

EELGRASS (*ZOSTERA MARINA*) POPULATION DECLINE IN MORRO BAY, CA: A
META-ANALYSIS OF HERBICIDE APPLICATION IN SAN LUIS OBISPO
COUNTY AND MORRO BAY WATERSHED

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PROJECT ADVISEMENT

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ABSTRACT

Eelgrass (*Zostera marina*) Population Decline in Morro Bay, CA: A Meta-Analysis of Herbicide Application in San Luis Obispo County and Morro Bay Watershed

Tyler King Sinnott

The endemic eelgrass (*Zostera marina*) community of Morro Bay Estuary, located on the central coast of California, has experienced an estimated decline of 95% in occupied area (reduction of 344 acres to 20 acres) from 2008 to 2017 for reasons that are not yet definitively clear. One possible driver of degradation that has yet to be investigated is the role of herbicides from agricultural fields in the watershed that feeds into the estuary. Thus, the primary research goal of this project was to better understand temporal and spatial trends of herbicide use within the context of San Luis Obispo (SLO) County and Morro Bay Watershed by analyzing data of application by mass, area, and intensity to identify herbicides with the highest potential for local environmental pollution. California Pesticide Use Annual Summary Reports (PUASR) from the years 2000 to 2017 were used to obtain data for conducting a meta-analysis to estimate total herbicide application by weight within every township, range, and section for each of the eight selected herbicides: oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin. A second goal was to select an analytical laboratory that would be best suited for herbicide analysis of estuary sediments to determine the presence, or lack thereof, of the eight selected herbicides. Criteria of consideration in laboratory selection included herbicides detection capabilities,

detection/reporting limits, testing prices, chain of custody protocols, turnaround times, and laboratory site locations.

The meta-analysis yielded results showing high herbicide application rates in SLO County with glyphosate, oxyfluorfen, and chlorthal-dimethyl being identified as three herbicides of elevated risk for local environmental contamination due high rates of use by mass, by area, and/or intensity during the study timeframe. Additionally, Morro Bay Watershed exhibited moderate rates of herbicide application with chlorthal-dimethyl and glyphosate being of highest risk for contamination and accumulation within the estuary because of high application rates by mass, by area, and/or intensity. Finally, Environmental Micro Analysis (EMA) and Primus Group, Inc. (PrimusLabs) were identified as the top candidates for analytical laboratory testing of Morro Bay Estuary sediment samples to be obtained and tested for the selected herbicides. These laboratories provide superior analytical capabilities of the eight herbicides, impressive reporting limits or lower detection limits, competitive testing prices for detecting multiple constituents in multiple samples, robust chain of custody protocols, options for quick turnaround times, and laboratory site locations within California.

Keywords: Morro Bay, Seagrass, Eelgrass, Estuary, Herbicide, Pollution.

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1. INTRODUCTION

1.1 Importance of Estuarine Environments

Estuaries around the world are critical natural habitats that offer a plethora of both environmental and economic benefits. Estuarine environments provide habitat and food sources for thousands of unique birds, mammals, fish, and other wildlife species. Estuaries improve water quality by filtering sediments and pollutants; provide protection of upland habitat and human settlements from coastal storm surges; and are the foundation for countless coastal economies with an estimated 1 billion people worldwide living within 50 km of estuaries (Waycott et al., 2009; USEPA, 2016). Estuaries are of high value to both humans and the environment; nonetheless, many challenges are associated with this boundary between land and sea. Estuaries persist in a delicate balance that can easily be disturbed by natural events as well as anthropogenic actions through changes in land use and development (National Parks Service, 2018). Coastal communities are growing approximately three times faster than any other region in the United States (USEPA, 2016); this rapid coastal growth threatens the fragile integrity of the nation's estuaries and associated ecosystems.

1.2 Importance and Degradation of Seagrass Meadows

Seagrasses serve as keystone species within shallow aquatic environments and can be found from the tropics to the Arctic Circle (Reynolds, 2018). Seagrass meadows are amongst the most important aquatic ecosystems, often dominating coastal and estuarine environments. Seagrass meadows are critical biogenic habitats that support

global and local ecosystem functions (Walter et al. 2018). Globally, seagrass meadows contribute approximately 1% of net marine productivity and approximately 20% of marine and estuarine carbon sequestration (Hughes et al., 2018). Seagrasses rooted in aquatic meadows are considered ecosystem engineers (Walter et al., 2018) by serving crucial roles in a given environment including the reduction of erosion, cycling of nutrients, improvement of water quality, and sequestration of carbon (Harenčár et al., 2018). Additionally, seagrasses are of great interest to environmental researchers and managers because they often serve as an indicator species of ecosystem health due to their sensitivity to environmental perturbations (Walter et al., 2018).

Despite the many reasons for proper stewardship of seagrass, seagrass meadows are considered one of the most threatened ecosystems on the planet and are often likened to rainforests due to similarities in global importance coupled with widespread decline (Harenčár et al., 2018). It is estimated that 29% of global seagrass populations have been heavily degraded or entirely lost because of a broad spectrum of anthropogenic and natural causes. Causes of seagrass degradation include, but are not limited to, decline in water quality, decrease in light availability, impacts from climate change, runoff of toxic contaminants into the environment, eutrophication due to nutrient enrichment, attack by harmful parasites, and destructive fishing practices (Waycott et al., 2009; Barbier et al., 2011).

1.3 Morro Bay Estuary and Eelgrass

A pertinent example of the seagrass meadow degradation in an estuarine environment is found in the Morro Bay National Estuary. The Morro Bay National Estuary, situated on the central coast of California, is an example of an estuary that supports a variety of marine habitats, commercial fishing, oyster farming, and a host of recreational activities (Muleta, 2010). The lagoon associated with the estuary has been degrading in recent history due to upstream land use and management. Poor erosion control and agricultural runoff from intensive cropping operations are two of the possible drivers of the estuarine degradation that have been identified (California State Water Resources Control Board, 2018). A negative response in the estuarine system to the anthropogenic disturbances is evident in the endemic community of eelgrass, which is a sea grass species native to the coast of California.

The Morro Bay Estuary eelgrass community has experienced an estimated decline of 95% in occupied area (reduction of 344 acres to 20 acres) from 2008 to 2017 for reasons that have not yet been identified (Figure 1). This rapid and severe decline in eelgrass is a concern for the overall stability of the Morro Bay Estuary because the eelgrass acts as a necessary component of the local ecology by providing aquatic life with crucial habitat, food source, improved water quality, and other ecosystem services (Harenčár et al., 2018). Abiotic factors that may be driving the decline that have been investigated include, but are not limited to, change in sedimentation rates, change in water depth, and change in water temperature. A possible driver of the eelgrass degradation, that has not been well studied, is the runoff from upland use of toxic herbicides in the croplands of the Chorro Creek and Los Osos Creek Subwatersheds of

the greater Morro Bay Watershed (Muleta, 2010). Herbicides are an area of interest to researchers because of potential impacts on seagrass species.



Figure 1. Aerial image of the Morro Bay lagoon that has been digitized using ESRI ArcMap to represent eelgrass presence in green. Image on the left shows approximately 344 acres of intertidal eelgrass detected in 2007, compared to image on the right of approximately 20 acres detected in 2015. See Figure 2 for location of Morro Bay in California, USA. (Excerpted from Morro Bay National Estuary Program, 2017).

1.4 Effects of Agricultural Herbicides on Seagrass

There is large body of evidence showing that agricultural herbicides can runoff into and be transported by surface waters (NCCOS, 2017) to persist in substantial concentrations within estuarine environments (Readman et al., 1993). Additionally, herbicides have been shown to harm and degrade seagrass species, such as eelgrass, in a

variety of direct and indirect ways. First, the presence of herbicides in sediments and water bodies can directly affect seagrass species via acute toxicity. Some herbicides have been shown to disrupt photosynthesis of aquatic plants through the inhibition of a key photosynthetic protein known as Photosystem II (Murata & Kuwabara, 1983). Additionally, researchers have found that a mixture of multiple herbicides present together may have a synergistic effect, essentially multiplying the negative impacts of each herbicides compared to each herbicide persisting in an environment individually (Nielsen & Dahllöf, 2007). This synergistic effect is of great concern because often a variety of herbicides are applied across an entire watershed, making the likelihood of multiple herbicides mixing in estuaries a realistic possibility. In contrast to direct toxicity to seagrasses, herbicides can also indirectly impact seagrasses through changes in phytoplankton populations. Herbicides that inhibit photosynthesis have been shown to decrease phytoplankton populations in shallow waters (DeNoyelles et al., 1982), which can have a cascading ecological effect that impacts seagrass meadows (Readman et al., 1993). As described above, agricultural herbicides can contribute to seagrass mortality in a multitude of ways; thus, it is critical that the possibility of herbicides persisting in the Morro Bay environment is investigated.

1.5 Project Goal and Objectives

The overarching goal of this research project is to provide information to inform future herbicide detection sampling in Morro Bay Estuary.

Two primary objectives have been identified to meet the project goal:

1. Perform a meta-analysis of California Pesticide Use Annual Summary Reports (PUASR) data (2000 – 2017) in San Luis Obispo County and Morro Bay Watershed to compile, analyze, summarize and visualize data describing the spatial distribution and temporal trends of local herbicide application. The analysis of application data will yield maps, summary tables, and figures of PUASR data that correspond to application by mass, area, and intensity (mass/area) of eight herbicides selected for this study: oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin.

2. Select an environmental analytical laboratory that provides services that capable of satisfying six selection criteria: herbicides detection capabilities in saturated sediment, detection or reporting limits, testing prices, chain of custody protocols, turnaround times, and laboratory site locations. The six selection criteria are considered to make an informed laboratory selection for the analysis of Morro Bay Estuary sediment samples that are to be collected for the next phase of research.

Understanding the trends and extent of herbicide application within Morro Bay Watershed is a crucial step of determining if herbicides are being transported to and persisting in the Morro Bay Estuary, potentially impacting the struggling eelgrass community.

It is hypothesized that there will be significant herbicide application within SLO County that increases with time because agricultural production of SLO County recently reached a record high totaling over \$1 billion in 2018 (Settevendemie, 2019). Morro Bay Watershed was estimated to have 68.2% agricultural land by area (SLO Watershed Project, 2014), so considerable herbicide application within the watershed is expected.

Finally, it is hypothesized that Chorro Creek will show more proximal herbicide application, therefore, higher potential for herbicide transport because it drains a larger land area than Los Osos Creek. Chorro Creek accounts for approximately 60% of total area that drains into Morro Bay Estuary (SLO Watershed Project, 2014).

2. LITERATURE REVIEW

This literature review will provide details on the eight herbicides of interest in this study. The details provided for each herbicide include information that is relevant to the environmental fate and behavior for the chemicals: chemical and physical properties, agricultural uses and application methods, and examples environmental detection and/or presence. Specific values or a range of values are presented for water solubility, half-life, organic carbon-water partition coefficient, and octanol-water partition coefficient for the herbicides oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin. Introductory information regarding the parameters of water solubility, organic carbon-water partition coefficient, and octanol-water partition coefficient is provided below:

(1) Water solubility is a parameter that describes the amount of mass of a given solute that can dissolve into a known volume of water before the point of saturation is reached. Low, moderate, and high water solubility of herbicides correspond to the values of less than 10 mg/L, 10 – 1,000 mg/L, and more than 1,000 mg/L, respectively (Ney, 1995).

(2) The organic carbon-water partition coefficient (K_{oc}) is a parameter that is used to understand environmental fate because it describes the mobility of a given substance in soil. A low K_{oc} value corresponds with high soil mobility, and K_{oc} values increase as soil mobility decreases (ChemSafetyPro, 2016). High K_{oc} values indicate that a chemical will exhibit significant partitioning into the solid phase. Ranges of K_{oc} values and the associated mobility class are provided by McCall et al. (1981) (Table 1).

Table 1. Summary of K_{oc} values and associated degree of soil mobility. Ranges of K_{oc} values and corresponding mobility classes provided by McCall et al. (1981).

K_{oc} (g OC/g soil)	Mobility
0 - 50	Very high
50 - 150	High
150 - 500	Medium
500 - 2000	Low
2000 - 5000	Slightly
> 5000	Immobile

(3) The octanol-water partition coefficient (K_{ow} , often reported as $\log K_{ow}$) is a parameter that is used to understand environmental fate because it describes the readiness of a given compound to sorb to organic matter. The degree of sorption to organic matter is used to determine the potential of sorption to soil or sediment surfaces and potential to bioconcentrate in biota (ChemSafetyPro, 2016). A chemical with a low $\log K_{ow}$ (a value of 1.0 or lower) tends to high water solubility, low potential to sorb to solids, and low risk of bioconcentration. A chemical with a high $\log K_{ow}$ (a value greater than 4.0) tends to have low water solubility, high potential to sorb to solids, and considerable potential to bioconcentrate in organisms (Yamamoto, 2011). Bioconcentration is a term used to describe the intake and retention of a given substance within a living organism via respiration of water or air (Alexander, 1999).

2.1 Oxyfluorfen: Chemical Properties, Agricultural Uses, and Environmental Fate

Oxyfluorfen ($C_{15}H_{11}ClF_3NO_4$) is a pre- and post-emergence herbicide primarily used for the control of annual broadleaf and grassy weeds (USEPA, 2002) that actively

works by inhibiting chlorophyll production (Rio et al., 1997). The chemical is widely applied in the agricultural industry because it can be used with a variety of trees, nuts, fruits, vines, and field crops. Oxyfluorfen is often mixed into a liquid and applied as a spray, resulting in a direct release into the environment via spray drift and surface runoff. The herbicide has potential to impact many levels of local ecological systems as it is toxic to terrestrial and aquatic plants, aquatic invertebrates, and fish (USEPA, 2002).

Once introduced into the environment, oxyfluorfen has an observed half-life of 7 to 17 days in estuarine sediment and 27.5 days in estuarine water (Walker et al., 1988). The extremely low water solubility of oxyfluorfen has been experimentally determined to be 1.16×10^{-1} mg/L (Tomlin, 2004). The chemical compound, with a K_{oc} value as high as 1.0×10^5 g OC/g soil, exhibits essentially no mobility in the soil profile due to strong adsorption to clay minerals and organic matter (Anatra-Cordone et al., 2005). A log K_{ow} value of 5.2 (Brudnell et al., 1995) indicates a high potential to sorb to solids and a moderate to high potential for bioconcentration. Thus, the main pathway of transport of oxyfluorfen in a water body is suspended solids and sediments in a water body, as opposed to being dissolved in the water column (USEPA, 2002).

2.2 Glyphosate: Chemical Properties, Agricultural Uses, and Environmental Fate

Glyphosate ($C_3H_8NO_5P$) is an agricultural herbicide that targets a broad range of weeds and is used for a wide variety of trees, fruits, vegetables, nuts, and other crops (USEPA, 2020). Glyphosate is the most used herbicide worldwide (Sanders & Lassen, 2015) making it an herbicide of great concern for environmental pollution. Often applied as a liquid, glyphosate can may be directly introduced into the environment via spray

drift and surface runoff (USEPA, 1993); runoff and spray drift of the herbicide allows for great potential of polluting nearby surface waters. During a field study conducted in the midwestern United States in 2002, glyphosate was found in 36% of water samples, while aminomethylphosphonic acid (AMPA), a glyphosate degradation product, was found in 69% of samples (USGS, 2019).

Once in the environment, the half-life of glyphosate is variable depending on environmental conditions, measured to persist in soil anywhere from 1.85 days (USEPA, 1993) to 127 days (Müller, 1981) and to persist in pond water anywhere between 12 – 70 days (Grossbard & Atkinson, 1985). Another glyphosate study showed that the chemical is susceptible to biodegradation in water and was observed to have an aquatic half-life ranging between 35 – 60 days (Sanders & Lassen, 2015). The chemical compound has an experimentally determined moderate to high water solubility of 1.05×10^4 mg/L (MacBean, 2008). Glyphosate measured K_{oc} values of 2,600 – 4,900 g OC/g soil (Glass, 1987) indicate slight mobility in the soil profile and high potential to partition in the solid phase when in water. Based on a $\log K_{ow}$ value of -3.40 (Grasso et al., 2018), glyphosate indicates high water solubility and minimal potential to bioconcentrate within organisms. Despite high water solubility, glyphosate is expected to mostly partition into the solid phase when in a water body due to the high range of measured K_{oc} values.

2.3 Diuron: Chemical Properties, Agricultural Uses, and Environmental Fate

Diuron ($C_9H_{10}Cl_2N_2O$) is an agricultural herbicide that is applied as a pre- and post-emergence herbicide for weed control in variety of environments. The herbicide is used in terrestrial and aquatic settings for food and non-food crops, ornamental trees, fish

aquariums and ponds. Diuron is often applied as a foliar spray, resulting in a direct release of herbicide into the surrounding environment primarily via surface runoff and spray drift (USEPA, 2003). Diuron is persistent, mobile, and is frequently found to contaminate both surface and groundwater (USEPA, 2001).

The herbicide diuron is expected to persist in the environment with relatively long field soil half-lives ranging from 133 – 657 days, with an average of 328 days (USEPA, 1982). Diuron has moderate to long persistence in aquatic environments with a half-life ranging between 5.5 – 67 days in water column depending on the degree of light attenuation (Australian Pesticides and Veterinary Medicines Authority, 2011). The herbicide exhibits a moderate solubility in water of 37.4 mg/L (MacBean, 2008) and a moderate to low soil mobility based on experimental K_{oc} values of 468 – 1,666 g OC/g soil (USEPA, 2003). Diuron indicates low to moderate potential for sorbing to solids and low to moderate potential of bioconcentration based on a $\log K_{ow}$ value of 2.68 (Hansch et al., 1995). Thus, with a low water solubility and low to moderate potential to sorb to solids, the constituent is expected to partition in both the solid and liquid phases.

2.4 Chlorthal-dimethyl: Chemical Properties, Agricultural Uses, and Environmental Fate

Chlorthal-dimethyl ($C_{10}H_6Cl_4O_4$) is a pre-emergence weed control herbicide primarily used for the control of dodder, a parasitic plant (White-Stevens, 1997). The chemical is widely used in the agricultural industry because it is approved for the use on common crops such as turf, ornamentals, alfalfa, strawberries, cotton, and soybeans (Meister, 1987). Chlorthal-dimethyl is often mixed into a liquid and applied as a foliar

spray (Glotfelty et al., 1984), resulting in a direct release into the environment via surface runoff and spray drift (Tomlin, 1994). Chlorthal-dimethyl, a possible human carcinogen (USEPA, 2018), has been known to be transported to aquatic environments downstream from agricultural regions. In two studies conducted in California to evaluate the presence of the herbicide, 39% of soil samples from Moss Landing and 47% from the Salinas and Carmel River Valley tested positive for chlorthal-dimethyl (concentrations ranging from not detected to 700 ug/kg soil dry weight) (Fleck et al., 1988).

Once introduced into the environment, chlorthal-dimethyl has a wide range of persistence depending on environmental conditions. The half-life may range from at least 7 days in sunlit, shallow, clear waters (Extonet, 1993a) to 100 days in low moisture soil (Choi et al., 1988). The low water solubility of chlorthal-dimethyl has been experimentally determined to be 0.5 mg/L at 25 °C (Danielsen & Yalkowsky, 1991). The chemical compound, with an experimentally determined K_{oc} of 5900 g OC/g soil, exhibits minimal mobility in the soil profile due to strong adsorption to clay minerals and organic matter (Lyman et al., 1990). The chemical has a high potential to sorb to solids and a moderate to high potential for bioaccumulation based on a log K_{ow} value of 4.40 (King, 2016). Thus, the main pathway of transport of chlorthal-dimethyl in a water body is characterized by being carried with suspended solids and sediments in a water body, as opposed to being dissolved in the water column (Swann et al., 1983).

2.5 Simazine: Chemical Properties, Agricultural Uses, and Environmental Fate

Simazine ($C_7H_{12}ClN_5$) is an agricultural herbicide used for the pre-emergence control of annual grasses, broad-leaved weeds (USEPA, 2007), and algae in ponds

(Humburg, 1989). Widely used in the United States, an estimated 5 to 7 million pounds are applied annually (USEPA, 2007). There is a direct release of simazine into the surrounding environment (Tomlin, 1997) via surface runoff (Frank et al., 1987) as the herbicide is applied to bare soil in the form of solid granules or mixed into a foliar spray (Humburg, 1989). Due to its potential to dissolve into and be transported by water, simazine was found in 79 of 104 wells in rural Ontario, Canada, some of which had to be abandoned due to unsafe levels of contamination (Frank et al., 1987).

Once introduced into the environment, simazine is expected to have moderate to long persistence in soil with a half-life ranging between 27 – 102 days (Tomlin, 1997). The half-life in water is variable and can range from 30 days in pond water to 700 days in lake water (Ghassemi et al., 1981). The chemical compound has a low water solubility of 6.2 mg/L (Tomlin, 1997). Based on measured K_{oc} values ranging from 78 g OC/g soil (Frank et al., 1987) to 3,559 g OC/g soil (Sukop & Cogge, 1992), simazine is expected to have anywhere from low to high mobility in the soil profile. Simazine has a low to moderate potential to sorb to solids and a low to moderate potential to bioconcentrate based on a measured $\log K_{ow}$ value of 2.18 (Grasso et al., 2018). Thus, with low solubility and low to moderate potential to sorb to solids, the main pathway of transport of simazine in a water body is characterized by being carried with suspended solids and sediments in a water body, with some dissolution into the water column.

2.6 Napropamide: Chemical Properties, Agricultural Uses, and Environmental Fate

Napropamide ($C_{17}H_{21}NO_2$) is an herbicide primarily used for the control of broadleaf weeds and annual grasses on a variety of fruits, nuts, vegetables, ornamentals,

turf, forestry sites and tobacco (USEPA, 2005). The herbicide is often mixed into a liquid and applied as a foliar spray (USEPA, 2013), resulting in potential for a direct release into the environment via surface runoff and spray drift.

Once introduced into the environment, napropamide is expected to have moderate to long persistence in soil with a half-life ranging between 15 – 446 days depending on water content. With long terrestrial persistence, the chemical has potential to reach aquatic environments via surface runoff (USEPA, 2005). In aquatic environments, napropamide was observed to be susceptible to photolysis have a half-life of 7 minutes in sunlit, shallow, clear water. The herbicide has a moderate water solubility of 73 mg/L (Extonet, 1993b). Napropamide measured K_{oc} values ranging from 218 (USDA ,1995) to 700 (Vogue, 1994) indicate moderate to low mobility in the soil profile and moderate potential to partition in the solid phase when in water. Based on a log K_{ow} value of 3.36 (Hansch et al., 1995), napropamide indicates moderate potential to sorb to solid particles and low to moderate potential to bioconcentrate within organisms (USEPA, 2005). Thus, napropamide expected to mostly partition into the solid phase when in a water body.

2.7 Trifluralin: Chemical Properties, Agricultural Uses, and Environmental Fate

Trifluralin ($C_{13}H_{16}F_3N_3O_4$) is an herbicide used for the control of broadleaf annual weeds and annual grasses and typically applied to a variety of crops, shrubs, and flowers (USEPA, 1984). Applied as a foliar spray, the herbicide has potential to contaminate nearby surface waters via surface runoff and spray drift (USEPA, 1996).

In terrestrial environments, the half-life in soil is variable depending on water content with anerobic half-lives ranging between 25 – 59 days and aerobic half-lives

between 116 – 201 days (USEPA, 1996). In aquatic environments, trifluralin was observed to be susceptible to photolysis with a half-life of 22 minutes in sunlit, shallow, clear water (Zepp & Cline, 1977). The chemical has a moderate water solubility of 18.4 mg/L (MacBean, 2008). Based on a K_{oc} value of 7,000 g OC/g soil (Extonet, 1993d), trifluralin is essentially immobile in soil and has a high potential to partition in the solid phase when in water. Trifluralin has a very high potential to sorb to solids and bioconcentrate in organisms due to a measured $\log K_{ow}$ of 5.34 (Hansch et al., 1995). Thus, the herbicide is expected to partition into the solid phase when transported by a water body.

2.8 Oryzalin: Chemical Properties, Agricultural Uses, and Environmental Fate

Oryzalin ($C_{12}H_{18}N_4O_6S$) is an agricultural herbicide that is applied as a pre-emergence herbicide for weed control (Meister, 2000), and is often mixed into a liquid and applied as a foliar spray (Hartley & Kidd, 1987). The foliar application method results in a direct release of herbicide into the surrounding environment primarily via surface runoff and spray drift. Once transported into a water body, oryzalin poses a risk to aquatic vegetative species in shallow waters adjacent to areas of application (USEPA, 1994).

The half-life of oryzalin exhibits a wide range of persistence depending on environmental conditions. The half-life may range from 0.34 – 4.35 months in anaerobic and aerobic soil conditions, respectively (Gingerich & Zimdahl, 1976). Additionally, in aquatic environments the chemical was observed to be susceptible to photolysis with a half-life of 1.4 hours in sunlit, shallow, clear water (USEPA, 2000).

The chemical compound has a low solubility in water of 2.5 mg/L. Oryzalin has a low soil mobility based on an experimental K_{oc} value of 600 g OC/g soil (Extonet, 1993c) and a field study showing that oryzalin stays in the top 7.5 cm of the soil profile (Ahrens, 1994). Based on a log K_{ow} value of 3.73 (Santa Cruz Biotechnology, 2009), oryzalin indicates moderate potential to sorb to solid particles and low to moderate potential to bioconcentrate within organisms. With a low solubility and moderate potential to sorb to solids, the constituent is expected partition into the solid phase and be transported by suspended solids and sediment in a water body; however, there is observed potential for it to partially dissolve into the water column (USEPA, 2000).

Table 2. Summary table of chemical and physical properties presented in the literature review of the selected herbicides.

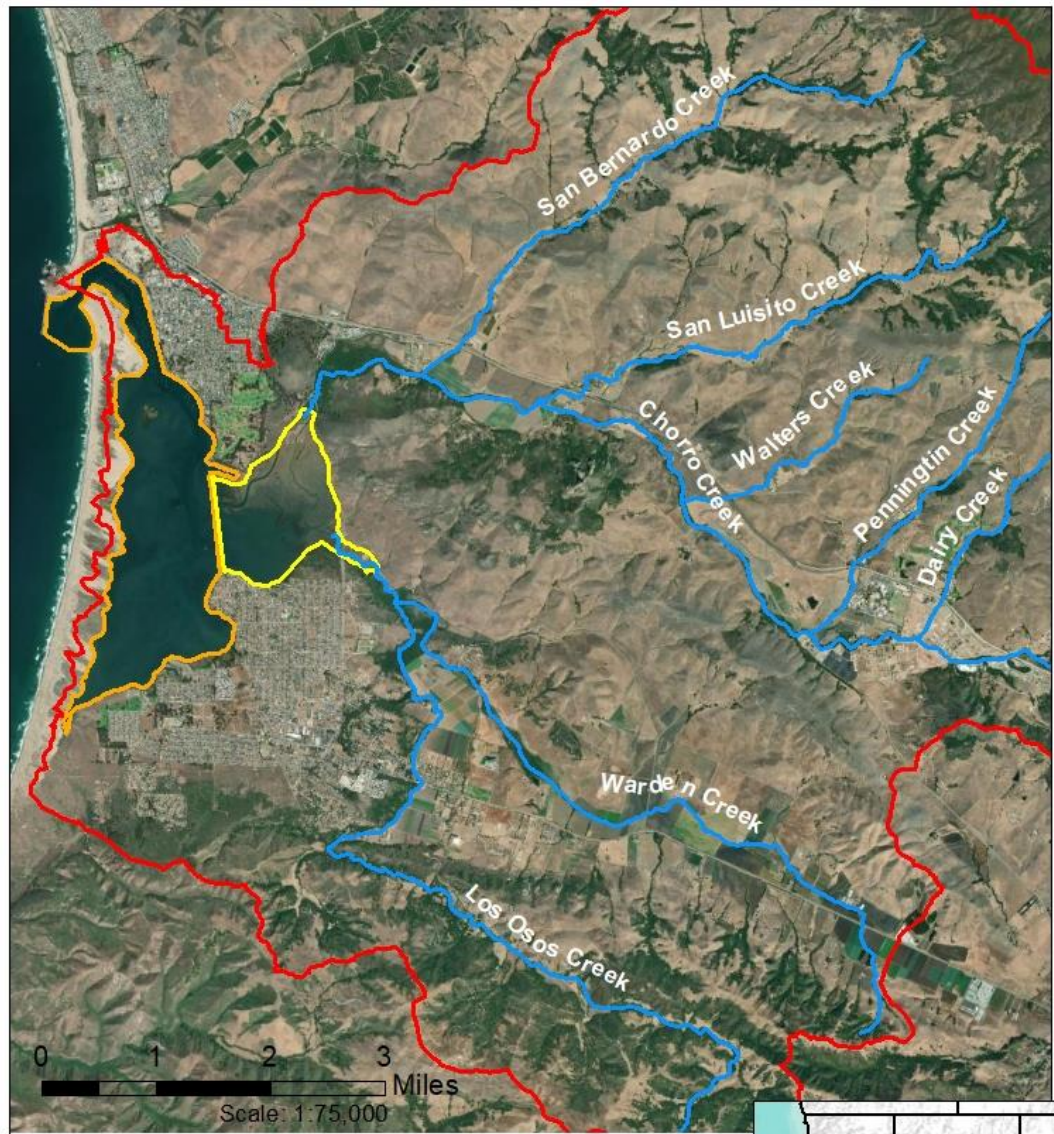
Herbicide	Water Solubility (mg/L)	Half-Life Range	K _{oc} (g OC/g soil)	log K _{ow}
Oxyfluorfen	1.16x10 ⁻¹ (Tomlin, 2004)	7 - 17 days in estuarine sediment (Walker et al., 1988) 27.5 days in estuarine water (Walker et al., 1988)	As high as 1.0x10 ⁵ (Anatra-Cordone et al., 2005)	5.2 (Brudnell et al., 1995)
Glyphosate	1.05x10 ⁴ (MacBean, 2008)	1.85 - 127 days in soil (USEPA, 1993; Müller, 1981) 35 - 60 days in water (Sanders & Lassen, 2015)	2,600 - 4,900 (Glass, 1987)	-3.40 (Grasso et al., 2018)
Diuron	37.4 (MacBean, 2008)	133 - 657 days in soil (USEPA, 1982) 5.5 - 67 days in water column (Australian Pesticides and Veterinary Medicines Authority, 2011)	468 - 1,666 (USEPA, 2003)	2.68 (Hansch et al., 1995)
Chlorthal-Dimethyl	0.5 (Danielsen & Yalkowsky, 1991)	100 days in aerobic soil (Choi et al., 1988) >7 days in sunlit, clear, shallow waters (Extonet, 1993a)	5900 (Lyman et al., 1990)	4.40 (King, 2016)
Simazine	6.2 (Tomlin, 1997)	27 - 102 days in soil (Tomlin, 1997) 30 days in pond water (Ghassemi et al., 1981) 700 days in lake water (Ghassemi et al., 1981)	78 - 3,559 (Frank et al., 1987; Sukop & Cogge, 1992)	2.18 (Grasso et al., 2018)
Napropamide	73 (Extonet, 1993b)	15 - 446 days in soil (USEPA, 2005) 7 minutes in sunlit, clear, shallow water (Extonet, 1993b)	218 - 700 (USDA, 1995; Vogue et al., 1994).	3.36 (Hansch et al., 1995)
Trifluralin	18.4 (MacBean, 2008)	25 - 59 days in anaerobic soil (USEPA, 1996) 116 - 201 days in aerobic soil (USEPA, 1996) 22 minutes in sunlit, clear, shallow water (Zepp & Cline, 1977)	7,000 (Extonet, 1993d)	5.34 (Hansch et al., 1995)
Oryzalin	2.5 (Extonet, 1993c)	0.34 months in anaerobic soil (Gingerich & Zimdahl, 1976) 4.35 months in aerobic soil (Gingerich & Zimdahl, 1976) 1.4 hours in sunlit, clear, shallow water (USEPA, 2000)	600 (Extonet, 1993c)	3.73 (Santa Cruz Biotechnology, 2009).

3. METHODS

3.1 Site Description

Morro Bay is located on the Central Coast of California in San Luis Obispo County. Morro Bay Estuary can be separated into three distinct components: freshwater creeks flowing into the estuary (Chorro and Los Osos Creeks), a salt marsh, and saline bay waters. Fresh water from the Chorro and Los Osos creeks mix with saltwater from the Pacific Ocean (Morro Bay National Estuary Program, 2017) in the intertidal zones located within the salt marsh, which separates the upland creeks from the bay (Figure 2). Chorro creek is a perennial stream that is 11.25 miles long and drains a 30,000-acre watershed. Los Osos Creek is a perennial stream that is also 11.25 miles long and drains a 18,000-acre watershed. The last portion of drainage into Morro Bay is contributed by the eastern slopes of the sand spit, which drains an area of around 450 acres. The Morro Bay drainage basin area totals to approximately 48,450 acres (Gerdes et al., 1974). According to the SLO Watershed Project, much of Morro Bay Watershed is used for agriculture and golf courses, which are two land uses that often rely on herbicides for weed control. It is estimated that 68.2% of the watershed land area is designated as agricultural (SLO Watershed Project, 2014), communicating the likelihood of widespread herbicide application and potential of herbicides draining into Morro Bay Estuary.

Morro Bay, CA Site Map



Legend

- Morro Bay Watershed
- Creeks
- Bay Waters
- Salt Marsh



Figure 2. A site map of Morro Bay, CA and three distinct components: streams of the Chorro and Los Osos creek watersheds, the Morro Bay Estuary salt marsh, and saline bay waters.

3.2 Criteria for Herbicide Selection

The eight herbicides selected for this study are oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin. These herbicides were selected for the study because they are among the top 10 most used herbicides by mass in San Luis Obispo County from 2009 to 2019, according to the Agricultural Pesticides Mapping Tool provided online by Tracking California (Tracking California, 2020). The exception to the previously mentioned selection criterion is diuron, which was picked for the study because it was detected in a Warden Creek water sample at 0.008 ug/L in 2013 (California State Water Resources Control Board, 2020) and has been shown to have a negative impact on eelgrass (Chesworth et al., 2004; Hughes et al., 2018). Warden Creek drains into Morro Bay Estuary via the Los Osos Creek inlet (Figure 2).

Scientific literature describing the effects of specific herbicides on seagrass species is limited (Price & Kelton, 2013). However, three of the herbicides selected for this study that have been shown to have impacts on seagrass species: diuron (Chesworth et al., 2004; Hughes et al., 2018), glyphosate (Kittle III & McDermid 2016), and simazine (Wilkinson et al., 2015). Another criterion of selection was the known tendencies of the eight herbicides to partition into the solid phase (adsorb to suspended solids and sediment) (Ameli, 2016), which is the ideal media of sampling for future research with the aim to detect the selected herbicides in Morro Bay Estuary.

3.3 Meta-Analysis of Herbicide Use Data in San Luis Obispo County

Pesticide Use Annual Summary Reports (PUASR) from the years 2000 – 2017 (California Department of Pesticide Regulation, 2020a), were used for herbicide application data acquisition. The annual summary reports were accessed through an online, open source database containing information from the state of California that was exported to MS Excel 2016. Information that was compiled for the eight selected herbicides (oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin) included chemical (herbicide) name, chemical mass of a given application in pounds, area treated by a chemical application in acres, application location data (county, township, range, and section), and date of application.

Once the PUASR data was downloaded and imported into MS Excel 2016, data pre-processing of the PUASR records was necessary to compile and display the pertinent data in ESRI ArcMap 10.7.1. First, all data records that did not correspond to the eight selected herbicides were removed for each given year (2000 – 2017). All records that were incomplete, missing data such as herbicide mass, area, and location of an application, were removed as these data are necessary for the selected analysis. Next, the total mass and area applied of all eight herbicides combined, as well as the mass applied for each given herbicide, were summed in MS Excel to determine herbicide application quantities for every section within SLO County (Table 3). Finally, the township, range, and section location data for all entries were converted to a format that was identical to that of a SLO County township, range, and section shapefile, provided online by the California Department of Pesticide Regulation (California Department of Pesticide Regulation, 2020b). The location data in MS Excel needed be in the same the format of

the SLO County township, range, and section shapefile so that the excel data table could be joined to the attribute table of a GIS shapefile and spatially displayed in ESRI ArcMap (Figure 3).

As a result of pre-processing, maps depicting combined total mass and spatial distribution of the PUASR herbicide application data within SLO County and Morro Bay Watershed were created with ESRI ArcMap using county, township, range, and section data provided by the annual reports to visually represent herbicide usage from 2000 to 2017 (Figure 3; Figure 7). Summary tables of application by mass of the selected herbicides were created to provide more detail of temporal trends in the PUASR data within SLO County and Morro Bay Watershed for the selected time frame (Table 3; Table 4). Additionally, time series depicting individual herbicide application by mass, area, and intensity (mass/area) were generated for both the SLO County and Morro Bay Watershed study areas (2000–2017) to visually represent the extent and temporal trends of application for each of the eight herbicides (Figure 4; Figure 5; Figure 6; Figure 8; Figure 9; Figure 10).

3.4 Laboratory Selection for Analysis of Sediments

Herbicide residue presence in the Morro Bay Estuary environment will be assessed in saturated marine and freshwater sediments. This portion of the project describes the selection of an environmental analytical laboratory that is most appropriate for this type of lab analysis. The selection of the most appropriate laboratory was based on several criteria: herbicides testing capabilities, detection or reporting limits, testing prices, chain of custody protocols, turnaround times, and laboratory site locations.

Analytical capabilities of laboratories that were considered include ability to test for all eight selected herbicides, ability to run tests on sediment samples saturated with saline and fresh water, and reporting limits and/or detection limits. Location was a criterion of consideration because sediments sampled from Morro Bay Estuary intended for herbicide detection will need to be collected, refrigerated, shipped, and analyzed by a laboratory within a short time frame for results to be considered valid. A laboratory that is closer to California Polytechnic State University, San Luis Obispo is preferred over one that is further away in effort to minimize shipping time.

Five environmental analytical laboratories were considered to determine the laboratory best suited for herbicide analysis Morro Bay Estuary sediments:

Environmental Micro Analysis Laboratories, Inc. (EMA); Primus Group, Inc.

(PrimusLabs); EMAX Laboratories, Inc.; BSK Associates; and Weck Laboratories, Inc.

4. RESULTS

4.1 San Luis Obispo County Herbicide Application Analysis

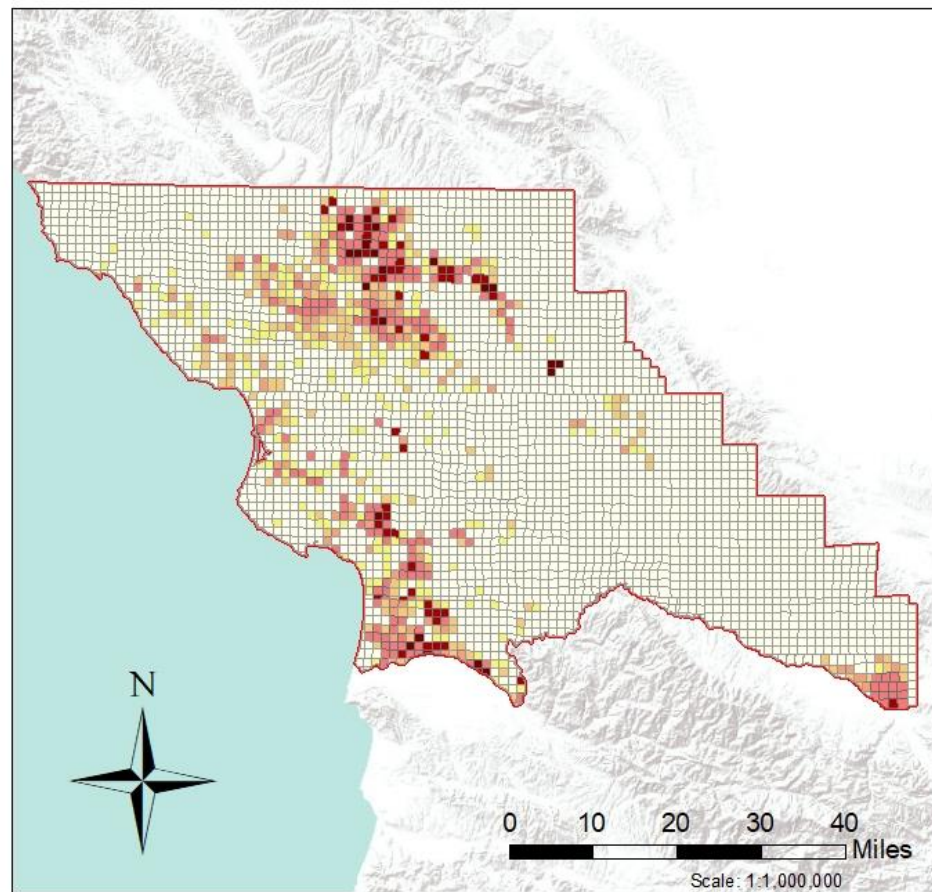
There was an estimated 1,226,949 lbs. of the herbicides oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin applied in SLO County from 2000 to 2017 (Table 3), with an average of 68,164 lbs. applied per year. The northern and southwestern regions of the county spatially showed the most significant rates of application, indicated by the highest densities of sections with over 10,000 lbs. applied (Figure 3). Glyphosate and oxyfluorfen were the two most heavily applied herbicides of interest in SLO County with 498,090 lbs. and 270,420 lbs. applied from 2000 to 2017, respectively. Conversely, diuron and napropamide exhibited the lowest rates of usage by mass within SLO County with 29,585 lbs. and 26,773 lbs., respectively (Table 3: Figure 4).

Glyphosate was the most and oxyfluorfen the second most applied herbicide by area in SLO County from 2000 to 2017. The remaining six selected herbicides had relatively low annual acreages of application with none of the remaining herbicides totaling over 10,000 acres within a given year (Figure 5).

From the list of selected herbicides, chlorthal-dimethyl and oryzalin showed the highest intensities of application in SLO County, with chlorthal-dimethyl being the most intense during the study time frame. Conversely, glyphosate and oxyfluorfen showed the lowest intensity of application despite the high rates of application by mass. The four remaining herbicides (trifluralin, simazine, napropamide, and diuron) had relatively low annual application intensities with no years exceeding an application intensity of

2 lbs./acre, with the exception of trifluralin in 2001 and 2002 and simazine in 2000 and 2003 (Figure 6).

Total Herbicide Application in San Luis Obispo County 2000-2017



Legend

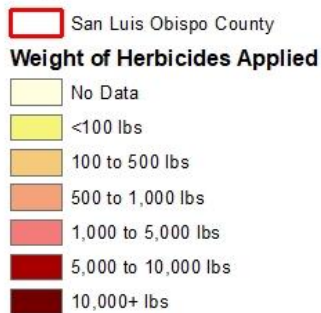


Figure 3. Herbicide application by mass in San Luis Obispo County by township, range, and section for the years 2000 – 2017. Eight herbicides were selected for mapping of total application weight: oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin. Locations with 'No Data' are assumed to have no herbicide application.

Table 3. Summary of total mass of application of eight herbicides of interest by year in San Luis Obispo County for the years 2000 – 2017.

Year	Mass of Herbicides(s) Applied (lbs.)								
	Glyphosate	Oxyfluorfen	Oryzalin	Chlorthal- dimethyl	Simazine	Trifluralin	Diuron	Napropamide	All Herbicides
2000	20,581	13,035	13,114	1,187	17,667	4,546	2,117	812	73,059
2001	19,440	15,426	3,045	6,424	10,498	8,589	1,825	1,318	66,565
2002	22,902	12,520	3,536	6,510	4,917	5,245	2,170	3,514	61,314
2003	27,018	15,179	18,823	10,273	11,213	3,496	2,359	1,128	89,489
2004	18,615	17,772	11,797	7,136	6,851	7,239	2,331	494	72,235
2005	28,480	12,982	5,004	6,705	6,699	4,227	3,802	1,298	69,197
2006	34,601	20,080	5,218	8,173	7,975	3,966	4,002	2,078	86,093
2007	26,267	14,817	5,882	5,933	6,683	2,549	2,220	2,160	66,511
2008	23,293	17,821	9,672	3,902	5,030	2,508	1,757	2,187	66,170
2009	24,236	13,707	4,716	4,255	3,326	2,537	1,035	757	54,569
2010	20,366	13,992	6,321	5,092	4,388	2,654	1,305	678	54,796
2011	25,361	11,455	3,769	4,747	4,236	2,932	1,202	442	54,114
2012	28,830	11,868	5,869	3,428	3,878	3,909	911	1,000	59,693
2013	32,759	12,511	9,537	6,505	1,895	2,738	1,047	1,400	68,392
2014	29,413	13,943	5,180	9,807	2,797	1,351	824	1,467	64,782
2015	33,225	17,407	8,717	6,838	2,368	902	128	1,556	71,141
2016	46,326	25,965	1,172	7,361	3,594	1,075	489	2,735	88,717
2017	36,377	9,940	1,977	7,151	1,391	1,436	61	1,749	60,082
All Years	498,090	270,420	123,349	111,427	105,406	61,899	29,585	26,773	1,226,949

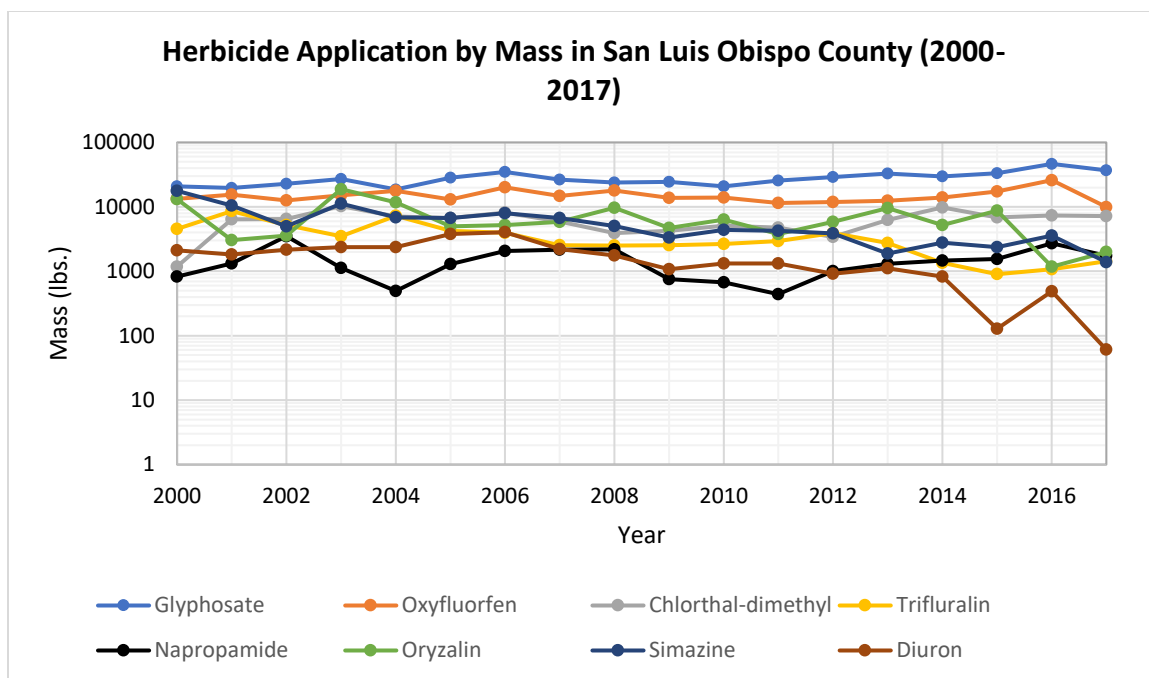


Figure 4. Time series of total masses (lbs.) of each of the eight herbicides of interest by year in San Luis Obispo County for the years 2000 – 2017.

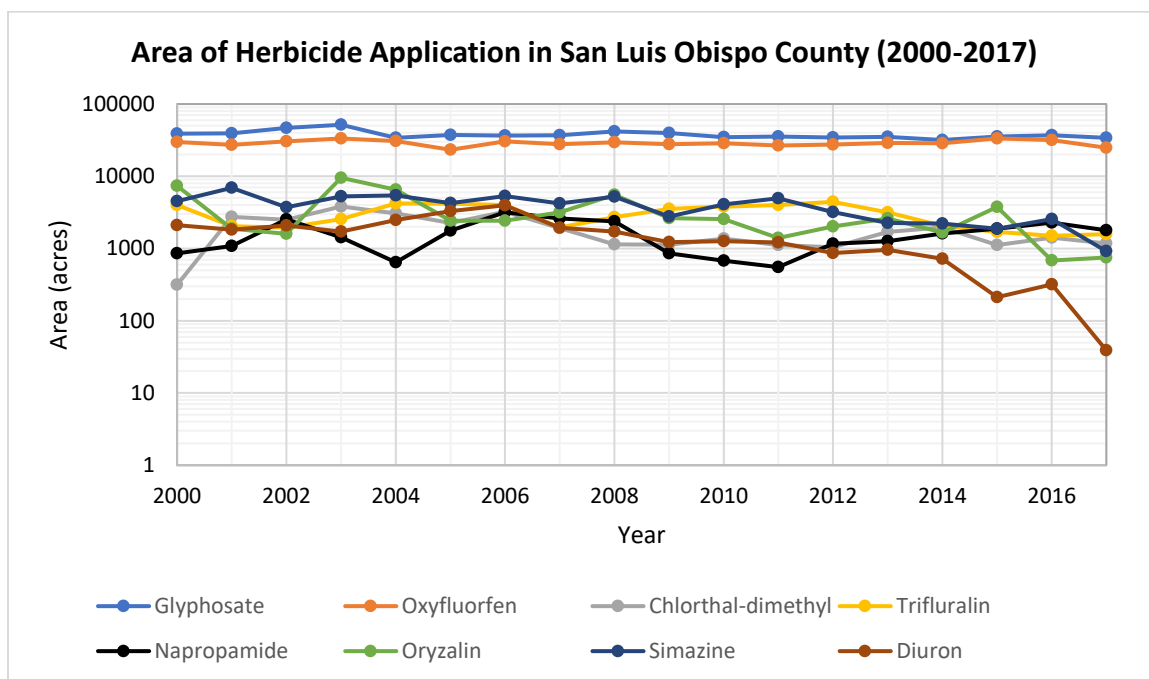


Figure 5. Time series of total area applied (acres) of each of the eight herbicides of interest by year in San Luis Obispo County for the years 2000 – 2017.

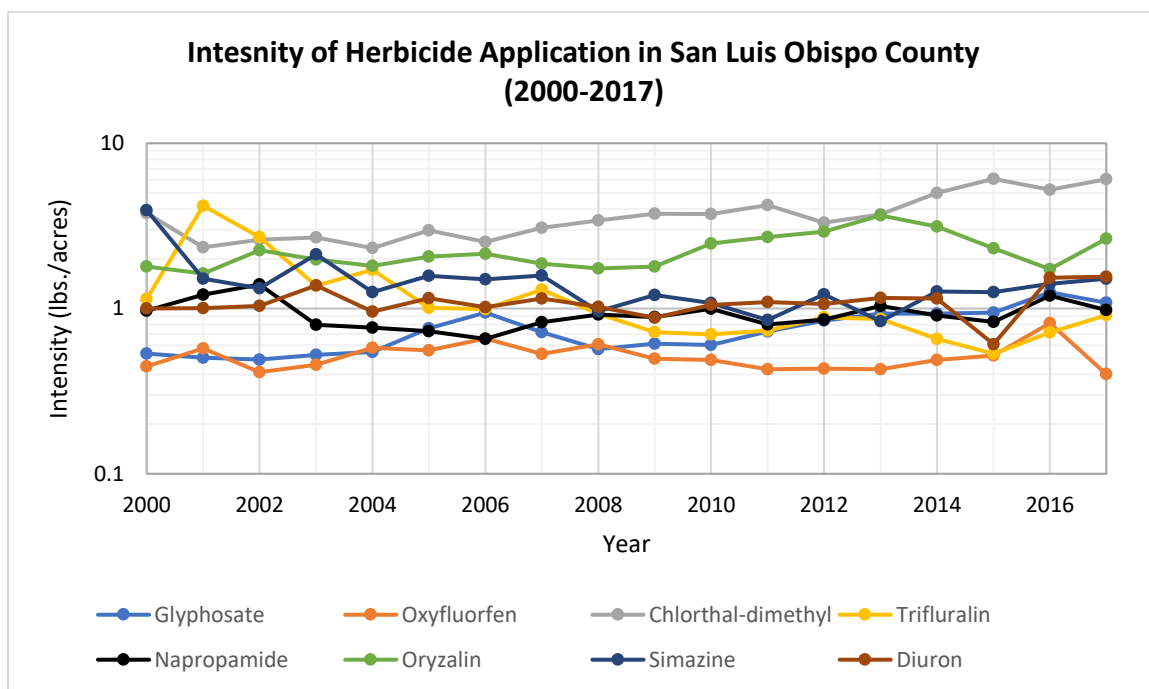


Figure 6. Time series of application intensity (lbs./acres) of each of the eight herbicides of interest by year in San Luis Obispo County for the years 2000 – 2017.

4.2 Morro Bay Watershed Herbicide Application

There was an estimated total application of 20,602 lbs. of the herbicides oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin within Morro Bay Watershed from 2000 to 2017 (Table 4), with an average of 1,145 lbs. applied per year. The southern region of the watershed showed the most mass of application, indicating substantial herbicide usage in proximity to Warden and Los Osos Creeks (Figure 2; Figure 7). Additionally, chlorthal-dimethyl, glyphosate, and oxyfluorfen were the three most heavily applied herbicides in Morro Bay Watershed. Chlorthal-dimethyl was by far the most used herbicide in with an estimated 10,284 lbs. applied. Glyphosate and oxyfluorfen also had significant usage with 4,815 lbs. and 2,674 lbs. applied from 2000 to 2017, respectively. Conversely, diuron was not applied at all

within the watershed with no recorded application during the selected time frame (Table 4; Figure 8).

Chlorthal-dimethyl, glyphosate, and oxyfluorfen exhibited relatively high rates of herbicide application by area in Morro Bay Watershed from 2000 to 2017 (Figure 9). The remaining five selected herbicides had relatively low annual acreages of application with none of the remaining herbicides totaling over 100 acres applied within a given year, except for trifluralin and napropamide in 2017 (Figure 9).

From the list of selected herbicides, chlorthal-dimethyl and simazine showed the highest intensities of application in Morro Bay Watershed, with chlorthal-dimethyl use being the most intense during the study time frame. The six remaining herbicides exhibited low annual application intensities apart from oryzalin in 2005 and 2010 (Figure 10).

Total Herbicide Application in Morro Bay Watershed 2000-2017

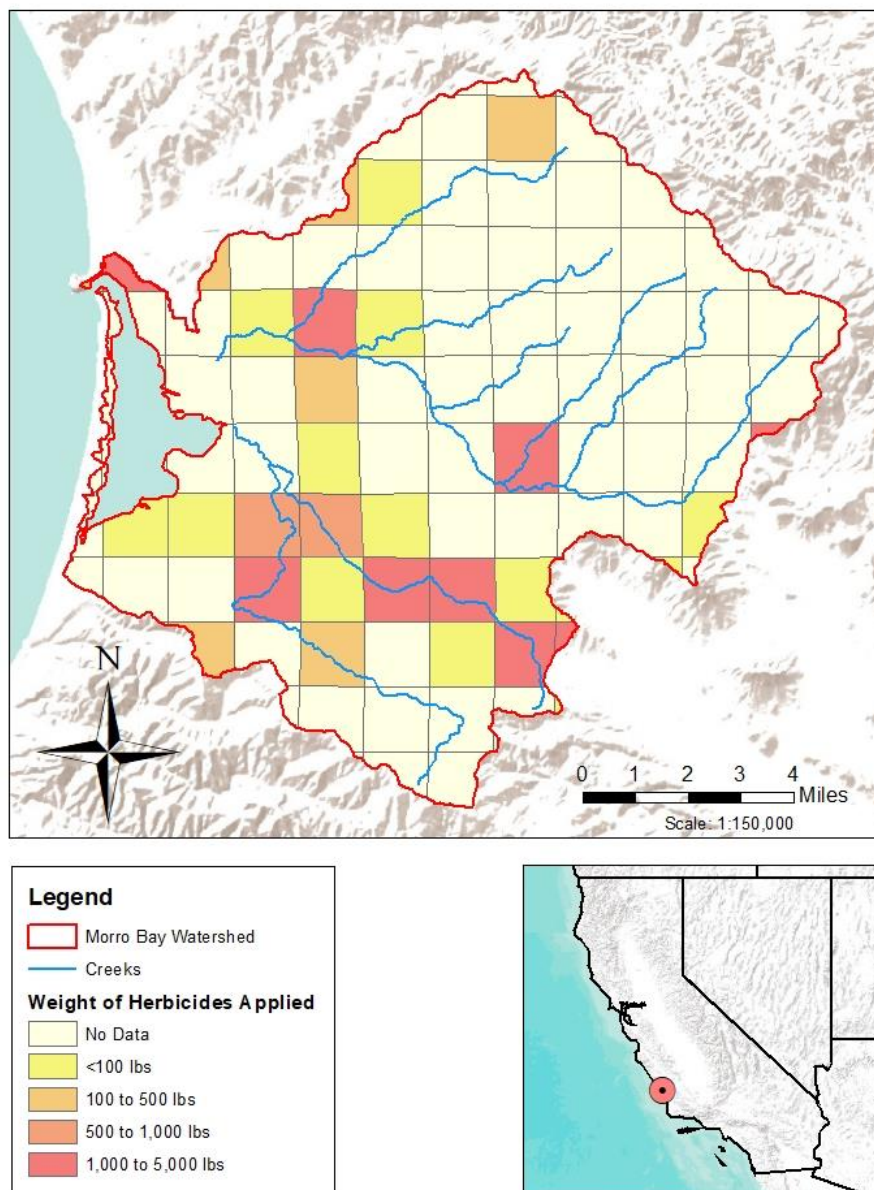


Figure 7. Herbicide application by mass in Morro Bay Watershed by township, range, and section for the years 2000 – 2017. Eight herbicides were selected for mapping of total application weight: oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin. Locations with 'No Data' are assumed to have no herbicide application.

Table 4. Summary of total mass of application of eight herbicides of interest by year in Morro Bay Watershed for the years 2000 – 2017.

Year	Mass of Herbicides(s) Applied (lbs.)								
	Glyphosate	Oxyfluorfen	Oryzalin	Chlorthal- dimethyl	Simazine	Trifluralin	Diuron	Napropamide	All Herbicides
2000	82	111	0	566	0	0	0	26	785
2001	230	54	0	750	0	6	0	51	1,091
2002	139	63	0	1,149	0	5	0	11	1,367
2003	324	75	0	1,081	0	0	0	0	1,480
2004	239	40	133	370	4	0	0	0	786
2005	281	79	161	693	324	0	0	0	1,538
2006	328	265	39	1,080	11	0	0	0	1,723
2007	334	223	33	1,030	226	0	0	5	1,851
2008	353	153	0	750	235	3	0	0	1,494
2009	398	182	0	688	114	3	0	0	1,385
2010	146	107	0	590	26	0	0	0	869
2011	318	181	28	503	0	0	0	24	1,054
2012	402	193	6	213	250	33	0	37	1,134
2013	730	181	0	610	0	68	0	91	1,680
2014	199	172	0	133	0	48	0	64	616
2015	112	238	0	0	0	56	0	76	482
2016	134	121	3	0	0	50	0	86	394
2017	66	236	26	78	0	214	0	253	873
All Years	4,815	2,674	429	10,284	1,190	486	0	724	20,602

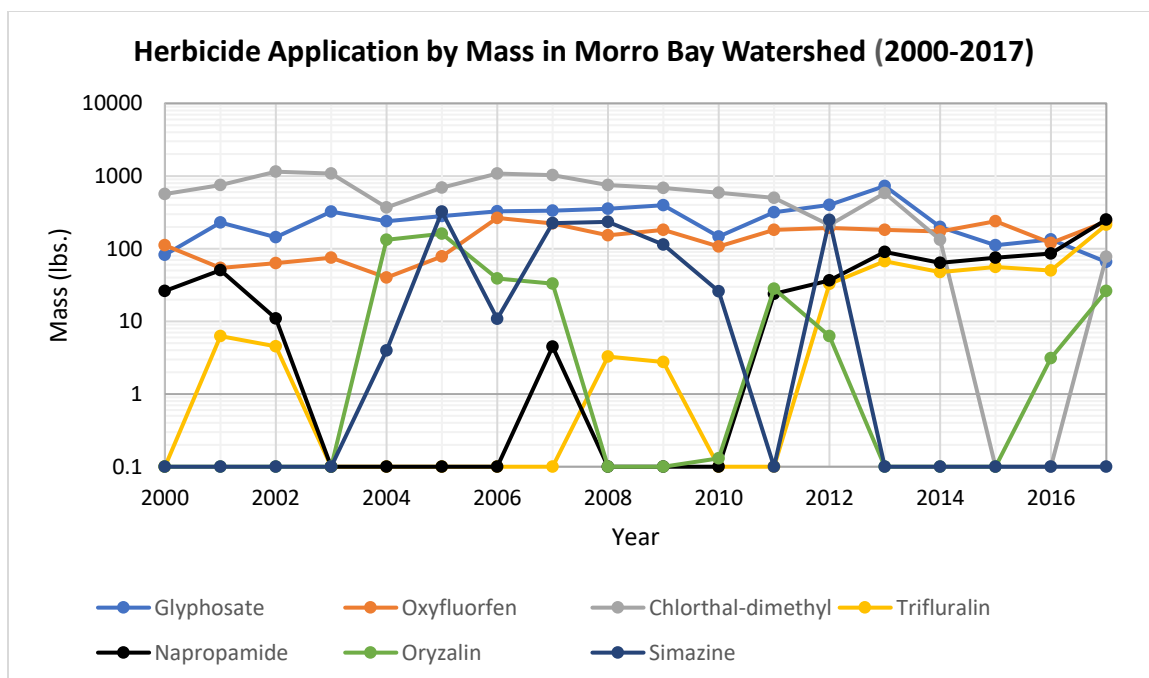


Figure 8. Time series of total masses (lbs.) of each of the eight herbicides of interest by year in Morro Bay Watershed for the years 2000 – 2017. Diuron is excluded from the time series because it was not applied in the watershed during the selected timeframe (California Department of Pesticide Regulation, 2020a).

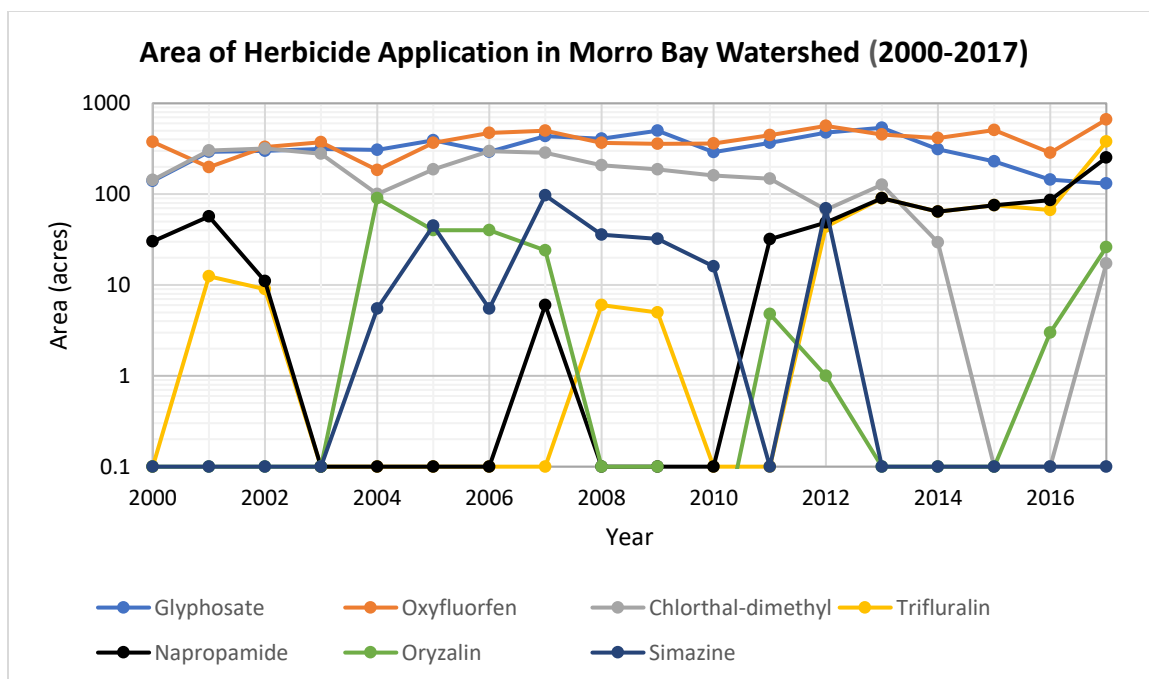


Figure 9. Time series of total area applied (acres) of each of the eight herbicides of interest by Morro Bay Watershed for the years 2000 – 2017. Diuron is excluded from time series because it was not applied in the watershed during the selected timeframe (California Department of Pesticide Regulation, 2020a).

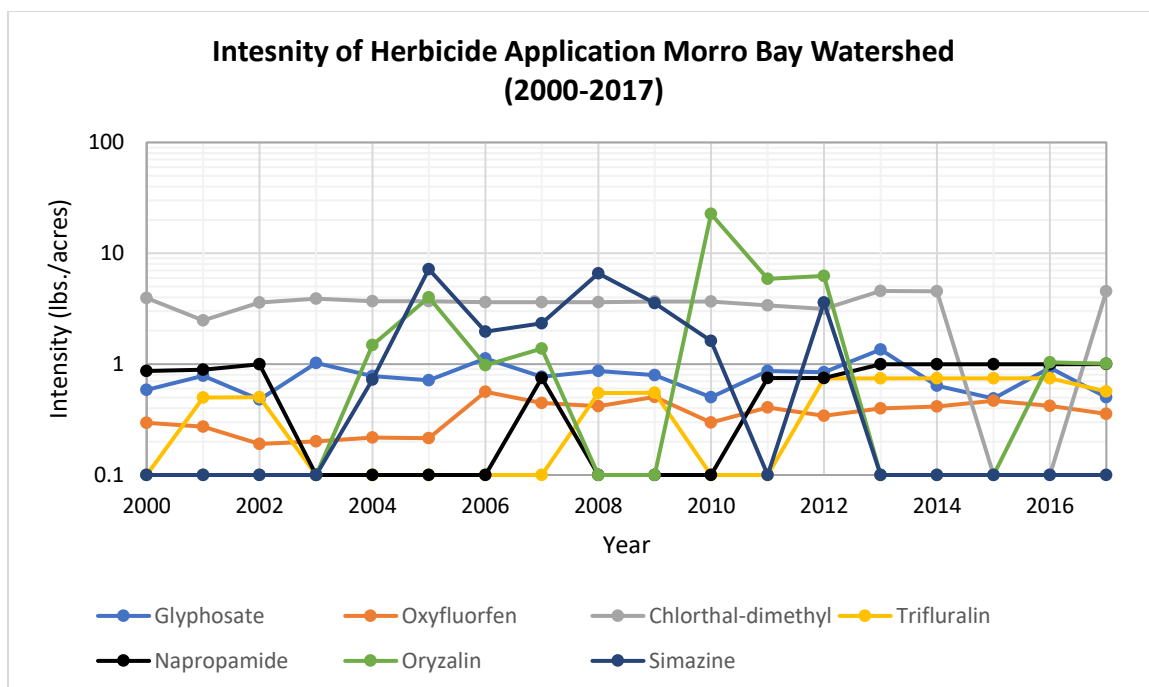


Figure 10. Time series of application intensity (lbs./acres) of each of the eight herbicides of interest by year in Morro Bay Watershed for the years 2000 – 2017. Diuron is excluded from time series because it was not applied in the watershed during the selected timeframe (California Department of Pesticide Regulation, 2020a).

4.3 Analytical Laboratory Selected for Herbicide Analysis of Morro Bay Sediments

EMA and PrimusLabs were identified as the top two candidates for herbicide analysis of Morro Bay Estuary sediments from the list of five analytical laboratories. EMAX Laboratories, Inc.; BSK Associates; and Weck Laboratories, Inc. do not qualify as top candidates for Morro Bay Estuary sediment analysis because of lacking capabilities in testing for all eight of the selected herbicides (Table 5).

4.3.1 Environmental Micro Analysis Laboratories, Inc.

Environmental Micro Analysis Laboratories, Inc. (EMA), located in Woodland, CA, was identified as a top candidate for herbicide analysis of Morro Bay Estuary sediments. EMA is capable of testing saline and fresh water saturated sediment samples

for oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin. In accordance with the California Department of Food and Agriculture regulations and standards, EMA utilizes USEPA official methods of herbicide detection for all the selected herbicides, except for glyphosate, which uses an American National Standards Institute (ANSI) certified proprietary method. (Table 6). Testing for any of the eight herbicides can be expected to yield a reporting limit of 0.04 – 0.05 ppm. The cost to analyze a single sediment sample for all eight herbicides may range between \$621 – \$690 per sample, depending on the total number of samples tested (EMA, Inc., 2020) (Table 8).

As for the chain of custody protocols required for EMA herbicide analysis, each sediment sampling location would require two sediment samples be obtained. One sample must be stored in a 1-liter Nalgene bottle intended for the analysis of glyphosate and one sample must be stored in a 1-liter amber glass bottle for the analysis of the seven remaining herbicides. Samples collected must be stored in darkness and shipped to EMA while remaining at a temperature around 4 degrees Celsius during transport by using chemical ice packs. The sediment must be analyzed within 14 days of collection to ensure validity of the laboratory analysis results; turnaround time for results is expected to be between 1 – 10 business days (EMA, Inc., 2020).

4.3.2 Primus Group, Inc.

PrimusLabs, located near Morro Bay Estuary in Santa Maria, CA, was identified as a top candidate for herbicide analysis of Morro Bay Estuary sediments. PrimusLabs has the means and capabilities to analyze saline and fresh water saturated sediment

samples for oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin using analytical methods provided by the Association of Official Analytical Chemists International (AOAC). All of the selected herbicides can be analyzed in-house with reported lower detection limits ranging between 0.01 – 0.05 ppm, except for glyphosate, which may be subcontracted out to several different laboratories with a range of possible lower detection limits (Table 6). The cost to analyze a single sediment sample for all eight herbicides is between \$675 – \$975 per sample, depending on the utilization of the Multi-Residue Screen Extended Plus option and the laboratory selected for subcontracting the analysis of glyphosate (Table 8). The Multi-Residue Screen Extended Plus is an analysis option offered by PrimusLabs that provides testing of multiple compounds at competitive group pricing (PrimusLabs, 2020b).

The chain of custody protocols required for PrimusLabs analysis are based upon the protocols for soil and sediment sampling provided by the California Department of Food and Agriculture’s Pesticide Enforcement Investigative Sampling Manual and the US Food and Drug Administration’s Investigations Operations Manual. Once sediment samples are collected, specimens must be placed into a polyethylene bag (a zip-lock bag can be used for smaller specimens) or a clean bucket marked with a specimen-specific identification, thoroughly mixed, and stored in a protective, insulated container. The container storing the samples must be kept cool with chemical ice during storage and transportation to the selected PrimusLabs laboratory site, where a temperature reading of no greater than 10 degrees Celsius is required upon arrival to ensure validity of test results (PrimusLabs, 2020a). Once sediment samples are received, PrimusLabs ensures an impressive turnaround time ranging between 1 – 5 business days (PrimusLabs, 2020b).

Table 5. Summary of the capabilities of the selected analytical laboratories to analyze the eight herbicides of interest. The ability for a laboratory to analyze a given herbicide is represented by "X," if capable.

Analyte	Laboratory Name				
	Enviroenmtnal Micro Analysis (EMA)	Primus Group, Inc.	EMAX Laboratories, Inc.	BSK Associates	Weck Laboratories, Inc.
Oxyfluorfen	X	X		X	X
Glyphosate	X	X		X	X
Diuron	X	X		X	X
Chlorthal- dimethyl	X	X			X
Simazine	X	X	X	X	
Napropamide	X	X			
Trifluralin	X	X		X	X
Oryzalin	X	X			

Table 6. Summary of the herbicide detection methods with reporting limit or lower detection limit for the testing of the eight selected herbicides in sediment used by Environmental Micro Analysis and Primus Group, Inc. (EMA, Inc., 2020; PrimusLabs, 2020).

Laboratory Information		Analyte							
		Oxyfluorfen	Glyphosate	Diuron	Chlorthal-dimethyl	Simazine	Napropamide	Trifluralin	Oryzalin
EMA	Method	EPA 8081	ANSI accredited proprietary method	EPA 632	EPA 8081	EPA 8141	EPA 8141	EPA 8081	EPA 632
	Reporting Limit	0.04 ppm	0.05 ppm	0.05 ppm	0.04 ppm	0.05 ppm	0.05 ppm	0.05 ppm	0.05 ppm
Primus Group, Inc.	Method	AOAC 2007.01, Group D (ORGANO-NITROGENS)	Consulting lab used TBD	AOAC 2007.01, Group E (OTHER COMPOUNDS)	AOAC 2007.01, Group A (ORGANO-HALIDES)	AOAC 2007.01, Group D (ORGANO-NITROGENS)	AOAC 2007.01, Group D (ORGANO-NITROGENS)	AOAC 2007.01, Group D (ORGANO-NITROGENS)	AOAC 2007.01, Group D (ORGANO-NITROGENS)
	Lower Detection Limit	0.01 ppm	TBD	0.01 ppm	0.01 ppm	0.01 ppm	0.01 ppm	0.01 ppm	0.05 ppm

Table 7. Summary of the pricing details for EMA analytical services. (EMA, Inc., 2020).

Number of Samples	Method of Detection				Total Cost for All Tests per Sample
	<i>EPA 632 (cost/sample)</i>	<i>EPA 8081 (cost/sample)</i>	<i>EPA 8141 (cost/sample)</i>	<i>Glyphosate (proprietary method) (cost/sample)</i>	
0 – 4	Water: \$140 Soil: \$145	Water: \$135 Soil: \$140	Water: \$135 Soil: \$140	Water: \$265 Soil: \$265	Water: \$675 Soil: \$690
5 – 9	Water: \$133 Soil: \$137.75	Water: \$128.25 Soil: \$135	Water: \$128.25 Soil: \$135	Water: \$251.75 Soil: \$251.75	Water: \$641.25 Soil: \$659.50
10 – 24	Water: \$126 Soil: \$130.50	Water: \$124 Soil: \$126	Water: \$124 Soil: \$126	Water: \$238.50 Soil: \$238.50	Water: \$612.50 Soil: \$621

Table 8. Summary of the pricing details for PrimusLabs analytical services (PrimusLabs, 2020b).

Sample Pricing Rate Type	Method of Detection					Total Cost per Sample
	<i>AOAC 2007.01, Group A (ORGANO-HALIDES)</i>	<i>AOAC 2007.01, Group D (ORGANO-NITROGENS)</i>	<i>AOAC 2007.01, Group E (OTHER COMPOUNDS)</i>	<i>Glyphosate (Sub-contracted)</i>	<i>AOAC 2007.01, Group A, D, and E</i>	
1 Sample (Base Price)	\$165	\$165	\$195	\$300 – 450	-	\$825 – 975
1 Sample with MULTI-RESIDUE SCREEN EXTENDED PLUS	-	-	-	\$300 – 450	\$375	\$675 – 825

5. DISCUSSION

5.1 Herbicide Application in San Luis Obispo County (2000 – 2017)

According to the PUASR data meta-analysis, there was significant herbicide application within SLO County from 2000 to 2017, therefore, indicating significant potential for environmental pollution of herbicides. The northern and southwestern regions of the county yielded the highest application rates, indicated by the most occurrences of sections where total herbicide application was greater than 10,000 lbs. (Figure 3). The southern region of SLO County had highest herbicide application rates and is in proximity to the Pacific Ocean, exhibiting potential for coastal pollution (Figure 3).

Glyphosate is a constituent of concern for environmental pollution and degradation in SLO County due to the highest estimated application rates by mass and by area of the eight selected herbicides from 2000 to 2017 (Table 3; Figure 4; Figure 5). Glyphosate can be persistent in the environment with a variable half-life ranging from 1.85 days to 127 days in soil (USEPA, 1993; Müller, 1981) and 12 – 70 days in pond water (Grossbard & Atkinson, 1985). Thus, glyphosate is identified as an herbicide with high potential to be introduced into and persist within the local environment of SLO. Furthermore, once introduced into the environment, glyphosate can have negative impacts on coastal aquatic habitats. A seagrass species known as beaked tasselweed (*Ruppia maritima*) was observed to have experienced a significant reduction in the chlorophyll absorbance and photosystem II (PSII) when exposed various concentrations of glyphosate (Kittle III & McDermid 2016).

In addition to glyphosate, oxyfluorfen shows considerable potential for environmental pollution within SLO County due to high application rates by mass and by area (Figure 4; Figure 5). Chlorthal dimethyl is another herbicide that exhibits potential for polluting the county showing moderate application by mass and by area exacerbated by the highest application intensities of the eight in the context of the county. Conversely, diuron and napropamide are of lower concern, despite long environmental persistence (USEPA, 1982; USEPA, 2005), because of low application by mass and by area at low intensity from 2000 to 2017 (Table 3; Figure 4; Figure 5; Figure 6).

The meta-analysis results indicate a temporal trend of rather consistent herbicide application from 2000 to 2017, except for three years of elevated total application occurring during 2003, 2006, and 2016 (Table 3). The observed temporal trend differs from the previously stated hypothesis, which anticipated a noticeable rise in herbicide application from 2000 to 2017 due to recent record-breaking agricultural production in SLO County in 2018 (Settevendemie, 2019).

5.2 Herbicide Application in Morro Bay Watershed (2000 – 2017)

According to the PUASR data meta-analysis, there was moderate herbicide application within Morro Bay Watershed from 2000 to 2017, indicating potential for environmental pollution of herbicides to Morro Bay Estuary. This result confirms one of the original hypotheses that Morro Bay Watershed would show considerable potential for herbicide pollution in the environment because agriculture is a dominant land use type within the watershed (SLO Watershed Project, 2014). The southern region of the watershed showed the highest rates of application and indicate that the Los Osos Creek

inlet to the Morro Bay Estuary may have higher potential for receiving and transporting herbicides than the Chorro Creek inlet (Figure 7). This finding refutes the original hypothesis that Chorro Creek Watershed would show more potential for herbicide reception and transport into Morro Bay Estuary due to the larger area drained compared to the Los Osos Creek Watershed (SLO Watershed Project, 2014).

Of the eight selected herbicides, chlorthal-dimethyl is the constituent of most concern for environmental contamination in Morro Bay Watershed due to high application rates by mass and by area at high intensities from 2000 to 2017 (Figure 8; Figure 9; Figure 10). Additionally, chlorthal dimethyl is persistent in the environment with a half-life ranging from at least 7 days in sunlit, shallow waters (Extonet, 1993a) to 100 days in low moisture soil (Choi et al., 1988) (Table 2). Thus, chlorthal-dimethyl exhibits the highest potential of the selected herbicides to be received by the Chorro and Los Osos Creeks, transported to the bay, and accumulate in the sediments of Morro Bay Estuary. Whether the presence of chlorthal-dimethyl in estuary sediments poses a great risk to Morro Bay eelgrass is unknown because current scientific literature describing the effects of this herbicide on seagrasses is minimal. However, potential degradation can be expected because many other herbicides have been proven to promote seagrass mortality (Nielsen & Dahllof, 2007).

In addition to chlorthal-dimethyl, glyphosate shows considerable potential to contaminate the Morro Bay Watershed environment due to relatively high application rates by mass and by area in the context of the watershed (Table 4; Figure 8, Figure 9). As stated previously, this is an area of concern because glyphosate may be persistent in both soil and water media (Müller, 1981; Grossbard & Atkinson, 1985) and pose a risk to

seagrass productivity and health (Kittle III & McDermid 2016). Conversely, diuron is of minimal concern for persisting in Morro Bay Estuary sediments because it was shown to have not been applied at all within the watershed during the selected time frame (Table 4) despite being detected in the Morro Bay Watershed from a Warden Creek water sample at 0.008 ug/L in 2013 (California State Water Resources Control Board, 2020). The discrepancy between PUASR application data and detection data for diuron may be attributed to the fact that the PUASR data only reports commercial uses of herbicides, typically related to agricultural operations, compared to other possible sources of herbicide pollution such as small-scale residential applications.

The meta-analysis conducted in this study indicates a recent decrease of total herbicide application within Morro Bay Watershed. A decrease of herbicide usage can be seen from 2014 to 2017, after a peak application year in 2013 (Table 4). This recent decrease in herbicide application may be cause for hope that potential herbicides inputs to Morro Bay Estuary is decreasing, which may allow for improved environmental conditions that are more conducive to the rebound of the endemic eelgrass community.

5.3 Analytical Laboratory Selection Rationale

EMA and PrimusLabs were identified as the top two candidates for herbicide analysis of Morro Bay Estuary sediments for satisfying six selection criteria: herbicides detection capabilities in saturated sediment, detection or reporting limits, testing prices, chain of custody protocols, turnaround times, and laboratory site locations.

(1) EMA and PrimusLabs have the capability to test for all eight of the herbicides in fresh and saline water saturated sediment samples using methods accredited and/or provided by the USEPA, ANSI, or AOAC.

(2) The various herbicide testing methods from both laboratories yield impressive detection levels for the constituents of interest. PrimusLabs can provide lower detection limits as low as 0.01 – 0.05 and while EMA can provide reporting limits as low as 0.04 – 0.05 ppm. The detection levels offered by each company are comparable considering that reporting limits are expected to be three to five times higher in value than lower detection limits due to differences in dilution calibrations between the two constituent concentration reporting methods (California Department of Public Health, 2005).

(3) Both laboratory companies offer competitive pricing structures for the testing of multiple samples for multiple constituents. The pricing structures of EMA and PrimusLabs both allow for the testing of multiple compounds in multiple samples at competitive group pricing points.

(4) EMA and PrimusLabs have robust chain of custody procedures that provide protocols for the responsible handling of sediment specimens from the time of sampling to the time of testing to ensure test validity. PrimusLabs has a chain of custody procedure based upon the protocols provide by the California Department of Food and Agriculture's Pesticide Enforcement Investigative Sampling Manual and the US Food and Drug Administration's Investigations Operations Manual, while EMA employs proprietary chain of custody protocols.

(5) Typical turnaround times from sample reception to sample testing and results reporting are expected to be up to 5 days and up to 10 days for PrimusLabs and EMA,

respectively. However, both companies can yield rapid turnaround times of one business day given proper notice, providing minimal opportunity for the degradation of the eight herbicides from the time of sampling to the time of detection. Reducing the potential of herbicide degradation before detection promotes accuracy of any results reported.

(6) Both companies have laboratory sites found in California with locations in Woodland, CA and Santa Maria, CA for EMA and PrimusLabs, respectively. A laboratory location in California is important when considering potential transportation time and costs for sediment samples. Reducing sample transportation times will reduce potential for herbicide degradation between time of sampling and time of herbicide detection. The Santa Maria site of PrimusLabs is in a rather ideal location for the sampling Morro Bay Estuary sediments because Morro Bay (San Luis Obispo County) and Santa Maria (Santa Barbara County) are in relatively close proximity being situated in neighboring counties on the central coast of California.

6. CONCLUSIONS

The northern and southwestern regions of SLO County exhibit significant total herbicide application of the chemicals oxyfluorfen, glyphosate, diuron, chlorthal-dimethyl, simazine, napropamide, trifluralin, and oryzalin from 2000 to 2017. The southeastern region of the county shows potential for coastal water habitat pollution of herbicides due to the high intensity of herbicide application and proximity to the Pacific Ocean. Out of the eight selected herbicides, glyphosate, oxyfluorfen and chlorthal-dimethyl have been identified as three chemicals of higher risk for local environmental contamination due high rates of use by mass, by area, and/or intensity during the study timeframe. Of these three higher risk herbicides, current scientific literature reflects that only glyphosate has been studied to characterize the impacts of the compound on seagrass and was shown to be harmful. However, literature suggests that oxyfluorfen and chlorthal-dimethyl may have negative impacts on seagrasses because a number of other herbicides have been observed to degrade seagrass species. In the context of the County, diuron and napropamide are of the lowest concern for contaminating local environments because of relatively low application rates by mass and area at low intensities.

Morro Bay Watershed exhibited moderate application of the selected herbicides, mostly concentrated in the southern region (Los Osos Creek Watershed) from 2000 to 2017. Chlorthal-dimethyl was by far the most applied herbicide by mass within the watershed with high application intensities throughout the study timeframe, presenting the highest risk for environmental pollution of any of the chemicals studied. Due to a combination of local application rates, environmental fate, and environmental

persistence, chlorthal-dimethyl was identified as the herbicide with the highest potential to transport to and accumulate within Morro Bay Estuary sediments, suggesting a potential role in the degradation of the local eelgrass community. Also, glyphosate presents considerable potential for environmental contamination in the watershed and possibly the estuary because of relatively high application rates by mass and by area. Conversely, diuron, which was not applied at all within the watershed during the selected time frame, has been identified as an herbicide that may pose minimal threat to the eelgrass of Morro Bay Estuary.

For when the sampling of Morro Bay Estuary sediments is executed, Environmental Micro Analysis and PrimusLabs have been identified as top candidates for analytical laboratory services to detect the eight herbicides in fresh and saline saturated sediments. These two laboratories have been selected as candidates because they effectively satisfy six main criteria for the analysis of Morro Bay Estuary sediments providing superior analytical capabilities of the eight herbicides, impressive reporting limits or lower detection limits, creative testing prices for detecting multiple constituents in multiple samples, robust chain of custody protocols, options for quick turnaround times, and laboratory site locations within California.

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