Assessing Bioremediation Capacities of Green Lipped Mussels – Measuring the Presence and Bioaccumulation of Heavy-Metals in Green Lipped Mussel Shells

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Abstract:

This experiment aimed to assess the bioremediation capacities of green-lipped mussel shells in marina environments, specifically in relation to heavy metal uptake. Mussels were deployed for a period of five months in three different marina locations in the Auckland region – Half Moon Bay, Orakei, and Hobsonville. The contents of the shells were assessed using LA-ICP-MS, and the accumulation tendencies were measured by comparing the metal levels of three fragments representing different areas of the shell's growth. Results showed no significant evidence of heavy metal accumulation, however presence of all of the 11 assessed metals (Si^{29}, Ti^{47}, V^{51}, Cr^{52}, Mn^{55}, Co^{59}, Ni^{60}, Cu^{63}, Zn^{66}, Ag^{107}, Cd^{111}, and Pb^{208}) was found in all shell fragments. Such a finding is a source for concern in terms of ecosystem health as well as an indicator of heavy metal pollution from some sort of human activity. Half Moon Bay consistently showed higher metal levels than the other two marinas most likely because of the historical location of intense industry in the catchment area and the current abundance of intense land use sites like quarries and landfills. Given the lack of data reflecting bioaccumulation trends there still are substantial opportunities to continue and further this research.

Introduction:

Of all marine environments, urban coastal environments experience heavy anthropogenic use and are usually the most susceptible to eutrophication and pollution. Water contamination is a serious issue not only for the stability of aquatic ecosystems and the environment, but also for human health. Most notably, human health is threatened with contamination of heavy metals, specifically lead, cadmium, mercury, arsenic, zinc, and copper (Järup, 2003; Auckland Regional Council, 2010). Heavy metals sources are mainly from industrial and commercial effluents as well as leaching from soils due to acid rain, which is becoming an increasingly important problem with the rise of climate change (Zheng et. al., 2012). Although adverse health effects of heavy metals have been known for quite some time, humans still experience exposure through a variety of pathways including surface water runoff (Järup, 2003). These threatening substances are toxic even at low concentrations, and also tend to accumulate throughout the food chain, or bioaccumulate (Auckland Regional Council, 2010). Heavy metals tend to bioaccumulate because they are very difficult to degrade or destroy, especially by normal metabolic processes. Furthermore, heavy metals have an impact on marine ecological health. Discovering methods to remove these contaminants from coastal waters, and the marine environment is therefore quite significant.

There are many ways to approach this issue, however one method in particular stands out – implementing the inherent skills of bivalves. These filter feeding and deposit-feeding animals live in benthic environments at the substrate and sediment-water interface of aquatic systems (Biachi et. al., 2014; Walker & Mac Askill, 2013). Because of their ability to filter large volumes of water, bivalves are capable of removing suspended solids desorbed from particulate matter, chemical contaminants (PAHs, PCBs, and TCCs), excess nutrients, heavy metals, and bacteria from the water column (Bianchi et. al., 2014; Walker & Mac Askill, 2013; Ismail et. al., 2014; Auckland Regional Council, 2010; Brown et. al., 2005; Gren et. al., 2008; Prieto et. al., 2003). The contaminants then accumulate either in the organism's tissues or shells, and can also be excreted as feces (Ismail et. al., 2014). The method by which this uptake occurs varies. Bivalves are a group of shelled organisms from the Phylum Mollusca, which also includes Gastropods (snails and slugs), Cephalopods (octopus and squid). Bivalves have shells that are composed of calcium carbonate and the biopolymer chitin – two substances that are known to have sorption capacities for contaminants (Auckland Regional Council, 2010). Chitin, and its deacetylated partner chitosan, have high proportions of free amine groups that are known to be quite effective at attracting and absorbing heavy metals (Auckland Regional Council, 2010). Additional contaminant uptake can occur through the ion exchange and conversion of calcium carbonates into mixed-metal, or inorganic carbonates that form an inorganic fraction of the shell structure (Auckland Regional Council, 2010). Yet another pathway of contaminant uptake would be the reactive sub-micron carbonate structure that provides extensive surface area and makes the shell material highly reactive (Auckland Regional Council, 2010).

Because water chemistry, especially in coastal ecosystems, can be highly variable in both space and time (i.e. plumes and tidal changes) species that are both bioindicators and bioremediators are quite useful. While quite a lot of research has been done with bioremediation and mussels in freshwater and storm water areas, there is somewhat of a lack of strictly marine studies (Brown et. al., 2005; Ismail et. al., 2014; Bianchi et. al., 2014; Auckland Council 2010). Research has been done in the Baltic Sea region, however this study focused on mussel's capacity to reduce eutrophication effects (Gren et. al., 2008). There has also been a sufficient amount of work done to simulate bivalve effectiveness in short-term laboratory settings, that mainly look at the effectiveness of the mussel shell components like chitin, chitosan, and crushed up shells (Prieto et. al., 2003; Auckland Regional Council, 2010). Investigating heavy metals specifically, and in the shells of a marine mussels species is a novel and intriguing venture. The ultimate goal of this experiment is assess the bioremediation capabilities of green-lipped mussels based on their bioaccumulation trends, and additionally to provide useful information on presence of lack thereof of various heavy metals in the water column.

Materials & Methods:

Mussels were deployed on ropes in December of 2015 in three separate marinas in the Auckland region – Orakei Marina, Half Moon Bay Marina, and West Park Marina. The locations of these sites were in the suburbs of varying distances from Auckland's CBD Orakei (closest), Half Moon Bay, and Hobsonville (farthest away) respectively (Figure 1). Mussels were collected after a period of 5 months, and 20 individuals from each marina were chosen from the deployed ropes. Heavy metals in the shell were assessed using Laser Ablation Inductively Coupled Plasma Mass Spectrometery (LA-ICP-MS). LA-ICP-MS is a favorable analytical tool because it can complete elemental analysis for a wide range of materials including organic biological samples (University of Auckland, Faculty of Science webpage). Using the ICP-MS data will allow for a strong understanding of what, and the amount substances the mussels are filtering out of the water and incorporating into their shell growth. Given the constraints of the LA-ICP-MS staging area, the shells had to be smashed into smaller fragments and placed onto microscope slides (Figure 2). Fragments were chosen from three portions of the shell – the rear, middle, and lip of the shell - in order to investigate bioaccumulation trends (Figure 3). Since This fragmentation meant that three 'spots' on the LA-ICP-MS were taken for each shell, allowing 20 replicates per shell portion per marina site. The levels of 14 elements were examined including the heavy metals Si^{29}, Ti^{47}, V^{51}, Cr^{52}, Mn^{55}, Co^{59}, Ni^{60}, Cu^{63}, Zn^{66}, Ag^{107}, Cd^{111}, and Pb^{208}. The LA-ICP-MS was run a total of three times because only 72 shell fragments were able to fit on the stage for each given run. Data was standardized against a metal-level standard known as NIST 610 and also against background calcium concentrations (Jochum et al 2011). Two shell fragments, HM2-Lip and HM18-Rear, were omitted from average calculations due to their consistent outlier characteristics across all assessed elements. Error in LA-ICP-MS sequencing is most likely the source of these abnormal data.

Results:

All shell fragments across all three marina sites displayed presence of the assessed metal elements. The changes in metal levels amongst the shell fragments showed quite a variety of trends, particularly metal levels that decreased (Figure 4a & 4i), peaked in the middle fragment (Figure 4c & 4h), or remained rather constant (Figure 4e & 4l). These trends differ from the expected accumulation development, however the standard deviation values for the metal level averages were rather high. Most importantly, the results of the t-test analysis of metal level averages between the shell fragments did not show any p-values less than the required 0.05 value, meaning the metal values amongst the shell fragments were not significantly different (Tables 1 & 2). This situation was the case for all three marinas and across all metals, and thus no metal accumulation was observed in any of the shells.

While detrimental to investigation in bioaccumulation capabilities, the data collected in this experiment does allow for simpler comparisons of metal level averages between the examined marinas. Titanium displayed, by multiple orders of magnitude, the highest metal levels across all three marinas, ranging from 3900 to 6600 µmol/mol Ca (Figure 6). The other eleven metals displayed levels that can be divided into three distinct groups. Si, V, Ag, and Cd showed levels in the 1-20 µmol/mol Ca range; Co, Ni, and Pb showed levels in the 20-100 µmol/mol Ca range; and Cr, Mn, Cu, and Zn showed levels in the 60-250 µmol/mol Ca range (Figure 5). Half Moon Bay consistently showed the highest average levels across all metal types, especially for chromium and nickel for which Half Moon Bay levels are approximately double those of Orakei and West Park (Figure 5).

Discussion and Conclusions:

While the main goal of this experiment was to assess the bioremediation capabilities of mussels, the resulting data provides more insight into the role of mussels as bioindicator species. The simple presence of metals that are normally indicative of heavy metal pollution (Pb, Mn, Cd, Cu, Zn) reveals that these substances are somehow making it into the examined marina ecosystem and exist at levels that make them available to bivalves. Many heavy metals do not normally occur in marine environments and thus their presence is indicative of human activity consequences that are affecting water catchment areas. The incredibly high levels of titanium seen in all marinas reflect this notion as it is a rather inert metal that cannot be easily broken down. Specifically, titanium is known to break down only in the presence of hydrofluoric acid. The acid rain induced soil-leaching pathway for heavy metal pollution seems rather impossible given this characteristic of titanium, thus the main source must be from human industrial and commercial wastes. Additionally, since Cu and Mn levels were some of the highest of the examined metals and are key metals associated with heavy metal pollution, this experiment's findings also reveal sources of concern for the health of these marina ecosystems. Knowing the level of metals in the mussel shells could possibly be translated into a measure of metal levels in other aspects of the marine environment such as mussel tissue or even the water itself. One study found a proportional relationship between lead levels in mussel shells and in both tissue and suspended particulate matter of water (Bourgoin et. al., 1990). Since this study focused on just lead, it could prove very worthwhile to study the same metal levels within the tissues of these mussels and investigate the resulting relationship.

While simple t-tests showed no significant difference between the metal fragments' metal levels, it is possible that a more complex statistical approach such as an analysis of variance (ANOVA) or a rec transformation could reflect differences between the values. Additionally, it could prove useful to eliminate more outlying data in order to reduce standard deviation values and generate more accurate average values. Such manipulations of the data could even possibly reveal some accumulation trends.

Despite the lack of significant difference between mussel fragment metal levels, discussing the various trends that were not the expected accumulation brings up important intricacies in the process of heavy metal uptake for mussels. The mussels deployed in this experiment began as juveniles of approximately 20-30 cm long, after collection the mussels measured about 70-100 cm in length. This period of rapid growth that occurs in the beginning of a mussel's lifetime causes mussels to take up just about anything that can help them grow and become incorporated into their shell. This sort of behavior could explain why metal levels appear to 'peak' in the middle fragments rather than display a linear increase. Additionally, heavy metal presence seems to vary seasonally with heavy metals appearing at higher levels in the summer and lower levels in the winter (Melaku et. al., 2007; Kaur & Mehra, 2012). The period for which the mussles were deployed was during New Zealand's summer and early fall, which could reflect why some metal levels appeared to decrease. As it got closer to fall metal levels might have been decreasing in the water column and thus showed decreases in the mussel shell fragments.

Lastly the results comparing the average metal levels across the three examined marina sites pose an important question as to why the sites showed differences – specifically why Half Moon Bay had such consistently higher metal levels. The most likely explanation for this occurrence would have to relate to land use in the catchment areas surrounding the marina sites. Notably, Half Moon Bay has a catchment area that was historically the site of intense industry and today is located near various industrial and commercial sites including multiple quarries, a landfill, and an airport (Figure 7). Orakei, however is located nearest Auckland CBD, which does not have very many industrial sites, and West Park Marina in Hobsonville is surrounded by forested area and 'rural coastal' habitation (Figure 7). Given this information, the reasoning behind Half Moon Bay's high metal levels becomes obvious, however it is intriguing that West Park's location in a more forested catchment area did not lead to it showing the lowest metal levels. With this initial analysis it would prove incredibly enlightening to study various land use qualities in the marina catchment areas. GIS visualization could be employed to assess the amount of impervious surface in the catchment area or current and historical proximity of the marinas to industrial sites in order to generate a more thorough understanding of where these heavy metals could be coming from.

Further areas of this experiment that offer opportunities for future research mostly include areas where methodology could be changed in order to obtain better results on the mussels bioaccumulation of heavy metals. For example allowing the mussels to grow over a longer period of time, or assessing more than three fragments of the shell could show greater accumulation of metals. Additionally the reliability of the University of Auckland LA-ICP-MS was rather questionable, and it is possible that using a different machine or sampling technique could provide results that show more obvious different between the levels of the various shell fragments.

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Figures and Tables:

Figure 1. Map of Auckland region and the three sites at which the mussels were deployed.

a **b**

Figure 2 (a, b). Layout of shell fragments on microscope slides (a) and on the LA-ICP-MS stage (b). Blue circles are the NIST 610 standards used to standardize ICP-MS values.

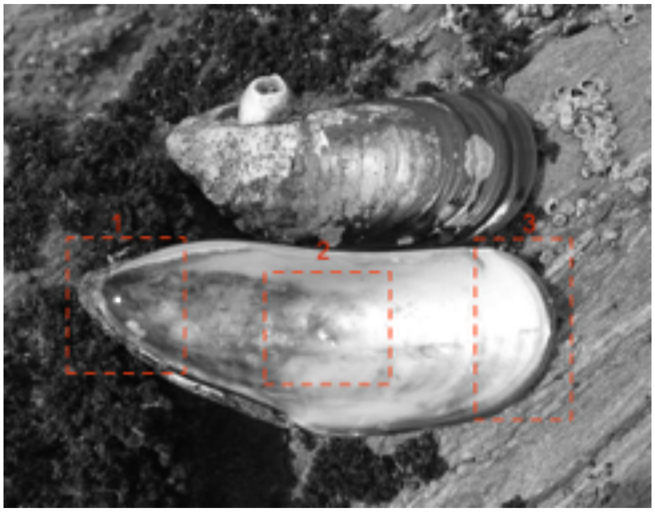


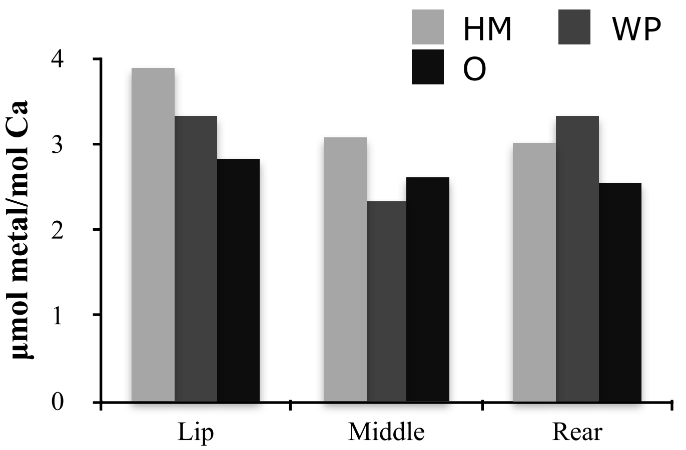
Figure 3. Rough boundaries depicting where shell fragments were chosen from each mussel shell. Area 1 encloses the rear of the shell, area 2 shows the middle of the shell, and area 3 includes the lip of the shell.

Table 1. Observed p-values for one-tailed t-tests between the average metal values of front and middle shell fragments for all three marinas. Compared p-value to prove significant difference was 0.05.

	Si^{29}	Ti^{47}	V^{51}	Cr^{52}	Mn^{55}	Co^{59}	Ni^{60}	Cu^{63}	Zn^{66}	Ag^{107}	Cd^{111}	Pb^{208}
Orakei	0.083	0.319	0.114	0.181	0.167	0.199	0.267	0.477	0.226	0.136	0.200	0.319
West Park	0.482	0.170	0.419	0.140	0.169	0.186	0.416	0.365	0.486	0.156	0.188	0.160
Half Moon Bay	0.162	0.122	0.383	0.119	0.497	0.178	0.392	0.285	0.221	0.166	0.168	0.180

Table 2. Observed p-values for one-tailed t-tests between the average metal values of middle and rear shell fragments for all three marinas. Compared p-value to prove significant difference was 0.05.

	Si^{29}	Ti^{47}	V^{51}	Cr^{52}	Mn^{55}	Co^{59}	Ni^{60}	Cu^{63}	Zn^{66}	Ag^{107}	Cd^{111}	Pb^{208}
Orakei	0.160	0.096	0.235	0.454	0.119	0.155	0.143	0.145	0.336	0.435	0.242	0.168
West Park	0.100	0.216	0.091	0.227	0.160	0.292	0.236	0.413	0.388	0.187	0.138	0.068
Half Moon Bay	0.151	0.187	0.160	0.153	0.113	0.163	0.106	0.198	0.391	0.165	0.165	0.161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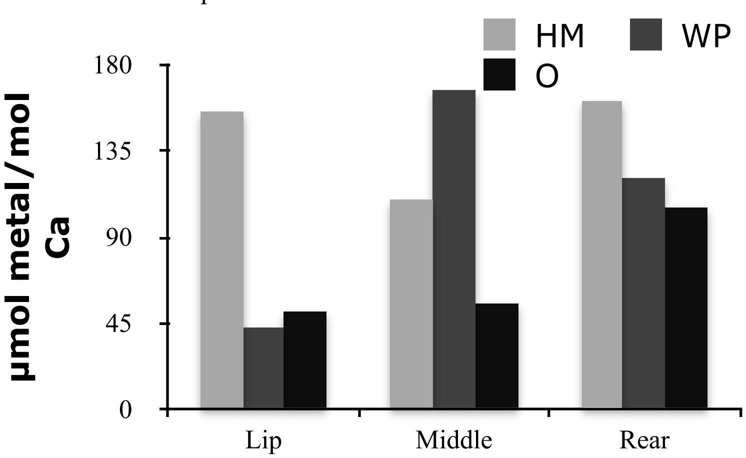
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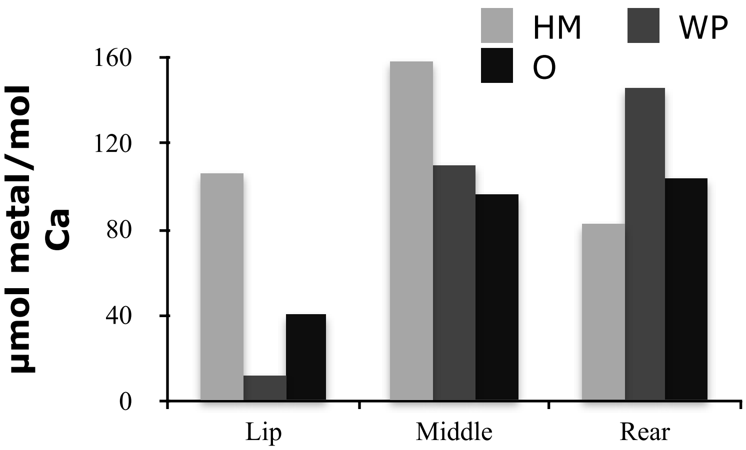
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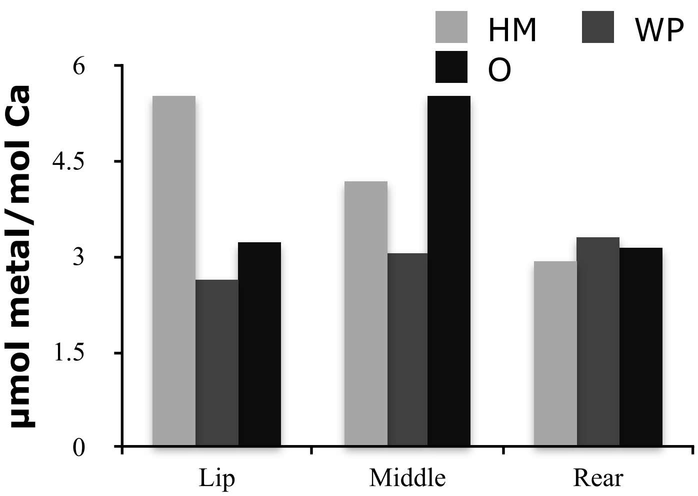
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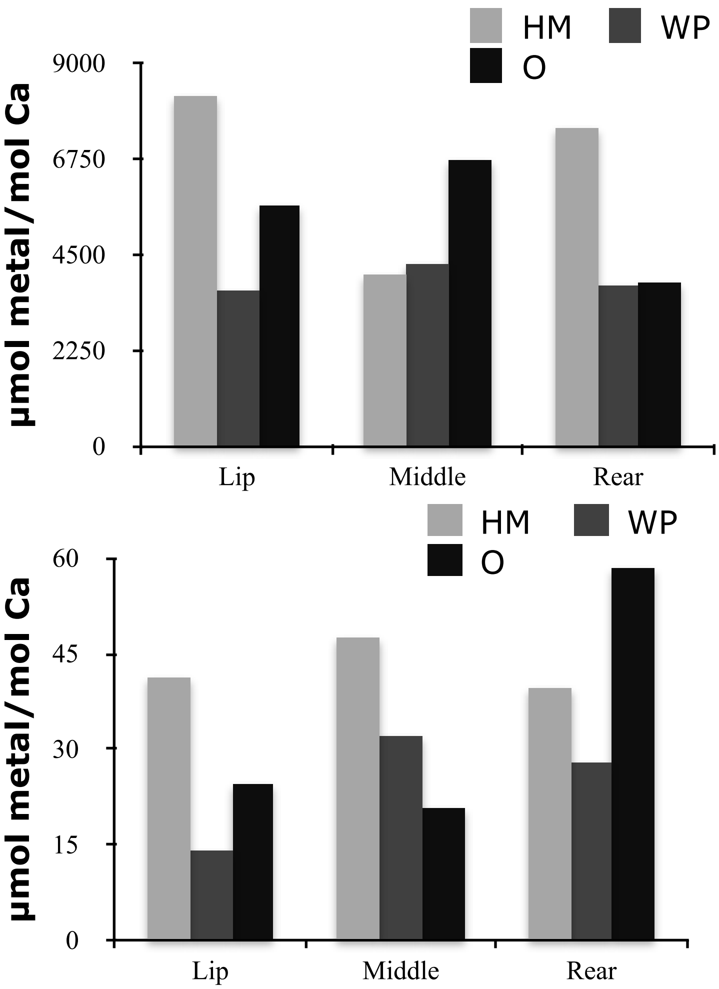
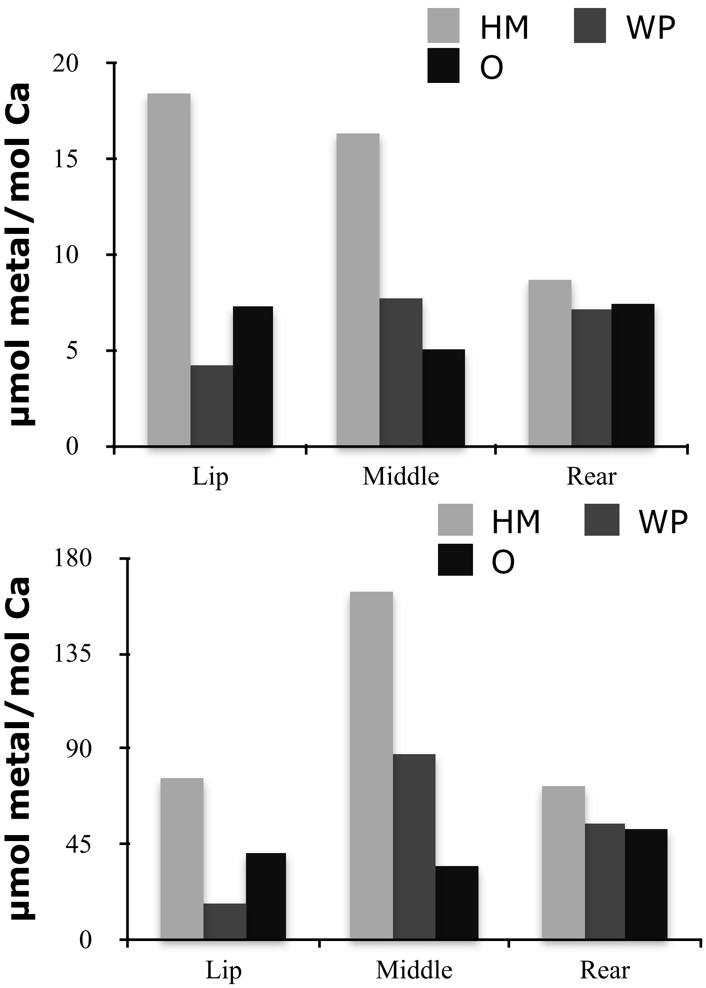


Figure 4 (a-f). Metal levels across shell fragments of all examined marinas for Si^{29} (a), Mn^{55} (b), Cu^{63} (c), Cd^{111}(d), Ti^{47} (e), and Co^{59} (f). Acronyms for the marinas are HM –Half Moon Bay, WP – West Park, and O – Orakei.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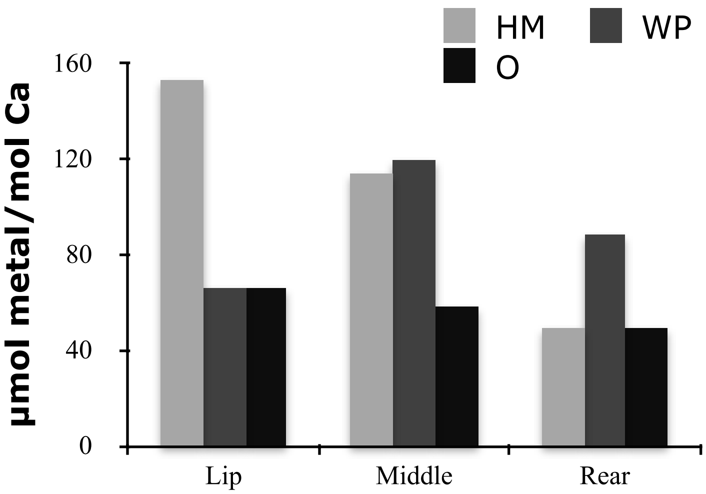
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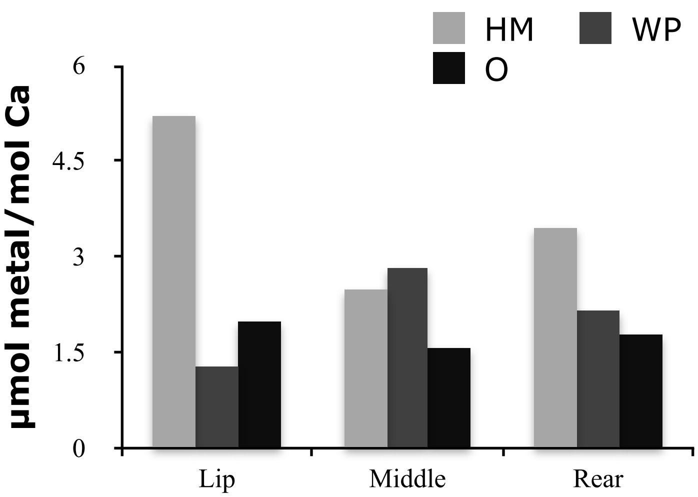
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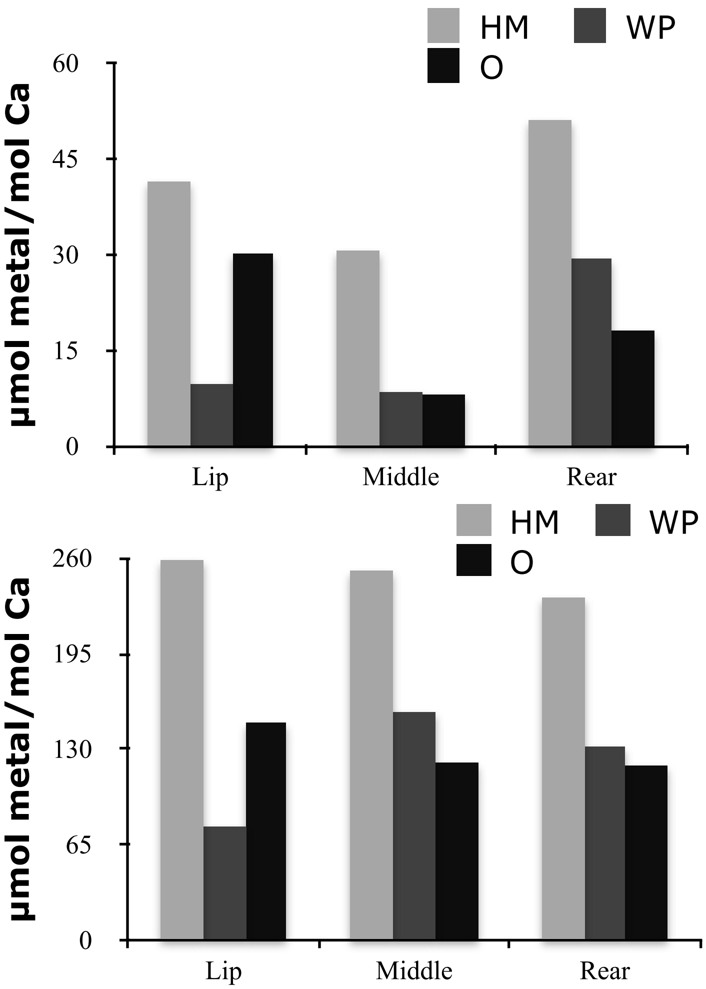

Figure 4 (g-l). Metal levels across shell fragments of all examined marinas for V^{51} (g), Ni^{60} (h), Zn^{66} (i), Ag^{107}(j), Pb^{208} (k), and Cr^{52} (l). Acronyms for the marinas are HM –Half Moon Bay, WP – West Park, and O – Orakei.

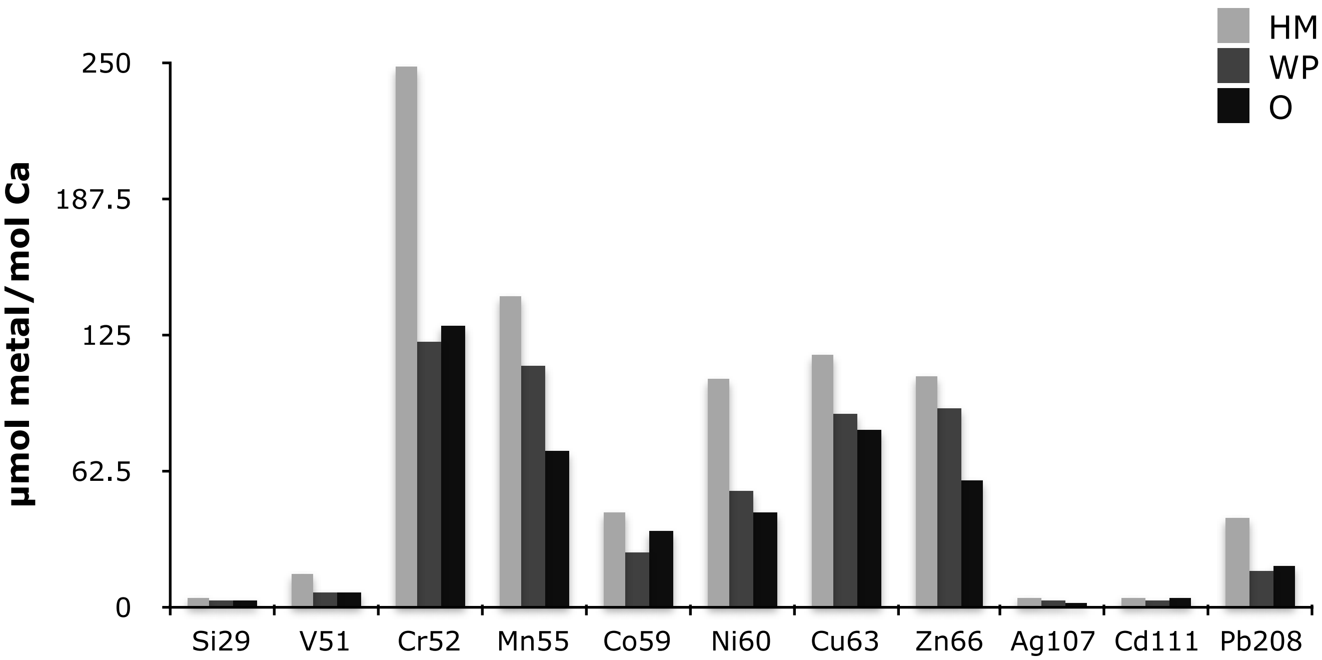

Figure 5. Average metal levels compared across the three examined marina sites. Titanium was omitted from the graph in order to facilitate a more detailed view of the other metals' trends.

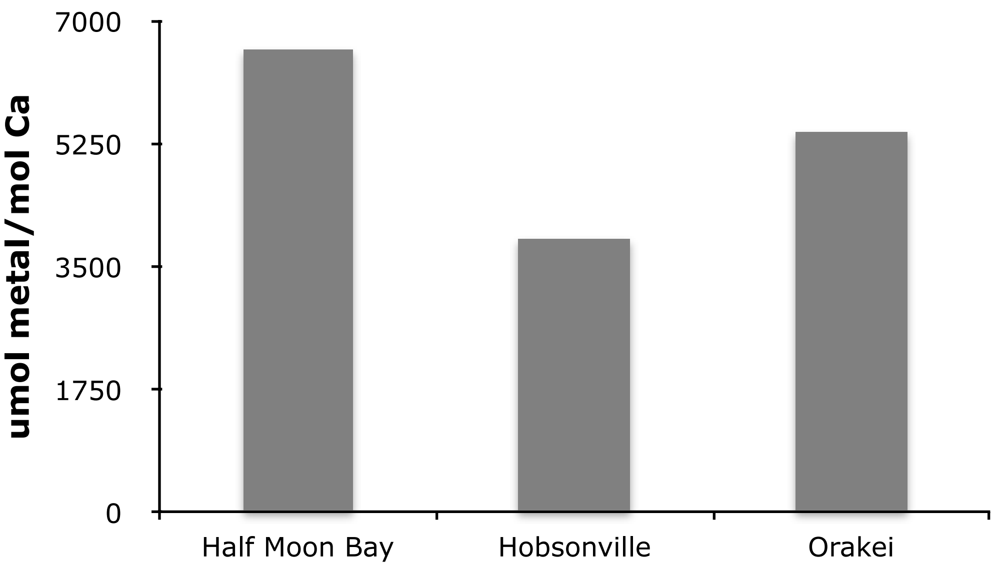

Figure 6. Average titanium levels compared across the three examined marina sites.

Figure 7. Map of Auckland region displaying the various types of land and living situations along with locations of intense land use facilities such as landfills and quarries. Map was taken from the Auckland Plan section on Auckland's Rural Strategy.