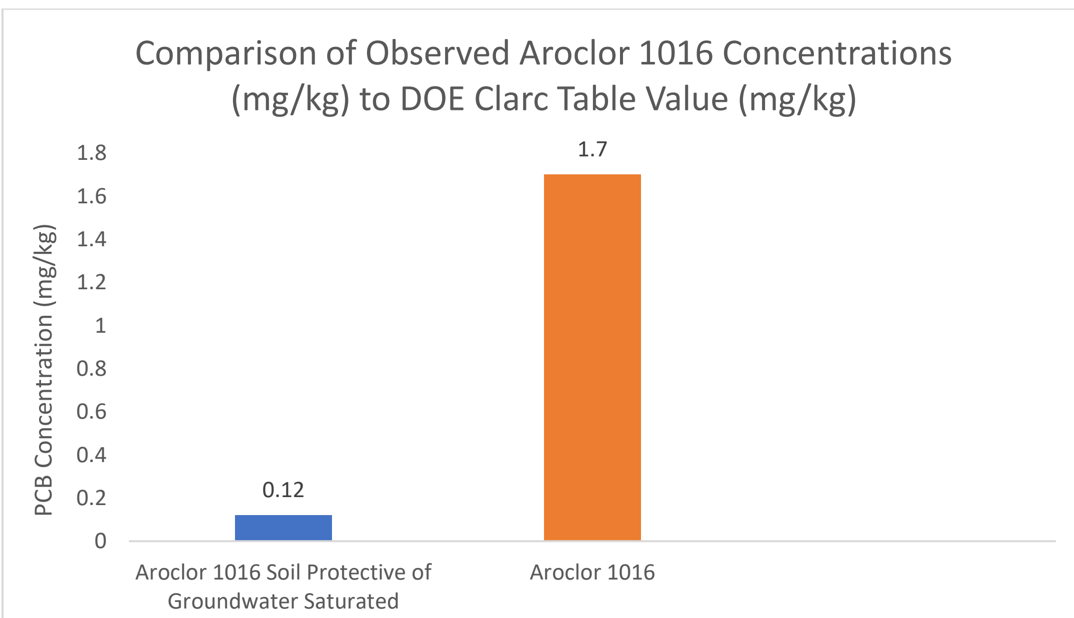

Figure 8. Comparison of observed Aroclor 1016 (color = orange) concentrations detected (Sample ID# = 5) to respective Washington Department of Ecology Clarc table value (color = blue) for soil protective of groundwater saturated.

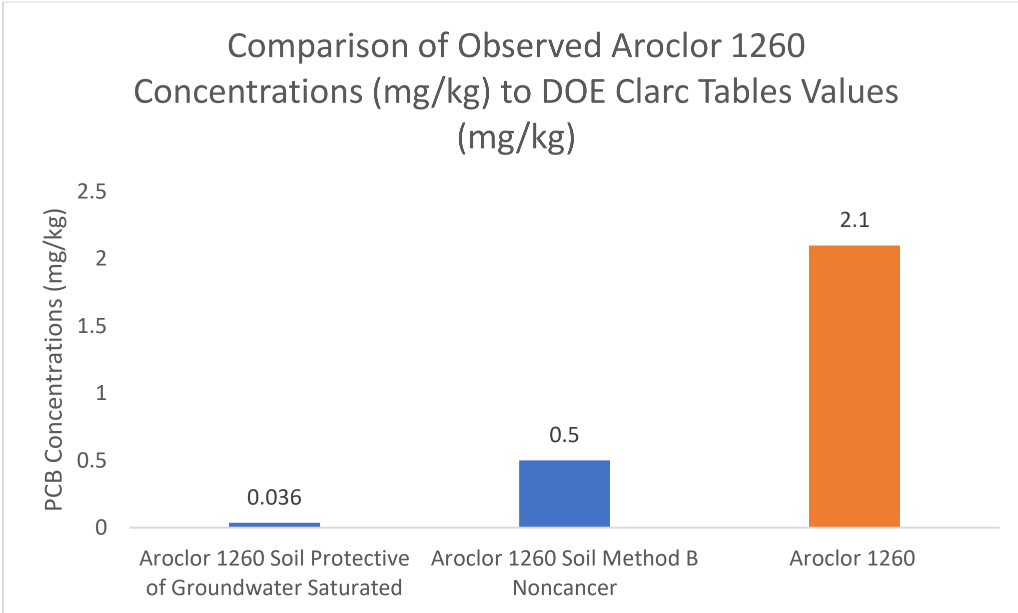

Figure 9. Comparison of observed Aroclor 1260 (color = orange) concentrations detected (Sample ID# = 5) to respective Washington Department of Ecology Clarc table value (color = blue) for soil protective of groundwater saturated and soil method B noncancer.

Total Al concentrations of diluted samples (Sample ID #3 = 6,720 mg/kg, Sample ID# 4 = 6,170mg/kg, Sample ID# 5 = 6,610 mg/kg, Sample ID# 6 = 692 mg/kg) were detected in four samples taken from three near-shore sediment samples and one ghost shrimp specimen sample during the second survey period, on April 17th – April 18th, 2021 (Figure 10).

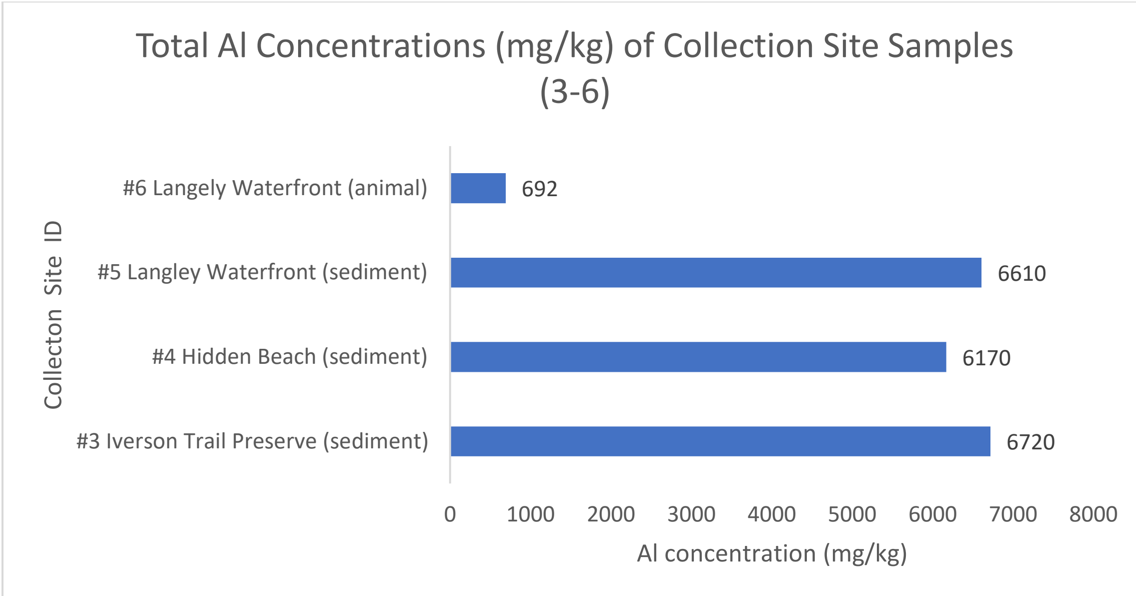

Figure 10. Total aluminum concentrations (mg/kg) detected in collection samples (3-6) during the second survey sampling period (April 2021).

Sample # 5 (sediment) & Sample #6 (animal) were both taken from an observed gray whale feeding pit on the Langley Waterfront, in Langley, WA. The values of total Al detected in the sediment and animal samples analyzed are of notable concentrations according to the Washington Department of Ecology's Clarc table (Table 4.) of soil method A unrestricted land use.

Table 4. Washington State Department of Ecology - CLARC Soil Unrestricted Land Use Table (Methods A, B, Groundwater Protection, and Soil Leaching Parameters) (WA DEPT of ECOLOGY February 2021)

Chemical Group	Chemical Name	Soil Method A Unrestricted Land Use (mg/kg)	Soil Method B Noncancer (mg/kg)	Soil Method B Cancer (mg/kg)	Soil Protective Groundwater Vadose @ 13 degrees C (mg/kg)	Soil Protective Groundwater Saturated (mg/kg)
Metals	Aluminum		80000		480000	24000
PCBs	Aroclor 1016		5.6	14		0.12
PCBs	Aroclor 1260			0.5		0.036

Chapter Five: Discussion

Comparison of Historic Puget Sound Sediment Contaminant Values to Survey Collection Values

Although there are still many unknowns on the topic of toxicology and contaminant effects to cetaceans, we do have historic tangible data available of their concentrations in Puget Sound marine benthic sediments. The Washington Department of Ecology monitors the concentrations of compounds in both the marine water-column and marine benthic sediments. The marine benthic sediment data was collected by Van Veen grab sampling, during the time period of 1989-2019. These values are not static and can fluctuate over time, and so they are subject to influence by factors such as seasonality, depth, temperature, circulation, and point and non-point source inputs (Washington Department of Ecology 2021). For the purpose of this research, the concentration values of Al, and PCB congeners Aroclor 1016 and Aroclor 1260 will be evaluated in Island County of Washington State only.

Total Al Concentrations

Total Al concentrations (mg/kg) of the Washington Department of Ecology's Van Veen grab sampling (n = 71) from Island County have been sorted in Table 5 by increasing value of concentration. The majority of samples in Island County were taken in the Saratoga Passage (n = 20). A histogram of the total Al concentrations is depicted in Figure 11. An R analysis of the total Al values is as follows: minimum value = 6030 mg/kg, 1st quartile = 18300 mg/kg, median = 21250 mg/kg, mean = 35821 mg/kg, 3rd quartile = 68000, maximum value = 87000.

The total Al values from the second survey collection (Sample ID #3 = 6,720 mg/kg, Sample ID# 4 = 6,170 mg/kg, Sample ID# 5 = 6,610 mg/kg, Sample ID# 6 = 692 mg/kg) are similiar to the minimum value (6030 mg/kg) found in the DOE database. These values are also well below the mean value of 35821 mg/kg. A spatial analysis (Figure 12) of hot spots (large clusters of high values) of the DOE total Al concentrations reveals survey sampling sites lie outside of the hot spot concentration zones.

Table 5. Total Al concentrations (mg/kg), in increasing value, of DOE Puget Sound benthic sediment data (1989-2019) by Van Veen grab sampling (Department of Ecology 2021).

Location Name	Field Collection Date	Total Al (mg/kg)	Latitude (DD)	Longitude (DD)
USELESS BAY	4/1/1992	6030	47.98517	-122.49149
USELESS BAY	6/30/1998	6820	47.98152	-122.50338
USELESS BAY	6/30/1998	7180	47.98152	-122.50338
USELESS BAY	6/30/1998	8500	47.96341	-122.50795
SKAGIT BAY	7/2/1997	9300	48.30833	-122.49163
SARATOGA PASSAGE	6/30/1997	9820	48.11163	-122.49257
SARATOGA PASSAGE	6/30/1997	10600	48.11163	-122.49257
OAK HARBOR	4/1/1993	11300	48.25617	-122.625

SKAGIT BAY	4/1/1991	12100	48.29533	-122.4885
SKAGIT BAY	7/2/1997	12200	48.26747	-122.51675
OAK HARBOR	4/1/1992	13400	48.25617	-122.625
OAK HARBOR	4/1/1991	14500	48.25617	-122.625
SKAGIT BAY	7/2/1997	14800	48.27085	-122.54587
OAK HARBOR	4/1/1989	15400	48.25617	-122.625
WEST BEACH, WHIDBEY ISLAND	4/1/1991	16800	48.39933	-122.671
OAK HARBOR	7/2/1997	17600	48.27445	-122.65198
OAK HARBOR	7/3/1997	17900	48.2839	-122.63665
OAK HARBOR	7/3/1997	18200	48.28528	-122.6372
PORT SUSAN	4/1/1993	18600	48.17317	-122.45775
SOUTH OF OAK HARBOR	7/2/1997	18600	48.2558	-122.64643
PORT SUSAN	4/1/1989	18700	48.17317	-122.45775
SARATOGA PASSAGE	4/1/1990	18900	48.09792	-122.47134
SARATOGA PASSAGE	4/1/1993	18900	48.09792	-122.47134
OAK HARBOR	7/3/1997	18900	48.28528	-122.6372
SARATOGA PASSAGE	4/1/1989	19100	48.09792	-122.47134

SARATOGA PASSAGE	4/1/1992	19400	48.09792	-122.47134
SARATOGA PASSAGE	6/24/1997	19600	48.05778	-122.39025
SARATOGA PASSAGE	6/30/1997	19600	48.2228	-122.55915
NORTHERN SARATOGA PASSAGE	7/1/1997	19600	48.23717	-122.58813
SARATOGA PASSAGE	6/30/1997	20300	48.15583	-122.53523
SARATOGA PASSAGE	4/1/1991	20600	48.09792	-122.47134
PORT SUSAN	4/1/1992	20600	48.17317	-122.45775
SARATOGA PASSAGE	6/30/1997	20600	48.06138	-122.42585
SARATOGA PASSAGE	6/30/1997	20800	48.13888	-122.5436
OAK HARBOR	4/1/1990	20900	48.25617	-122.625
PORT SUSAN	6/23/1997	21600	48.16963	-122.4178
PENN COVE	7/1/1997	21600	48.23615	-122.6658
HOLMES HARBOR, WHIDBEY ISLAND	4/1/1992	21700	48.08833	-122.55051

SARATOGA PASSAGE	6/30/1997	21700	48.06138	-122.42585
PORT SUSAN	6/23/1997	22200	48.16963	-122.4178
PORT SUSAN	4/1/1990	22300	48.17317	-122.45775
PORT SUSAN	4/1/1991	23000	48.17317	-122.45775
MOUTH OF PENN COVE	7/1/1997	23000	48.24283	-122.62218
PENN COVE	7/1/1997	23100	48.22472	-122.71052
PENN COVE	7/1/1997	23800	48.23168	-122.69357
SARATOGA PASSAGE	6/30/1997	40600	48.11163	-122.49257
SARATOGA PASSAGE	6/30/1997	44000	48.11163	-122.49257
USELESS BAY	6/30/1998	46500	47.96341	-122.50795
SOUTH OF OAK HARBOR	7/2/1997	53300	48.2558	-122.64643
USELESS BAY	6/30/1998	58300	47.98152	-122.50338
SKAGIT BAY	7/2/1997	59200	48.30833	-122.49163
SARATOGA PASSAGE	6/30/1997	64100	48.15583	-122.53523
SARATOGA PASSAGE	6/30/1997	69300	48.13888	-122.5436
SKAGIT BAY	7/2/1997	69300	48.30833	-122.49163

PENN COVE	7/1/1997	69400	48.22472	-122.71052
PENN COVE	7/1/1997	70300	48.23168	-122.69357
SKAGIT BAY	7/2/1997	72200	48.27085	-122.54587
SARATOGA PASSAGE	6/24/1997	72700	48.05778	-122.39025
SKAGIT BAY	7/2/1997	74400	48.26747	-122.51675
NORTHERN SARATOGA PASSAGE	7/1/1997	74500	48.23717	-122.58813
SARATOGA PASSAGE	6/30/1997	74600	48.06138	-122.42585
SARATOGA PASSAGE	6/30/1997	76400	48.2228	-122.55915
PENN COVE	7/1/1997	76500	48.23615	-122.6658
OAK HARBOR	7/3/1997	77000	48.28528	-122.6372
OAK HARBOR	7/3/1997	77300	48.2839	-122.63665
OAK HARBOR	7/2/1997	77900	48.27445	-122.65198
OAK HARBOR	7/3/1997	78200	48.28528	-122.6372
MOUTH OF PENN COVE	7/1/1997	78600	48.24283	-122.62218
PORT SUSAN	6/23/1997	85700	48.16963	-122.4178
PORT SUSAN	6/23/1997	87000	48.16963	-122.4178

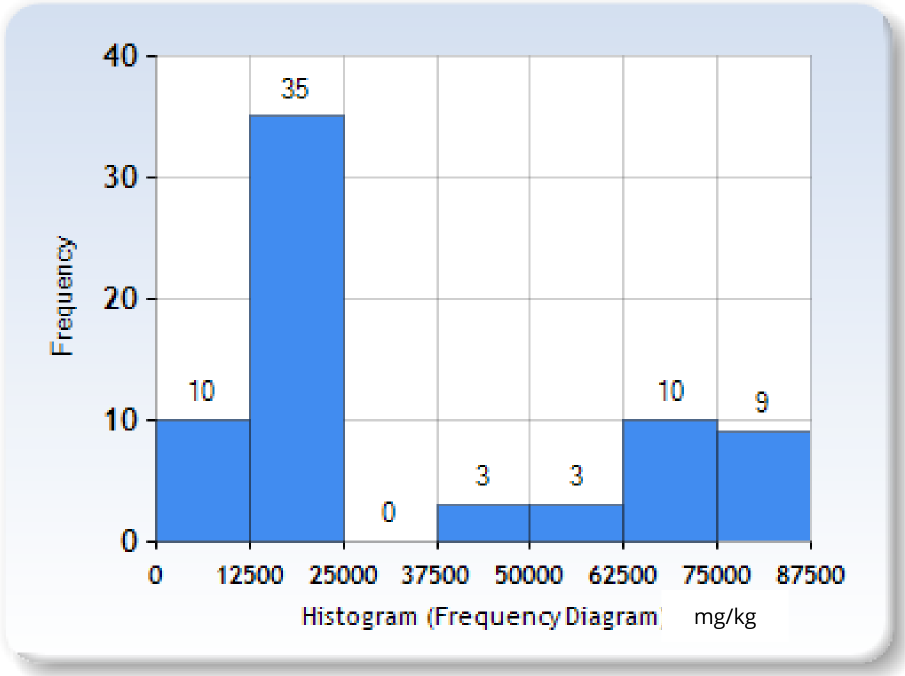

Figure 11. Histogram of Total Al concentrations of DOE Puget Sound benthic sediment data (1989-2019) by Van Veen grab sampling (Washington Department of Ecology 2021) generated by Social Science Statistics. For reference, results in this study varied from 692 mg/kg – 6720 mg/kg.

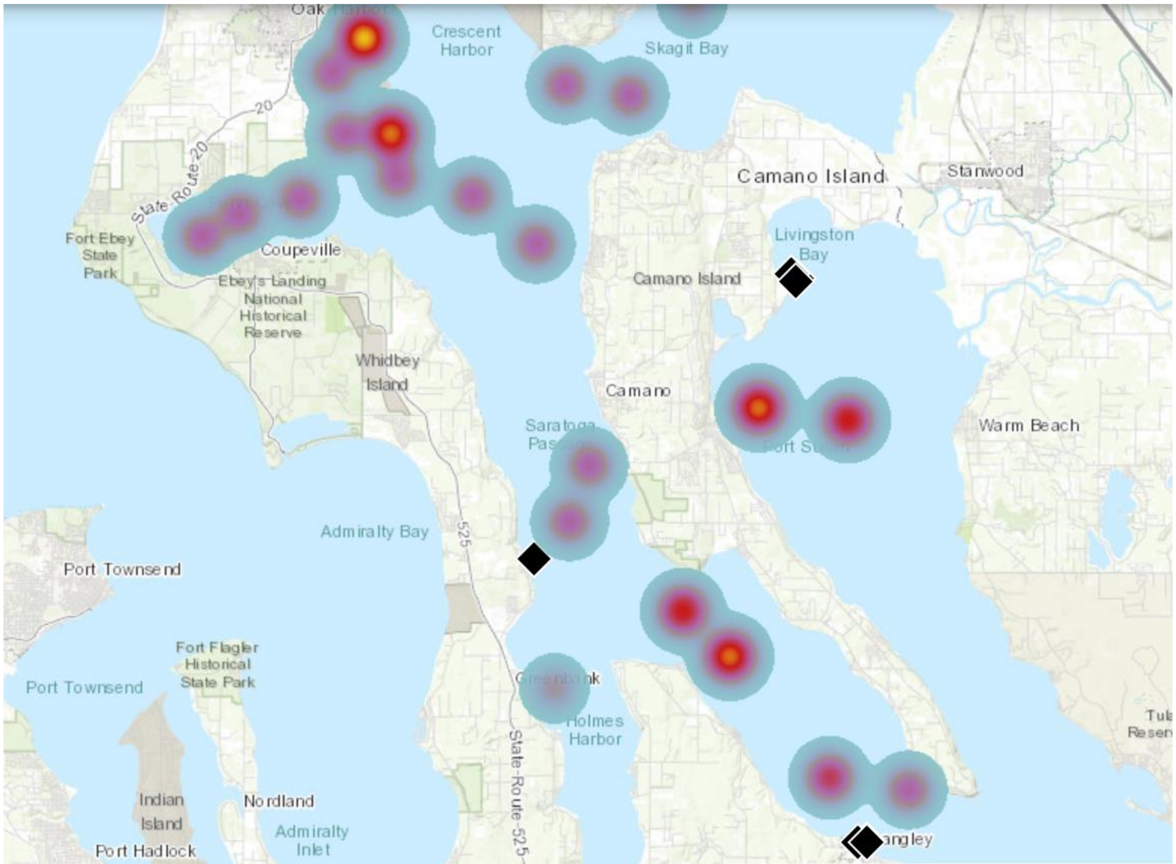

Figure 12. Survey sample collection sites (collection sites = black diamonds) and hot spots (blue = low, purple = medium, red = high, yellow = very high) of total Al concentrations from DOE Puget Sound benthic sediment data. Generated with ArcGIS.

Aroclor 1016 concentrations

Aroclor 1016 concentrations (mg/kg) of the Washington Department of Ecology's Van Veen grab sampling (n = 78) from Island County have been sorted in Table 6 by increasing value of concentration. A histogram of the Aroclor 1016 concentrations is depicted in Figure 13. An R analysis of the Aroclor 1016 values is as follows: minimum value = 0.001200 mg/kg, 1st quartile = 0.005100 mg/kg, median = 0.009765 mg/kg, mean = 0.008627 mg/kg, 3rd quartile = 0.010175 mg/kg, maximum value = 0.019000 mg/kg.

The Aroclor 1016 value from the second survey collection (Sample ID# 5 = 1.7 mg/kg) is remarkedly above both the DOE database mean (0.008627 mg/kg) and maximum value (0.019000 mg/kg) of this area. This value also exceeds the DOE Clarc value of soil protective of groundwater saturated (0.12 mg/kg) and is therefore both abnormally high and potentially concerning. A spatial analysis (Figure 14) of hot spots of the DOE Aroclor 1016 concentrations reveals survey sampling sites lie outside of the known hot spot concentration zones.

Table 6. Aroclor 1016 concentrations (mg/kg), in increasing value, of DOE Puget Sound benthic sediment data (1989-2019) by Van Veen grab sampling (Department of Ecology 2021).

Location Name	Field Collection Date	PCB Aroclor 1016 Concentration (mg/kg)	Latitude (DD)	Longitude (DD)
OAK HARBOR	7/2/1997	0.0012	48.27445	-122.65198
PSEMP_LT-40007	4/30/2018	0.00126	48.22609563	-122.5437651
SARATOGA PASSAGE	6/30/1997	0.0016	48.15583	-122.53523
PSEMP_LT-40015	4/22/2019	0.0017	48.08877563	-122.448545
USELESS BAY	6/6/2003	0.0048	47.936462	-122.447223
PSAMP_SP-3855	6/18/2014	0.0048	47.950614	-122.47886
PSAMP_SP-3187	6/19/2014	0.0048	47.999232	-122.566721

SOUTH OF POSSESSION PT.	6/13/2008	0.0049	47.847345	-122.418419
USELESS BAY	6/17/2002	0.005	47.945124	-122.471458
USELESS BAY	6/17/2002	0.005	47.945124	-122.471458
MUTINY BAY	6/24/2002	0.005	47.989749	-122.554923
PSAMP_SP-783	6/18/2014	0.005	47.943468	-122.482832
PSAMP_SP-2063	6/19/2014	0.005	47.927478	-122.448443
PSAMP_SP-3203	6/19/2014	0.005	47.987084	-122.561603
PSAMP_SP-1443	6/19/2014	0.005	47.965792	-122.509161
EAST OF POSSESSION PT.	6/13/2008	0.0051	47.894683	-122.362001
PSAMP_SP-1807	6/18/2014	0.0051	47.950305	-122.465638
PSAMP_SP-3343	6/18/2014	0.0051	47.976061	-122.50084
PSAMP_SP-2319	6/18/2014	0.0051	47.97828	-122.483775
PSAMP_SP-2319	6/18/2014	0.0051	47.97828	-122.483775
PSAMP_SP-2831	6/18/2014	0.0051	47.961127	-122.487157
PSAMP_SP-1571	6/19/2014	0.0051	47.959997	-122.501224
POSSESSION SOUND	6/1/2009	0.0052	47.939653	-122.336727
USELESS BAY	6/30/1998	0.0054	47.96341	-122.50795
USELESS BAY	6/30/1998	0.0054	47.98152	-122.50338
USELESS BAY	6/30/1998	0.0054	47.98152	-122.50338
USELESS BAY	6/30/1998	0.0056	47.96341	-122.50795

SKAGIT BAY	7/2/1997	0.0057	48.30833	-122.49163
SKAGIT BAY	7/2/1997	0.0065	48.26747	-122.51675
SARATOGA PASSAGE	6/30/1997	0.0068	48.11163	-122.49257
SKAGIT BAY	7/2/1997	0.007	48.27085	-122.54587
PORT SUSAN	6/23/1997	0.0078	48.16963	-122.4178
PORT SUSAN	6/23/1997	0.0079	48.16963	-122.4178
SARATOGA PASSAGE	6/6/2007	0.0092	48.15079	-122.54696
SARATOGA PASSAGE	6/6/2007	0.0094	48.139553	-122.544899
SARATOGA PASS	6/11/2007	0.0094	48.067587	-122.449544
HOLMES HARBOR	6/6/2007	0.0096	48.043554	-122.51557
SARATOGA PASS	6/11/2007	0.0096	48.041723	-122.378578
SARATOGA PASS (NORTH)	6/5/2007	0.0097	48.237088	-122.63562
PSEMP_LT-209R	5/3/2016	0.00983	48.29533	-122.4885
PSEMP_LT-19	4/27/2016	0.00985	48.09792	-122.47134
SKAGIT BAY	6/5/2007	0.0099	48.291669	-122.486003
HOLMES HARBOR	6/6/2007	0.0099	48.036152	-122.526755
SARATOGA PASS	6/11/2007	0.0099	48.108076	-122.451612
SARATOGA PASS	6/11/2007	0.0099	48.0584	-122.417132
OAK HARBOR	7/3/1997	0.01	48.28528	-122.6372

SKAGIT BAY	6/5/2007	0.01	48.275939	-122.520873
CRESENT HARBOR	6/5/2007	0.01	48.286562	-122.579053
SARATOGA PASS (NORTH)	6/5/2007	0.01	48.231155	-122.583858
SARATOGA PASSAGE	6/5/2007	0.01	48.200165	-122.548387
SARATOGA PASSAGE	6/6/2007	0.01	48.178308	-122.575625
HOLMES HARBOR	6/6/2007	0.01	48.107718	-122.56206
HOLMES HARBOR	6/6/2007	0.01	48.062865	-122.520724
POSSESSION SOUND	6/7/2007	0.01	47.989157	-122.343898
PORT SUSAN	6/8/2007	0.01	48.168488	-122.41852
SARATOGA PASS	6/11/2007	0.01	48.067092	-122.443248
PORT SUSAN	6/11/2007	0.01	48.057499	-122.35367
PSEMP_LT-209R	5/3/2016	0.0101	48.29533	-122.4885
PSEMP_LT-209R	5/3/2016	0.0102	48.29533	-122.4885
PENN COVE	7/1/1997	0.011	48.23615	-122.6658
MOUTH OF PENN COVE	7/1/1997	0.011	48.24283	-122.62218
OAK HARBOR	7/3/1997	0.011	48.28528	-122.6372
OAK HARBOR	7/3/1997	0.011	48.2839	-122.63665

POSSESSION SOUND	6/7/2007	0.011	48.001263	-122.352855
PORT SUSAN	6/8/2007	0.011	48.117441	-122.399167
PSEMP_LT-19	4/27/2016	0.0113	48.09792	-122.47134
PSEMP_LT-19	4/27/2016	0.0117	48.09792	-122.47134
SARATOGA PASSAGE	6/24/1997	0.012	48.05778	-122.39025
NORTHERN SARATOGA PASSAGE	7/1/1997	0.012	48.23717	-122.58813
SARATOGA PASSAGE	6/30/1997	0.013	48.2228	-122.55915
PENN COVE	7/1/1997	0.013	48.23168	-122.69357
SOUTH OF OAK HARBOR	7/2/1997	0.013	48.2558	-122.64643
SARATOGA PASSAGE	6/30/1997	0.015	48.06138	-122.42585
OAK HARBOR	4/1/1993	0.016	48.25617	-122.625
SARATOGA PASSAGE	6/30/1997	0.016	48.13888	-122.5436
PENN COVE	7/1/1997	0.016	48.22472	-122.71052
PORT SUSAN	4/1/1993	0.018	48.17317	-122.45775

SARATOGA PASSAGE	4/1/1993	0.019	48.09792	-122.47134

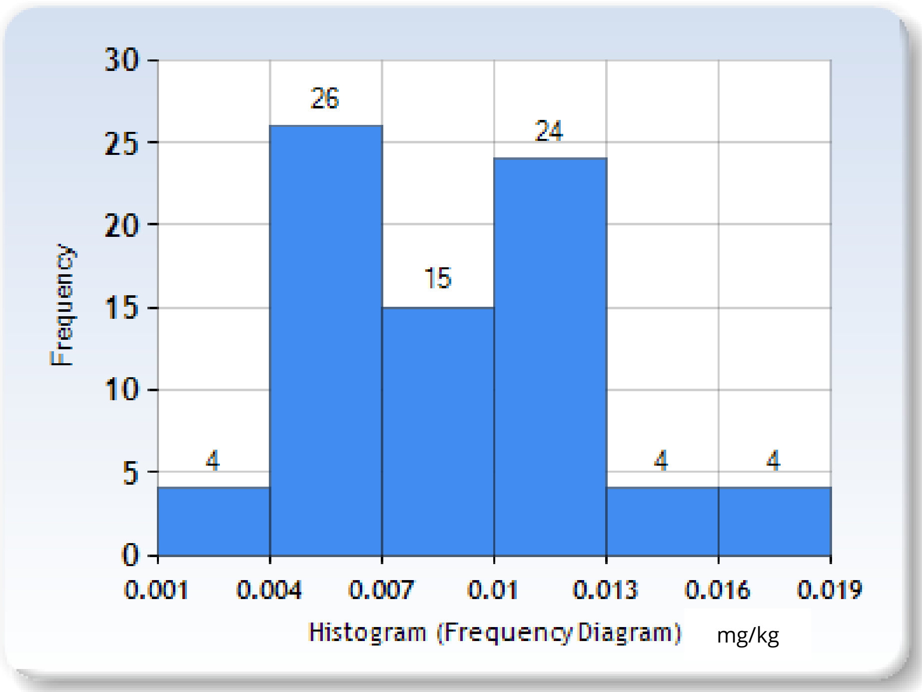

Figure 13. Histogram of Aroclor 1016 concentrations of DOE Puget Sound benthic sediment data (1989-2019) by Van Veen grab sampling (Washington Department of Ecology 2021) generated by Social Science Statistics. For reference, the Aroclor 1016 value observed in this study is 1.7 mg/kg.

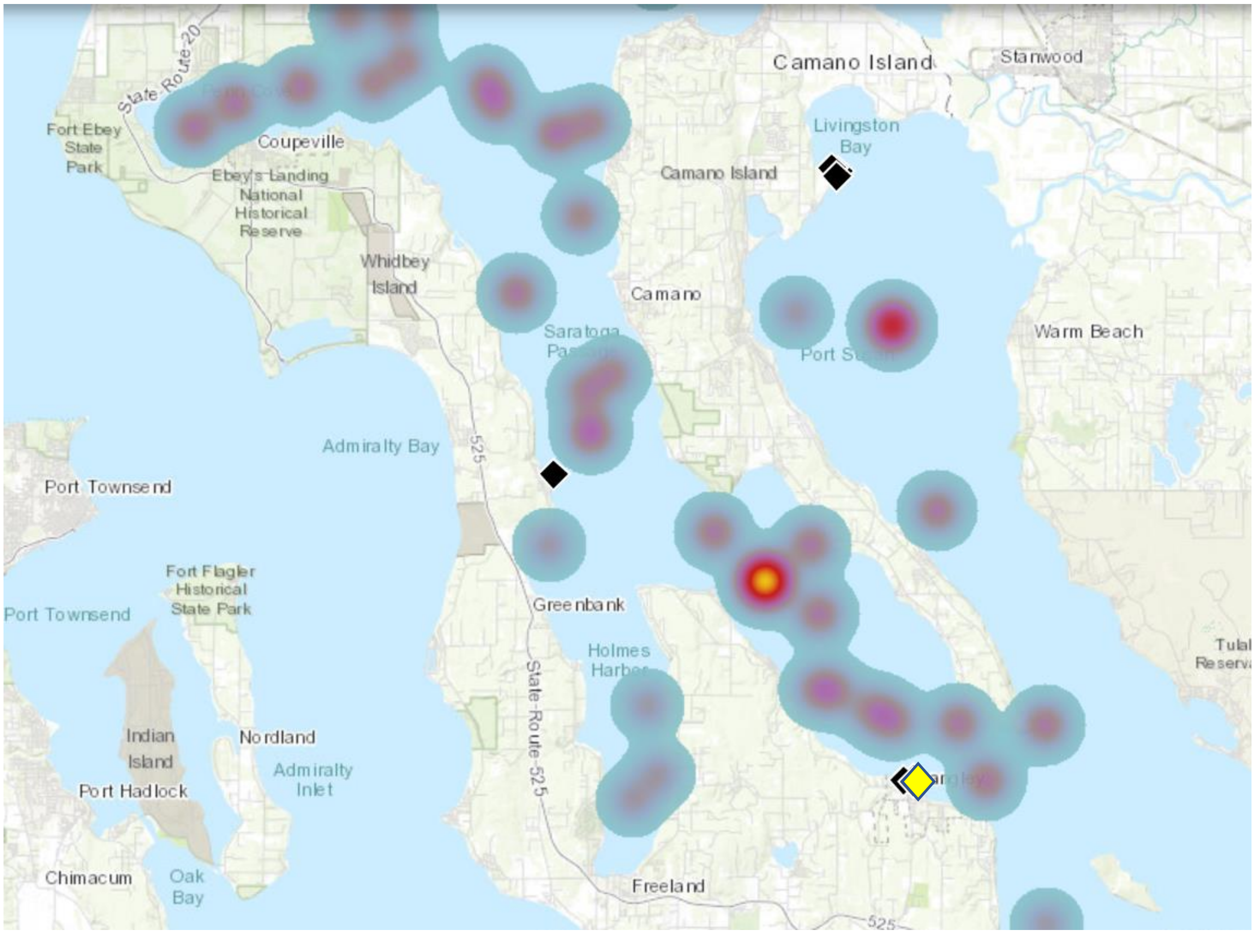

Figure 14. Survey sample collection sites (collection sites = black diamonds, high observed value = yellow diamond) and hot spots (blue = low, purple = medium, red = high, yellow = very high) of Aroclor 1016 concentrations from DOE Puget Sound benthic sediment data. Generated with ArcGIS.

Aroclor 1260 concentrations

Aroclor 1260 concentrations (mg/kg) from the Washington Department of Ecology's Van Veen grab sampling (n = 95) have been sorted in Table 7 by increasing value of concentration. A histogram of the Aroclor 1260 concentrations is depicted in Figure 15. An R analysis of the Aroclor 1260 values is as follows: minimum value = 0.001200 mg/kg, 1st quartile = 0.005100 mg/kg, median = 0.00990, mean = 0.01054 mg/kg, 3rd quartile = 0.01200 mg/kg, maximum value = 0.06000 mg/kg.

The Aroclor 1260 value from the second survey collection (Sample ID# 5 = 2.1 mg/kg) is also substantially above two Clarc Table values, the soil method B noncancer (0.5 mg/kg) and soil protective of groundwater saturated (0.036 mg/kg). This value (2.1 mg/kg) is also well above the mean value (0.01054 mg/kg) and the maximum value (0.06000 mg/kg) of the DOE study area. The PCB values found during this survey study require further investigation and survey sampling to ensure safe parameters for humans and cetaceans alike. A spatial analysis (Figure 16) of hot spots of the DOE Aroclor 1260 concentrations reveals survey sampling sites lie outside of the known hot spot concentration zones. It is noted, however, that both hot spots for Aroclor concentrations lie in the same geographic area. This area is located ~5.15 nautical miles NW from Sample ID #5. The current also flows in the direction from the noted hot spot towards where Sample ID #5 was collected. It is possible that a point or non point source of PCBs in the form of Aroclor 1016 and 1260 is located near this hot spot.

Table 7. Aroclor 1260 concentrations (mg/kg), in increasing value, of DOE Puget Sound benthic sediment data (1989-2019) by Van Veen grab sampling (Department of Ecology 2021).

Location Name	Field Collection Date	Aroclor 1260 Concentration (mg/kg)	Latitude (DD)	Longitude (DD)
OAK HARBOR	7/2/1997	0.0012	48.27445	-122.65198
PSEMP_LT-40007	4/30/2018	0.00126	48.22609563	-122.5437651

SARATOGA PASSAGE	6/30/1997	0.0016	48.15583	-122.53523
USELESS BAY	6/6/2003	0.0048	47.936462	-122.447223
PSAMP_SP-3855	6/18/2014	0.0048	47.950614	-122.47886
PSAMP_SP-3187	6/19/2014	0.0048	47.999232	-122.566721
SOUTH OF POSSESSION PT.	6/13/2008	0.0049	47.847345	-122.418419
PSEMP_LT-209R	5/3/2016	0.00491	48.29533	-122.4885
PSEMP_LT-19	4/27/2016	0.00492	48.09792	-122.47134
USELESS BAY	6/17/2002	0.005	47.945124	-122.471458
USELESS BAY	6/17/2002	0.005	47.945124	-122.471458
MUTINY BAY	6/24/2002	0.005	47.989749	-122.554923
PSAMP_SP-783	6/18/2014	0.005	47.943468	-122.482832
PSAMP_SP-2063	6/19/2014	0.005	47.927478	-122.448443
PSAMP_SP-3203	6/19/2014	0.005	47.987084	-122.561603
PSAMP_SP-1443	6/19/2014	0.005	47.965792	-122.509161
PSEMP_LT-209R	5/3/2016	0.00505	48.29533	-122.4885
PSEMP_LT-209R	5/3/2016	0.0051	48.29533	-122.4885
EAST OF POSSESSION PT.	6/13/2008	0.0051	47.894683	-122.362001
PSAMP_SP-1807	6/18/2014	0.0051	47.950305	-122.465638
PSAMP_SP-3343	6/18/2014	0.0051	47.976061	-122.50084
PSAMP_SP-2319	6/18/2014	0.0051	47.97828	-122.483775

PSAMP_SP-2319	6/18/2014	0.0051	47.97828	-122.483775
PSAMP_SP-2831	6/18/2014	0.0051	47.961127	-122.487157
PSAMP_SP-1571	6/19/2014	0.0051	47.959997	-122.501224
POSSESSION SOUND	6/1/2009	0.0052	47.939653	-122.336727
PSEMP_LT-40015	4/22/2019	0.00533	48.08877563	-122.448545
USELESS BAY	6/30/1998	0.0054	47.96341	-122.50795
USELESS BAY	6/30/1998	0.0054	47.98152	-122.50338
USELESS BAY	6/30/1998	0.0054	47.98152	-122.50338
USELESS BAY	6/30/1998	0.0056	47.96341	-122.50795
PSEMP_LT-19	4/27/2016	0.00565	48.09792	-122.47134
SKAGIT BAY	7/2/1997	0.0057	48.30833	-122.49163
PSEMP_LT-19	4/27/2016	0.00585	48.09792	-122.47134
SKAGIT BAY	7/2/1997	0.0065	48.26747	-122.51675
SARATOGA PASSAGE	6/30/1997	0.0068	48.11163	-122.49257
SKAGIT BAY	7/2/1997	0.007	48.27085	-122.54587
SKAGIT BAY	4/1/1991	0.0075	48.29533	-122.4885
PORT SUSAN	6/23/1997	0.0078	48.16963	-122.4178
PORT SUSAN	6/23/1997	0.0079	48.16963	-122.4178
SARATOGA PASSAGE	6/6/2007	0.0092	48.15079	-122.54696

SARATOGA PASSAGE	6/6/2007	0.0094	48.139553	-122.544899
SARATOGA PASS	6/11/2007	0.0094	48.067587	-122.449544
HOLMES HARBOR	6/6/2007	0.0096	48.043554	-122.51557
SARATOGA PASS	6/11/2007	0.0096	48.041723	-122.378578
SARATOGA PASS (NORTH)	6/5/2007	0.0097	48.237088	-122.63562
SKAGIT BAY	6/5/2007	0.0099	48.291669	-122.486003
HOLMES HARBOR	6/6/2007	0.0099	48.036152	-122.526755
SARATOGA PASS	6/11/2007	0.0099	48.108076	-122.451612
SARATOGA PASS	6/11/2007	0.0099	48.0584	-122.417132
PORT SUSAN	4/1/1992	0.01	48.17317	-122.45775
USELESS BAY	4/1/1992	0.01	47.98517	-122.49149
OAK HARBOR	7/3/1997	0.01	48.28528	-122.6372
SKAGIT BAY	6/5/2007	0.01	48.275939	-122.520873
CRESENT HARBOR	6/5/2007	0.01	48.286562	-122.579053
SARATOGA PASS (NORTH)	6/5/2007	0.01	48.231155	-122.583858
SARATOGA PASSAGE	6/5/2007	0.01	48.200165	-122.548387
SARATOGA PASSAGE	6/6/2007	0.01	48.178308	-122.575625
HOLMES HARBOR	6/6/2007	0.01	48.107718	-122.56206

HOLMES HARBOR	6/6/2007	0.01	48.062865	-122.520724
POSSESSION SOUND	6/7/2007	0.01	47.989157	-122.343898
PORT SUSAN	6/8/2007	0.01	48.168488	-122.41852
SARATOGA PASS	6/11/2007	0.01	48.067092	-122.443248
PORT SUSAN	6/11/2007	0.01	48.057499	-122.35367
PENN COVE	7/1/1997	0.011	48.23615	-122.6658
MOUTH OF PENN COVE	7/1/1997	0.011	48.24283	-122.62218
OAK HARBOR	7/3/1997	0.011	48.28528	-122.6372
OAK HARBOR	7/3/1997	0.011	48.2839	-122.63665
POSSESSION SOUND	6/7/2007	0.011	48.001263	-122.352855
PORT SUSAN	6/8/2007	0.011	48.117441	-122.399167
SARATOGA PASSAGE	6/24/1997	0.012	48.05778	-122.39025
NORTHERN SARATOGA PASSAGE	7/1/1997	0.012	48.23717	-122.58813
SARATOGA PASSAGE	6/30/1997	0.013	48.2228	-122.55915
PENN COVE	7/1/1997	0.013	48.23168	-122.69357

SOUTH OF OAK HARBOR	7/2/1997	0.013	48.2558	-122.64643
OAK HARBOR	4/1/1991	0.015	48.25617	-122.625
SARATOGA PASSAGE	4/1/1991	0.015	48.09792	-122.47134
PORT SUSAN	4/1/1991	0.015	48.17317	-122.45775
WEST BEACH, WHIDBEY ISLAND	4/1/1991	0.015	48.39933	-122.671
SARATOGA PASSAGE	6/30/1997	0.015	48.06138	-122.42585
OAK HARBOR	4/1/1993	0.016	48.25617	-122.625
SARATOGA PASSAGE	6/30/1997	0.016	48.13888	-122.5436
PENN COVE	7/1/1997	0.016	48.22472	-122.71052
PORT SUSAN	4/1/1993	0.018	48.17317	-122.45775
SARATOGA PASSAGE	4/1/1993	0.019	48.09792	-122.47134
PORT SUSAN	4/1/1989	0.02	48.17317	-122.45775
OAK HARBOR	4/1/1990	0.02	48.25617	-122.625
SARATOGA PASSAGE	4/1/1990	0.02	48.09792	-122.47134
OAK HARBOR	4/1/1992	0.02	48.25617	-122.625

SARATOGA PASSAGE	4/1/1992	0.02	48.09792	-122.47134
HOLMES HARBOR, WHIDBEY ISLAND	4/1/1992	0.02	48.08833	-122.55051
OAK HARBOR	4/1/1989	0.026	48.25617	-122.625
SKAGIT BAY	4/11/1994	0.027	48.29533	-122.4885
SARATOGA PASSAGE	4/1/1989	0.036	48.09792	-122.47134
PORT SUSAN	4/1/1990	0.06	48.17317	-122.45775

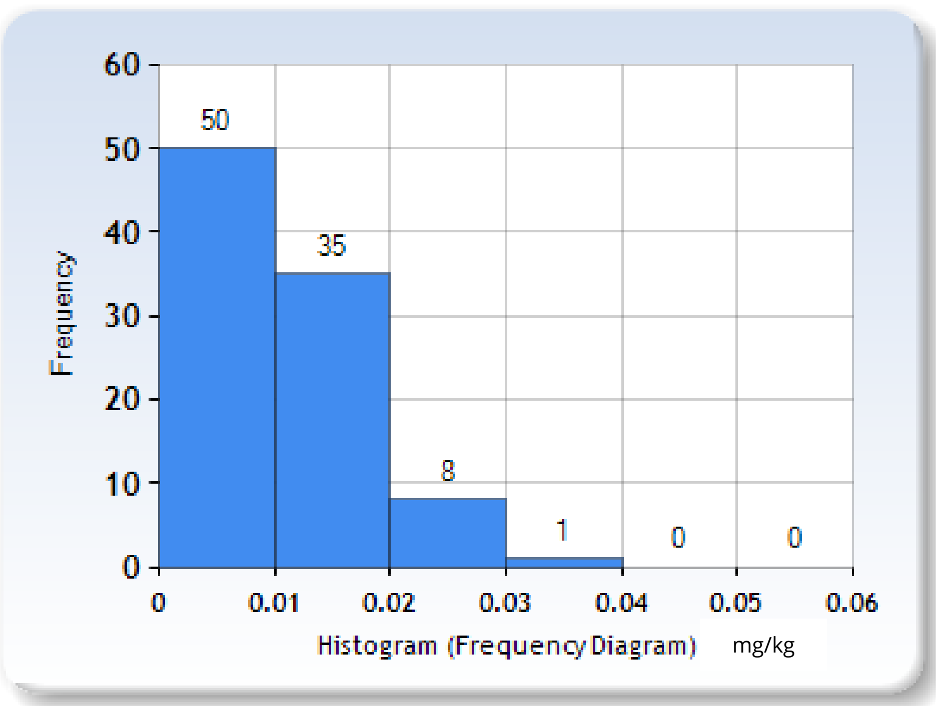

Figure 15. Histogram of Aroclor 1260 concentrations of DOE Puget Sound benthic sediment data (1989-2019) by Van Veen grab sampling (Washington Department of Ecology 2021)

generated by Social Science Statistics. For reference, the value of Aroclor 1260 observed in this study is 2.1 mg/kg.

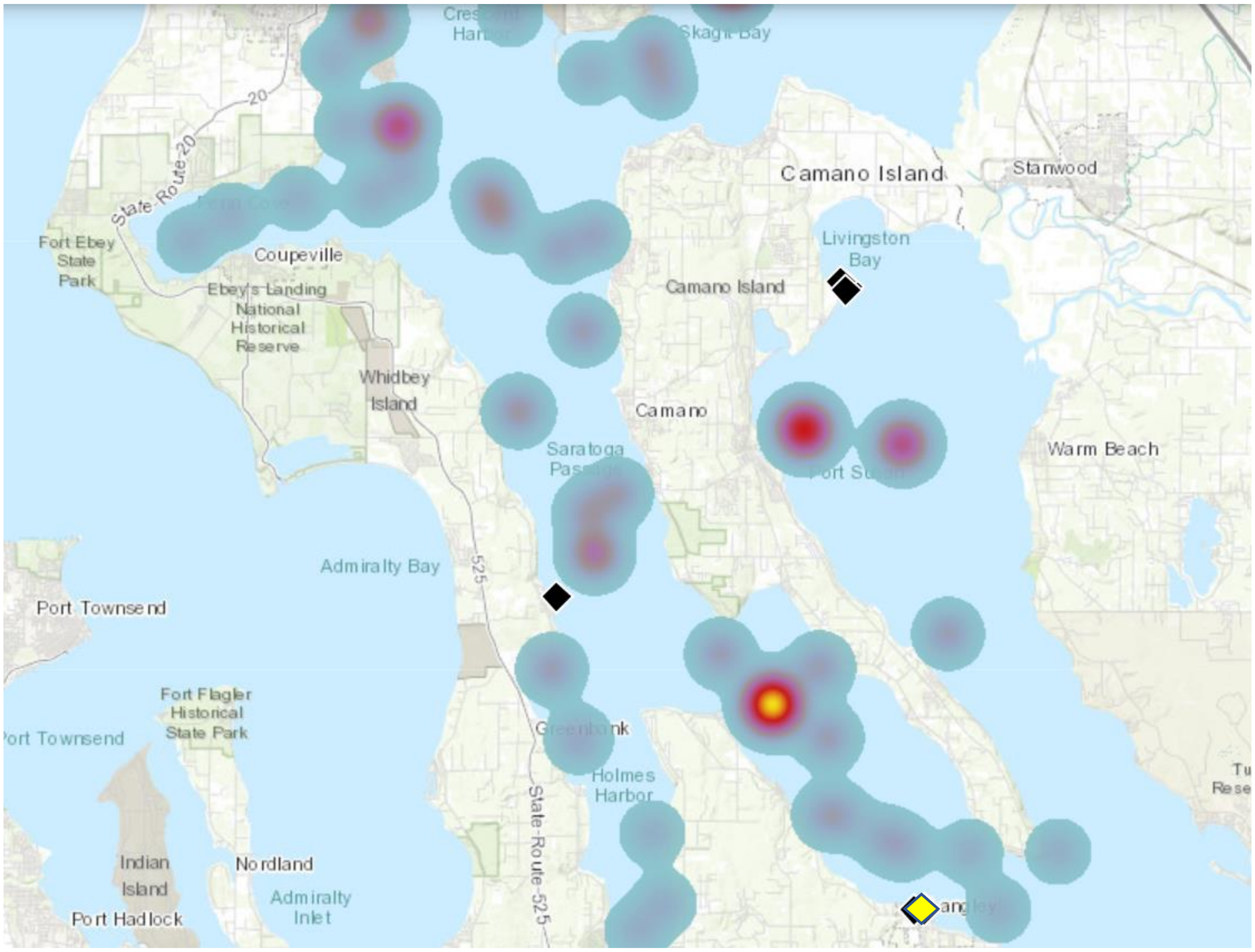

Figure 16. Survey sample collection sites (collection sites = black diamonds, high observed value = yellow diamond) and hot spots (blue = low, purple = medium, red = high, yellow = very high) of Aroclor 1260 concentrations from DOE Puget Sound benthic sediment data. Generated with ArcGIS.

Conclusion

In conclusion, the current Unusual Mortality Event (UME) and overall increase in Eastern Pacific gray whale stock strandings continue without an identifiable source(s). Of the many hypotheses proposed, the contamination and pollution of marine benthic sediments, and their communities, remains in the forefront. Benthic sediments are important biogeochemical sinks for substances, and the unique feeding strategy of the Eastern Pacific gray whale may therefore predispose them to an exceptional risk for contamination and mortality. The concentrations of PCBs in the form of Aroclor 1016 (Sample ID# 5 = 1.7mg/kg) and Aroclor 1260 (Sample ID# 5 = 2.1mg/kg) taken from a known gray whale feeding site, which is visited almost annually, raise concerns of potential soil and groundwater contamination. The concentrations of Al detected in this study (Sample ID #3 = 6,720 mg/kg, Sample ID# 4 = 6,170mg/kg, Sample ID# 5 = 6,610 mg/kg, Sample ID# 6 = 692 mg/kg) also raise concerns of potentially hazardous metals detected for unrestricted land use. The continued anthropogenic altering of oceanic chemistry, and increased acidification of our oceans, may lead to an increased release of benthic heavy metal compounds, such as Al. There is currently no known toxicology of Al, Hg, and PCBs to either gray whales or their ghost shrimp prey. Further research into these topics is needed to continue to understand and support the viability of these key species.

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Appendix

Combined Lab Results

Near-shore sediment sampling period (1) February 2021

Libby Environmental, Inc.

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1A + 2A PROJECT
Megan Bungum
Island County, Washington
Libby Project # L210208-3

Analyses of PCB (Polychlorinated Biphenyls) in Soil by EPA Method 8082

Sample Description	PQL	Method Blank	1A	2A
Date Sampled		N/A	2/6/2021	2/7/2021
Date Analyzed		2/10/2021	2/10/2021	2/10/2021
	(mg/kg)	(mg/L)	(mg/kg)	(mg/kg)
Aroclor 1016	0.1	nd	nd	nd
Aroclor 1221	0.1	nd	nd	nd
Aroclor 1232	0.1	nd	nd	nd
Aroclor 1242	0.1	nd	nd	nd
Aroclor 1248	0.1	nd	nd	nd
Aroclor 1254	0.1	nd	nd	nd
Aroclor 1260	0.1	nd	nd	nd
Surrogate Recovery				
TCMX		111	117	119
DCBP		98	107	108

"nd" Indicates not detected at listed detection limit.
"int" Indicates that interference prevents determination.

ACCEPTABLE RECOVERY LIMITS FOR SURROGATE 65% TO 135%
ACCEPTABLE RECOVERY LIMITS FOR MATRIX SPIKES: 75%-125%
ACCEPTABLE RPD IS 20%

ANALYSES PERFORMED BY: Sherry Chilcutt

Libby Environmental, Inc.

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1A + 2A PROJECT
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Island County, Washington
Libby Project # L210208-3

Analyses of PCB (Polychlorinated Biphenyls) in Soil by EPA Method 8082

Sample Description	PQL	LCS	L210209-3 MS	L210209-3 MSD
Date Sampled		N/A	2/9/2021	2/9/2021
Date Analyzed		2/10/2021	2/10/2021	2/10/2021
	(mg/kg)	(mg/L)	(mg/kg)	(mg/kg)
Aroclor 1016	0.1	113%	98%	107%
Aroclor 1221	0.1			
Aroclor 1232	0.1			
Aroclor 1242	0.1			
Aroclor 1248	0.1			
Aroclor 1254	0.1			
Aroclor 1260	0.1	109%	98%	110%
Surrogate Recovery				
TCMX		95	102	117
DCBP		73	81	88

"nd" Indicates not detected at listed detection limit.
"int" Indicates that interference prevents determination.

ACCEPTABLE RECOVERY LIMITS FOR SURROGATE 65% TO 135%
ACCEPTABLE RECOVERY LIMITS FOR MATRIX SPIKES: 75%-125%
ACCEPTABLE RPD IS 20%

ANALYSES PERFORMED BY: Sherry Chilcutt

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Libby Project # L210208-3

Analyses of Total Mercury in Soil by EPA Method 7471

Sample Number	Date Analyzed	Mercury (mg/kg)
Method Blank	2/10/2021	nd
1A	2/10/2021	nd
1A Dup	2/10/2021	nd
2A	2/10/2021	nd
Practical Quantitation Limit		0.5

"nd" Indicates not detected at the listed detection limits.

ANALYSES PERFORMED BY: Sherry Chilcutt

QA/QC for Total Mercury by EPA Method 7471

Sample Number	Date Analyzed	Mercury (% Recovery)
LCS	2/10/2021	119%
1A MS	2/10/2021	104%
1A MSD	2/10/2021	119%
RPD	2/10/2021	13%

ACCEPTABLE RECOVERY LIMITS FOR MATRIX SPIKES: 75%-125%
ACCEPTABLE RPD IS 20%

ANALYSES PERFORMED BY: Sherry Chilcutt

Near-shore & ghost shrimp specimen sampling period (2) April 2021

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MEGAN BUNGUM PROJECT
Megan Bungum
Island County, Washington
Libby Project # L210419-2

Analyses of PCB (Polychlorinated Biphenyls) in Soil by EPA Method 8082

Sample Description	PQL	Method Blank	LCS	1A	2A	3A	4B
Date Sampled		N/A	N/A	4/17/2021	4/18/2021	4/18/2021	4/18/2021
Date Analyzed		4/21/2021	4/21/2021	4/21/2021	4/21/2021	4/21/2021	4/21/2021
	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Aroclor 1016	0.1	nd	113%	nd	nd	1.7	nd
Aroclor 1221	0.1	nd		nd	nd	nd	nd
Aroclor 1232	0.1	nd		nd	nd	nd	nd
Aroclor 1242	0.1	nd		nd	nd	nd	nd
Aroclor 1248	0.1	nd		nd	nd	nd	nd
Aroclor 1254	0.1	nd		nd	nd	nd	nd
Aroclor 1260	0.1	nd	115%	nd	nd	2.1	nd
Surrogate Recovery							
TCMX		114	79	130	107	Dil	126
DCBP		114	83	129	127	Dil	126

"Dil" Indicates that the compound was not recoverable due to a dilution.
"nd" Indicates not detected at listed detection limit.
"int" Indicates that interference prevents determination.

ACCEPTABLE RECOVERY LIMITS FOR SURROGATE 65% TO 135%
ACCEPTABLE RECOVERY LIMITS FOR MATRIX SPIKES: 75%-125%
ACCEPTABLE RPD IS 20%

ANALYSES PERFORMED BY: Sherry Chilcutt

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MEGAN BUNGUM PROJECT
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Libby Project # L210419-2

Analyses of PCB (Polychlorinated Biphenyls) in Soil by EPA Method 8082

Sample Description	PQL	L210409-4 MS	L210409-4 MSD
Date Sampled		N/A	N/A
Date Analyzed		4/21/2021	4/21/2021
	(mg/kg)	(mg/kg)	(mg/kg)
Aroclor 1016	0.1	89%	87%
Aroclor 1221	0.1		
Aroclor 1232	0.1		
Aroclor 1242	0.1		
Aroclor 1248	0.1		
Aroclor 1254	0.1		
Aroclor 1260	0.1	85%	87%
Surrogate Recovery			
TCMX		127	106
DCBP		114	133

"nd" Indicates not detected at listed detection limit.
"int" Indicates that interference prevents determination.

ACCEPTABLE RECOVERY LIMITS FOR SURROGATE 65% TO 135%
ACCEPTABLE RECOVERY LIMITS FOR MATRIX SPIKES: 75%-125%
ACCEPTABLE RPD IS 20%

ANALYSES PERFORMED BY: Sherry Chilcutt

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MEGAN BUNGUM PROJECT
Megan Bungum
Island County, Washington
Libby Project # L210419-2

QA/QC for Total Mercury by EPA Method 7471

Sample Number	Date Analyzed	Mercury (% Recovery)
LCS	4/22/2021	92%
1A MS	4/22/2021	85%
1A MSD	4/22/2021	90%
RPD	4/22/2021	5.7%

ACCEPTABLE RECOVERY LIMITS FOR MATRIX SPIKES: 75%-125%
ACCEPTABLE RPD IS 20%

ANALYSES PERFORMED BY: Sherry Chilcutt

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MEGAN BUNGUM PROJECT
Megan Bungum
Island County, Washington
Libby Project # L210419-2

Analyses of Total Mercury in Soil by EPA Method 7471

Sample Number	Date Analyzed	Mercury (mg/kg)
Method Blank	4/22/2021	nd
1A	4/22/2021	nd
1A Dup	4/22/2021	nd
2A	4/22/2021	nd
3A	4/22/2021	nd
4B	4/22/2021	nd
Practical Quantitation Limit		0.5

"nd" Indicates not detected at the listed detection limits.

ANALYSES PERFORMED BY: Sherry Chilcutt

Fremont Analytical

Analytical Report

Work Order: **2104265**
Date Reported: **4/28/2021**

CLIENT: Libby Environmental
Project: Megan Bungum

Lab ID: 2104265-001 **Collection Date:** 4/17/2021
Client Sample ID: **1A** **Matrix:** Sediment

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
Total Metals by EPA Method 6020B				Batch ID: 32053		Analyst: EH
Aluminum	6,720	159	D	mg/Kg	20	4/27/2021 5:35:20 PM

Lab ID: 2104265-002 **Collection Date:** 4/18/2021
Client Sample ID: **2A** **Matrix:** Sediment

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
Total Metals by EPA Method 6020B				Batch ID: 32053		Analyst: EH
Aluminum	6,170	157	D	mg/Kg	20	4/27/2021 5:40:54 PM

Lab ID: 2104265-003 **Collection Date:** 4/18/2021
Client Sample ID: **3A** **Matrix:** Sediment

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
Total Metals by EPA Method 6020B				Batch ID: 32053		Analyst: EH
Aluminum	6,610	153	D	mg/Kg	20	4/27/2021 5:46:28 PM

Lab ID: 2104265-004 **Collection Date:** 4/18/2021
Client Sample ID: **4B** **Matrix:** Animal

Analyses	Result	RL	Qual	Units	DF	Date Analyzed
Total Metals by EPA Method 6020B				Batch ID: 32053		Analyst: EH
Aluminum	692	7.87		mg/Kg	1	4/22/2021 6:22:36 PM

Fremont Analytical

Date: 4/28/2021

Work Order: 2104265
CLIENT: Libby Environmental
Project: Megan Bungum

QC SUMMARY REPORT

Total Metals by EPA Method 6020B

Sample ID: **MB-32053** SampType: **MBLK** Units: **mg/Kg** Prep Date: **4/21/2021** RunNo: **66749**
Client ID: **MBLKS** Batch ID: **32053** Analysis Date: **4/22/2021** SeqNo: **1344000**

Analyte	Result	RL	SPK value	SPK Ref Val	%REC	LowLimit	HighLimit	RPD Ref Val	%RPD	RPDLimit	Qual
Aluminum	ND	7.87									

Sample ID: **LCS-32053** SampType: **LCS** Units: **mg/Kg** Prep Date: **4/21/2021** RunNo: **66749**
Client ID: **LCSS** Batch ID: **32053** Analysis Date: **4/22/2021** SeqNo: **1344001**

Analyte	Result	RL	SPK value	SPK Ref Val	%REC	LowLimit	HighLimit	RPD Ref Val	%RPD	RPDLimit	Qual
Aluminum	389	8.00	400.0	0	97.4	80	120				

Sample ID: **2104286-006AMS** SampType: **MS** Units: **mg/Kg-dry** Prep Date: **4/21/2021** RunNo: **66749**
Client ID: **BATCH** Batch ID: **32053** Analysis Date: **4/22/2021** SeqNo: **1344004**

Analyte	Result	RL	SPK value	SPK Ref Val	%REC	LowLimit	HighLimit	RPD Ref Val	%RPD	RPDLimit	Qual
Aluminum	8,730	9.03	451.6	8,559	36.7	75	125				ES

NOTES:
S - Analyte concentration was too high for accurate spike recovery(ies).
E - Estimated value. The amount exceeds the linear working range of the instrument.

Sample ID: **2104286-006AMSD** SampType: **MSD** Units: **mg/Kg-dry** Prep Date: **4/21/2021** RunNo: **66749**
Client ID: **BATCH** Batch ID: **32053** Analysis Date: **4/22/2021** SeqNo: **1344005**

Analyte	Result	RL	SPK value	SPK Ref Val	%REC	LowLimit	HighLimit	RPD Ref Val	%RPD	RPDLimit	Qual
Aluminum	10,200	9.03	451.6	8,559	361	75	125	8,725	15.5	20	ES

NOTES:
S - Analyte concentration was too high for accurate spike recovery(ies).
E - Estimated value. The amount exceeds the linear working range of the instrument.

Fremont Analytical

Date: 4/28/2021

Work Order: 2104265
CLIENT: Libby Environmental
Project: Megan Bungum

QC SUMMARY REPORT

Total Metals by EPA Method 6020B

Sample ID: **2104286-006APDS**	SampType: **PDS**			Units: **mg/Kg-dry**		Prep Date: **4/21/2021**			RunNo: **66749**			
Client ID: **BATCH**	Batch ID: **32053**					Analysis Date: **4/22/2021**			SeqNo: **1344008**			
Analyte	Result	RL	SPK value	SPK Ref Val	%REC	LowLimit	HighLimit	RPD Ref Val	%RPD	RPDLimit	Qual	
Aluminum	12,000	11.3	564	8,560	605	75	125				ES	

NOTES:
S - Analyte concentration was too high for accurate spike recovery(ies).
E - Estimated value. The amount exceeds the linear working range of the instrument.