# “Use of LA-ICP-MS to analyse dental enamel in order to locate the geographical origin of teeth”

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# Abstract

The investigation reported here involved the analysis of possum tooth samples by Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) to determine whether or not the data obtained from the elemental composition of the dental enamel was sufficient to differentiate samples according to their geographical origin.

The possum jaws were processed, and the teeth removed and cleaned and mounted on slides before analyses could be carried out. A LA-ICP-MS method was developed in order to obtain data for the tooth enamel. This involved optimising specific laser parameters, such as laser spot size, laser power, and acquisition time. It was also essential to determine the best calibration standard for the project, which proved to be NIST 612.

Data was collected from samples from across ten different regions. The data was statistically analysed by Principal component analysis (PCA) and Linear Discriminant Analysis (LDA). It was found that some regions overlapped in the PCA, meaning they could not be 100% separated however, many regions could be distinguished, and it was determined, by that using LDA, 75.75% of the samples could be correctly grouped into their regions of origin.

A further analysis was conducted once several regions were removed (for historical or statistical reasons), which greatly improved the results. 82.9% of the samples could be placed into their region of origin.

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## 1.1 Aims

This thesis aims to examine the validity of a method to identify the origin of human remains found on archaeological sites, via the analysis of teeth by ICP-MS (Inductively Coupled Plasma-Mass Spectrometry). The work completed will determine if it is possible to use laser ablation (LA) coupled to ICP-MS to narrowly place archaeological remains within the different New Zealand regions. To assess the viability of this, the current work is a survey of possum teeth, collected from different regions within New Zealand, using LA-ICP-MS to analyse the tooth enamel and relate this to the underlying geology of the regions from which the teeth were sourced. It is not possible to use humans for this survey as most modern humans do not eat locally, as their diet is sourced worldwide.

### 1.1.1 General Aim

Develop a method using LA-ICP-MS to analyse possum tooth enamel, in order to identify the region of origin of the possum teeth samples.

### 1.1.2 Ultimate Aim

To develop a method that can be used to identify the origin of human remains found on archaeological sites. This method may be applied in such situations as, the problematic repatriation of Māori preserved heads to their homelands.

## 1.2 Upoko Tuhi (preserved Māori Heads)

In the ancient Māori world, tattooing was the norm. Ancient Māori engraved the body using ink to form moko. This adornment covered most of the body from head to legs, with particular attention to the face. The practice of cutting off and preserving the heads of the dead (who were tattooed) resulted in upoko tuhi. Māori were not the only people that participated in this practice; Jivaro Indians of the Amazon and Papua New Guineans also partook in this ancient ritual. However, Vandyke-Lee, asserts that New Zealand's upoko tuhi are the best examples of this pratice[3].

Traditionally, these heads were preserved for two reasons.[4] Firstly, they were preserved as a comfort to the grieving widows and family left behind, and a reminder to the young of their ancestors. Upoko tuhi represented a great amount of mana or prestige, status and authority. They could enhance an iwi's mana or could deplete it. Thus, great importance was placed on the upoko tuhi being kept within their whānau. Secondly, upoko tuhi were taken and preserved by the dead person's enemies after a battle as a way to ridicule conquered enemies. Often these would be traded back to the dead person's iwi, as it was considered a great shame if the upoko tuhi were not in the hands of their own people.

Early European voyagers quickly recognised the practice described above, and admired the fine craftsmanship of not only the preserved head, but the moko (tattoo on the face) itself. As a result a trade between Māori and Europeans in the upoko tuhi was established as early as 1770 and continued until the 1830s. Pomare and Hone Hika of Ngāpuhi were two northern war leaders, to whom is attributed the acquisition of the majority of upoko tuhi traded. Pomare focused his attention in the North Island, whereas Hone Hika raided as far as the east coast of the South Island. However, both died in the late 1820s, and with their deaths came the decline of this trade[5].

Upoko tuhi were often taken back to Europe to be placed in museums or in private collections. It is estimated by the Mokomokai Education trust that around 250 heads were traded during this trading period. In recent years there have been requests made to return these heads back to New Zealand, and to their iwi, to restore mana.[4]

More than 120 institutions around the world are known to house Māori human remains, but it is suggested that many more might possess upoko tuhi and other human remains.[6] Since 1992, Te Papa (New Zealand's national museum in Wellington) has made requests to museums and institutions around the world to return these human remains, to restore dignity to the dead and to their tribes.[7] Many institutions have heeded this request, and most during the 1960s, according to the New Zealand Herald, accepted that it was inappropriate to display the remains.[6]

Between 2004 and 2007 several Scottish museums and universities returned toi moko and other human remains to Te Papa.[6, 8] In 2009, 33 human remains were returned to Te Papa from Europe, becoming the second largest return ever, after 45 remains were returned in 2007.[9, 10] This brings the total of returned remains to 332, from 12 different countries; however, over 500 remains are still overseas, needing to be returned.[10] From these 332, only 91 have been returned to the land they came from, as the information used to identify the specific homelands, is often not sufficient,[10] hence the need for this type of research.

## 1.3 Teeth

### 1.3.1 Composition of teeth

Teeth are made up of three layers; enamel, dentine and cementum; dentine representing the bulk constituent. Dentine and cementum are highly calcified like enamel. However, dentine and cementum differ from enamel by the fact they can form throughout life, whereas enamel does not regenerate after a certain point.[11] Enamel is a composite mineral with a crystalline structure.[12] It is the most mineralized of the three layers , as it is 96-97% inorganic, and predominately consists of hydroxyapatite, $Ca_5(PO_4)_3OH$ crystals.[11, 13]

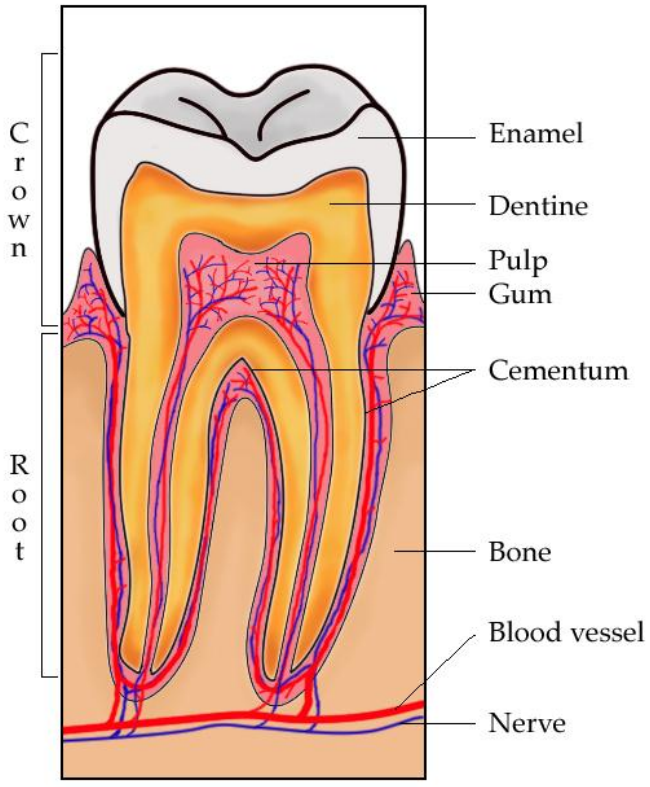


**Figure 1: Typical tooth[2]**

These hexagonal crystals pack together, at right angles to the tooth surface, to form rods with a diameter of approximately 4µm. The 3% of enamel that is not inorganic hydroxyapatite type compounds is found within the rod layers, and generally consist of either water or proteins and lipids.[11]

Calcium and phosphate are the main elements found within teeth, and correspond to around 34-39% of a tooth's composition. These elements can be substituted by a range of trace elements, which are then incorporated into the enamel (at either the cationic centre ($Ca^{2+}$) or the anionic centres ($PO_4^{3-}$ or $OH^-$)) at the time of exposure.[11, 12] The major cations that are usually incorporated are $Na^+$, $K^+$ and $Mg^{2+}$, whereas the common anions are $CO_3^{2-}$, $F^-$ and $Cl^-$. However, 40 more ions have been reported to be found in the 1000 ppm region including zinc, iron, aluminium and strontium, and more again in the 100 ppb region, including nickel, lithium, silver, and arsenic.[11, 13]

### 1.3.2 Formation of teeth

Enamel forms in a controlled and regular manner to produce rings as in trees.[11] The formation of enamel of the deciduous teeth (or 'milk' teeth, typical of mammals, including humans and possums) starts three months after conception and ends one month after birth, according to Uryu.[14] After these deciduous teeth fall out, the mature, adult teeth that replace them, have already formed the enamel before they emerge from the gum. Thus, the enamel of adult teeth, (which does not undergo significant change, once formed), represents the chemical exposure between the first month of birth to the time when the adult tooth emerges. Enamel formation, according to Humphrey *et al*., can be split into two stages; secretion and maturation.[12] During the secretion stage enamel crystallites are 'planted' in a protein matrix. Eventually thin crystalline ribbons are produced that extend the length of the enamel. Enamel crystallites account for around 14% of the mineral content in mature enamel.[12]

Maturation is the stage in which degradation and removal of growth inhibiting enamel matrix proteins occurs. This converts the partially mineralised enamel produced in the secretion stage into robust and mature enamel. Enamel produced in the maturation stage accounts for 86% of the mineral content of enamel found in mature teeth.[12] Thus, deciduous teeth can be examined to unlock the chemicals exposed in utera, whereas the mature adult teeth can be examined to show what chemical exposure occurred in early childhood.

## 1.4 Information from elemental analysis of bones and teeth by instrumental methods other than LA-ICP-MS

Instrumental methods can be adapted, by altering the introduction (LA), ionisation (ICP) or detection (MS) methods systems of a particular instrument. This section discusses work previously carried out using methods other than LA-ICP-MS.

### 1.4.1 Solution ICP-MS

Solution ICP-MS is a method that involves sample introduction, ionisation and detection. The introduction method is via a solution*, introduced into the system by a peristaltic pump. A nebulizer converts liquid samples into an aerosol, which is suspended in argon carrier gas. This aerosol is transported into the plasma for atomisation and ionisation. Temperatures in the plasma can reach up to 6,000k-10,000k, which causes the aerosol to ionize to its smallest components (elements) which can be detected by Mass Spectrometry. MS quantifies/detects the elements present by calculating their mass to charge ratio (m/z). This is done by, firstly, sorting the ions according to their masses using electromagnetic fields (this section of a mass spectrometer is called the mass analyser), and then, secondly, the detector of the MS quantifies the ions present.

Solution ICP-MS has been used to assess the nutritional status of children.[15] The results emphasise the type of data that can be collected from solution ICP-MS. This research compared the elemental composition of children's teeth from the United Kingdom and Uganda. This was done to discover whether factors such as disease and poor nutrition affected the elements found, and their relative concentrations in the teeth. The study found that while the majority of elemental concentrations did not differ, some, such as the essential element zinc, did[15].

Solution ICP-MS was also used to investigate $^{87}Sr/^{86}Sr$ isotope ratio measurements of prehistoric bone and teeth to assess migration patterns.[16] One disadvantage of using solution ICP-MS, is that it can lead to microstructural changes to the remains after the digestion and nebulisation processes occur. This can lead to data that is not representative of the sample. Thus, the work described focused on generating a procedure that allowed for strontium to be quantitatively

* For samples that are originally solid, a method involving digestion, often with harsh solvents ($HNO_3$) must first take place.

separated from rubidium (prior to the ICP-MS) to enable strontium isotope ratios to be measured, free of interference.[16]

Solution ICP-MS was also used to good effect when used to determine the lead, zinc and strontium concentrations of modern, deciduous teeth from Mexico and Egypt, and comparing the data with that collected from permanent teeth from the Bronze Age found in the United Arab Emirates, and from 18$^{th}$ century New York City.[17] The results found that the teeth from Egypt contained high concentrations of lead, which can be linked to diet. The work carried out also established that solution ICP-MS is an important technique to unravel elemental information that can be used to analyse diet, nutritional and environmental history of individuals.[17]

Another study that involved archaeological samples was the study conducted by Bentley *et al.*, seventeen individuals' tooth enamel was investigated using isotope analysis data collected from solution ICP-MS. The samples were taken from a grave site in Vanuatu to determine if any of the individuals were immigrants.[18] Data for $^{87}Sr/^{86}Sr$, $^{18}O$ and $^{13}C$ isotope ratios, as well as the concentrations of barium and strontium were collected. The results showed that the majority of the individuals had similar Ba/Sr ratios, consistent with a marine based diet. However, four individuals were found to have higher Ba/Sr ratios and $^{13}C$, which is consistent with a terrestrial based diet, indicating that these four individuals were most probably immigrants. Further evidence to support this finding were, that three of the four outlier individuals were buried in a different manner to the majority, with their heads facing Southward.[18]

Shaw *et al.*, used solution ICP-MS to obtain $^{87}Sr/^{86}Sr$ data from both human and pig tooth samples, to attempt to determine migration patterns of the Lapita people of 3300–2200 BP.[19] The data collected was able to determine that the strontium levels were higher in the archaeological samples, than that of modern day samples, indicating the uptake of strontium from a marine based diet. However, the researchers noted that there is large variation in strontium concentrations between the Pacific islands, so more research is needed.[19]

Arruda-Neto *et al.* carried out work in the largest city of Brazil, Sao Paulo, where the concentrations of lead, copper, zinc and cadmium in sediment were found by

the World Health Organisation to be higher than internationally accepted limits.[20] The study analysed 74 teeth, taken from this area, by solution ICP-MS and found that the concentration of lead was 40% higher in the teeth taken from this area, than the control area. It also found that lead concentration was up to 70% higher in carious molars of boys, than boys from the control area, concluding that this type of tooth, and gender was the most efficient contamination pathway for lead.[20]

### 1.4.2 Solution ICP-AES/OES (Atomic emission spectroscopy)

This instrument contains the same introduction (solution) and ionisation (ICP) systems as discussed above for solution-ICP-MS; however, it differs in the detection method. AES (Atomic Emission Spectroscopy, also known as OES-Optical Emission Spectroscopy), is a method which quantitatively measures the optical emission of excited atoms (excited by the ICP ionisation section of the instrument set-up), to determine the concentration of an analyte. The atoms that are excited by the ICP, decay from the promoted energy levels to their ground state, whilst emitting light which is detected and analysed.

Solution ICP-AES was used in the work carried out by Webb *et al.*, that was discussed above.[17] Similar results were achieved using this technique as already discussed for solution ICP-MS, as the project aimed to assess the validity of both methods. It was further noted that solution ICP-AES is a valuable analytical tool for analysing tooth samples, as there is elemental information archived that allows for nutritional information and thus geographical information to be elucidated.[17]

Another study carried out using solution ICP-AES, gathered data from 47 teeth, taken from the adult population of Malaysia, and compared that data to data already collected from other populations. The teeth were analysed for zinc, copper and lead by solution ICP-AES.[21] The study found that zinc was the element in highest concentration, and copper was the lowest. It also found that lead concentrations varied from $1.7\mu g^{-1}$ to $40.5\mu g^{-1}$. Chew *et al.*, concluded that these values for lead, were neither considered high or low in terms of what had previously been found.[21] The study did, however, find that in this group the concentrations of zinc were lower than levels found in other studies. The authors expressed concern that zinc deficiency can lead to degenerative diseases such as

inflammatory joint disease, and that further work should be carried out across the Malaysian population to determine whether the deficiency is widespread.[21]

### 1.4.3 LA-ICP-AES

This method has the same ionisation and detection methods as solution ICP-AES, but differs in the introduction method. Here, a laser is used to ablate solid samples. The laser is fired at the sample; the material absorbs the energy from the laser, and then evaporates to form an aerosol, which is then transported to the ionisation section of the instrument.

LA-ICP-AES, is not a very commonly used analytical technique. Thus, much of the work carried out to date involves trialling the method to see if the results obtained are satisfactory. One such study used LA-ICP-AES to analyze elemental concentrations of magnesium and aluminium in pharmaceutical tablets.[22] The results found that detection limits were $70\mu g^{-1}$ for aluminium and $40\mu g^{-1}$ for magnesium. The authors concluded that these detection limits were satisfactory, and therefore LA-ICP-AES was a reasonable technique to use for pharmaceutical analysis.[22]

### 1.4.4 Isotope Analysis

Isotope analysis is a destructive technique that allows for the identification of isotope signatures measured by MS. The analysis can be applied to a food web, allowing direct estimations to be drawn regarding the diet of the individual from whom the sample originates. This has been used to good effect, by Walter and Buckley from the University of Otago, to distinguish whether ancestral Māori remains, excavated from Wairau Bar, were originally from that region, or if the individuals had spent their childhood elsewhere. Buckley has also been involved in several different projects using this analytical technique and samples taken from the Pacific islands, two of which used solution ICP-MS and which have been discussed above.[18, 19]

Valentin *et al.*, analysed bone collagen of both humans and animals for their isotopic signatures via IRMS (Isotope Ratio Mass Spectrometry, which is a

specialisation of MS, in which the relative abundance of isotopes in a sample are measured).[23] Carbon and nitrogen ratios were used to indicate whether the collagen being analysed was of good quality, which could then be related to the type of foods consumed. From these signatures several conclusions were made about the people of Lapita, such as: that food was consumed from both marine and terrestrial sources; that food was foraged from close by marine and terrestrial environments; and that animals and plant food sources were farmed and grown by the people themselves.[23]

## 1.5 Applications of LA-ICP-MS for elemental analysis

LA-ICP-MS, includes introduction (LA), ionisation(ICP) and detection(MS) steps. All three of these steps have been described above. This section examines the work done previously using this analytical technique.

### 1.5.1 Bones and teeth

Bones and teeth are common samples used in both modern and archaeological applications, as outlined below.

#### 1.5.1.1 Archaeological applications

In 2007, LA-ICP-MS was allowed to be used to analyse the tooth , hair and bone samples from what was thought to be the bodies of the musician Mozart and his family.[24] Bone fragments from the skulls and femurs were analysed. Similar results were concluded for the bone, hair and teeth samples, for example the levels of Pb were found to be high in all the sample types. However, it was deduced that bone, did not give as useful results as teeth. This is due to the possibility of diagenetic alteration[†], which occurs when bone is buried in soil. Although in this study this alteration was minimal, it still leads to the conclusion that tooth enamel, which is somewhat 'immune' to diagenetic alteration, due to the composition of the material (see below) is superior.

In the same study of Mozart's family that analysed bones, teeth were also analysed. Teeth were fractured for analysis. A select group of elements was chosen to be analysed, including, mercury, as it was thought Mozart may have

[†] Diagenetic alteration is a term used to describe chemical, biological or physical change to skeletal remains, after they are buried.

been poisoned by mercury which resulted in his death. The investigation was able to conclude from the levels of mercury in the teeth samples that the conjectured 'Mozart' did not die of mercury poisoning. However, the analysis could not prove whether the remains were in fact Mozart's or not.

LA-ICP-MS has also been applied in the work carried out by Horstwood *et al*. who examined the $^{87}Sr/^{86}Sr$ ratio in archaeological samples of cattle teeth, by removing a section of the tooth. The study was able to obtain low levels of uncertainty (0.1%-0.2%).[25]

Other projects have used LA-ICP-MS to analyse ancient animal teeth. Galiova *et al.*, investigated the strontium, sodium, magnesium, iron, barium, calcium and phosphorus content of prehistoric bear tooth dentine.[26] It was concluded that LA-ICP-MS can be used to quickly and successfully analyse archaeological samples. The results were able to show seasonal fluctuations and the migration pattern of the bear and these results were used to conclude that the particular bear specimen had been hunted near a human settlement, when it was foraging before hibernation.[26]

Uranium was used in the work carried out by Grün *et al*. to determine the age of a Neanderthal tooth by LA-ICP-MS.[27] The results showed that the concentration of uranium in the enamel ranged from 1-1500 ppb, and that the majority of the uranium had migrated internally through the dentine into the enamel. However, it was concluded that the areas studied were too small to obtain any definite conclusion on how uranium acted within the particular sample.[27]

Bellis *et al*. have carried out several projects using LA-ICP-MS, for bone and tooth samples, usually with an emphasis on the lead content.[28] An archaeological project was carried out by the group that analysed the lead content in two teeth (by LA-ICP-MS) excavated from an archaeological burial site in Manhattan, New York, U.S.A. The results showed that the lead content in the dentine was significantly high (2000 $\mu g\ g^{-1}$), and in the cementum (700 $\mu g\ g^{-1}$ ). It was also noted that there was a large amount of cementum present, which suggests the teeth came from African-American slaves, as hypercementosis (excess cementum formation) was widespread among the slave population.[28]

The work carried out by Budd *et al*., used both ancient and modern teeth for analysis by LA-ICP-MS.[29] This was in order to determine whether significant diagenetic alteration occurs in enamel of ancient tooth samples. Six archaeological tooth samples were taken from three different time periods (Iron-age, 300AD, 1450AD) from UK burial sites. Lead was analysed as this is a good indicator of alteration. The results showed that there were significantly higher concentrations of lead in the outer enamel of both the ancient and modern teeth, and consistently low lead concentrations in core enamel tissue.[29] This suggests that diagenetic alteration is not an issue when analysing tooth enamel, as the tissue is extremely mineralized and robust in terms of undergoing chemical change.

Tooth and bone samples were used from First and Second World War burial sites in an analysis by LA-ICP-MS to determine whether or not individuals could be discriminated using the data collected from these samples.[13] Tooth samples from 14 individuals were able to be discriminated; however, one individual's teeth samples could not be associated all together, as the variation between teeth was significant.[13] The bone samples were found to be less useful when discriminating, with only 42.7% correct classification when using all the bones provided. However, this could be improved by considering the femur and humerus bones separately, where correct classification increased to 75.2% and 63.1% respectively.[13]

Work was carried out on twelve archaeological teeth from Mexico, in order to determine whether cut or broken surfaces of enamel afforded significantly different data. It was concluded that the data collected from the different type of surfaces gave data that was not significantly different. This work was carried out in order to further the work on these twelve samples in future projects.
Bone and tooth samples taken from three individuals from an excavation site in Austria were analysed by LA-ICP-MS.[16] The site is dated to 5-6$^{th}$ century, and is of importance to assess how far the cultural practice of artificial deformation of the skull, spread geographically. Strontium ratios were used, and it was concluded that the relative standard deviation was 0.1-0.2%. The data gathered from the bone samples and tooth samples of an individual were compared, and the

difference in some of the data was attributed to the individual migrating after early childhood.[16]

#### 1.5.1.2 Modern applications

Bellis *et al*. also completed work on two modern projects. In the first, bone (from goats) was analysed for its lead content, to allow for calibration with standard reference materials.[30] The study determined that the lead values from the SRMs (NIST 1486 – bone meal, and NIST 1440 – bone ash) were in good agreement with the certified reference values. This then allowed for the bone from a lead dosed goat to be analysed. Results showed that the lead content was distributed, so that the inner bone had higher concentrations, indicating that lead preferentially accumulates in the 'spongy' parts of bone.[30]

LA-ICP-MS was used to analyse goat's teeth, in the second modern project carried out my Bellis *et al.*[31] The study focused on obtaining lead concentration in tooth enamel of goats, where the amount of lead exposure to the goats was also known. Whole tooth, and just dentine samples were analysed, and it was determined that dentine was the better sampling area in this particular study.[31] This is due to the fact that dentine, unlike enamel, contains elements that an individual has been recently exposed to. In this case the lead the goats were recently exposed to would be found in dentine, but not in their enamel (refer to section 1.3).

Lead was also analysed by LA-ICP-MS in tooth samples in the work carried out by Uryu *et al.*[14] Lead can adversely affect foetuses in utero, however, the problem caused by the exposure might not show up till much later in life; therefore, it is important to monitor its exposure. As the tooth enamel from deciduous teeth incorporates elements that were present in utero, tooth enamel was well suited for this project. It was concluded that LA-ICP-MS had high enough sensitivity to allow for lead to be analysed.[14] Similar work was carried out by Arora *et al.*, which also analysed lead in enamel, dentine and blood to monitor exposure of children. The lead content of paint and soil of the participants surroundings was also examined.[32] The work carried out in this study showed that lead content of dentine was reflected in the lead content of the blood samples. Arora et al.,

concluded that the method they developed was effective in monitoring lead exposure.[32]

Dolphin *et al*., have carried out several projects involving LA-ICP-MS and teeth.[33, 34] In one, the different layers of deciduous teeth were analysed to determine whether the elemental composition of the different layers varied significantly. The results showed (from 38 samples) that the variation between the layers of the same tooth, were more than the variation between teeth of the sample person.[34] However, the 38 tooth samples came from 36 people, meaning the comparison within a jaw was not as extensive as within the tooth. Kang *et al.* also carried out a similar project investigating the elemental distribution of selected elements within the different regions of human deciduous teeth.[11] The study concluded the elemental intensities of elements followed the order : Sr>Mg>Zn>Pb>Fe>Cu.[11] The elements found in different levels of the tooth were also examined in earlier work (1999) carried out by Lochner *et al.,* which determined that LA-ICP-MS showed promise to be used in this type of research if a suitable standard could be produced[35].

In another project carried out by Dolphin *et al.,* zinc was examined in order to determine whether the information LA-ICP-MS yields was able to give any insight into the individual the tooth came from.[33] Samples were taken from individuals living in Mexico. The results showed that zinc content of pre-natal enamel (i.e the enamel formed while the child was still in the womb) correlated with food, like tortillas, known to influence zinc bioavailability. However, the same data did not correlate with the meat consumed.[33] The authors note, however, that interestingly, the mothers that had the poorest zinc enriched diets gave birth to the children with the highest Zn/Ca ratio. The overall conclusion made, was that zinc is not a good indicator for this type of study.[33]

Teeth of herbivores (zebra, impala and sable antelope) from the Kruger National Park in South Africa were analysed by LA-ICP-MS to examine the mobility of mammals through the elemental ratios found in their tooth layers.[36] It was found that the $^{87}Sr/^{86}Sr$ ratio variations can be reconstructed to give the mobility of the animals for over a year, with resolution of ten days.[36] The work carried out also

dealt with calibration methods involving hydroxyapatite standards which will be examined in section 1.6.1.

Rodent teeth were analysed by both LA-ICP-MS and solution ICP-MS, in order to compare the two methods, in the work carried out by Copeland *et al.*[37] The data collected did not show significant difference between the two methods, meaning both methods are effective when analysing tooth for elemental composition.

Strontium intensities in modern day deciduous teeth were again analysed using LA-ICP-MS in the work carried out by Humphrey *et al.*[38] The data collected was able to determine when babies were weaned from breastfeeding. The aim for this research is to use this method on archaeological samples, to determine the breastfeeding patterns of ancient peoples.[38]

### 1.5.2 Other Archaeological applications

LA-ICP-MS has been used to analyse samples, other than the common bone and teeth types. For instance in 2008, the technique was used to investigate carbonate deposits, on the sides of a water tower in the ancient city of Ostia, 20km from Rome.[39] In this study, elemental ratios were used to identify whether there were any changes in the carbonate deposits that could indicate seasonal variations, i.e. through water supply.[39] This study was the first to use LA-ICP-MS to examine seasonal changes via land deposits. The research concluded that there were two water supplies evident, and that the trace element variations can be correlated to the seasonal variations causing changes in the amount of rain, and ambient temperatures.

Another type of sample that can be analysed using LA-ICP-MS is hair. Hair was analysed in the work previously discussed by Stadlbauer *et al.*[24] The hair samples were initially cleaned to remove surface contamination, and both the exterior and interior of the sample was analysed to examine whether any contamination had occurred. This study concluded that hair samples are difficult to quantify, as reference materials are not easily made, or available. From the data that was collected, it was concluded that the element patterns were not comparable, as they could vary up to 30%.

Hair was also used in the work carried out by Byrne et al., which analysed 46 ancient mummified hair samples, from the people known as Chinchorros, from the modern day Chile region.[40] Due to the drinking water being rich in arsenic, the hair strands were tested for arsenic. Results showed that arsenic ranged from <0.8 to ~696 µg g -1, and due to this large range, the overall conclusion was that a broader sample size was needed to draw any insight into the arsenic exposure of these people.[40]

Although not an archaeological application, another study which should be mentioned, (previously referred to in section 1.4.3) analyzed elemental concentrations of magnesium and aluminium in pharmaceutical tablets.[22] The results found that detection limits were $40\mu g^{-1}$ for aluminium and $6\mu g^{-1}$ for magnesium. The authors concluded that these detection limits were pleasing, so LA-ICP-MS was a reasonable technique to use for pharmaceutical analysis.[22] This study also illustrates that detection limits for LA-ICP-MS, are lower than those for LA-ICP-AES.

## 1.6 Techniques used in studies that have used LA-ICP-MS to sample bones and teeth

### 1.6.1 Calibration

Calibration when using LA-ICP-MS is important, according to Cucina *et al*.[41] because if the matrix is not matched to the standard, then the ablated sample volumes may be different, thereby causing both poor sensitivity and poor correlation coefficients. Calibration is achieved by using solid standards. These range from readymade standards such as the NIST (the US National Institute of Standards and Technology) glass standards, to purpose made standards produced from materials such as $CaCO_3$.

In 2000, an article was published describing how better repeatability and accuracy could be obtained for the determination of trace elements in mussel shells, comprising of calcium carbonate type materials, by using a series of multielement calibration standards.[42] This was achieved by a method involving co-precipitation, which was in contrast to the previous methods. Although the previous method

gave reasonable correlation coefficients, it suffered from two significant problems. Firstly, the standards were produced by adding one powder to another powder then pressing them into pellets. This produced standards that were inhomogeneous. By using co-precipitation this inhomogeneity is overcome. The authors therefore, asserted that at present, co-precipitation is the most effective way of producing standards for carbonate containing materials undergoing LA-ICP-MS.

An article in 2007, further supported the use of carbonate based standards.[43] The article dealt with attempting to produce standards that could be used for determining the density of bone, which, like mussel shells, is made up of calcium carbonate. Although the primary objective of this work was not the determination of trace elements, it was still concluded that when using LA-ICP-MS as an analytical tool to analyse carbonate based samples, using carbonate based standards is advantageous.

However, purpose made standards can often be difficult to produce, and not as reliable as those produced by companies or institutes specialising in standard production. Thus, the other type of standard that requires consideration in this type of research, is pre-made NIST glass standards.[44] NIST glass standards, prior to 2000 according to Bellotto *et al*., were the most frequently employed procedure for the analysis of carbonate materials by LA-ICP-MS. Work prior to this study has used both NIST glass standards, as well as purpose made standards as an internal calibration method.[42] The work detailed in this thesis only used NIST glass standards, for reasons explained in the method development section.

Although purpose made standards can be difficult to produce and are not as reliable as NIST glass standards, Durrant explains that glass standards can lead to complications in the data collected by LA-ICP-MS.[44] Bellotto *et al*., elaborates on this by explaining that when using the same laser conditions, the shape and depth and therefore the mass of the sample ablated, differs between glass and carbonates. This can lead to an overestimation of trace element's concentrations in carbonate samples.[42] However, for the data collected in this work, which will only be compared to the other data collected by the method employed here, this overestimation will be consistent across all the data, and thus irrelevant when drawing data into groups.[45]

### 1.6.2 Teeth and bone sampling

Cucina *et al*.[41] compared the data collected from teeth cut with a diamond saw with data collected from broken tooth samples. It was concluded that there was no difference in the data collected from a smooth area created by a diamond saw, and the data collected from broken tooth samples. This method affords the benefit that the surface does not have to be re-cleaned or polished after fracturing; the tooth is not contaminated as it can be from a diamond saw. This is advantageous as cleaning teeth samples can be time consuming.[41]

Another way in which sampling occurs is by using a vibration saw. The work conducted by Stadlbauer *et al.* used a vibration saw to create smaller fragments of bone, which could then be more easily analysed by LA-ICP-MS.[24] These fragments were then stored until analysis, at which point they were then broken to produce a fresh cleavage area.

### 1.6.3 Sample acquisition

There are five factors that need to be controlled to obtain usable data, laser power, spot size, gas flow rate, acquisition time, and repetition rate. Table 1 summarises the various values for these factors that have been used in the literature.

#### 1.6.3.1 Laser power

The laser power employed largely depends on the type of sample being analysed, and the machine being used. For a small, fine sample such as hair, weaker laser strength such as 6 J cm $^{-2}$ would be used.[24] However for bone or tooth samples laser strengths of 10 J cm $^{-2}$ may be appropriate, as was the case in Stadlbauer *et al*.[24] However, laser power differs greatly from project to project. Schweizer *et al.,* when ablating tooth samples used 7 J cm$^{-2}$ [43], whereas Copeland *et al.,* used 4.35 J cm$^{-2}$ [37] and Prohaska *et al.,* applied only 0.6 J cm$^{-2}$ of energy[16], (as this method employed a large spot size of 200µm). Thus, there is a large range in which laser power can fall, depending on the type of sample being analysed, and the spot size being used.

#### 1.6.3.2 Spot Size

Spot size also varied greatly in earlier research. Spot sizes varied from 200 µm[23,] 100 µm[25], 70 µm[24, 36] to as small as 40 µm by Schweizer *et al.*[43] However, Horstwood *et al*., explained that smaller spot sizes can be used in order to preserve the sample, which may be beneficial for archaeological work.[16, 25] However as explained above, spot size and laser power strength need to be determined in conjunction to ensure the best sample regime for the particular sample type.

#### 1.6.3.3 Gas Flow rate

Gas flow rate, unlike laser strength and spot size, does not differ greatly between projects. Gas flow involves the carrier gas (typically helium, which sweeps the sample into the plasma chamber 1.0 L/min[24]) and the Nebuliser gas (argon, which is used to nebulise the sample into an aerosol 0.65 L/min). Usually each individual instrument has a specific optimized flow rate, as will be explained in the method development section.

#### 1.6.3.4 Acquisition time

Again, acquisition time is a factor that can be varied greatly for the specific needs of a project. Acquisition time, is the time taken to run one spot, that is, the time it takes to record background levels before ablation starts, ablation time (to record

signal) and the time after ablation to record background levels returning to pre-ablation levels. Anywhere between 34 seconds[42] and 180 seconds[37] is common.

#### 1.6.3.5 Repetition rate

Repetition rate is the rate at which the laser is fired. In the majority of the references, the repetition rate is 10Hz,[14, 27, 29, 42] so this will be used in this study.

Table 1- Parameters for LA-ICP-MS from work previously carried out.

| *Reference* | *Laser Type* | *Sample Type* | *Laser power* | *Spot Size* | *Gas Flow Rate* | *Acquisition time* | *Repetition rate* |
|---|---|---|---|---|---|---|---|
| Balter *et al.*[36] | 157 nm $F_2$/He excimer laser | Tooth (Zebra, impala, sable antelope) | | 70-100 µm | | | |
| Bellis et al. [28] | | Tooth (Human) | 1.0 mJ | Line ablation 20 µm $s^{-1}$ | | | 10 |
| Bellis et al.[31] | | Tooth (Goat) | 1.0 mJ | Line ablation 20 µm $s^{-1}$ | | | 10 |
| Bellis et al. [30] | | Bone (Human) | 1.0 mJ | Line ablation 20 µm $s^{-1}$ | | | 10 |
| Bellotto *et al* .[42] | Nd:YAG 266nm | Mussel Shells | 3.5 mJ | Line ablation 100 µm $s^{-1}$ | 0.85 L $min^{-1}$ | 59 seconds | 10 Hz |
| Budd *et al* [29] | Nd:YAG 266nm | Tooth (Human) | 1.2 mJ | 20 µm | | | 10 Hz |
| Byrne *et al.*[40] | Nd:YAG 266nm | Hair (Human) | 0.85-1.0 mJ | | 1.0 L $min^{-1}$ | | 5 |
| | Nd:YAG 213nm | Hair (Human) | 0.045-0.95 mJ | 8 µm | 1.0 L $min^{-1}$ | | 20 |
| Carlut *et al.*[39] | Nd:YAG 266nm | Carbonate deposits | | 50 µm | | | |
| Castro *et al.*[13] | Nd:YAG 213nm | Tooth/ Bone (Human) | 2.8 mJ | 100 µm | 0.80-0.95 L $min^{-1}$ | 60 seconds | 10 Hz |
| Copeland *et al* .[37] | Nd:YAG 213nm | Tooth (Rodent) | 1.35 mJ | Line ablation 200 µm | 0.5-0.55 L $min^{-1}$ | 210 seconds | 20 Hz |
| Copeland et al.[46] | Nd:YAG 213nm | Tooth (Rodent) | 1.35 mJ | Line ablation 200 µm | 0.5-0.55 L $min^{-1}$ | 210 seconds | 20 Hz |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Cucina *et al.*[41] | Nd:YAG 213nm | Tooth (Human) | | | | | |
| Dolphin et al.[33] | Nd:YAG 266 nm | Tooth (Human) | 1.53 mJ | | 1.2 L $min^{-1}$ | | 10 Hz |
| Dolphin et al.[34] | Nd:YAG 266 nm | Tooth (Human) | 1.53 mJ | | 1.2 L $min^{-1}$ | | 10 Hz |
| Galiova *et al.*[26] | Nd:YAG 213nm | Tooth (Ancient bear) | 12 J $cm^{-2}$ | 100 µm | 1.01 L $min^{-1}$ | | 10 Hz |
| Grun *et al* .[27] | Nd:YAG 193nm | Tooth (Human) | 10 J $cm^{-2}$ | | | | 10 Hz |
| Horstwood *et al.*[25] | Nd:YAG 193nm | Tooth (cattle) | | | | | |
| Kang *et al*.[11] | Nd:YAG 266 nm | Tooth (Human) | 0.85-1.02 mJ | 10 µm | 1.2 L $min^{-1}$ | | 10 Hz |
| Lam et al.[22] | Nd:YAG 266 nm | Pharmaceutical tablet | 1.8-.08 mJ | 100 µm | 1.2 L $min^{-1}$ | 120 seconds | 10 Hz |
| Lochner *et al.*[35] | Nd:YAG 1064nm | Tooth (Human) | 0.293 J $cm^{-2}$ | | 0.9751 L $min^{-1}$ | 45 seconds | 100 Hz |
| Prohaska *et al* . [16] | Nd:YAG 213nm | Tooth/Bone (Human) | 0.2 mJ | 200 µm | | | 10 Hz |
| Schweizer *et al*.[43] | Geolas C laser unit | Phantom calibration materials (Carbonate based) | 7 J $cm^{-2}$ | 40 µm | | | |
| Stadlbauer *et al* .[24] | Nd:YAG 213nm | Hair (Human) | 6 J $cm^{-2}$ | µm | 1.0 L $min^{-1}$ | 2mins 18 sec | 10 Hz |
| Stadlbauer *et al* .[24] | Nd:YAG 213nm | Tooth/Bone (Human) | 10 J $cm^{-2}$ | 70 µm | 1.0 L $min^{-1}$ | 2mins 18 sec | 10 Hz |
| Uryu *et al* .[14] | Nd:YAG 213nm | Tooth (Human) | 0.75-3 mJ | 100 µm | 0.5 L $min^{-1}$ | 65 seconds | 10 Hz |

## 1.7 Justification of the decision to analyse possum teeth using LA-ICP-MS

### 1.7.1 Teeth

Teeth are considered to be a bioarchive of an individual's nutritional life. For this project it was essential that a sample type was chosen that would be able to give data that could trace an individual's geographical origin. As teeth contain substituted elements, such as sodium and strontium, it is possible to associate the elements present, and the quantities in which they are present, to an individual's environment. Thus, teeth are an obvious sample choice.

The idea of 'unlocking' nutritional information from teeth has been investigated (see section 1.4 and 1.5). The nutritional information that can be determined from the different areas within the tooth, and the trends of any elements which increase or decrease with age have also been investigated[12]. The method used in the latter instance was to section the tooth, polish it, and attach it (using resin) to a glass slide. A similar survey was conducted which demonstrated, that, by using LA-ICP-MS, one is able to map the micro-spatial distribution of elements in dental tissues.[11]

Within teeth there are three types of materials that could be examined. However, the most valuable for this case study is enamel. This is due to two reasons. Firstly, enamel, forms at known stages of life, and stops once an individual reaches a certain age.[13, 29] This means that enamel can be used to examine the elements an individual was exposed to in childhood, thus giving their geographical region of origin. Dentine or cemtentum, conversely, may provide data that can be used to identify an individual's final geographical region. However, for the purposes of this study, the origin is needed, thus enamel is best suited to the study.

Secondly, enamel is known to be relatively stable after death, even if the sample is buried.[13, 24, 29] This is important when dealing with archaeological samples, as not only are the samples old, they may have also been exposed to conditions that could degrade other types of samples, such as bone or dentine. Thus, when

sampling tooth enamel, one can be confident that the elements identified within the sample correspond to the historical environment of the individual the tooth comes from, not the conditions it has been exposed to post-mortem.

### 1.7.2 Possums

The ultimate goal of this project is to develop a method that can be used to determine the New Zealand geographical region of origin of archaeological remains, including Māori preserved heads. To develop this method, a type of sample was needed that would provide data that could correlate to human remains. Thus, a mammal species was needed that could easily be obtained.

Possums have a localised food source, which is comparable with early Māori, who also had a localised food source. Possums' 'home ranges' vary between 1.3-1.9 hectares, and on average a juvenile possum only moves 5 km from its place of birth.[47] As a result of this, the food a juvenile possum eats (i.e when the tooth enamel is being formed, see section 1.4.2.2) will represent the area from which it is caught. The data collected from the enamel will therefore, hopefully, indicate the region from which the possum was collected.

According to the Department of Conservation (DOC), possums are a pest, therefore, DOC, along with other organisations trap possums in an attempt to reduce their numbers. Consequently, large numbers of already dead possums were available for research, avoiding ethics consents needing to be granted for the project.

Along with the ease of obtaining samples from possums, other factors such as their distribution were beneficial. Possums, according to DOC, have spread to every region in the country, apart from isolated islands. Thus, samples taken and analysed would potentially give an overview of the entire country.

### 1.7.3 LA-ICP-MS

Solution ICP-MS until recently was the most common way of introducing samples into the inductively coupled plasma. However, development of laser

ablation methods has meant that, laser ablation is now the preferred sample introduction method for many applications, particularly when chronological information can be obtained. Some view laser ablation with apprehension when it is used for analysing tooth enamel, as it has been suggested it was not accurate enough.[37] One such paper that views LA sceptically, concludes that, although LA can provide relatively precise data, it is not precise enough to rely on this information to interpret historical population information.[48] However, this paper was written in 2007, and much development has occurred even in the last three years, such as the work completed by Copeland *et al.*[37] This work concluded that when comparing solution and laser ablation, the degree of precision is within the margin necessary for investigating geographical origins of both animals and humans. This was determined by analysing 30 rodent teeth by both laser ablation and solution ICP-MS. He established that the two introduction methods were comparable as the average difference between measurements made by solution ICP-MS and LA ICP-MS was small.[37]

Copeland *et al*. also suggest that LA is perhaps the superior introduction method. This is due to it being generally quicker and less expensive than solution ICP-MS as well as being able to analyse rare, small and fossil type samples, without causing as much damage. It also requires a smaller amount of sample to achieve the same results as solution ICP-MS.[37] This assertion is supported by other articles, such as the earlier work of Prohaska *et al.*, which concluded that LA-ICP-MS is a very useful method for analysing bone and tooth tissues.

Lochner *et al*. made similar conclusions when comparing solution ICP-AES and LA-ICP-MS.[35] This work was completed in 1999, so it was relatively early work using LA-ICP-MS, thus the research focused primarily on examining what sort of data the laser system could acquire and what affected it. Lochner *et al.* found that the data was dependent on four factors: the power setting of the laser, the gas flow rate, the number of shots/size of spot size and the acquisition time.[35] Due to this, the method development section of this thesis will focus on these four factors. Data was able to be acquired that indicated time lines/chronological elemental differences, which is important for analysing samples which contain such characteristics, for example, teeth and trees. This is not possible when using solution ICP-AES, as it requires solution sample introduction, therefore not

allowing different layers to be analysed. Hence, LA-ICP-MS is a much more effective technique, when compared to solution-ICP-MS and solution-ICP-AES.[35]

Due to LA-ICP-MS being uncommon, it is often perceived as a difficult technique to use effectively, and one that may require special training. As a consequence of this, little is written about LA-ICP-MS exclusively, often work involves comparing LA-ICP-MS with other methods, or using the data collected from this method to support evidence gathered from other instrumentation. Although included in many journal articles as the technique used for the research, LA-ICP-MS has few specific articles written about it. However, Thomas completed a series of journal articles on the general functions of solution ICP-MS[‡], which has become well known as a comprehensive guide to understanding ICP-MS.[49]

Thomas argues that LA-ICP-MS has much to offer the analytical world and asserts that two of the main benefits of using LA-ICP-MS, is that it offers the ability to analyse numerous elements, not only rapidly (similar to the ICP-AES), but also within excellent detection limits (like GFAA- Graphite Furnace Atomic Absorption).[49] In Thomas's view then, LA-ICP-MS should be transformed from merely a research technique, to a more routinely used analytical instrument, which can be found and easily used in many laboratories.[49]

Another benefit identified in many of the studies conducted using LA-ICP-MS, is its ability to target not only specific areas of a sample, but small areas also, that are often invisible to the naked eye.[37, 41, 49] This is in contrast to solution ICP-MS which requires whole sections of sample to acquire data. This, consequently, leads to another benefit of LA-ICP-MS, its non-destructiveness as only a small amount of sample is needed to obtain the same amount of data as solution ICP-MS.[35, 37] This allows for samples to be analysed without the destruction of potentially rare or important artefacts.

Thomas, along with others, also notes that there are many limitations to LA-ICP-MS.[48, 49] Almost certainly, the disadvantage, which inhibits the use of, or discourages people the most from using LA-ICP-MS, is the price. Although it

[‡] The article also featured a chapter on LA-ICP-MS

offers the benefits of solution ICP-AES and GFAA combined, it costs twice and three times as much as these techniques respectively. The price tag associated with the LA-ICP-MS often results in the technique being uncommon. Accordingly, when the technique is used, frequently a specially trained technician is needed to operate it. Again this can add to the overall cost of the techniques and consequently make it even less desirable. Therefore, for a LA-ICP-MS to be cost effective the institution implementing it must fully understand the technique as well as use it regularly. Also, and probably most significant, is the fact that a technician is needed fulltime to keep the instrument calibrated and optimized. This is due to the fact that it is notoriously hard to keep running accurately without a dedicated technician, a problem of which manufacturers are not only aware but about which they also warn potential users.

Although many perceive this technique as not the first point of call, in recent years (the last decade) LA-ICP-MS has been found to be integral in analysing samples, such as hair, teeth, bone and glass, especially if the samples are of archaeological significance i.e. solid materials that may only have a small amount of practicable sampling area. LA-ICP-MS was considered as the best analytical technique for this project due to previous research (discussed in the section 1.3) on similar projects, along with the following reasons:

- Tooth enamel samples are not only small and difficult to remove, but are also often of the 'precious' or Taonga archaeological type of sample. Consequently, an analytical technique is required, that uses the smallest amount of sample possible, but which still gives accurate and reliable results.

- LA-ICP-MS is also an effective technique for this type of research as it provides elemental concentrations within the sample, not just molecular information. This allows for trace elemental analyses, which again is well suited to tooth enamel samples. Trace elemental analyses in tooth samples, allows for the determination of the type of elements that would have been present in the food/soil (of the mammal the tooth came from) at an early age.

- The excellent detection limits, resulting from using the LA-ICP-MS also are beneficial for this project.[22, 49] Small trace amounts of certain elements may hold the key into the likely source of a mammal. It also may be a useful way of separating two different locations, where the trace elemental composition of the soil is very similar.

- Other research has established the utility of this particular method.[11-14, 16, 24, 25, 27, 29, 35-37, 39, 41-43, 48]

# 2 Experimental

## 2.1 Sample collection

In order to get a representative set of data, samples were collected from throughout New Zealand. The following section describes these areas, and how they differ. It also details the samples taken from each region, and how these were collected. Although, upoko tuhi, are likely to have originated predominately from the North Island and the East Coast of the South Island (section 1.2), samples were collected from the lower South Island also, but the emphasis was on collecting many samples from the North Island, and the East Coast of the South Island.

Samples were obtained in several different ways. Initially, the Department of Conservation (DOC) was contacted, as one of their roles is pest control in native bush areas. If this failed, friends or family from particular regions were contacted, to see whether they could obtain samples. Along with these means, the media was contacted, and an article was distributed among several newspapers, such as the Waikato Times. This article asked for help from the public, and several samples were collected this way. Samples were still needed from some areas, thus, other research organisations, and pest controllers were contacted.

### 2.1.1 Northland


Figure 2- Map of Northland1

#### 2.1.1.1 The Land

Northland was one of the first areas of New Zealand to be settled by Europeans; accordingly, it is an important region in terms of this study, as many of the Toi Moko may have come from, or passed through this region.[50]

The Northland region spans an area of 12,600km$^2$, much of which is coastal land; it is only 100km wide, at its widest point.[50] It is different from other regions of New Zealand, as it lacks mountain ranges, and is mainly made up of rolling hill country.

According to the New Zealand Land Inventory (NZMS 290 map series), there are over 100 different soil types in the Northland region. The difference in soils, can chiefly be correlated to the original indigenous forest.[51]

Areas that contained trees such as totara, kauri and kahikatea, are now areas of leeched and infertile soils. These soils now need superphosphate and potassium fertilizers. Whereas areas that were home to trees like puriri, kohekohe, taraire and tawa, have higher fertilities, as these trees are more able to retain nutrients, and therefore, only need nitrogen fixing fertilizers.[51] Most soils in this region, depending on the amount of time they have been farmed, may also need injections of potassium, molybdenum and copper.[51] Also important to note is that cadmium is monitored in this region as it has been found to be in slightly higher concentrations than in other regions. Overall, the majority of the land has been subjected to high levels of weathering, due to the warm, moist, subtropical climate, and thus, the soils contain a lot of clays.[51]

#### 2.1.1.2 The Samples

Four samples were sourced from this region. These were obtained from Mr and Mrs Searle from Whangarei (Mangapai Caves Road). The samples were sourced through the media, after the couple read about the project in the 'Northern Advocate' (17/12/08). This area is just outside of Whanagarei centre, and is predominately farm land, thus it is expected to see a deficiency in copper and other metals in the soil, and therefore the possum tooth enamel.

According to GNS the type of soil found specifically in the area where the samples were collected from varies. Whangarei/Northland region contains a number of diverse soils in close proximity to each other. There are two possible soil types in this specific area: Waipara group soils, which are predominately made up of volcanic sandstone, basalt and argillite soils; and the Waitemata group soils, which are made up of Mangakahia complex mudstones. Thus, the possums collected from this region could have been exposed to different types of soils. Also important to note is that these samples came from an area in close proximity to the sea, thus, one could expect some salt influence present in the soils.

### 2.1.2 Auckland

Figure 3-Map of Auckland1

#### 2.1.2.1 The Land

The Franklin district, of the Auckland region, from which many of the samples for this region were obtained, contains some of the most productive farm land in New Zealand.[52] The soils found in this region are loam soils, which are known to be free draining and easily cultivated, thus, are ideal for intensive dairying, crops, or horticulture. They derive from volcanic ashes of varying ages. These soils strongly retain phosphate and sulphate, but are generally deficient in potassium and, increasingly, cobalt.[52]

#### 2.1.2.2 The Samples

Four jaws were obtained from Waiuku through the Animal Health Board, and AsureQuality. These had the following GPS coordinates:
(E2660374 N6433557) (E2660579 N6434542) (E2660822 N6433810) (E2660374 N6433557)

Four more jaws were sourced from Awhitu, a small community about 40km South West of Waiuku. These jaws were sourced from Andrew Pye Road, and were obtained through friends.

The samples collected from the two different regions both come from coastal sediment type soils. These soils differ greatly from the typical soils found in the greater Auckland region due to the proximity to coastlines. The soils are made up of basalt sandstone and marine pumice. Iron sands on the western side of Waiuku could also contribute minerals such as Fe and Mn to soils which could therefore be incorporated into the tooth enamel of possums.

### 2.1.3 Waikato

#### 2.1.3.1 The Land

The soils found around the Waikato region are similar to those found in the Auckland region that is free draining loam soils. However, due to the higher rain fall that occurs in the Waikato, the soil also contains a fine clay, called allophone.[52] This allophone soil is known to absorb phosphate and sulphate, so fertilizers are often added to the soil to promote pasture growth. The Waikato River is a contributing factor to the soil found in this region. Along the river, a mixture of ash material is deposited to form complexes of poorer drained grey soils, sandy soils, or gravelly soils, which are prone to drying out over the summer months.[52]

Figure 4-Map of Waikato1

Due to the peaty loam and peat soils formed from poor drainage, many elements are considered deficient in the Waikato. Once the soils are drained (usually for dairying) a lot of lime is usually added. Phosphorus, potassium, sulphur, copper, selenium and boron are all elements regarded as deficient in these soils.[52]

#### 2.1.3.2 The Samples

One sample was obtained within Hamilton city, from the Hillcrest area (Morrinsville Road). These samples were obtained through family.
Five other samples were sourced by a University staff member, and had the GPS coordinates of: (E2840925 N 6291380).

### 2.1.4 Coromandel/ Bay of Plenty

Figure 5-Map of Bay of Plenty1

#### 2.1.4.1 The Land

Soils in this region are predominantly loam soils, which originate from volcanic ash (See Auckland region for more details section 2.1.2). Generally, they crumble easily and are free draining. These types of soils are known for their ability to retain phosphate and sulphate; however, they are deficient in potassium and increasingly cobalt. 90% of the land in this region is covered by indigenous forest, introduced plants, or pastures.[52]

#### 2.1.4.2 The Samples

Five samples were obtained from Ron Vautier of Auckland, from his holiday home in the Coromandel. Three of the five were sourced from Waitete Bay (GPS coordinates of: (E 2730200 N 6501678)) whereas the other two were from Papaaroha (GPS coordinates: 9E 2733000 N 6498150)). These samples were again obtained via media exposure, as Vautier had read about the project in the Waikato Times.

The samples come from an area where the soil type derives from the Coromandel Volcanic zone, marine sediment rock is also present. The specific area fits into the Te Mata subgroup which contains imbedded sandstone and mudstone. However, like the Whangarei region, there are many different soil types, so it is possible the possums could have fed across several different types of soil.

### 2.1.5 Central North Island

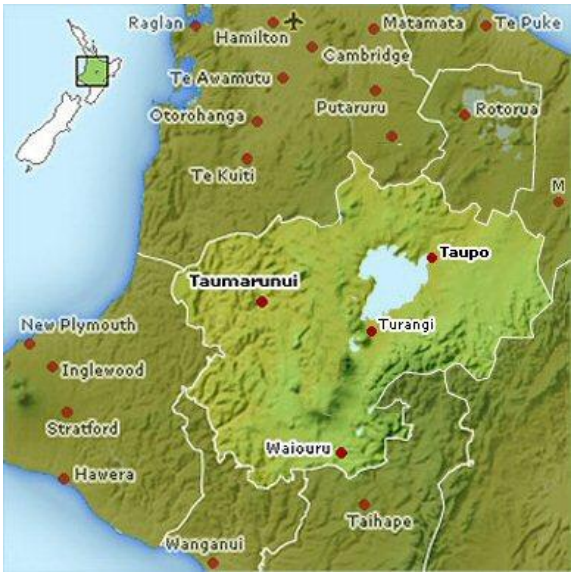

Figure 6-Map of the Central North Island1

#### 2.1.5.1 The Land

The soils found in this region are derived from the volcanic activity of the area. Volcanic pumice showers from the Taupo and Kaharoa eruptions, (which occurred in the last 4000 years), form the basis of today's soils.[53] Pumice soils are best suited for beef and sheep farming. However, this was initially not the case, as farming this region in the early 1900s was impossible due to 'bush sickness' later discovered to be due to cobalt deficiency. Moreover, cobalt is not the only element deficient in this region. Potassium, boron, magnesium, cobalt, copper, sulphur and phosphate may also be in low concentrations.[53]

#### 2.1.5.2 The Samples

Six samples were received from Shirley Porter, from the South East side of Lake Taupo, with GPS coordinates of (S 385457 E 1755427). These were sourced, again, via media exposure of the project.

### 2.1.6 Taranaki

#### 2.1.6.1 The Land

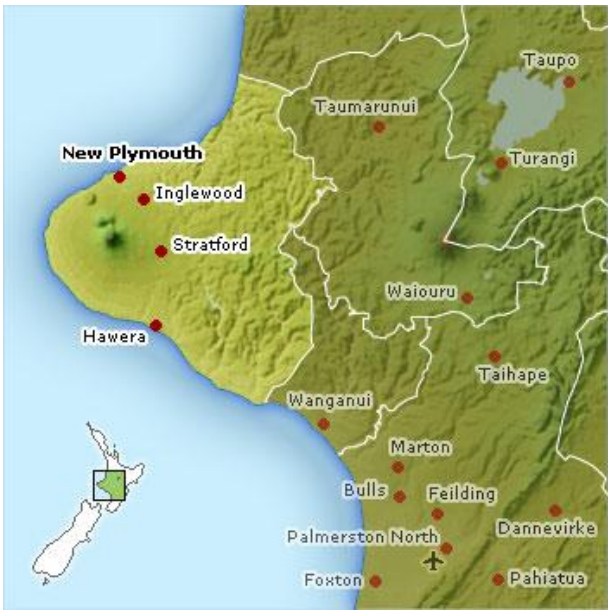

Figure 7-Map of Taranaki1

The soils found here are very similar to those found in the central North island, that is, derived mainly from volcanic activity.[53] Known also as a dairying region, the Taranaki region's soils are made up of mainly volcanic andesitic ash from Mount Taranaki. This is true for the Western parts of the region, and these soils are stony, and tend to impede drainage. To the east (where the samples for this region have been sourced) the soils are very much less stony, and allow for drainage, but may be prone to erosion.[53] Typical of ash derived soils, phosphorus, sulphur and potassium fertilisers are used in this region.

#### 2.1.6.2 The Samples

Six jaws were obtained from Tauramanui (which borders the east side of Taranaki), through the Animal Health Board, and AsureQuality. These had the following GPS coordinates:
(E 2697724 N 6258592) (E2697697 N6259392) (E2697697 N6259392) (E2695908 N6257754) (E2697656 N6254048) (E2697436 N6259488) (E2696279 N6253599) (E2698362 N6259367) (E2702951 N6255754) (E2695797 N6255479) (E2697255 N6259285)

The samples were taken from an area where the soils are predominately from the Taumaranui group formation. These soils are thin to medium bedded, and are made up of graded sandstone, mudstone, volcanic basalt and local imbedded limestone.

### 2.1.7 Nelson

#### 2.1.7.1 The Land

Old Moutere gravels are the predominant soil in the hill country soils. As in the top of the North Island, many fertilizers are used to promote farming and crop production.[54] These soils are generally deficient in copper, cobalt, nitrogen, phosphorus, boron, and magnesium.[54]


Figure 8-Map of Nelson1

#### 2.1.7.2 The Samples

Three jaws were sourced from this region. The first three jaws were obtained from the Nelson Department of Conservation, with GPS coordinates of (2505820 5991690) (2580320 5990160) (2580320 5990160).

The samples were taken from an area close to Blenheim, where the soil types are mostly made up of Volcanic Basalt rocks, specifically Mandamus igneous complexes. There may also be present sandstone and mudstone soils, as well as some soils commonly found near coastal areas.

### 2.1.8 West Coast

Figure 9-Map of the West Coast1

#### 2.1.8.1 The Land

Soils found in the west coast are considered to be a mixture of alluvial soils. The soils on flat areas are recently formed from flood gravels and silts, or conversely, from very old pakihi soils, which result from very compacted, impermeable subsoil. The newer soils are easily cultivated, and usually only require small amounts of phosphorus and sulphate fertilizers. However, the older soils can be deficient in many elements, including copper, magnesium, boron, molybdenum, cobalt and potassium.

#### 2.1.8.2 The Samples

Six samples were sourced from this region by the West Coast Department of Conservation. Four were sourced from Otira, west of Hokitika. Two were also obtained from Whitcombe, North of Hokitika.

The area from which these samples were taken is strongly influenced by the numerous rivers around this area, and thus tend to be river gravel and sand soils also known as Greywacke soils.

### 2.1.9 Canterbury

Figure 10- Map of Canterbury1

#### 2.1.9.1 The Land

Irrigation is most widely used in this region, and much of the farming done in this region is only possible due to this process. The soils found here are alluvial, and closer to the Southern Alp mountain region, the soils become shallow, stony and friable. In contrast to this, in the flat areas, the soil is considered 'fluffy'. Element deficiency in the soils of this region is less of an issue than that of other regions. Usually only phosphorus, sulphur, and molybdenum are needed, in order to cultivate the land.

#### 2.1.9.2 The Samples

Five jaws were obtained from this region, again via the media, from Andrew Were. The GPS coordinates for the five samples are: (E 2414975 N 5763475), (E 2434025 N 5755550), (E 2470650 N 5748275), (E 2476550 N 5768975), (E 2473125 N 5771300).

This area is within the plains of the Christchurch region, thus the soils are very similar to those found from the region from which samples were collected in the West Coast. The soils are Greywacke soils, made up of sand and mud, strongly influenced by nearby rivers and seas.

### 2.1.10 Fiordlands/Southland

Figure 11- Map of Fiordlands1

#### 2.1.10.1 The Land

Moist, brown soils, requiring drainage, are the soils type found in this region. Here, the soils are thought to be as fertile as those found in the Waikato. Usually, only the addition of phosphorus and sulphur fertilizers are needed, though, in some areas, molybdenum can only be found in low concentrations.

#### 2.1.10.2 The samples

Five samples were sourced by the West Coast department of Conservation from this region, specifically from Okuru, North of Fiordland National Park.

As Okuru is North of Fiordland National park, it's soils tend to be Greywacke also, rather than the granite type of soils found further south in this region. Thus, the soils found in the area, where these samples were taken from are similar to those of the Christchurch samples and the West Coast samples.

## 2.2 Jaw processing

Jaws were cut away from deceased possums, and flesh was removed using an autoclave. Jaws were placed in beakers (250ml) with distilled water (200ml), covered (tin foil) and boiled (300$^{o}$C for 2 hours). Flesh and teeth were then removed. Teeth were then cleaned (acetone, distilled water) and left to dry, to remove any surface impurities.

## 2.3 Sample preparation

Teeth were mounted on slides using Blu-Tack®. Rows of approximately 5 across and three down were used, to maximise sample throughput. Three teeth from each possum were analysed. Space was allowed at the bottom of each slide to permit the attachment of the general NIST 612 standard and NIST 1400 standard.

## 2.4 Standard preparation

An attempt was made to prepare a calibration standard by adapting the method found in Stadlbauer *et al.*, using pure hydroxyapatite ($Ca_5(PO_4)_3(OH)$) (6g).[24] Merck liquid standards, which contain single elements in nitrate form, were added (MERCK 21-10mg/L and MERCK 17-100mg/L)(1ml). The mixture was then completely dissolved ($HNO_3$ conc.). A rotatory evaporator was used to evaporate the solution to dryness, then a freeze drier was used overnight to remove any residual liquid. However, this method resulted in liquid still being present. Therefore, a new standard solution was made up and dried in a vacuum oven at 60$^{o}$C for 48hours. This standard was then dry enough to use, however the data obtained from the standard was inconsistent (see method development for results). Thus, only the NIST 612 standard was used for data collection of the main data set.

NIST 1400 Bone Ash was purchased from the National Institute of Standard Technology, and was pressed into a pellet (~4g) by a KBr disk press. NIST 1400 (as well as NIST612) was then used to collect a small section of data as a comparison (to NIST 612) as an internal standard.

## 2.5 Laser Ablation procedure and parameters

A New Wave Research UP-213 Laser Ablation system with a 213nm neodymium yttrium aluminium garnet (ND-YAG) laser was used to ablate the possum teeth. A mixture of helium and argon gas carried the ablated enamel to a Perkin Elmer DRCII ELAN 6000 inductively coupled mass spectrometer.

A range of elements was analysed, based upon previous work reported in the literature[17, 24, 35, 42]. These included: Silicon ($Si^{28}$), phosphorus ($P^{31}$), calcium ($Ca^{43}$), chromium ($Cr^{52}$), manganese ($Mn^{55}$), iron ($Fe^{57}$), cobalt ($Co^{59}$), copper ($Cu^{63}$), zinc ($Zn^{64}$), selenium ($Se^{82}$), cadmium ($Cd^{114}$), mercury ($Hg^{202}$), lead ($Pb^{208}$), arsenic ($As^{75}$), tin ($Sn^{118}$) sodium ($Na^{23}$), magnesium ($Mg^{24}$), barium ($Ba^{138}$), and strontium ($Sr^{88}$).

Table 2 details the settings used for the LA-ICP-MS of the possum tooth enamel:

| | ***Tooth Sample*** | ***NIST 612*** | ***NIST 1400*** | ***Purpose made standard*** |
|---|---|---|---|---|
| ***Laser power*** | 40% | 60% | 80% | 40% |
| ***Spot Size*** | 60 µm | 60 µm | 60 µm | 60 µm |
| ***Repetition rate*** | 10 Hz | 10 Hz | 10 Hz | 10 Hz |
| ***Acquisition time*** | 2.59 sec | 2.59 sec | 2.59 sec | 2.59 sec |
| ***Internal Standard (wt %)*** | CaO (53.587) | CaO (11.928) | CaO (38.18) | CaO (53.587) |
| ***Nebuliser Gas flow*** | 1.00L $min^{-1}$ | 1.00L $min^{-1}$ | 1.00L $min^{-1}$ | 1.00L $min^{-1}$ |
| ***Plasma Gas flow*** | 0.65L $min^{-1}$ | 0.65L $min^{-1}$ | 0.65L $min^{-1}$ | 0.65L $min^{-1}$ |
| ***Wavelength*** | 213nm | 213nm | 213nm | 213nm |

**Table 2- LA-ICP-MS parameters**

Procedures were adapted from Blair *et al.*, where the work carried out was on the same instrument and on similar materials.[45] The sample slide (section 2.3) was placed in the sample chamber of the LA-ICP-MS, and the following procedure was carried out, to ensure consistency between laser sessions. The sample chamber was purged for at least ten minutes after every introduction of a new slide to allow background levels to stabilize. A consistent start up protocol was used before each laser ablating session, and the power readings were recorded

everyday to ensure comparable data from day to day. The laser was fired, at 60% power with a spot size of 60µm and with a repetition rate of 10Hz. This was completed with the shutter closed, for 30 to 60 minutes, to warm the laser up, until constant power readings were obtained. Finally, the sample chamber was flushed with helium and argon, (to clear the oxygen from the tubing) for several minutes.

Before laser ablation can begin, optimization of the instrument needs to occur. This was done in two parts. Firstly, a liquid standard was used. This consisted of 2ppm Sc, 2ppm Ga, 20ppb Te 40 ppb Rh and 20 ppb Lu in Milli-Q water and nitric acid. After this, the instrument was optimized using the solid reference NIST 612 standard. This involved a line scan (60% power, 60µm spot size, $5\mu m^{-1}$ scanning speed) of the NIST 612 standard.[45] This standard, was used for all analyses, and the concentrations are those published in Pearce *et al*.[55]

Once the instrument had been optimized, two spots of the NIST 612 standard were ablated (60% power, 60µm)[§]. After this was completed, the analyses of the tooth samples could be started. The background levels of the elements being analysed were measured by analysing a gas blank (obtained by firing the laser with the shutter closed) for 60s before the laser ablation of the enamel took place.[45] Four spots on each tooth were ablated, and three teeth from each possum jaw were analysed (section 3.5.1). The enamel was analysed using 40% laser power (section 3.3.2). Instrument drift for each session was measured by analysing another two spots on the NIST 612 standard after all the tooth samples on one slide had been measured.

Once analyses were complete, the data was processed using the programme GLITTER (GEMOC Laser ICP-MS Total Trace Element Reduction).[56] $Ca^{42}$ was used as an internal standard, and thus, in order to calculate the elemental concentrations of the unknown elements, the counts in each analysis were standardised to the stoichiometric abundance of CaO in hydroxyapatite, which is 53.587%, [45] (see section 3.2.4). GLITTER gives the mean concentration of each element in ppm. The GLITTER data used is MDL (minimum detection limit) filtered. This means, using background readings and Poisson counting statistics,

[§] In the case where NIST 1400 was also used, two spots were ablated (80% power, 60µm) after the NIST 612 data had been obtained.

that the data is calculated by GLITTER at the 99% confidence interval using the formula:

$$MDL = 2.3 \times \sqrt{2B}$$

Where B is the total counts in the background interval.[45] The data can be altered slightly by the user, by reviewing the data signal, and selecting the area of the signal from which the data should be obtained. This allows for the first and last few seconds of laser data to be discarded, in case surface contamination is still present on the possum teeth (the first few seconds), and as the signal declines as laser firing ends (in the last few seconds).[37, 45]

# 3 Method Development

## 3.1 Slide preparation

### 3.1.1 Adhesive

#### 3.1.1.1 Superglue

Initially the tooth samples were attached to the microscope slide using superglue. However, the glue was difficult to work with, and often covered the tooth. If this occurred, the laser fired on the adhesive, and the data collected represented this, rather than the enamel. Also, slides could not be reused when superglue was the adhesive applied. Thus a second adhesive was trialled.

#### 3.1.1.2 Blu-Tack®

Blu-Tack® was applied in a thin layer on the sample slide, and teeth were pressed into it. This proved to be the most effective form of adhesive, as slides could be reused, and the sample's position could be changed without the cleaning process needing to be repeated. Also it did not cover the teeth, so, the data collected was not compromised by accidently gathering data from the adhesive rather than the enamel.

### 3.1.2 Preparation of tooth for LA-ICP-MS

Several different ways of sampling the teeth were examined to determine which was the most effective

#### 3.1.2.1 Cut teeth

A common method of sampling teeth when analysing by LA-ICP-MS is cutting using a diamond saw before the ablation process.[11, 14, 16, 35, 46] Small sections of tooth enamel (5-10mm) were cut off the entire tooth using a diamond saw. Samples were then washed (acetone and then double distilled water). The small fragments were subsequently attached to the glass microscope slide using resin. However, the diamond saw tended to be inconsistent when cutting, therefore the size, shapes and places the samples were being cut from were not consistent enough to give reliable, meaningful data, as shown by the data collected. The samples, that this method yielded, were also very small, so small that the adhesive appeared to 'overtake' the sample, making it difficult to acquire data from the actual enamel. Therefore other methods were considered.

### 3.1.2.2 Other methods

#### 3.1.2.2.1 Fractured teeth

This method was attempted, by securing the tooth in pliers and fracturing using a chisel. Once fractured, the extract was cleaned (acetone then distilled water), left to dry, and then mounted on microscope slides using Blu-Tack®.

#### 3.1.2.2.2 Whole teeth

Whole teeth were attached to microscope slides using Blu-Tack®. Teeth were washed (acetone and then double distilled water), and left to dry before attaching. Fig. 13 shows that the percentage coefficient of variation across all the elements is consistently higher for the fractured teeth.

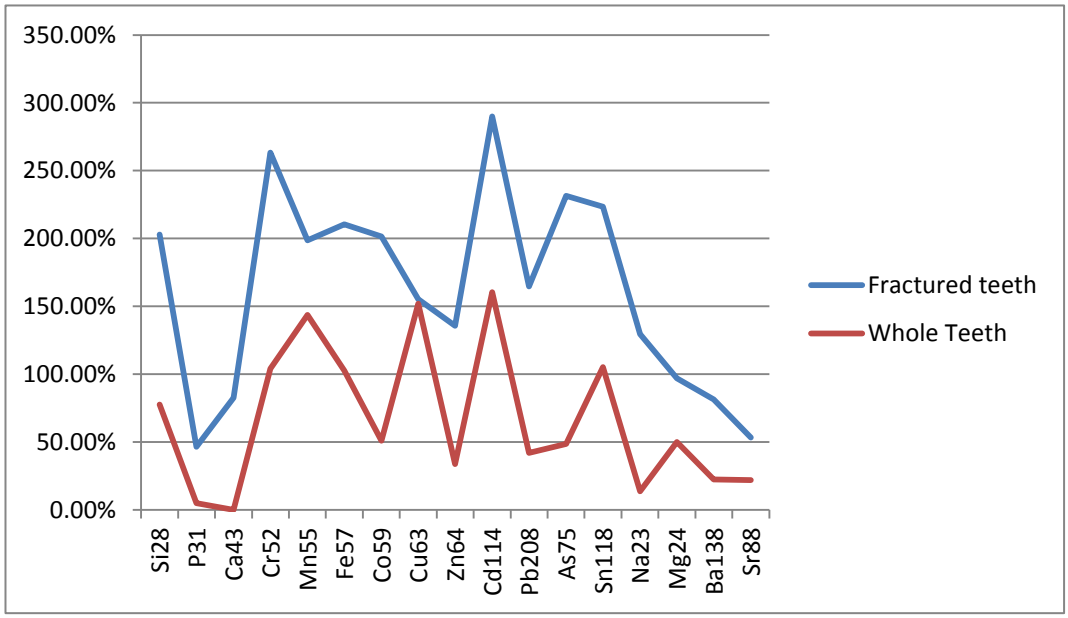


Figure 12- Percentage coefficient of variations for each element (calculated from the data collected from three spots, on three teeth from the same jaw)compared for whole teeth and fractured teeth.

Tables 3 and 4 compare the percent coefficient of variation of fractured teeth and whole teeth respectively. The data shows that fractured teeth have a higher percentage coefficient of variation than that of the whole teeth. The figure for fractured teeth across all the elements analysed in this project, was 162.83%, whereas, for whole teeth it was only 66.76%. Thus whole teeth, were chosen as the sample type used in this project.

| Sample | Si28 | P31 | Ca43 | Cr52 | Mn55 | Fe57 | Co59 | Cu63 | Zn64 | Cd114 | Pb208 | As75 | Sn118 | Na23 | Mg24 | Ba138 | Sr88 | Average % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***TF1S1*** | 204.65 | 534410.7 | 382986.8 | 7.24 | 28.22 | 1106.68 | 0.25 | 10.88 | 759.67 | 0.79 | 1.28 | 1.58 | 0.69 | 10603.26 | 13255.36 | 1286.47 | 958.33 | |
| ***TF1S2*** | 939.43 | 466690.3 | 382986.8 | 94.68 | 98.67 | 185.51 | 2.36 | 6.09 | 444.13 | 6.87 | 1.48 | 20.04 | 7.61 | 7115.01 | 2050.1 | 164.76 | 463.65 | |
| ***TF1S3*** | 447.54 | 460386 | 382986.8 | 24.61 | 135.07 | 124.35 | 0.7 | 1.58 | 364.96 | 2.2 | 0.5 | 5.59 | 2.42 | 5339.76 | 1474.61 | 139.86 | 403.59 | |
| ***TF2S1*** | 67269.54 | 486513.2 | 382986.8 | 815.68 | 103.71 | 6628.95 | 19.05 | 554.7 | 8816.51 | 72.83 | 122.38 | 193.34 | 120.15 | 54354.88 | 23040.89 | 1203.37 | 806.05 | |
| ***TF2S2*** | 173879.6 | 112991.4 | 1700842 | 14409.64 | 886.13 | 25496.9 | 58.45 | 907.69 | 4984.7 | 2689.79 | 361.64 | 1087.09 | 1609.94 | 7577.54 | 413.33 | 24.92 | 249.27 | |
| ***TF2S3*** | 16974.81 | 36300.04 | 382986.8 | **** | 25.27 | **** | **** | 1063.54 | 8808.69 | 0 | 273.84 | **** | 395.98 | **** | 948.8 | 78.89 | 125.17 | |
| ***TF3S1*** | 139.49 | 487124.2 | 382986.8 | 2.39 | 1.33 | 49.79 | 0.259 | 0.23 | 208.93 | 0.22 | 0.324 | 0.55 | 0.22 | 6373.89 | 8799.59 | 1082.92 | 993.42 | |
| ***TF3S2*** | 186.56 | 481218.9 | 382986.8 | 3.52 | 4.32 | 113.43 | 0.282 | 0.87 | 258.18 | 0.19 | 1.38 | 0.34 | 0.18 | 5609.2 | 9556.5 | 1153.03 | 973.3 | |
| ***TF3S3*** | 200.94 | 512881.4 | 400446.9 | 2.57 | 1.26 | 60.67 | 0.182 | 0.36 | 226.34 | 0.2 | 0.247 | 0.63 | 0.32 | 6831.64 | 9482.34 | 1123.77 | 1022.34 | |
| ***SD*** | 58655.27 | 185463.8 | 438595.5 | 5054.28 | 283.3374 | 8886.654 | 20.54491 | 439.1282 | 3750.074 | 893.44 | 139.6681 | 378.973 | 530.9833 | 16798.21 | 7444.628 | 566.8252 | 355.2555 | |
| ***Average*** | 28915.83 | 397612.9 | 531355.1 | 1920.041 | 142.6644 | 4220.785 | 10.19163 | 282.8822 | 2763.568 | 308.1211 | 84.78567 | 163.645 | 237.5011 | 12975.65 | 7669.058 | 695.3322 | 666.1244 | |
| ***CV %*** | 202.85 | 46.64 | 82.54 | 263.24 | 198.60 | 210.55 | 201.59 | 155.23 | 135.70 | 289.96 | 164.73 | 231.58 | 223.57 | 129.46 | 97.07 | 81.52 | 53.33 | 162.83 |

Table 3-The data collected for specified elements from three spots on three fractured teeth from one jaw (TF=Tooth fractured). That data was then used to calculate the standard deviation of each element (SD), the average of each element, the percent coefficient of variation of each element, and then the overall percentage coefficient of variation, which is the average of the percent coefficient of variation of all elements.

| Sample | Si28 | P31 | Ca43 | Cr52 | Mn55 | Fe57 | Co59 | Cu63 | Zn64 | Cd114 | Pb208 | As75 | Sn118 | Na23 | Mg24 | Ba138 | Sr88 | Average % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***TW1S1*** | 145.72 | 270703.3 | 382986.7 | 6.01 | 9.21 | 68.41 | 0.3 | 1.11 | 425.02 | 0.84 | 0.22 | 4.24 | 1.73 | 5208.52 | 2391.33 | 149.74 | 617.85 | |
| ***TW1S2*** | 188.84 | 255485.7 | 382986.7 | 5.12 | 8.24 | 110.32 | 0.28 | 3.71 | 443.99 | 0.78 | 0.191 | 3.5 | 1.47 | 4916.69 | 2057.85 | 123.7 | 565.6 | |
| ***TW1S3*** | 299.12 | 257131.3 | 382986.8 | 5.38 | 8.04 | 40.18 | 0.35 | 0.79 | 382.89 | 0.93 | 0.157 | 4.03 | 1.58 | 4977.11 | 2271.62 | 150.43 | 605.26 | |
| ***TW2S1*** | 1331.23 | 254070.7 | 382986.7 | 53.9 | 221.86 | 499.1 | 0.75 | 20.2 | 481.48 | 17.46 | 0.46 | 12.92 | 16.53 | 4406.85 | 675.5 | 91.66 | 423.32 | |
| ***TW2S2*** | 447.14 | 255734.5 | 382986.7 | 11.09 | 354.48 | 77.75 | 0.7 | 1.56 | 491.8 | 1.51 | 0.38 | 7.08 | 3.31 | 4133.35 | 702.75 | 96.42 | 427.45 | |
| ***TW2S3*** | 231.46 | 251572.4 | 382986.8 | 9.72 | 148.97 | 98.32 | 0.69 | 1.53 | 487.39 | 1.21 | 0.38 | 8.28 | 3.04 | 4137.7 | 646.17 | 86.49 | 412.57 | |
| ***TW3S1*** | 694 | 241084.1 | 382986.7 | 12.14 | 14.05 | 89.7 | 0.82 | 2.65 | 919.51 | 2.24 | 0.34 | 7.64 | 3.54 | 5939.81 | 1270.26 | 99.77 | 371.49 | |
| ***TW3S2*** | 348.35 | 236402.7 | 382986.7 | 10.37 | 11.74 | 94.74 | 0.75 | 1.63 | 731.87 | 2.27 | 0.38 | 7.63 | 3.05 | 5939.55 | 1202.62 | 91.02 | 364.86 | |
| ***TW3S3*** | 589.28 | 230247.7 | 382986.7 | 18.45 | 15.84 | 141.89 | 1.38 | 3.19 | 815.46 | 2.75 | 0.63 | 14.22 | 6.28 | 5366.25 | 1208.17 | 104.05 | 390.55 | |
| ***SD*** | 369.3788 | 12325.68 | 0.018028 | 15.28067 | 126.5414 | 139.1686 | 0.341337 | 6.137132 | 193.9787 | 5.345259 | 0.146933 | 3.767662 | 4.741324 | 688.9736 | 693.161 | 24.93525 | 102.0805 | |
| ***Average*** | 475.0156 | 250270.2 | 382986.7 | 14.68667 | 88.04778 | 135.6011 | 0.668889 | 4.041111 | 575.49 | 3.332222 | 0.348667 | 7.726667 | 4.503333 | 5002.87 | 1380.697 | 110.3644 | 464.3278 | |
| ***CV%*** | 77.76 | 4.92 | 0.00 | 104.04 | 143.72 | 102.63 | 51.03 | 151.87 | 33.71 | 160.41 | 42.14 | 48.76 | 105.28 | 13.77 | 50.20 | 22.59 | 21.98 | 66.76 |

Table 4- The data collected for specified elements from three spots on three whole teeth from one jaw (TW=Tooth whole). That data was then used to calculate the standard deviation of each element (SD), the average of each element, the percent coefficient of variation of each element, and then the overall percent coefficient of variation, which is the average of the entire element's percent coefficient of variation of all elements.

The main reason research reported in the literature has used cut or fractured teeth, is purely a size issue. The teeth used in other projects are larger, as they belong to humans, or other larger mammals.[16, 24] However, possum teeth are approximately a quarter of the size of human teeth, and thus, they can fit onto a microscope slide, and in the ablation chamber, without being altered, whereas this would not be the case for human teeth. Copeland *et al*. used whole teeth, as the sample type when analysing rodent teeth by LA-ICP-MS.[37, 46] Rodent teeth are much closer in size to possum teeth, thus, this method, is the most suited to this project.

Another reason why other projects choose to fracture, or cut the teeth is attributable to the area from which data is being collected. Lochner *et al*., for example, focused on the neo-natal line, and thus required the tooth to be cut using a diamond blade.[35] This differs from this project, as only the information contained within the enamel is analysed, not the information found within the different areas of the tooth. Thus, teeth in this project do not require a diamond saw.

Kang *et al*. also used the diamond saw as they wished to observe the dentine growth layers. Thus cutting to expose this area is needed, otherwise a large amount of pre-ablation would need to take place, which can be time consuming and costly.[11] Sectioning of the tooth samples also took place in the work done by Prohaska *et al.,* (by an electric drill-similar to using a diamond saw); one reason for this was that the dentine and enamel had to be separated in order to subject the samples to solution ICP-MS.[16] As solution ICP-MS is not needed for this project, fracturing is also not a suitable method for tooth sampling.

Also important to note is that whole teeth are more suited to archaeological samples. This is because by cutting or fracturing teeth, the rare, precious samples can be destroyed. One of the benefits of LA-ICP-MS (instead of solution ICP-MS) is its non-destructiveness; breaking the tooth, negates this benefit.

## 3.2 LA-ICP-MS parameters

### 3.2.1 Power

The power of the laser is an important factor in the acquisition of data from LA-ICP-MS. To obtain the most significant data, different laser power strengths must be trialled, and statistically analysed to ensure the most suitable laser power is used for the particular sample being analysed. Most commonly for teeth samples (using this particular instrument) laser power strengths of around 60% are used, with a spot size of ~60µm. However, previous research generally focuses on human teeth which are bigger then possum teeth, therefore the laser strength needed for possum teeth is likely to be less as the enamel layer will be thinner. Also earlier work also requires the data from dentine or other parts of the teeth, whereas for this project, data is only needed from the surface enamel section of the tooth.

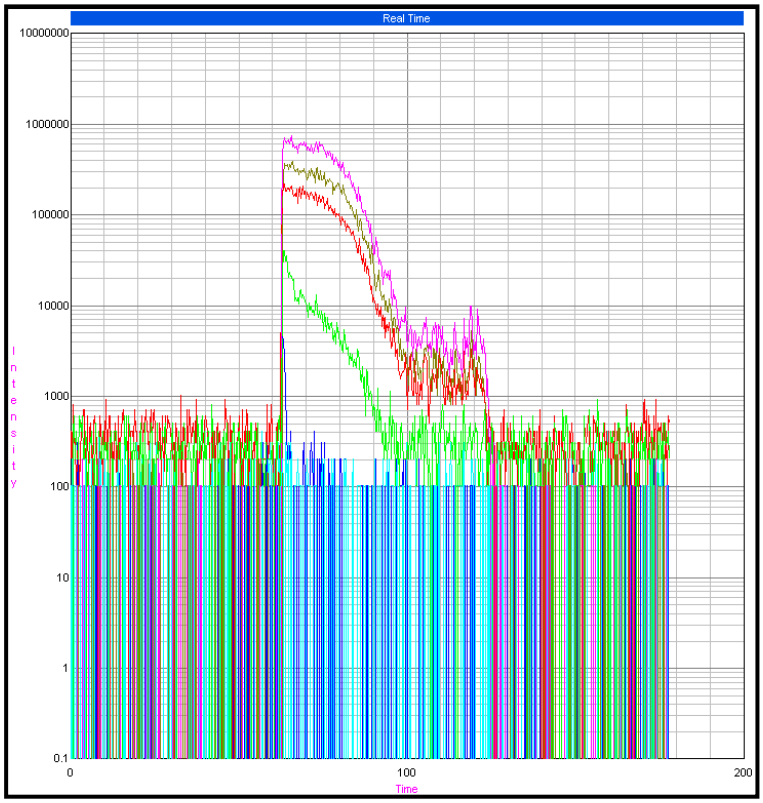


Figure 13 - 60% laser power, 60µm spot size concentration profile

Laser powers ranging from 20-80% were tested (using the common 60µm, spot size). Both 60% and 80%, were found to be too powerful for the enamel, and as a result the laser ablated through the enamel layer and into the dentine. This was

concluded following the concentration profiles, which changed sharply during the ablation process, indicating the dentine layer had been breached. Fig. 14 is an actual signal concentration profile seen when laser ablation occurs, and the power is too strong for the enamel (60% and 80%).

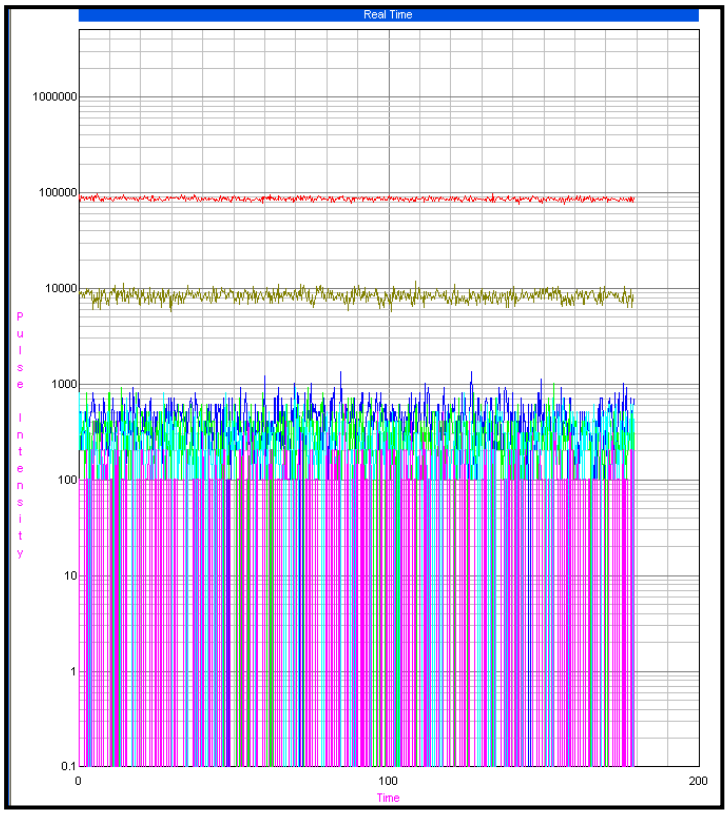


Figure 14 - 20% laser power, 60µm spot size concentration profile

20% strength proved to be too weak for the enamel. This was established due to concentration levels for all elements not increasing significantly above background levels, Fig. 15.

However, 40% was strong enough to gather data, but not so strong that it ablated through the enamel into the underlying dentine layer, Fig. 16. This also gave a good flat topped area for analysis, which is needed when analysing by LA-ICP-MS. If the area of analysis is too sloped or inconsistent, the data obtained is not reliable.

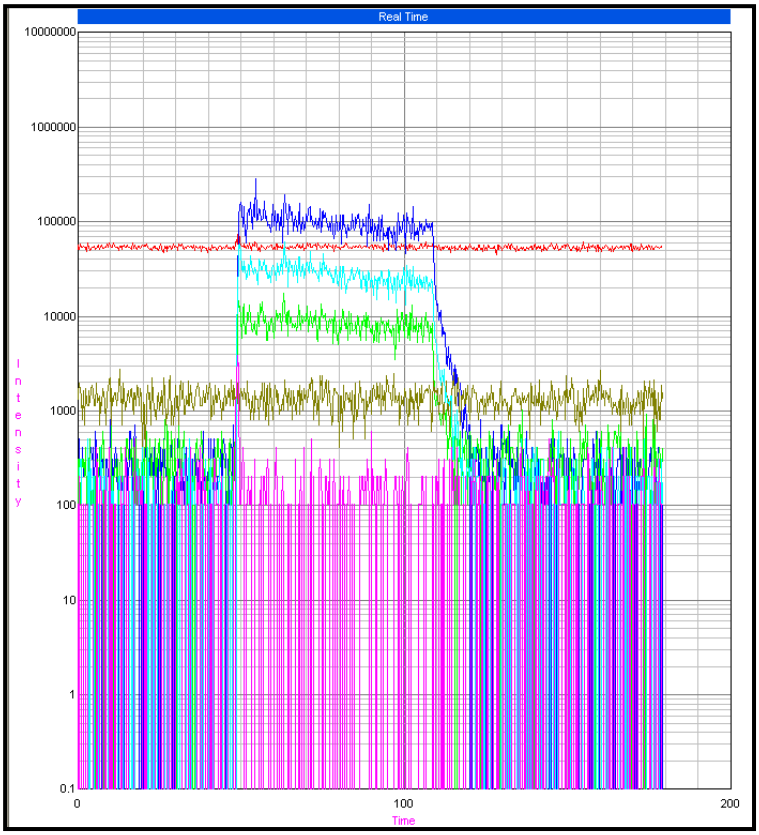


Figure 15 - 40% laser power, 60µm spot size concentration profile

### 3.2.2 Spot size

As discussed in section 1.3.2.1.1, spot size can vary greatly depending on the type of sample being ablated. Thus, different spot sizes were trialled on three different teeth to determine the best suited spot size, for this type of tooth sample. 15µm, 40µm, 60µm and 80µm diameter spot sizes were trialled at 40% laser power. It was concluded, from the concentration profiles, that the 15µm (Fig. 17) and 40µm (Fig. 18) spot sizes were too small to gather data above the background levels.

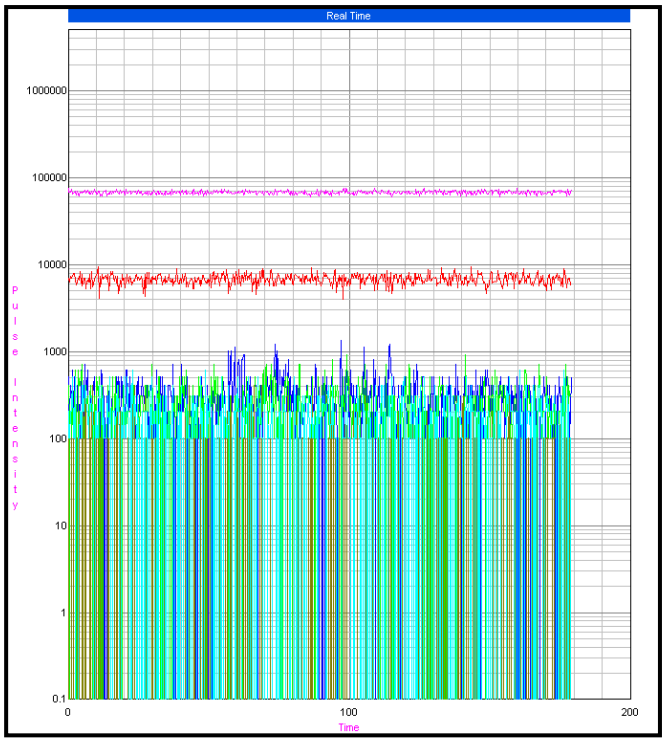

Figure 16 -40% laser power, 15µm spot size concentration profile

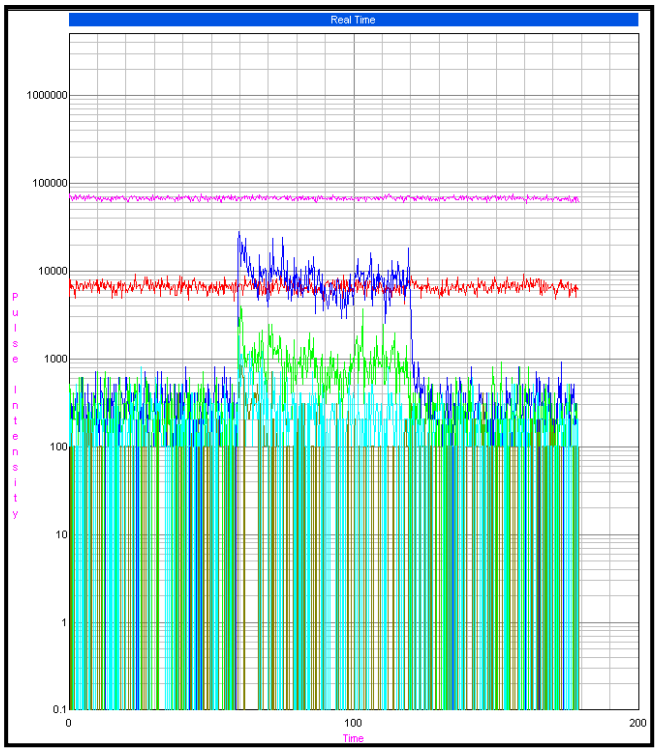

Figure 17 – 40% laser power, 40µm spot size concentration profile

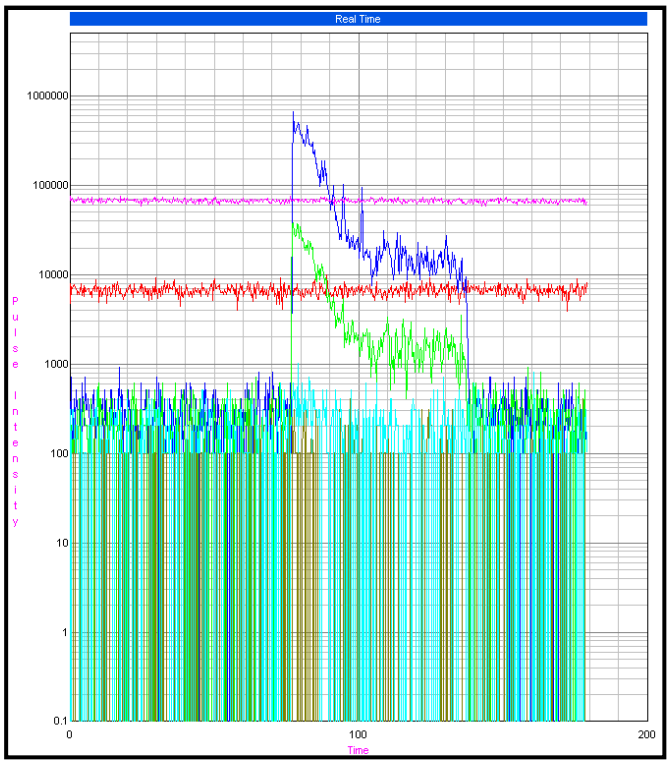

Figure 18 - 40% laser power, 80µm spot size concentration profile

The 80 µm (40% power) caused breaching of the enamel, Fig. 19. In comparison to the previous laser power and spot size combinations, the 60µm provided good quality data, shown in Fig.20. Thus, from these concentration profiles, it is obvious that 40% laser power and 60µm spot size is the most effective combination for gathering information from possum tooth enamel.

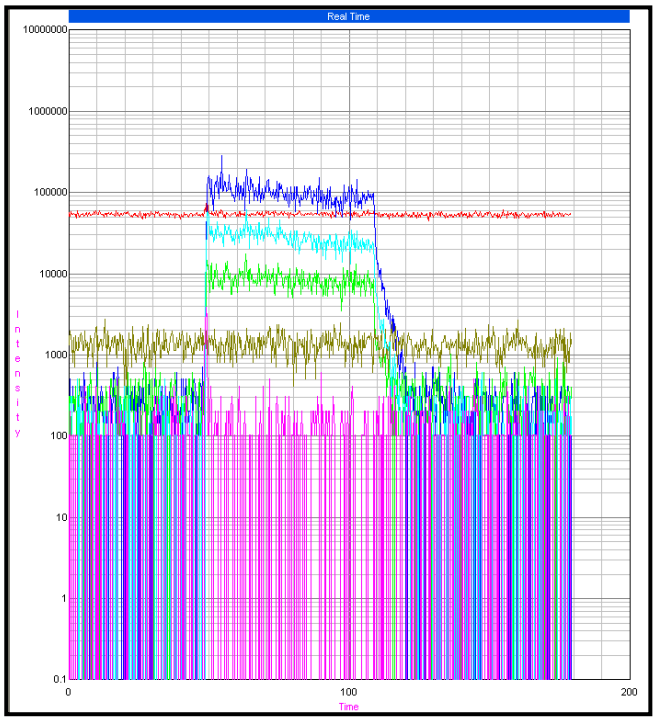

Figure 19 - 40% laser power, 60µm spot size concentration profile

### 3.2.3 Flow gas

Flow gas is set at 1.00 L $min^{-1}$, which is optimized for the specific instrument in use.

### 3.2.4 Internal standard value

An internal standard value is needed for the Glitter programme to calculate the unknown values relative to this standard value given. The internal value of CaO in hydroxyapatite was used (53.587%). Previous work done on the same machine used 56.03% which is the percentage of CaO in $CaCO_3$.[45] However, this value was not relevant in this method, as the work involved fish otiliths, which unlike humans, and mammals bones, are primarily calcium carbonate based.[45] Thus, the value used in this work was calculated using the molecular weight of calcium hydroxyapatite instead of calcium carbonate:

$$Mr\ (Ca_5(PO_4)_3OH = 502.3069\ gmol^{-1}$$

$$5CaO = 280.385$$

$$\text{Therefore \% of CaO} = 280.385/502.3069 \times 100$$

$$= 53.819\%$$

However, enamel is only made up of approximately 96.000% of hydroxyapatite, therefore:

$$(53.819/100) \times 96 = 53.587\%.$$

This number was therefore used, rather than the original 56.03% used in Blair.[45]

## 3.1 Calibration Standard

### 3.1.1 NIST 612

Initially, a purpose made standard was thought to be of benefit to the data analysis for this project.[24] This was thought to be the case as the shape and size of the craters left by the laser ablation on teeth, compared to the glass standard were found to be slightly different.[42] However, many problems were encountered during the preparation of the standard, mainly due to the $HNO_3$ not evaporating as easily as outlined in the method of Stadlbauer *et al*.[24] When a small amount of standard was able to be dried enough to place in the laser ablation chamber (after several methods of drying), the data collected from this standard was very

variable, Table 5. The large degree of variation is shown when comparing the average percent coefficient of variation (85.88%) with that of the average percent coefficient of variation of the NIST612 standard (24.00%), Table 6. Although 24.00% appears to be quite high, this was calculated from 71 samples, whereas the purpose made standard was calculated from only 17 samples.

| | *Si28* | *P31* | *Ca43* | *Cr52* | *Mn55* | *Fe57* | *Co59* | *Cu63* | *Zn64* | *Cd114* | *Pb208* | *As75* | *Sn118* | *Na23* | *Mg24* | *Ba138* | *Sr88* | *Average Coefficient Variation %* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Average*** | 1446.214 | 290024.8 | 382986.8 | 23.69412 | 62.10588 | 182.7347 | 3.8 | 3.428235 | 16.87882 | 5.326471 | 4.52 | 9.394118 | 22.41529 | 226.6306 | 1377.473 | 29.39235 | 424.4082 | |
| ***SD*** | 1560.492 | 155768.1 | 0.069393 | 48.3019 | 12.6535 | 179.6505 | 3.047805 | 3.956749 | 32.57014 | 3.325919 | 3.602006 | 17.25279 | 9.207038 | 174.7799 | 589.0607 | 14.75008 | 213.3852 | |
| ***Coefficient Variation %*** | 107.90 | 53.71 | 0.00 | 203.86 | 20.37 | 98.31 | 80.21 | 115.42 | 192.96 | 62.44 | 79.69 | 183.66 | 41.07 | 77.12 | 42.76 | 50.18 | 50.28 | 85.88 |

Table 5- The average, SD and percent coefficient of variation calculated for the data collected from the purpose made standard. Calculated from 17 trials.

| | *Si28* | *P31* | *Ca43* | *Cr52* | *Mn55* | *Fe57* | *Co59* | *Cu63* | *Zn64* | *Cd114* | *Pb208* | *As75* | *Sn118* | *Na23* | *Mg24* | *Ba138* | *Sr88* | *Average Coefficient Variation %* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Average*** | 335162.8 | 67.06 | 85262.45 | 43.39414 | 36.59757 | 48.77171 | 37.443 | 37.60871 | 36.47114 | 32.54943 | 42.87771 | 43.66643 | 41.26157 | 117040.9 | 76.21881 | 41.51329 | 78.10086 | |
| ***SD*** | 65510.06 | 29.02789 | 0.060491 | 8.668048 | 3.863834 | 21.50239 | 6.034808 | 5.341549 | 8.926797 | 10.12496 | 7.721256 | 10.80776 | 7.426127 | 25776.56 | 60.46761 | 6.41809 | 5.455338 | |
| ***Coefficient Variation %*** | 19.55 | 43.29 | 0.00 | 19.98 | 10.56 | 44.09 | 16.12 | 14.20 | 24.48 | 31.11 | 18.01 | 24.75 | 18.00 | 22.02 | 79.33 | 15.46 | 6.98 | 24.00 |

Table 6- The average, SD and percent coefficient of variation calculated for the data collected from the NIST612 standard. Calculated from 71 trials.

However, it was concluded that the purpose made standard may not be necessary for the work being carried out, as previous work has been carried out without a purpose made standard, or even an internal standard.[11, 41, 45] This was decided after referring to the recent thesis work completed by Jennifer Blair.[45] This work was carried out on the same LA-ICP-MS instrument and did not use a purpose made standard, and was still able to draw significant conclusions. Because this project is looking at the difference between teeth from different regions, that all rely on the same standard, the data collected is all relative to one another and to the standard, and does not rely on any outside data. Thus, because of these reasons, only the NIST 612 standard was initially used.

### 3.1.2 NIST 1400 comparison with NIST 612

Once all the data was collected using just the NIST 612 standard, some data (from chosen regions, jaws, teeth) were collected using this standard along with another NIST standard, NIST 1400 Bone Ash standard, for purposes of comparison**. NIST 1400 has been used in many previous studies involving LA-ICP-MS, bone and teeth.[14, 28, 31, 36]

One tooth from each region was analysed (four spots per tooth) as well as both standards (2 spots) at the beginning and the end of the session. This was done to assess whether there was a notable difference between the two standards, and if one was better suited than the other. Initially, it appeared the only difference between the two standards was that when using the NIST 1400 standard, the ppm of each element was approximately ~20ppm higher. That meant the range between the lowest ppm of one spot to the highest was the same for both standards. However, before any conclusions could be drawn from this, statistical analysis had to be carried out on the corresponding data. It was found that the result depended on what type of statistical analysis is used.

** This standard, as it was bought from overseas, was only obtained half way through the research. Thus, the comparison done here was to determine whether the data already collected, using the NIST 612, needed to be recollected using the new standard.

By analysing the data by the concentrations of elements in ppm, an obvious difference in the standards can be identified. This was done by taking the mean difference between the concentrations of one element and comparing these for both standards. It was concluded that there was a significant difference between the two standards, as shown by Fig.21.

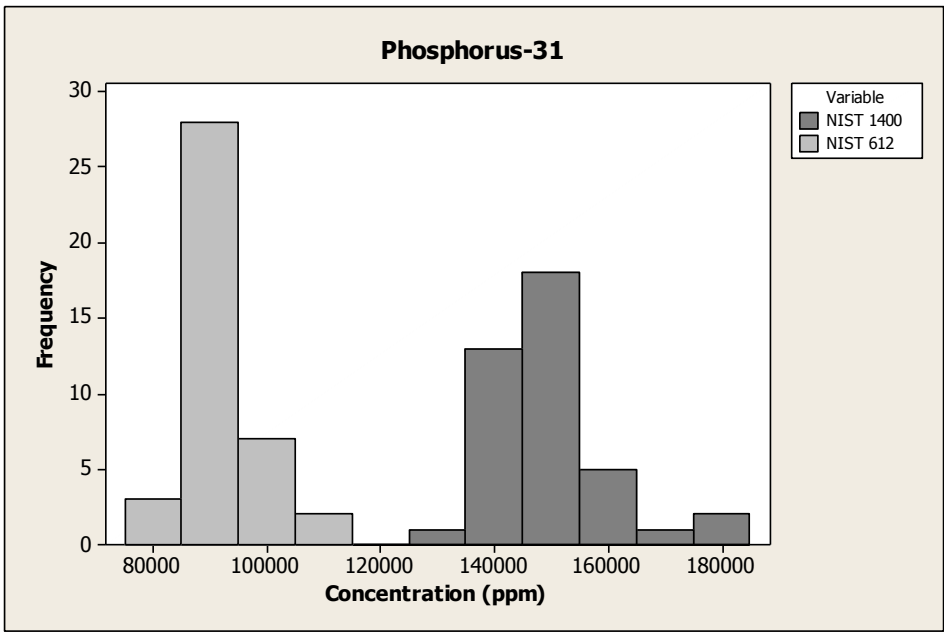


Figure 20 - Ppm for phosphorus when comparing NIST 612 and NIST 1400

However, the important information to gain from the data, in terms of this project, is the proportion of elements to each other, and if this varies across the region. Six elements' proportions (P,Ca, Mg, Mn, Na and Sr) were analysed by the same method for both standards. The proportion was divided by the mean, and then PCA (principal components analysis) was performed. By performing PCA, the variation of each data set is examined (see section 4.6 for further explanation of PCA). Each region was able to be plotted in terms of the concentration variation, Fig 22 and 23.

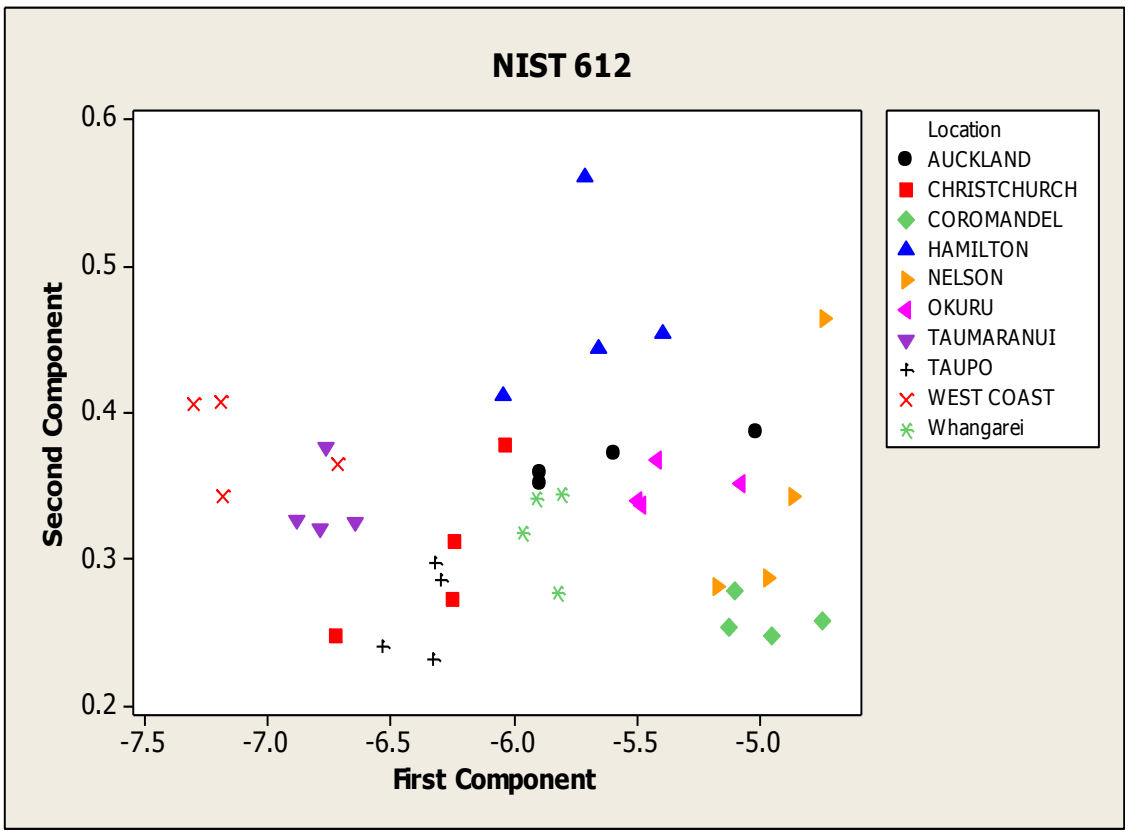


Figure 21 – PCA 1st component vs 2nd component for NIST 612

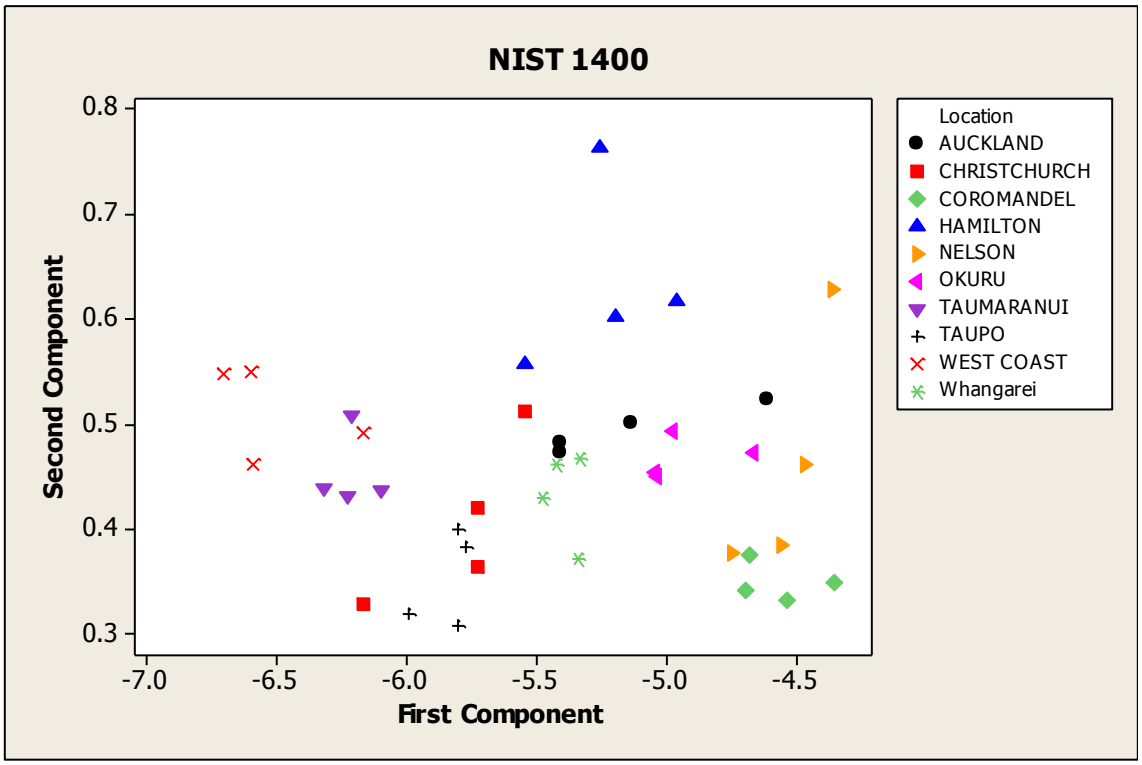


Figure 22 - PCA 1st component vs 2nd component for NIST 1400

From Fig. 22 and 23, it is apparent that the two standards cannot be separated, thus, as PCA is one of the main types of analysis that is to be used for this project (along with other similar types of statistical analysis) there appears to be little difference between the two standards. Thus, the NIST 612 standard was retained throughout the project.

## 3.2 Statistical considerations

### 3.2.1 Sample number

After consultation with a statistician it was concluded that at least three jaws from each region, three teeth per jaw, and four spots per tooth would be the minimum needed to afforded significant data. Thus, each region has between three and eight jaws analysed.

# 4 Results and discussion

As explained in section 3.1.2 these results were generated using the NIST612 standard.

Much of the previous research that has been discussed here (Chapter 1) had a different focus in terms of both the type of information gathered, and the type of analysis carried out on that information. Earlier investigations have used exact elemental concentrations,[24] or specific isotope ratios[16, 25, 37] whereas, this project centred on using the relative elemental composition of tooth. From the data collected this project then aimed to group samples into specific regions/groups, whereas previous studies tended to determine particular issues, such as the amount of lead in a specific tooth enamel sample.[14, 28-31]

## 4.1 Standard drift

The standard (as explained in section 2.4, 2.5, 3.2.4 and 3.1) was used to monitor the instrument drift rate during a laser ablation session. To establish if the results obtained from the samples were significant, two spots were analysed before and after each session to determine if any change could be observed. Overall there appeared to be a slight increase in the concentration from the beginning to the end of a session, but over all the sessions there appears to be little change. The GLITTER programme takes any increase or decrease in concentration of a standard per sessions into account and adapts the data accordingly. Fig. 24 illustrates the drift across all standard data obtained, for randomly selected elements that demonstrate typical drift.

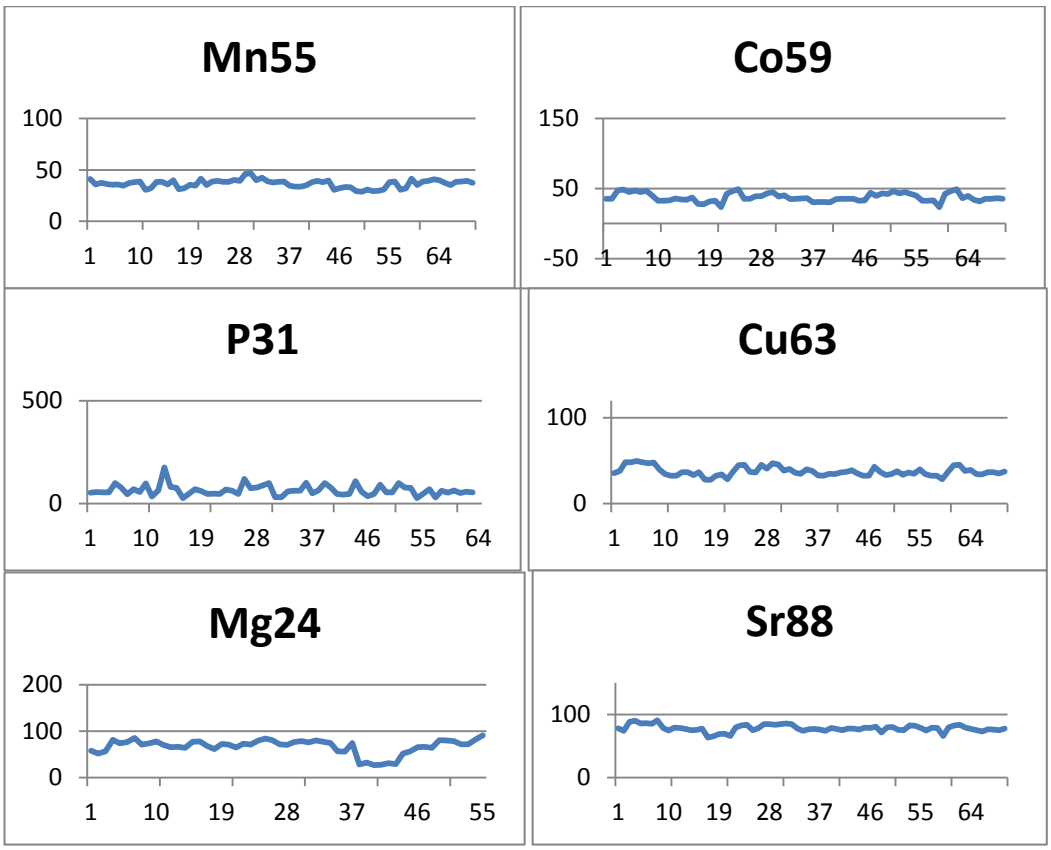


Figure 23- Standard drift rate of elements across data collection period. The X axis represents the sample number. Each lasering session had at least two NIST 612 samples taken at the beginning and the end. The data above represents the standard data collected from all lasering sessions over a two month period. The Y axis represents the intensity of the signal in ppm.

These graphs illustrate that the measurement of NIST 612 standard did not change significantly throughout the data collection process. The validity of NIST 612 was further discussed in section 3.1, where the percent coefficient of variation was included, showing little variation across the data obtained for the NIST 612 standard.

## 4.2 Minimum detection limits (MDL)

Minimum detection limits are the minimum concentrations that the LA-ICP-MS is able to detect for each element. Campbell *et al.*, have investigated the best ways of statistically dealing with data of this nature.[57] When faced with values lower than the MDL, Campbell *et al.*, assert that if the majority of the values for a particular element are below the MDL that element should be removed from the analysis. If the majority of the element's values are above the MDL, but contain a few below, then these values should not be removed, or replaced as zero[††], but

[††] If replaced with zero, this is considering the value to be a true zero value, which is incorrect.

rather they should be considered to be half the MDL. However, the detection limit for each element, on any given day, for any given sample acquisition is not consistent, due to changing background noise.[57] Thus, a way to deal with this, is to take the lowest value which is still above the MDL, halve it, and replace the values below MDL with this value. As many of the elements (as explained above) are mostly below the MDL, the elements most consistently above the MDL will be examined in the following sections for this project.

Minimum detection limits were different for each element. Table 5 shows the percentage of observations that were below the MDL, and thus not used for analysis. This information can be further broken down into measurements below MDL per region, Table 6.

| *Element* | *Si* | *P** | *Ca* | *Cr* | *Mn* | *Fe* | *Co* | *Cu* | *Zn* | *Cd* | *Pb* | *As* | *Sn* | *Na* | *Mg* | *Ba* | *Sr* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *% of observations below MDL* | 77.9 | 10.6 | 0 | 93.5 | 2.4 | 62.2 | 81.9 | 69.0 | 2.3 | 79.5 | 42.5 | 92.2 | 78.4 | 0.15 | 0.61 | 0.30 | 0.00 |

Table 5 - Percentage of observations below MDL. * Note the majority of P readings below MDL come from one region, Hamilton, which is unusual. The lack of phosphorus in Hamilton affected further analysis, particularly the Linear Discriminant Analysis in section 4.7.

| | *Si* | *P* | *Ca* | *Cr* | *Mn* | *Fe* | *Co* | *Cu* | *Zn* | *Cd* | *Pb* | *As* | *Sn* | *Na* | *Mg* | *Ba* | *Sr* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Hamilton*** | 58.75 | 85.00 | 0.00 | 98.75 | 5.00 | 81.25 | 77.50 | 58.75 | 3.75 | 61.25 | 62.50 | 93.75 | 92.50 | 0.00 | 1.25 | 0.00 | 0.00 |
| ***West Coast*** | 52.63 | 2.63 | 0.00 | 96.05 | 9.21 | 80.26 | 75.00 | 76.32 | 2.63 | 72.37 | 55.26 | 88.16 | 94.74 | 1.32 | 3.95 | 2.63 | 0.00 |
| ***Taumaranui*** | 34.21 | 0.00 | 0.00 | 96.05 | 1.32 | 75.00 | 88.16 | 84.21 | 1.32 | 82.89 | 61.84 | 92.11 | 88.16 | 0.00 | 0.00 | 0.00 | 0.00 |
| ***Nelson*** | 86.11 | 0.00 | 0.00 | 100.00 | 8.33 | 69.44 | 61.11 | 61.11 | 16.67 | 52.78 | 41.67 | 75.00 | 86.11 | 0.00 | 0.00 | 0.00 | 0.00 |
| ***Okuru*** | 48.21 | 0.00 | 0.00 | 83.93 | 0.00 | 57.14 | 85.71 | 67.86 | 0.00 | 85.71 | 66.07 | 94.64 | 57.14 | 0.00 | 0.00 | 0.00 | 0.00 |
| ***Christchurch*** | 40.00 | 0.00 | 0.00 | 96.70 | 0.00 | 88.30 | 93.30 | 83.30 | 1.70 | 85.00 | 20.00 | 93.30 | 86.70 | 0.00 | 0.00 | 0.00 | 0.00 |
| ***Auckland*** | 60.00 | 0.00 | 0.00 | 88.42 | 0.00 | 54.74 | 89.47 | 58.95 | 0.00 | 83.16 | 28.42 | 95.79 | 71.58 | 0.00 | 0.00 | 0.00 | 0.00 |
| ***Whangarei*** | 22.92 | 0.00 | 0.00 | 97.92 | 0.00 | 22.92 | 77.08 | 54.17 | 0.00 | 79.17 | 10.42 | 91.67 | 70.83 | 0.00 | 0.00 | 0.00 | 0.00 |
| ***Coromandel*** | 17.54 | 0.00 | 0.00 | 96.49 | 1.75 | 24.56 | 77.19 | 73.68 | 1.75 | 94.74 | 28.07 | 92.98 | 57.89 | 0.00 | 0.00 | 0.00 | 0.00 |
| ***Taupo*** | 58.33 | 0.00 | 0.00 | 87.50 | 0.00 | 54.17 | 84.72 | 70.83 | 0.00 | 93.06 | 38.89 | 97.22 | 73.61 | 0.00 | 0.00 | 0.00 | 0.00 |

Table 6 - Percentage of observations, per region, below MDL.

Table 6 can be used to deduce some key points concerning this data set:

- Some regions may have significant levels of trace elements. For example, Fe, for most regions has concentrations which are frequently below the MDL. However, in Whangarei and Coromandel Fe is above the MDL in ~80% of samples. Thus, these detection limits may be used to identify elements of interest for each region.

- Na, Mg, Ba and Sr are detectable to almost 100% in every region.

- Elements such as Zn and Mn are also mostly detectable in every region.

- Various elements are mostly undetectable across all regions- Cr, Co, Cu, Cd, As and Sn.

Due to these findings it was determined that statistical analysis would be carried out on a select group of elements, which had consistently the majority of their data above the MDL. That group is Mn, Zn, Na, Mg, Ba, and Sr. Ca and P, the major elements in calcium hydroxyapatite, are also included in some analysis, mainly where they are used as internal standards. Although, the absence of elements, (that is to say that they were not able to be detected above the MDL) may be significant to distinguish between each region, their absence cannot be quantified, and therefore cannot be compared. Also, often the elements that were mostly non-detectable, were so across all regions, thus not a useful distinguisher. Therefore, further analysis will not take into account elements that were mostly non-detectable using LA-ICP-MS.

Initially it was thought Fe may be a useful distinguishing element. However, Fe suffers from difficulties in analysis because of interfering ions ($^{40}Ar^{16}O$and $^{40}Ca^{16}O$- as a large amount of Ca would be present in the hydroxyapatite, this particular interfering ion would have a significant effect) in the plasma. This results in data that is often varied and unreliable for Fe. Thus, Fe is not suitable to be used to help identify regions, and thus was also omitted from further analysis.

## 4.3 Preliminary Results

Campbell *et al.*, also suggests that before any complex statistical analysis takes place, that simple visualisation analysis should occur, to assess whether any outliers exist, and if any conclusions or predictions can be drawn from simple analysis.[57]

### 4.3.1 Individual value plots

Data was initially processed using the direct readings the GLITTER programme gave (although the programme gives these as ppm, they are more an arbitrary value calculated from the signal intensity ratios).[58] The elemental concentrations of the elements with the most consistent detection across all regions (section 4.1.2) were used to generate individual value plots to determine whether any conclusions could be drawn visually.

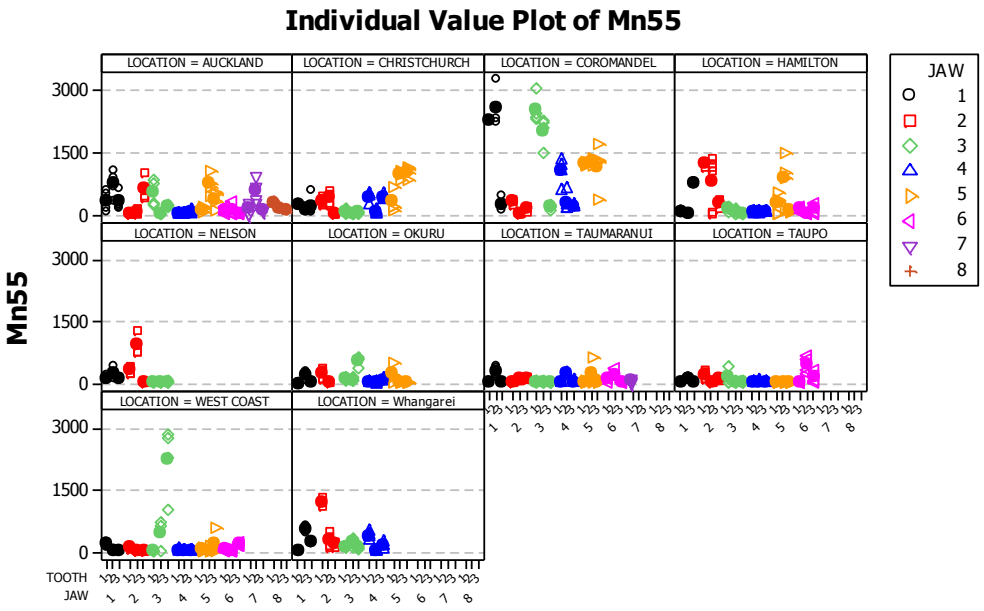


Figure 24 - Individual value plot of the concentration of each spot (4 spots per tooth, 3 teeth per jaw, 3-8 jaws per region) of Mn (ppm)

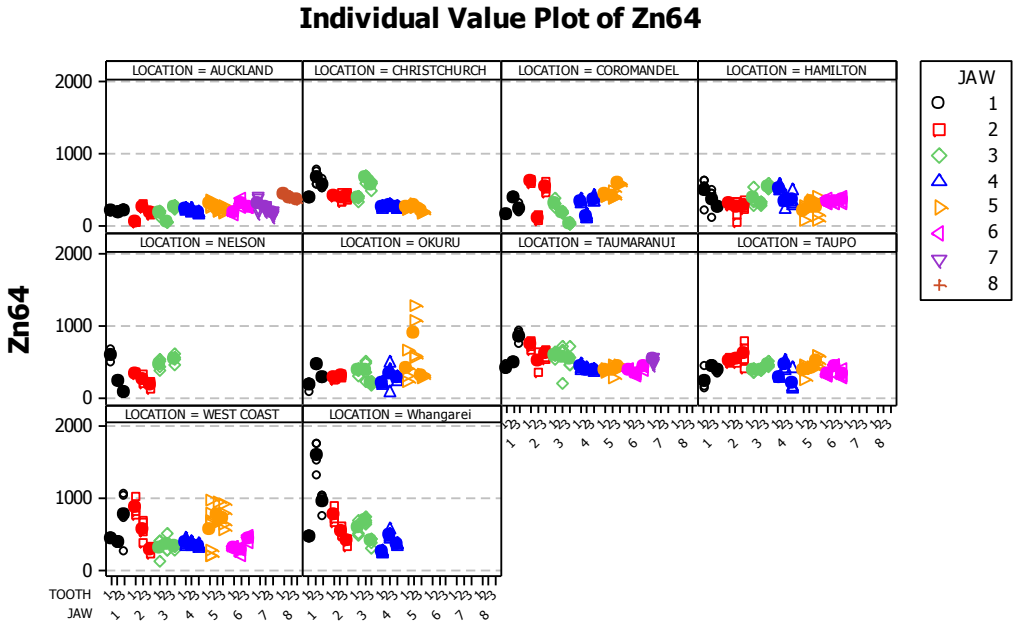


Figure 25 - Individual value plot of the concentration of each spot (4 spots per tooth, 3 teeth per jaw, 3-8 jaws per region) of Zn (ppm)

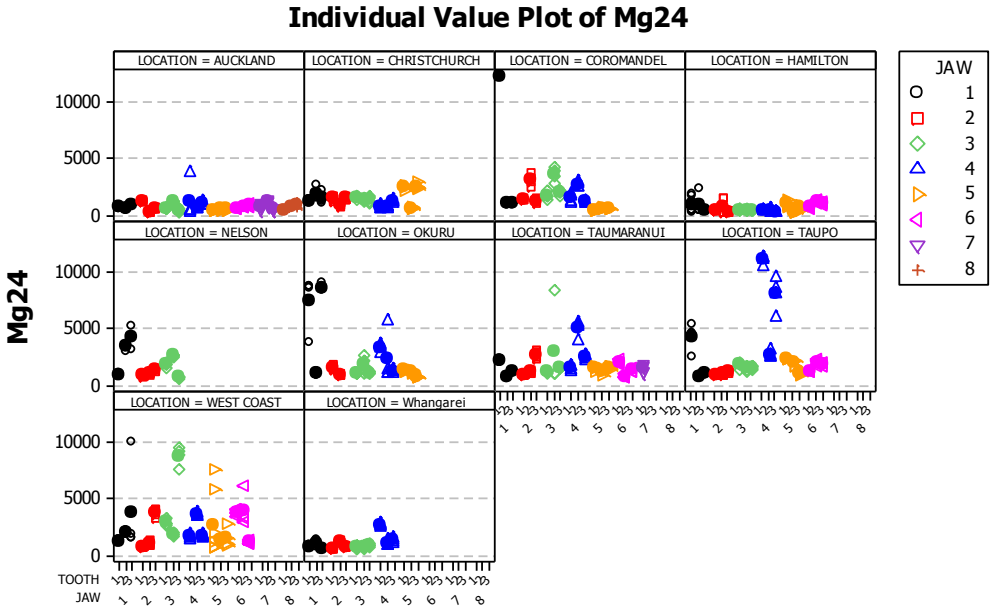


Figure 26- Individual value plot of the concentration of each spot (4 spots per tooth, 3 teeth per jaw, 3-8 jaws per region) of Mg (ppm)

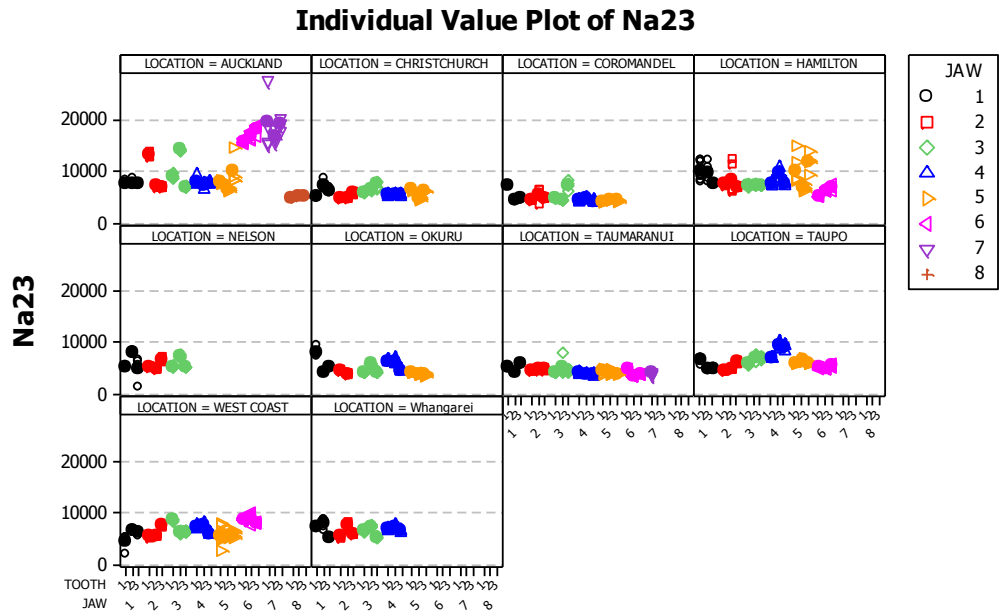


Figure 27 - Individual value plot of the concentration of each spot (4 spots per tooth, 3 teeth per jaw, 3-8 jaws per region) of Na (ppm)

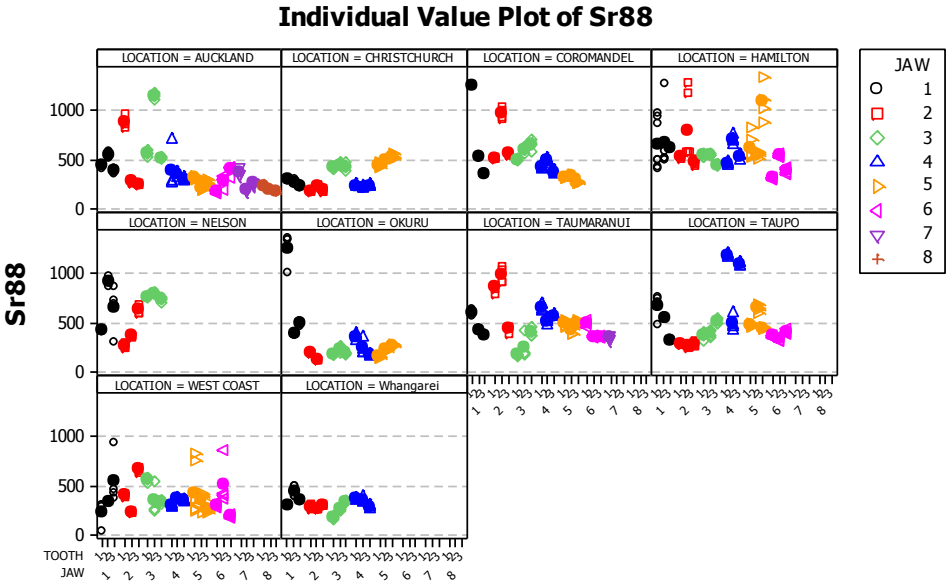


Figure 28 - Individual value plot of the concentration of each spot (4 spots per tooth, 3 teeth per jaw, 3-8 jaws per region) of Sr (ppm)

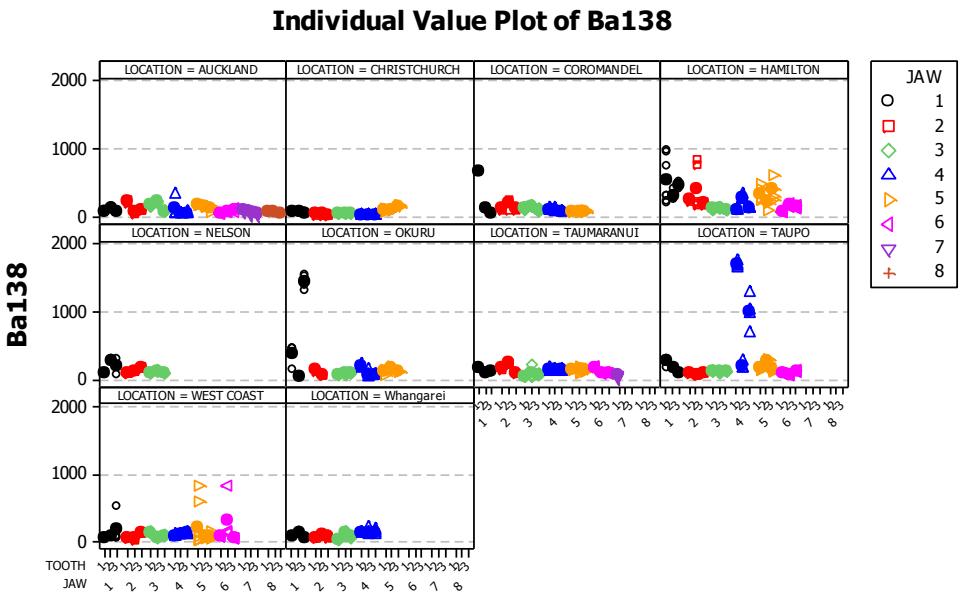


Figure 29 - Individual value plot of the concentration of each spot (4 spots per tooth, 3 teeth per jaw, 3-8 jaws per region) of Ba (ppm)

These individual value plots yielded little usable information; however, a few conclusions could be drawn:

- The amount of Mn in Coromandel and the West Coast varied slightly within the region.
- Zn levels in Whangarei were varied
- The amount of Na present in Auckland possum teeth was slightly higher, than other regions.
- The amount of Sr in most regions varied within the region
- Apart from a few outliers, Ba was relatively consistent within each region.
- There appeared to be some outliers that were checked on the original signal output on the LA-ICP-MS instrument to determine whether they were representative of the sample, or if they were simply contamination or reflecting possible problems with the instrument. A few spots were removed this way, including spots 1 and 2 from the first tooth of the first jaw from Hamilton, as it appeared these spots were not representative of the tooth (as the signal output was not flat, or consistent with the other spots collected from that tooth and jaw).

Thus, by producing these box plots, it was established that further statistical interpretation and transformation was needed to yield any significant conclusions.

## 4.4 Compositional transformation

The GLITTER programme used in this project requires the input of an internal standard value to calculate the value of all the other elements analysed. In this case, CaO was used. The value was calculated, (section 3.2.4), however, there is no way to know the exact concentration of CaO in every sample (without analysing every sample also by solution ICP-MS). Thus, there may be a slight deviation from this value from tooth to tooth.

As a consequence of this, the concentrations of the other elements are calculated as a ratio of this first internal standard value. In theory all the data collected should add up to one million (as the elements are measured in ppm). However, due to the slight inaccuracy of the internal standard value they do not. In order to draw relevant conclusions from the data, therefore, the data is converted into ratios in relation to calcium, so that all the data from one sample is equal to 1. This data is then used throughout the following calculations.[‡‡]

## 4.5 Compositional Comparison

A compositional comparison analysis was carried out to see whether it was possible to draw any conclusions about the relative concentrations of elements to one another, i.e. to see which elements are the biggest contributors to the enamel. To do this the concentrations of each element was compared with the concentrations of every other element to give ratios that describe the proportion of each element to one another. This is important to confirm that whether calcium and phosphorus are in the highest proportions, as would be expected in a hydroxyapatite sample. It also indicates which other elements are in the biggest concentrations, i.e. which elements apart from calcium and phosphorus may influence the results of further statistical analysis.

Matrices below summarise the key descriptors $\left\{\log\left(\frac{x_i}{x_j}\right)\right\}$ for compositional variables $x_i$, where $x_i$ is the data value for a particular element and $x_j$ is the corresponding data value for calcium. Table 7 takes the mean of each element's compositional transformation data (section 4.4), and then uses this mean to

[‡‡] It is important to note that calcium, once used to calculate the other elements' relative values, is then measured exactly, by back calculation. Thus, the value for Ca is not a constant.

generate a ratio of each individual element, with every other element that is analysed, and then takes the log of this number. If the number is large and positive, this indicates that the element in the left hand column is in greater proportion than that of the corresponding element in the top row. If the number is small, and close to zero, this signifies that the elements in question are close to equal in proportion. Thus, one can conclude from this data collection that calcium is in the greatest proportion, but is of similar proportion to phosphorus, which is expected, as both elements are the main constituents of hydroxyapatite, which is the compound of which teeth are comprised.

Table 7- Summarises the mean log ratios of each two element combination. Every element is analysed with every other element to give the full table.

| | Ca | Na | Mg | P | Mn | Zn | Sr | Ba |
|---|---|---|---|---|---|---|---|---|
| Ca | 0 | 4.17 | 5.66 | 0.35 | 8.4 | 7.05 | 6.93 | 8.45 |
| Na | -4.17 | 0 | 1.5 | -3.82 | 4.24 | 2.88 | 2.77 | 4.28 |
| Mg | -5.66 | -1.5 | 0 | -5.32 | 2.74 | 1.39 | 1.27 | 2.78 |
| P | -0.35 | 3.82 | 5.32 | 0 | 8.05 | 6.7 | 6.59 | 8.1 |
| Mn | -8.4 | -4.24 | -2.74 | -8.05 | 0 | -1.35 | -1.47 | 0.04 |
| Zn | -7.05 | -2.88 | -1.39 | -6.7 | 1.35 | 0 | -0.11 | 1.4 |
| Sr | -6.93 | -2.77 | -1.27 | -6.59 | 1.47 | 0.11 | 0 | 1.51 |
| Ba | -8.45 | -4.28 | -2.78 | -8.1 | -0.04 | -1.4 | -1.51 | 0 |

Table 8 takes the standard deviation of each element's compositional transformed data, and then uses this standard deviation to generate a ratio of each combination of elements, and then takes the log of this number. If the number is large (positive, and above ~3) this indicates that for that particular combination of elements, the proportion of these elements to each other is significantly variable. Whereas, if the number is small, this indicates that the variation of the proportion is small also.

Table 8- Summarises the SD log ratios of each element in relation to every other element being analysed.

| | Ca | Na | Mg | P | Mn | Zn | Sr | Ba |
|---|---|---|---|---|---|---|---|---|
| Ca | 0 | 0.34 | 0.68 | 0.29 | 1.51 | 0.6 | 0.47 | 0.82 |
| Na | 0.34 | 0 | 0.76 | 0.46 | 1.6 | 0.79 | 0.55 | 0.85 |
| Mg | 0.68 | 0.76 | 0 | 0.64 | 1.79 | 0.99 | 0.61 | 0.72 |
| P | 0.29 | 0.46 | 0.64 | 0 | 1.58 | 0.59 | 0.52 | 0.86 |
| Mn | 1.51 | 1.6 | 1.79 | 1.58 | 0 | 1.63 | 1.67 | 1.84 |
| Zn | 0.6 | 0.79 | 0.99 | 0.59 | 1.63 | 0 | 0.86 | 1.11 |
| Sr | 0.47 | 0.55 | 0.61 | 0.52 | 1.67 | 0.86 | 0 | 0.62 |
| Ba | 0.82 | 0.85 | 0.72 | 0.86 | 1.84 | 1.11 | 0.62 | 0 |

Table 9 takes the mean log ratio and divides it by the SD log ratio calculated above for each combination to give the mean/SD log ratio. If the number is large this means that the relative concentrations are most certainly not equal.

Table 9- Summarises the mean/SD log ratios of each two element combination. Every element is analysed with every other element to give the full table.

| | Ca | Na | Mg | P | Mn | Zn | Sr | Ba |
|---|---|---|---|---|---|---|---|---|
| Ca | * | 12.13 | 8.37 | 1.21 | 5.57 | 11.75 | 14.84 | 10.34 |
| Na | -12.13 | * | 1.97 | -8.26 | 2.64 | 3.67 | 5.02 | 5.05 |
| Mg | -8.37 | -1.97 | * | -8.3 | 1.53 | 1.4 | 2.09 | 3.85 |
| P | -1.21 | 8.26 | 8.3 | * | 5.11 | 11.41 | 12.59 | 9.4 |
| Mn | -5.57 | -2.64 | -1.53 | -5.11 | * | -0.83 | -0.88 | 0.02 |
| Zn | -11.75 | -3.67 | -1.4 | -11.41 | 0.83 | * | -0.13 | 1.26 |
| Sr | -14.84 | -5.02 | -2.09 | -12.59 | 0.88 | 0.13 | * | 2.44 |
| Ba | -10.34 | -5.05 | -3.85 | -9.4 | -0.02 | -1.26 | -2.44 | * |

## 4.6 Principal Component Analysis

Campbell *et al*., proposes the next step when analysing a large data set like this, is to apply a dimension reducing technique such as Principal Component Analysis (PCA).[57] PCA generates linear combinations of the elements, which according to Campbell *et al*., rotates the axis to represent the maximal variation. These linear combinations explain the maximal variance of the overall data set, "data cloud". PCA can generate a number of axes. The first, referred to as PC1 (or scor1), illustrates the most variation. There can be as many PCs as there are variables/elements, however, frequently between 1-4 (as in this project) are used.

Before a PCA can be performed, the data needs to be transformed into centred log-ratio (crl) coordinates. This is done to avoid spurious correlations caused by the constraints of using raw data with common denominators or numerators.[57] This transformation also allows for the data to be plotted in a Euclidean[§§] (rather than a simplex[***]) space.[57] Crl are calculated by taking the logarithm of the ratio of each element to the geometric mean of the sample, to give the following results, Table 10.

[§§] "Any n-dimensional space with notions of distance and angle that obey the Euclidean relationships"[57]

[***] "A simplex is a subset of Euclidean space within which the values of each variable are constrained to sum to a constant value"[57]

| *Variable/Element (crl form)* | *PC1* | *PC2* | *PC3* | *PC4* |
|---|---|---|---|---|
| ***Ca*** | 0.052 | 0.548 | 0.240 | -0.032 |
| ***Na*** | 0.163 | 0.368 | 0.623 | 0.125 |
| ***Mg*** | 0.436 | -0.108 | -0.377 | 0.586 |
| ***P*** | 0.097 | 0.494 | -0.326 | 0.297 |
| ***Mn*** | -0.537 | -0.276 | 0.186 | 0.175 |
| ***Zn*** | -0.211 | 0.373 | -0.494 | -0.528 |
| ***Sr*** | 0.489 | -0.048 | 0.166 | -0.296 |
| ***Ba*** | 0.446 | -0.303 | -0.008 | -0.394 |

Table 10 - PCA results for Ca, Na, Mg, P, Mn, Zn, Sr and Ba, using the transformed crl data values.

These values can then be plotted, Fig. 31, by region. The plot below shows that the variance is primarily due to the first component (PC1) as the data generally is more spread when compared to that of the second component (PC2), which by PCA definition is expected.

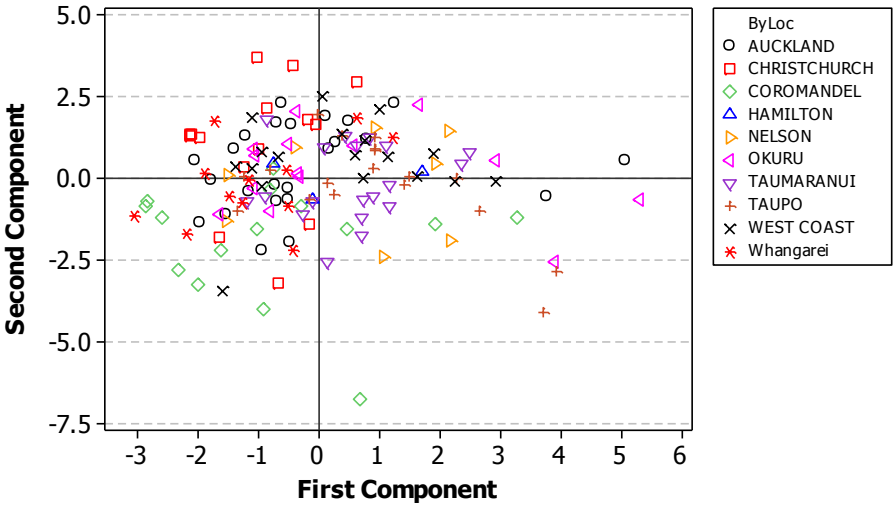


Figure 30 - PC1 vs. PC2 by region

The data can also be plotted as a box plot, Fig 32., which again illustrates that the majority of the variance can be accounted for in the first 2 PCs; note the larger y axis scale for PC1 and PC2.

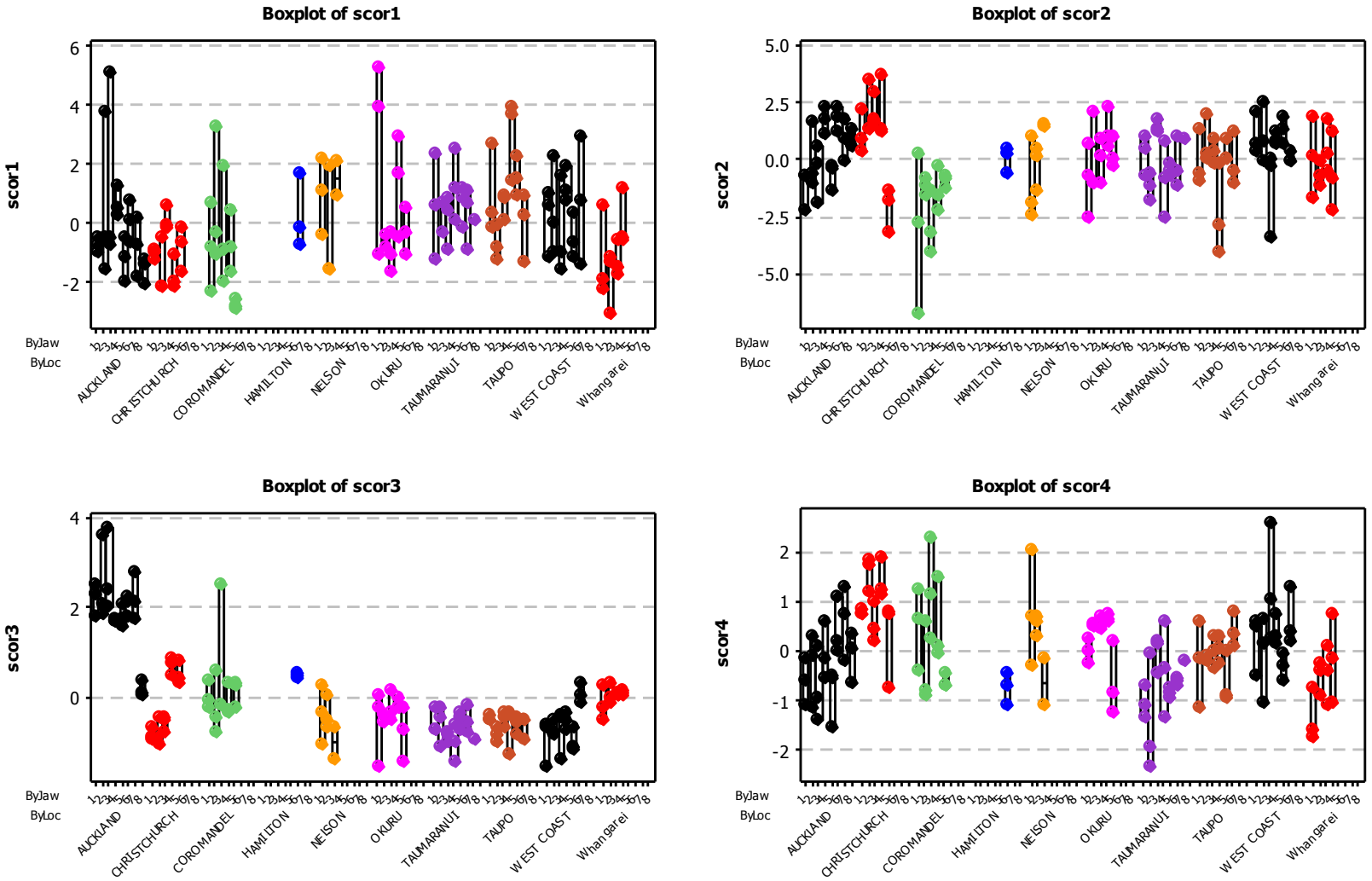


Figure 31 - Boxplot of PC1 (scor1) PC2 (scor2) PC3 (scor3) and PC4 (scor4)

From section 4.5 it is obvious that the major elements in the teeth are calcium and phosphorus. As calcium is the constant, one needs to consider whether or not phosphorus on its own was significantly different enough between regions to be able to discriminate. Thus, the additive logarithm ratio of P was established, multiplied by the geometric mean, and this was taken to the power of 10, to give the additive log ratio which was then plotted per region as a boxplot to give figure 33. From this box plot some of regions can be distinguished from each other, however, significant overlap still occurs.

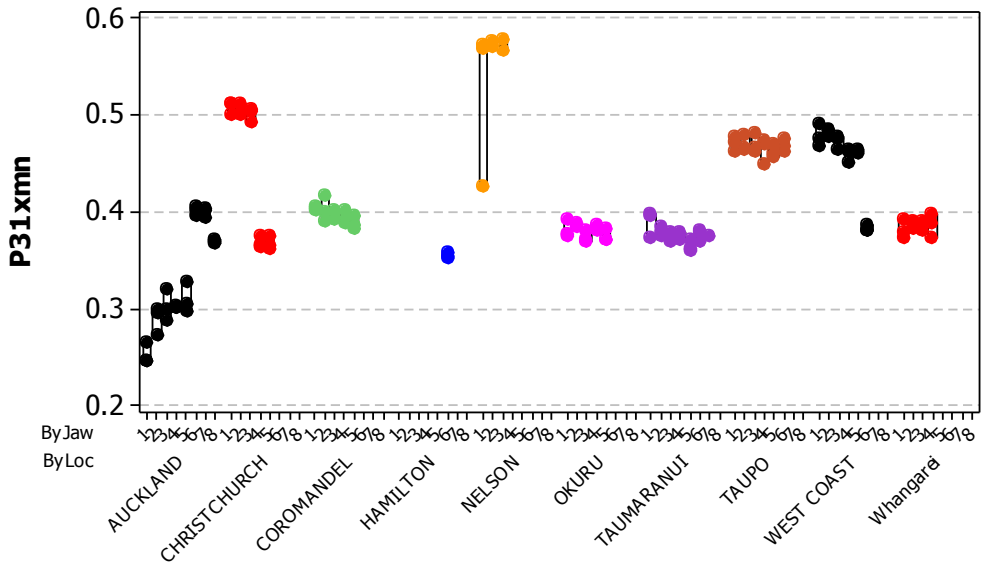


Figure 32 - Boxplot of Pxmn (PCA values for P) per region

The variance between locations, jaws and teeth can also be examined from the data obtained from PCA, Table 11. This description shows that the major variance comes from between teeth in the same jaw, rather than between jaws or location, which one might expect. Possible reasons for this will be discussed in the section 5 (discussion and conclusion).

| | **Score 1/ PC1** | | | **Score 2/ PC2** | | |
|---|---|---|---|---|---|---|
| | *Variance components* | *% of total* | *SD* | *Variance components* | *% of total* | *SD* |
| *Location* | 0.307 | 11.99 | 0.554 | 0.373 | 14.73 | 0.611 |
| *Jaw* | 0.381 | 14.88 | 0.617 | 0.746 | 29.43 | 0.864 |
| *Tooth* | 1.872 | 73.13 | 1.368 | 1.415 | 55.84 | 1.190 |

Table 11 - Variance components calculated from PCA of PC1 and PC2

An earlier project (section 1.5.1.1) carried out similar data analysis to discriminate between tooth and bone samples of human individuals.[13] By using PCA the majority of the samples could be discriminated. However, overlapping occurred for some of the samples. All the samples were able to be correctly grouped, however, some of these groups overlapped with each other. These findings are similar to those reported here

## 4.7 Initial Linear Discriminant Analysis (LDA)

Linear Discriminant Analysis is a way to organise samples into groups. It does this by producing a linear combination from a discriminant function.[57] Before LDA can be used, the data must be transformed from crl to arl (as explained in section 4.6). The arl is calculated, by taking the log ratios of each element over a common denominator value.[57] According to Campbell *et al*., the denominator choice is arbitrary, however, a element that has only a small amount of variability is a preferable choice.[57] However, as already explained (section 4.2) phosphorus in Hamilton is mainly below the MDL, thus as phosphorus was used in the LDA, Hamilton was excluded.

Tables 12 and 13 contain the results of the LDA for all regions apart from Hamilton. Hamilton was excluded from the LDA due to the low levels of detection of phosphorus. Phosphorous is one of the main constituents of calcium hydroxyapatite, therefore even if the soils were low in phosphorus, the levels within teeth should still be significant enough, to be detected. Thus, it is unknown what caused the unusual data obtained from Hamilton. However, as the possum samples for Hamilton came from within city limits, one would also expect that their diet would be made up of human rubbish, which could have come from any place in the world. Thus, the elemental composition of the possum teeth from this Hamilton region, are quite possibly not very useful for the project, regardless of the phosphorus levels. Thus, Hamilton was excluded from the LDA.

Table 12 contains the results for the non-cross validated[†††] LDA with an overall proportion of correctly identified samples for their regions of 0.811, i.e 116 samples from 143 were correctly placed in the region of origin.

| | ***Auckland*** | ***Christchurch*** | ***Coromandel*** | ***Nelson*** | ***Okuru*** | ***Taumaranui*** | ***Taupo*** | ***West Coast*** | ***Whangarei*** |
|---|---|---|---|---|---|---|---|---|---|
| *Auckland* | **21** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Christchurch* | 0 | **9** | 0 | 0 | **1** | 0 | **1** | **2** | 0 |
| *Coromandel* | 0 | **3** | **13** | 0 | 0 | **2** | 0 | 0 | 0 |
| *Nelson* | 0 | **1** | 0 | **8** | 0 | 0 | 0 | 0 | 0 |
| *Okuru* | **1** | 0 | 0 | 0 | **12** | **2** | 0 | **1** | 0 |
| *Taumaranui* | 0 | 0 | **2** | 0 | **1** | **15** | 0 | 0 | 0 |
| *Taupo* | 0 | 0 | 0 | 0 | 0 | 0 | **15** | **3** | 0 |
| *West Coast* | 0 | **2** | 0 | 0 | 0 | 0 | **2** | **11** | 0 |
| *Whangarei* | **2** | 0 | 0 | 0 | 0 | 0 | 0 | **1** | **12** |
| *Total N* | 24 | 15 | 15 | 8 | 14 | 19 | 18 | 18 | 12 |
| *N correct* | 21 | 9 | 13 | 8 | 12 | 15 | 15 | 11 | 12 |
| *Proportion correct* | ***0.875*** | ***0.600*** | ***0.876*** | ***1.000*** | ***0.857*** | ***0.789*** | ***0.833*** | ***0.611*** | ***1.000*** |

Table 12 - LDA results for all regions apart from Hamilton (see explanation above) (non-cross validated)

[†††] A set of data in LDA is considered cross validated when the data is grouped by a set of rules not used to firstly create the groups. Thus, cross validated LDA is the more reliable/true analysis.

Table 13 includes the results for cross-validated LDA, with an overall proportion of correctly identified samples for their regions of 0.748; 107 samples from 143 were correctly placed in their region of origin.

| | ***Auckland*** | ***Christchurch*** | ***Coromandel*** | ***Nelson*** | ***Okuru*** | ***Taumaranui*** | ***Taupo*** | ***West Coast*** | ***Whangarei*** |
|---|---|---|---|---|---|---|---|---|---|
| *Auckland* | **21** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Christchurch* | 0 | **9** | **1** | 0 | **1** | 0 | **1** | **2** | 0 |
| *Coromandel* | 0 | **3** | **10** | **1** | 0 | **3** | 0 | 0 | 0 |
| *Nelson* | 0 | **1** | 0 | **7** | 0 | 0 | 0 | 0 | 0 |
| *Okuru* | **1** | 0 | 0 | 0 | **12** | **2** | 0 | **1** | 0 |
| *Taumaranui* | 0 | 0 | **2** | 0 | **1** | **13** | 0 | 0 | 0 |
| *Taupo* | 0 | 0 | **1** | 0 | 0 | 0 | **13** | **3** | 0 |
| *West Coast* | 0 | **2** | 0 | 0 | 0 | 0 | **4** | **10** | 0 |
| *Whangarei* | **2** | 0 | **1** | 0 | 0 | **1** | 0 | **2** | **12** |
| *Total N* | 24 | 15 | 15 | 8 | 14 | 19 | 18 | 18 | 12 |
| *N correct* | 21 | 9 | 10 | 7 | 12 | 13 | 13 | 10 | 12 |
| *Proportion correct* | ***0.875*** | ***0.600*** | ***0.667*** | ***0.857*** | ***0.857*** | ***0.684*** | ***0.722*** | ***0.556*** | ***1.000*** |

Table 13 - LDA results for all regions apart from Hamilton (see explanation above) (cross-validated)

Another way in which this LDA data can be visualized is by plotting it as canonical variates as in Fig. 34

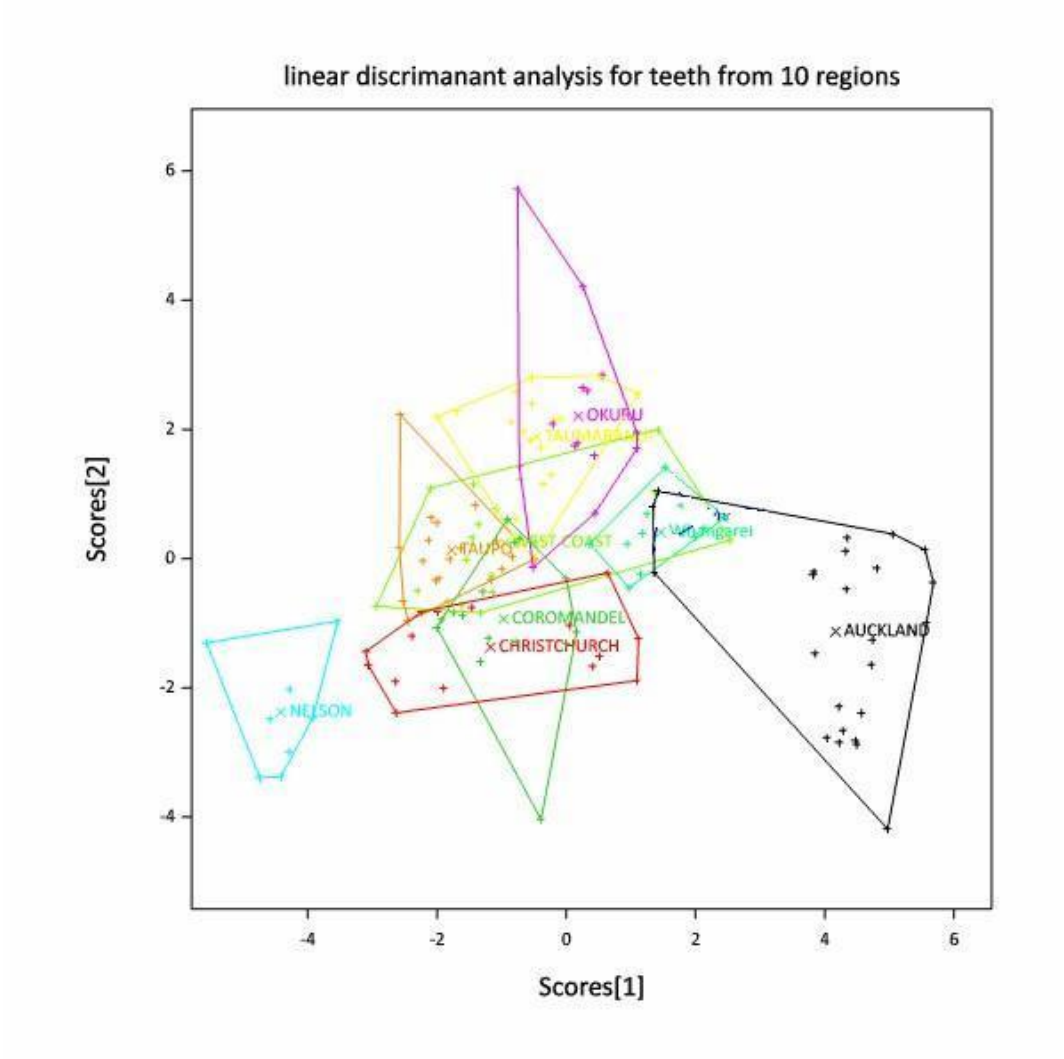


Figure 33 - LDA canonical variates for all ten regions

From this plot and using LDA several conclusions can be drawn:

- The Nelson region can be distinguished from the all other regions present using LDA.
- Auckland can be distinguished from Taumaranui, Christchurch, Okuru (Fiordlands), Taupo, and the Coromandel.
- Okuru (Fiordlands) overlaps significantly with several regions; Taumaranui, West Coast, Coromandel, Taupo, but can be separated from Auckland, Whangarei, Christchurch and Nelson.
- Christchurch and Coromandel overlap significantly, and Christchurch also slightly overlaps with the West Coast and Taupo. However it can be

distinguished from Auckland, Nelson, Taurmaranui, Okuru, and Whangarei.

- Coromandel overlaps significantly with Christchurch, Taupo, West Coast, Okuru and Taumaranui, but can be distinguished from the other regions.
- Whangarei overlaps with Auckland and the West Coast.
- Taumaranui overlaps with Okuru, West Coast, Taupo and Coromandel.
- The West Coast overlaps with all regions, apart from Nelson.
- Taupo can be distinguished from Nelson, Auckland, and Whangarei.
- On average the proportion of samples that could be correctly identified was 0.7575

Although there is significant overlap, these results are still useful as they can be used to narrow the possible regions from which samples could originate.

Canonical Discrimination Analysis (CDA) (a type of LDA) was also carried out on human bone and tooth samples by Castro *et al.*[13] The result from this analysis shows that 42.7% of the samples could be correctly classified, without cross validation. Approximately 50% of samples when analysed 'blindly' (cross validated) could be correctly grouped.[13] In comparison, in the current project, 81.1% of samples could be correctly grouped (without cross validation) and 74.8% of the samples could be correctly classified when cross validation was used. This shows that the samples analysed in this project are more distinct from each other, than those used in the project carried out by Castro *et al.*[13] and also illustrates the promising nature of this project.

## 4.8 Soil type in relation to initial results

The soil types per region were discussed in chapter 2. This section examines whether any conclusions can be made relating the types of soils present in a particular region, and the results of the LA-ICP-MS analysis of tooth enamel from that same region.

### 4.8.1 Area type

The type of area from which samples were taken, such as farmland, bushland, or city areas plays an integral part in the types of results obtained.

- Fertilization of soils appears to be important in determining which elements would be expected to be found in the tooth enamel. If samples came from bush lands, that would have undergone little addition of fertilization, it would be expected that some trace elements would be deficient, i.e. below the MDL, unless the region in question had high natural levels of a particular trace element.

- However, if the samples were taken from farmlands, one could expect a number of trace elements to be present, which under natural bush conditions would not be. Cd and P for example are elements present in fertilizers, and As, Pb and Cu are present in insecticides.

- If samples were obtained within city areas the elements found in the enamel of these possums could reflect a number of possibilities: the true natural elemental composition of the soils; modified elemental composition of soils created through composting; or completely altered elemental composition due to possums feeding on human rubbish that could be sourced from all over the world.

### 4.8.2 Whangarei/Northland

When the possum tooth enamel samples from this region were analysed using LDA, 100% of the samples could be correctly grouped into the Whangarei region. This definite differentiation of Whangarei from the other regions could be due to the fact that Whangarei region incorporates many diverse types of soils, many more than any other region in New Zealand; thus, the elements found in the possum tooth enamel are more diverse than other regions.

However, the LDA (when plotted against other regions) also indicated that the elements found in the possum tooth enamel is significantly similar to samples taken from the Auckland/ Waiuku region, and West Coast region region (see Fig.

34), as these regions had samples incorrectly identified as being from the Whangarei region. The West Coast, Auckland and Whangarei regions are all close to the sea, thus, Na, which was a large contributing element, could be similar for these regions which may have influenced these results. Also because Whangarei has many different soil types the plotted LDA may be reflecting the fact that the Whangarei possums were exposed to many different soils, some of which happen to be the same as the overlapping regions.

Although some overlap occurred, possums taken from the Whangarei region could be significantly distinguished from other regions.

### 4.8.3 Auckland/Waiuku region

According to the LDA of this region 87.5% (21 of 24 samples) of possums taken from the Auckland/Waiuku region could be correctly grouped in that region. 2 of the samples that could not be correctly grouped were grouped into the Whangarei region, which as explained above (see section 4.8.2) could be due to the diverse soils types of the Whangarei region and/or the proximity to the sea which influences the amount of Na present, and this has a significant contribution to analyses. The one other sample that was not correctly identified as being from the Auckland/Waiuku region was grouped as being from the Okuru/Fiordland region. This again could be due to the proximity to the sea.

However, when the LDA is plotted with all the other regions (Fig. 34) the Auckland region is almost completely separated from the other regions with just a slight overlap with Whangarei (see explanation above). The separation of the Auckland/Waiuku region could be due to the fact that the soils are considered geologically different from many of the other regions analysed[59]. This is due to the contribution from the sea and the basalt soils found there.

Although some elements were not included in further analysis after the MDL analysis, due to their low levels of detection, one can still draw some conclusions using the raw MDL data. However, no definite conclusions can be drawn from using this raw, non-statistically analysed data. It is interesting to note that this

region is considered to be cobalt deficient which is supported by the MDL data which shows that only 10.5% of readings could detect the presence of Co.

### 4.8.4 Coromandel Region

The initial LDA for the Coromandel showed that it was one of the least successful regions in terms of grouping into the correct area. Only 66.7% of the samples (10 from 15) could be correctly identified as being from this region. The five samples not able to be correctly identified as being from Coromandel were classified into four different regions; Taumarunui (2 samples), Taupo, Whangarei and Christchurch. However, this could be due to the fact that the Coromandel contains diverse soil types (see section 2.1.4), and as explained for the Whangarei samples this could mean samples taken from the Coromandel may have fed from soil types similar to or the same as other regions. Also as the Coromandel region is known to have soil originating from volcanic activity, as do the Taumarunui and Taupo regions, one might expect some similarities between samples from these regions. The similarity between Coromandel and Christchurch could be attributed to the influence of the nearby rivers and seas in both regions' collection areas as previously discussed in section 5.3.2.

When the LDA is plotted, overlap mainly occurs between Coromandel and Christchurch, with slight overlapping occurring with Taumarunui, Taupo, which can be explained by the same reasons given above. Overlap is also present with the West Coast, which again could be indicative of the contribution of the sea to elemental composition.

This region is considered to be cobalt deficient which is supported by the MDL data which shows that only 22% of readings could detect the presence of Co.

### 4.8.5 Hamilton/Waikato region

As already discussed (section 2.1.3) the Waikato/Hamilton region contains allophone soils, which are known to absorb phosphorus readily. Low phosphorus in Hamilton could, therefore, be attributed to these soils. Although fertilization is usually applied to overcome this type of problem, within the city (where many of

the samples came from) fertilization may not have taken place. This could have resulted in lower phosphorus levels in the soils, and therefore in the possum tooth enamel samples taken from this region. However, as phosphorous is one of the main constituents of calcium hydroxyapatite, even if the soils were low in phosphorus, the levels within teeth should still be significant enough, to be detected.

Also, as the possum samples came for within city limits, one would also expect some of their diet to be made up of human rubbish, which could have come from any place in the world. Thus, the elemental composition of the possum teeth from this Hamilton region, are quite possibly not very useful for the project. However, one should also note here that the PCA variation within the region appeared to be quite small. However, this could be due to all the possums from this region eating foods sourced from around the world, thus representing the same collection of food sources, rather than the same soil type. It also could mean that the few values that were detected, were of similar values. However, it is impossible to know if the phosphorus levels in all the Hamilton samples (those detected and those that were not) were similar. Due to its low phosphorus readings Hamilton was excluded from the both LDA as the data obtained was most probably not representative of the soils in this region.

### 4.8.6 Central North Island/Taupo region

The LDA of the Taupo samples correctly identified 72.2% (13 of 18) of samples as being from the Taupo region. Four of the five samples not correctly identified as being from Taupo were classified as being from the West Coast region. As the West Coast tends to be Greywacke soils (which are similar to $SiO_2$ type soils of the Taupo region) influenced by the proximity to the sea, this overlap of the two regions does appear to be soil related. There are also mountain ranges and lakes in both regions, which may greatly influence the elements present in both regions soils. This may have caused some samples from both regions to be incorrectly identified as being from the other region in question[‡‡‡]. When the LDA was

[‡‡‡] Interesting to note is that both the West Coast region and the Taupo region have samples incorrectly identified as being from the other region. Both also had one sample incorrectly classified as being from the Christchurch region. One could therefore conclude that these three

plotted, overlap of the Taupo region was predominately with the West Coast region, the possible reasons for this being explained above.

The MDL data showed that both Co and Cd have low detection in this region, with only 15.3% and 6.94% respectively being detected. This is expected as this region is known for its Co deficiency, and the region is often fertilized with Co to try and combat this. However, the area that these samples were taken from were not farm land, but were bushland, thus the addition of fertilizer to the land is unlikely, hence the low levels of detectable Co. However, what was unexpected was the low level of arsenic present. As Taupo is in a volcanic area, one would expect high levels of As present. However, unexpectedly As was detected only 2.7% of the time for the Taupo region (i.e. As was below the MDL ~97% of the time).

### 4.8.7 Taranaki/ Taumarunui region

The LDA for Taumarunui only correctly identified 68.4% (13 of 19) samples as being Taumarunui samples. Three samples were identified as being from the Coromandel region, and one was identified as being from the Whangarei region. This may be due to the diverse soil types of these two regions as explained previously (5.3.2 and 5.3.4). Two samples were also identified as being from the Okuru region. This is most probably due to the fact that the Okuru region has predominately Greywacke soils present which are similar to pumice, $SiO_2$ soils, which are prevalent in the Taumarunui region.

When the LDA was plotted, Taumarunui overlaps Okuru significantly, further suggesting the similarity of the soils from these regions. Thus one could conclude that it is not possible to successfully distinguish samples between these two regions. Overlap also occurred with the West Coast which is expected as the soil types of Okuru and the West Coast region are almost identical.

regions are significantly similar when LA-ICP-MS data is processed using LDA (as in this project) is carried out.

Co and Cu, like the Taupo region, were mainly undetectable as only 13.9% and 17.1%, respectively, were above the MDL. This is expected as the Taupo region and Taumarunui regions are close together, and are geologically similar in terms of the deficient elements.

### 4.8.8 Nelson/Marlborough region

In the LDA, Nelsons samples were correctly grouped 85.7% of the time (7 of 8 samples). Only one sample was incorrectly identified as being from the Coromandel region. Nelson was the region with the least amount of samples analysed, thus, the results may not be as comprehensive and informative as the other regions. The similarity between Nelson and the Coromandel, which may explain why one sample was incorrectly identified as being from there, could be due to two factors. Firstly, the proximity of both areas to the sea, and secondly the diversity of the Coromandel soils as explained above (5.3.4). When the LDA was plotted, the Nelson region had no overlap with any other regions, and is the most separated region.

This region is known for its orchards, some of which have been in the region for many decades. As a result of this, insecticides containing heavy metals such as As, Pb and Cu were used in this region before the environmental impacts of using heavy metals were known. As a consequence of this, the Nelson region had the highest rate of As detection (25%) and reasonably high detection of Cu (38.9%) and Pb (58.4%). Again, no definite conclusions can be drawn (see section 4.8.3), using the MDL raw data, but the trends are interesting to note. However, these elements were not included in the LDA analysis, thus the pesticides, and agriculture of this area will not affect the outcome of the LDA. This is important to note, as modern interferences with soil and the environment would not have been present when upoko tuhi were produced, thus obtaining results that are uninfluenced by modern interferences is imperative.

An unexpected finding was the low detection of Si in this region. As the Nelson region is close to the sea and is mainly made of Greywacke, $SiO_2$ pumice like soils, the Si content should be high. However, only 13.9% of the time was Si detected. This may have greatly affected the analysis of the Nelson region,

causing the region to be separated from the other regions, which mostly have high Si levels.

### 4.8.9 Christchurch region

The LDA of Christchurch revealed that 60% (9 of 15) of samples could be correctly identified as being from this region. Three were identified as being from the Coromandel region, which, again, is most probably due to the diverse nature of the soils present in the Coromandel and the proximity to the sea of both regions. The West Coast, Nelson and Christchurch regions contain Greywacke soils, are close to the sea and are in close proximity to each other. Thus, it is not surprising that two samples were incorrectly identified as being from the West Coast and one was grouped as being from Nelson rather than from Christchurch.

When the LDA was plotted Christchurch was reasonably separated from the other regions, apart from the Coromandel, the reason for which is explained above.

### 4.8.10 West Coast of South Island

The LDA of the West Coast region gave the worst results of all the regions for correctly grouping the samples. Only 55.6% (10 of 18) were correctly grouped into the West Coast region. Two samples were classified as Christchurch, reasons for which are already explained (5.3.9). One sample was identified as being from the Okuru region, and as already explained (5.3.7) is also expected. Two samples were grouped as Whangarei, which could be due to the diverse soil types of the Whangarei region and its proximity the sea, similar to the West Coast. Three samples were classified as being from the Taupo region which is probably a result of the volcanic influenced soils of both regions.

When the LDA was plotted, the West Coast was the most overlapped of all regions, and thus was the most undistinguishable. Consequentially, the West Coast region does not afford any useable information for separating regions by elemental composition.

In the West Coast only small amounts of fertilizers are used, and it was shown that the West Coast was the second lowest region for P detection. It also had low

detection of Mg, Na, Ba, and Cu in comparison with other regions which may be a consequence of the small amount of fertilization occurring.

### 4.8.11 Fiordland/ Okuru region

The Okuru region was reasonably well classified when analysed using LDA. 87.5% (12 of 14) of the samples were correctly classified. One sample was grouped into the Christchurch region and the other into the Taumarunui region. As already explained (5.3.9 and 5.3.7) Christchurch and Okuru share similar soil types and proximity, not only to each other but also to the sea, which may explain the mis-grouping in this case. Taumarunui region and Okuru are both close to mountain ranges, thus, the pumice-like soils may have contributed to the grouping of one of the Okuru samples into the Taumarunui region. When the LDA was plotted, the same result occurred where overlap between Okuru, Christchurch and Taumarunui occurred.

## 4.9 LDA of selected regions

It was decided that the results discussed above could be further narrowed. Firstly, by again removing regions where the data obtained is of questionable quality (Hamilton- See section 4.7 and 4.8.5 for further explanation). Secondly, if historical information is taken into account (Sections 1.2 and 2.1), two more regions can be removed, Okuru/Fiordland and the West Coat of the South Island, as it is unlikely upoko tuhi would have originated from these areas.[5]

Tables 14 and 15 contain the results of the LDA for selected regions (Auckland, Christchurch, Coromandel, Nelson, Taumaranui, Taupo, and Whangarei). Table 14 contains the results for the non-cross validated LDA with an overall proportion of correctly identified samples for their regions of 0.901, which is a significant improvement to the initial LDA of 0.811.

| | *Auckland* | *Christchurch* | *Coromandel* | *Nelson* | *Taumaranui* | *Taupo* | *Whangarei* |
|---|---|---|---|---|---|---|---|
| ***Auckland*** | 21 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Christchurch*** | 0 | 11 | 0 | 0 | 0 | 0 | 0 |
| ***Coromandel*** | 0 | 3 | 13 | 0 | 1 | 1 | 0 |
| ***Nelson*** | 0 | 1 | 0 | 8 | 0 | 0 | 0 |
| ***Taumaranui*** | 0 | 0 | 1 | 0 | 18 | 0 | 0 |
| ***Taupo*** | 0 | 0 | 1 | 0 | 0 | 17 | 0 |
| ***Whangarei*** | 3 | 0 | 0 | 0 | 0 | 0 | 12 |
| ***Total N*** | 24 | 15 | 15 | 8 | 19 | 18 | 12 |
| ***N correct*** | 21 | 11 | 13 | 8 | 18 | 17 | 12 |
| ***Proportion correct*** | ***0.875*** | ***0.733*** | ***0.876*** | ***1.000*** | ***0.947*** | ***0.944*** | ***1.000*** |

**Table 14- LDA results for selected regions (non-cross validated)**

Table 15 includes the results for cross-validated LDA of selected regions, with an overall proportion of correctly identified samples for their regions of 0.829, which again is a significant improvement to the initial cross validated LDA, which had a correctly identified proportion of samples of 0.748.

| | *Auckland* | *Christchurch* | *Coromandel* | *Nelson* | *Taumaranui* | *Taupo* | *Whangarei* |
|---|---|---|---|---|---|---|---|
| ***Auckland*** | 21 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Christchurch*** | 0 | 11 | 2 | 0 | 0 | 0 | 0 |
| ***Coromandel*** | 0 | 3 | 9 | 1 | 2 | 1 | 0 |
| ***Nelson*** | 0 | 1 | 0 | 7 | 0 | 1 | 0 |
| ***Taumaranui*** | 0 | 0 | 2 | 0 | 16 | 0 | 0 |
| ***Taupo*** | 0 | 0 | 1 | 0 | 0 | 16 | 0 |
| ***Whangarei*** | 3 | 0 | 1 | 0 | 1 | 0 | 12 |
| ***Total N*** | 24 | 15 | 15 | 8 | 19 | 18 | 12 |
| ***N correct*** | 21 | 11 | 9 | 7 | 16 | 16 | 12 |
| ***Proportion correct*** | ***0.875*** | ***0.733*** | ***0.600*** | ***0.875*** | ***0.842*** | ***0.889*** | ***1.000*** |

**Table 15-LDA results for selected regions (cross validated)**

Again, this information can be plotted. By removing the selected regions from the original LDA, less overlap occurs and the results obtained are illustrated in Fig. 35.

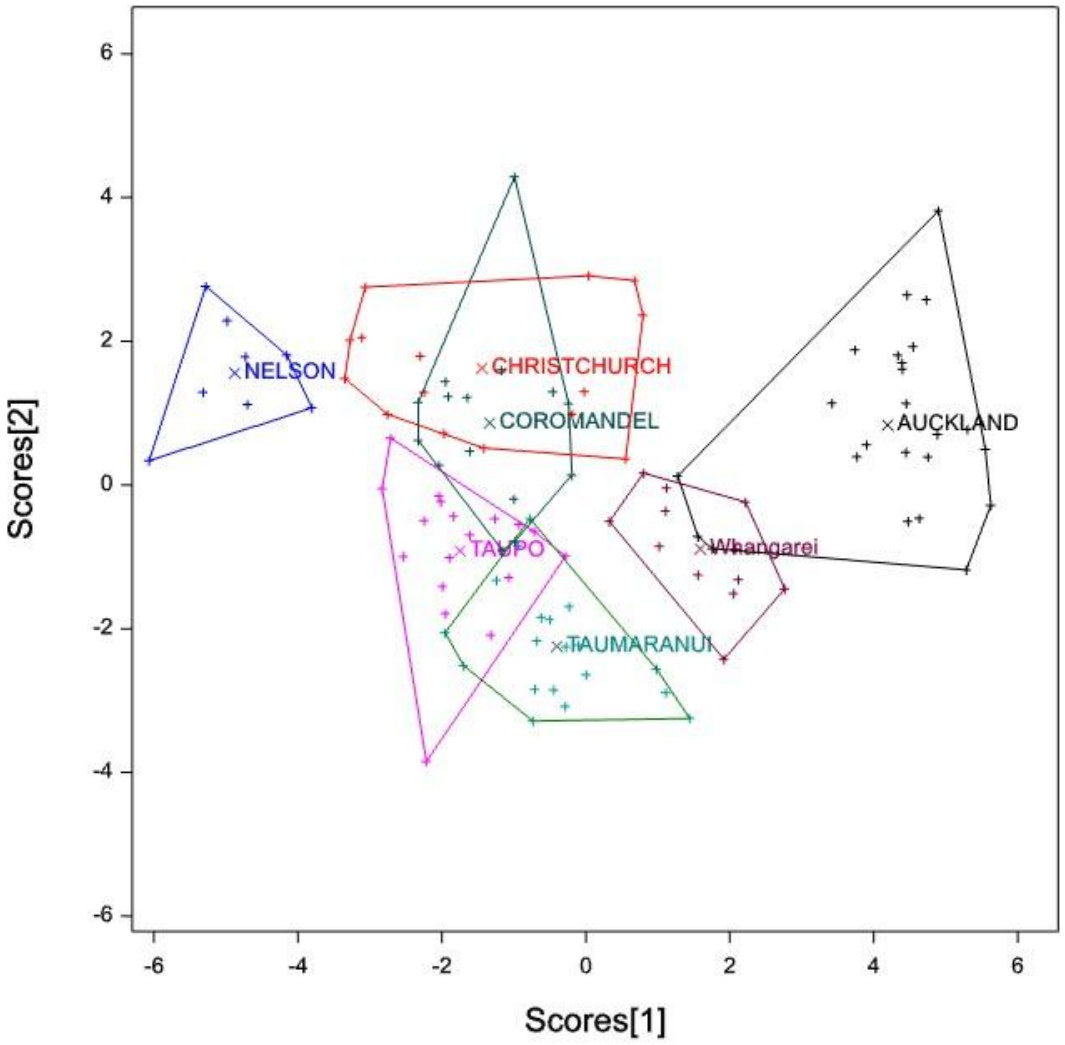


**Figure 34- LDA canonical variates for selected regions**

From this plot several conclusions can be drawn:

- Nelson is still able to be separated from all the other regions
- Whangarei and Auckland are mostly separated, apart from slight overlap with each other.
- Taupo and Taumaranui still overlap with each other, which is to be expected as their geology is similar.
- Coromandel and Christchurch are significantly overlapped.

- There is a slight overlap of the Coromandel and Taupo regions.
- Overlap in this LDA is significantly decreased compared with that of the initial LDA (see section 4.7).

The most important conclusion that can be drawn from this plot is that one is able to place an unknown sample into its correct region the majority of the time if further information is taken into account.

- If a sample was known to be from the North Island, but fell into the overlap between Christchurch and the Coromandel regions, one could eliminate Christchurch automatically and therefore deduce the sample originated from Coromandel.
- The overlap between Whangarei and Auckland is not important as the two areas from which samples were collected fall under the same tribal governance of Te Taitokerau[60].

The only problem that remains is that the Taupo and Taumaranui regions still overlap. Although, these regions are adjacent to each other geographically, there are two different tribal areas governing these two areas.[60] If applied to the upoko tuhi situation and the unknown upoko tuhi sample fell into the overlap of these regions, it could not be determined which iwi the upoko tuhi belongs. However, as the overlap is slight, one could conclude that that situation is unlikely to occur, and if it did, other information, like the type of moko present on the upoko tuhi might be used to distinguish between the regions.

As already discussed (section 4.7) CDA was also carried out on human bone and tooth samples by Castro *et al.*[13] The result from this analysis, shows that 42.7% of the samples could be correctly classified, without cross validation. Approximately 50% of samples when analysed 'blindly' (cross validated) could be correctly grouped.[13] In comparison, in the current project, once historical information is taken into account, 90.1% of samples could be correctly grouped (without cross validation) and 82.9% of the samples could be correctly classified when cross validation was used. This shows that the samples analysed in this project are more distinct from each other, than those used in the project carried out by Castro *et al.*[13] and also illustrates the promising nature of this project and

illustrates how by taking into account historical information, the success rate of placing samples into their geographical region of origin increases significantly.

## 4.10 Suggestions for further research

As the work carried out for this thesis was the first step in what could be an overall larger project, there are many areas that could be improved, or further examined in order to gain further conclusions. One area that needs a lot of additional assessment is the sample type. While it has been discussed previously (Chapters 1, 2 and 3) that possum tooth enamel is well suited to this project, this may be an over simplified way of viewing the sample type, and further experimental work could be done:

- Most experiments benefit from large sample number analyses. Thus, an improvement on the work carried out here is obviously to increase the number of samples collected and analysed. This is in the hope of decreasing the variation within a region, and increasing the variation between regions, to give more definite conclusions of where samples originated from.

- Fertilization of the land in areas from which possums were collected is also a key issue when discussing sample type. Research should be conducted into whether the elemental composition of tooth enamel from possums differs notably if samples of enamel are analysed that were formed shortly after fertilization of soil occurred, in comparison to enamel formed a long time after fertilization. One would expect that the enamel would reflect the timeframe of fertilization, so that samples should only be taken from a select timeframe occurring around soil fertilization. However, it should be further noted that collecting possums tooth samples was difficult enough without this requirement, so this restriction may not be feasible.

- One notable conclusion that can be made from the data collected is the significant variation between teeth in the same jaw, within the same region.

This could be due to different types of teeth within the same jaw being used for analysis. Possums, like other rodents, contain open rooted incisors, which grow throughout life. For this study these were not examined, as they would only reflect what the possum has been eating lately, not what its diet was made up of at birth and during infancy, which is what this project focused on. However, as three molar teeth were used from each jaw, teeth from different positions within the jaw were analysed. Additional research could be conducted that analyses the difference between molar teeth of the same jaw, to determine whether it would be beneficial to only analyse one certain position of tooth per jaw. This would hopefully decrease the amount of variation between teeth of the same jaw. Also, when choosing which tooth to analyse, it would be beneficial to examine the amount of wear on each tooth, and choose the one that looks the least worn. One would expect that this would correspond to the tooth with the most enamel intact, thus making analysis by laser ablation more successful.

- An Examination of whether the post-mortem treatment of sample affects the results obtained is also needed. Similar previous work has been carried out, and showed that enamel, due to its highly mineralized state, undergoes little change once the mammal has died, and been buried (see section 1.5.1.1).[29] However, this research was not carried out on possums, so further research would be needed to confirm this result. If it was determined there was significant difference between freshly deceased samples, and older, buried samples, then only one type would be used for further research. However, as the overall aim of this project is to use this method to identify the region of origin for Toi Moko, which are all old, archaeological samples, and none of which have been buried, this factor would not affect analysis of the Toi Moko.

Improvement can also be made to the method. An issue that was identified with the experiment was that data from different regions was analysed on different days. Although the NIST 612 standard was used to monitor instrument drift over each day, it is possible that even slight change to the NIST 612 (which may

appear trivial when using the NIST 612 as a marker) may influence the results of the enamel samples significantly. Thus, if one day of analysis is considerably influenced, this would correlate to one whole region being considerably influenced, as each region was analysed on a different day. An improvement to the method, therefore, would be to analyse a tooth from every region on a given day, rather than a whole region at a time. This would overcome the problem, as if one day was affected by the instrument, it would affect all regions equally.

Another improvement to the study would be to also analyse soil and plant samples by LA-ICP-MS from each region samples were taken from. This was done in the work carried out by Copeland *et al.*, which analysed rodent teeth, and the plants from the same region.[37] That study found a 2-sigma correlation for 29 of the 30 tooth samples between the strontium levels in tooth enamel and strontium levels in the plants. By analysing the soils and plants (specifically native flora) by the same method, one could then make correlations between the elemental compositions of:

- enamel and soils
- enamel and plants
- soils and regions
- soils and plants
- regions and plants

Also tribal areas may be quite different to the areas analysed here. For example, the Ngai Tahu tribal area spans the majority of the South Island, however, four different regions were analysed. Further discussion needs to be made on how much separation is needed- merely between Iwi (tribe), or further still to the Hapu (sub tribe) or even Whanau.

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